Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

Gwangju Institute of Science and Technology

2020

한국생체분자과학 연합학회학술대회

Time *Jan. 09~11, 2020*

Venue *GIST 오룡관*

한국생물물리학회
The Korean Biophysical Society

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

Contents

Organizing Committee

1. 조직위원회 (위원장 : 박철승 , GIST**)**

위원 : **김명희** (KRIBB), **이지오** (POSTECH),
최선 (이화여대), **정상전** (성균관대)

2. 운영위원회 (위원장 : 임현호, KBRI**)**

위원 : **이상호** (성균관대), **진미선** (GIST),
차선신 (이화여대), **이현수** (서강대)

3. 학술위원회 (위원장 : 황선욱, 고려대 의대**)**

위원 : **이형호** (서울대), **최희정** (서울대),
김승중 (KAIST), **이현수** (서강대)

4. 재무위원회 (위원장 : 이은희 , 가톨릭 의대**)**

위원 : **이상호** (성균관대), **진미선** (GIST),
이준혁 (KOPRI), **김낙균** (KIST)

5. 행정/문의 : 한혜민 (GIST, 062-715-2566)

한국결정학회 Korean Crystallographic Association
한국구조생물학회 KOREAN SOCIETY FOR STRUCTURAL BIOLOGY
한국단백질학회 Korean Society for Protein Science
KCS 대한화학회 KOREAN CHEMICAL SOCIETY
한국생물물리학회 The Korean Biophysical Society

Thursday (Jan. 9)

Registration	10:00 – 13:00
Opening Remarks (303호) 한국생물물리학회 회장 박철승 (GIST)	13:00 – 13:10

Plenary Lecture 1 (303호) (Chair :김명희, 한국생명공학연구원)		
오병하 (KAIST)	Computational protein design and its application to drug discovery	13:10 – 13:55

Parallel Symposia		
Symposium I (303호)	**Symposium II** (101호)	
GIST-Created New Horizon (Chair : **이광록**, GIST)	**Computational Approaches for New Molecular Findings** (Chair : **유우경**, DGIST)	
김정욱 (GIST) Structural basis for th activation and the activity of phosphatidylserine decarboxylase in the biosynthesis of phospholipids	**김윤학** (부산대) ESurv: a user-friendly web server for integrative survival analysis	
남호정 (GIST) AI-powered drug discovery and development	**최정모** (KAIST) The stickers and spacers framework for describing phase behavior of multivalent intrinsically disordered proteins	14:00 – 15:40
진미선 (GIST) The structural basis of the low catalytic activities of the two minor β-carbonic anhydrases of the filamentous fungus Aspergillus fumigatus	**천무경** (KBRI) A practical application of generative adversarial networks for RNA-seq analysis to predict the molecular progress of Alzheimer's disease	
이광록 (GIST) Chemical friction along the minor groove of DNA facilitates enzymatic translocation of λ exonuclease via electrostatic ratchet	**최선** (이화여대) Comprehensive mechanistic studies for the structural and functional elucidation of a tetraspanin arginine sensor	
한국생물물리학회 (101호), **구조생물학회** (201호)**총회 및 Poster session**		15:40 – 16:50

Parallel Symposia		
Symposium III (303호)	**Symposium IV** (101호)	
New Horizons in Membrane Signaling and Trafficking (Chair : **엄수현**, GIST)	**Ionic Transport Mechanism** (Chair : **강동묵**, 성균관대)	
최희정 (서울대) Biophysical and functional characterization of Frizzled4-mediated Norrin signaling	**임현호** (KBRI) Coupled ion-transport mechanism in a CLC-type Cl-/H+ antiporter	
임영준 (전남대) Structural Mechanism of Intracellular Sterol Distribution by Lipid Transfer Proteins	**이병철** (KBRI) Ion and lipid transport via TMEM16 scramblases	16:50 – 18:30
김한성 (부경대) A Catalytic Trisulfide in Human Sulfide Quinone Oxidoreductase Catalyzes Coenzyme A Persulfide Synthesis and Inhibits Butyrate Oxidation	**강경진** (성균관대) Analysis of Phototoxin Taste Closely Correlates Nucleophilicity to Type-I Phototoxicity	
정가영 (성균관대) Time-resolved Conformational Analysis during GPCR-Gs Coupling	**홍규상** (KIST) Cytoskeleton dependent activation of Tentonin 3/TMEM150C, a novel mechanosensitive channel	
Reception & Poster Session		18:30 – 20:00

Friday (Jan. 10)

Parallel Symposia		
Symposium V (303호)	**Symposium VI** (101호)	
New Approaches in Structural Biology (Chair : **이원태**, 연세대)	**Biomolecular Engineering** (Chair : **이현수**, 서강대)	
우재성 (고려대) Cryo-EM Structure of Human Cx31.3/GJC3 Hemichannel	**정용원** (KAIST) Supramolecular Biochemistry: engineering artificial protein assemblies	09:00 - 10:40
류제경 (TU Delft) AFM imaging of open and collapsed states of yeast condensin suggest a scrunching model for DNA loop extrusionHemichannel	**조규봉** (서강대) Visualization of A/T Specific Sequence on a Large DNA Molecule	
박석열 (포항가속기연구소) BL11C: a High-flux Micro-MX beamline for micron-sized protein crystals at PAL	**강세병** (UNIST) Development of Recombinant Secondary Antibody Mimics as Signal Amplifiers in Immunoassays	
김경현 (고려대) Time-resolved pH-induced protein dynamics using serial femtosecond crystallography	**안대로** (KIST) A library-based approach to develop tumor-targeted drug carriers	
Coffee Break		10:40 - 11:00

Plenary Lecture 2 (다산홀) (Chair : **김경규**, 성균관대)		
Genji Kurisu (PDBj, Osaka Univ.)	Structural Basis for the Ferredoxin-dependent Photosynthetic Electron Transfer	11:00 - 11:55

Luncheon Seminar		
PDBj (303호)	**Tomocube** (101호)	12:00 - 12:50
Dr. Atsushi Nakagawa (PDBj, Osaka Univ) OneDep: Unified System for Deposition, Biocuration, and Validation of Macromolecular Structures **Dr. Nobutoshi Ito (PDBj, Tokyo Medical and Dental Univ.)** Mandatory submission of PDBx/mmCIF files for crystallographic depositions to the PDB	**이수민 (토모큐브)** Holotomography Techniques For Non-invasive Label-free 3D Imaginig Of Live Cells And Materials	12:00 - 12:50

Parallel Symposia		
Symposium VII (303호)	**Symposium VIII** (101호)	
Cutting-Edge Technologies for Next-Generation Biomedicine (Chair : **정상택**, 고려대)	**Young Scientist Session** (Chair : **최명환**, 성균관대 **강진영**, KAIST)	
유동원 (서울대) Nanomaterials for Bioimaging and Therapeutics	**박성준** (KAIST) Special Talk: Multifunctional Neural Interfaces Technologies	
서지원 (GIST) Engineering natural peptides for discovery of novel anti-infectives	**유다슬이** (KAIST) Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins	13:00 - 15:05
예경무 (DGIST) Translational application of infectious libraries of human antibodies	**김지원** (POSTECH) Application of anti-helix antibodies in protein structure determination	
정우재 (성균관대) Multivalent Sialyl Ligands and Their Use in Influenza A Virus Entry Inhibitors	**이수빈** (POSTECH) A Small-Molecule Identified via Forward Chemical Genetics Selectively Kills Cancer ells	
김찬혁 (KAIST) Towards next-generation T cell therapy for Cancer	**허수경** (이화여대) The Effects of Tumor Suppressor INPP4B Oxidation on Actin Polymerization in Glioma Cells	

	김희령 (성균관대) Conformational Analysis of Gi/o α Subunits in GPCR Coupling	
	강민희 (고려대) Solution Structure of the Nucleotide hydrolase BlsM: Importance of its Substrate Specificity	
	민경진 (서울대) Structural and functional characterization of a peptidoglycan reshaping peptidase in intestinal pathogens	
	홍승곤 (KBIO) Structural investigation of Micromonospora echinospora GenB1; relationship between productivity and substrate preference	
Coffee Break		15:05 – 15:25

Plenary Lecture 3 (다산홀) (Chair : **정상전**, 성균관대)		
장영태 (POSTECH, IBS)	Strategy for Cell Discrimination using Fluorescent Probe	15:25 – 16:10

Parallel Symposia		
Symposium IX (303호)	**Symposium X** (101호)	16:15 – 17:55
Emerging Technologies on Protein Science (Chair : **김승중**, KAIST)	**Neuroscience and Imaging** (Chair : **김성현**, 경희대)	
박지용 (KAIST) Advanced MD Simulations that Quantify Target–Inhibitor Interactions: Case Studies of EGFR Kinase Inhibitors and Androgen Receptor Antagonists	**장재범** (KAIST) Expansion microscopy techniques and their applications to reveal fine molecular detailss	

김진해 (DGIST) The structural features of transthyretin in its aged aggregation–prone state	**최명환** (성균관대) Comprehensive functional screening of taste sensation in vivo	
송은주 (이화여대) Phosphorylation of USP15 and USP4 Regulates Localization and Spliceosomal Deubiquitination	**김진섭** (성균관대) Anatomy and connectivity of the cerebellum revealed by electron microscope images	
김민규 (KAERI) Inhibitory Activity of Nucleotide–based Metabolites Against Class C β–Lactamases	**김성현** (경희대) Distinct property of presynaptic physiology between excitatory and inhibitory neurons	
Special Remarks (다산홀)		18:00 – 18:30
박철승(GIST) 한국생물물리학회 회장 **나명수**(UNIST) 한국결정학회 회장		

Saturday (Jan. 11)

Networking & Small Group Discussion	09:00 – 12:00

| M | E | M | O |

Federation
of the Korean Societies for
Biomolecular Sciences
(FKSBS)

Federation
of the Korean Societies for
Biomolecular Sciences
(FKSBS)

2020

한국생체분자과학 연합학회학술대회

Plenary Lectures

Byung-Ha Oh, Ph.D.

Professor

Department of Biological Sciences, KAIST

Phone: +82- 42-350-2648 Email: bhoh@kaist.ac.kr

Education

1983 B.S., Food Science & Engineering, Seoul National University, Korea

1985 M.S., Protein Engineering, Seoul National University, Korea

1989 Ph.D., Biophysics, University of Wisconsin-Madison, USA

Work Experience

2018.02 – 2019.02 Visiting professor, Institute of Protein Design, University of Washington, USA

2016.07 – Present Member, Board of directors, Seo Kyung Bae Science Foundation

2015.05 – 2017.08. Director, KAIST BK21+ Biological Research Center

2015.05 – 2017.06. Chairman, Department of Biological Sciences, KAIST

2009.09 – Present Professor, Department of Biological Sciences, KAIST

2000.10 – 2009.05. Director, Center for Biomolecular Recognition (Designated by Ministry of Science and Technology)

1994.09 – 2009.08. Assistant/Associate/ Full Professor, Dept. of Life Sciences, POSTECH

1993.03 – 1994.08. Senior Investigator, SmithKline Beecham Pharmaceuticals, USA,

1990.03 – 1993.02. Postdoctoral fellow, University of California-Berkeley

Research Interest

Protein engineering and biophysics

Selected Publications

1. Kwak, M.-J.#, Kim, D. G., Kim, H. M., Kim, C. H., James W. B., Kim, S. H., Joo, K. H., Lee, J. Y., Jin, K. S., Kim, Y.-G., Lee, N. K., Jung, J. U., Oh, B.-H.* Architecture of the Type IV coupling protein complex of Legionella pneumophila. Nat. Microbiol. 2, 17114 (2017).

2. Marie-Laure, D.-D.#, Lee, H. S.#, Laura, B. R. A.#, Noh, H. M., Shin, H.-C., Im, H. R., Florian P. B., Frank, B., Alexandre, D., Alrun, B., Ham, S. H., Jérôme, B., Oh, B.-H.*, Stephan, G.*, Structure of full-length SMC and rearrangements required for chromosome organization, Mol. Cell 67, 334-347 (2017). [#co-first, *co-corresponding authors]

Computational protein design and its application

Byung-Ha Oh

Department of Biological Sciences, KAIST, Daejoen, 34141, Korea
**E-mail: bhoh@kaist.ac.kr*

In the endless protein fold space, nature has sampled only a “spot” of the space. Protein design with the ROSETTA software suite enables sampling of enormous protein fold space that has not been explored by nature. Since the function of a protein is determined by the three-dimensional structure of the protein, billions of new proteins with a novel function, in principle, can be generated by designing new protein folds. I will introduce a number of examples of de novo protein design and protein-protein interface design by this computational approach with aims to create novel proteins for therapy or diagnosis. I will also discuss computational exploration of the amino acid sequences of the ‘complementary determining regions’ of antibodies that are hardly accessible by conventional library search or in vivo hyper mutations.

References

Rosetta:MSF: a modular framework for multi-state computational protein design.
Löffler P et al. PLoS Comput Biol. (2017)

Genji Kurisu, Ph.D.

Professor

Institute for Protein Research, Osaka University, Japan

Phone: +81-(0)-6-6879-8604 Email: gkurisu@protein.osaka-u.ac.jp

Education

1992 B.S., Applied Chemistry (Crystallography), Osaka University, Japan

1997 Ph.D., Applied Chemistry (Crystallography), Osaka University, Japan

Work Experience

1995-1997	JSPS Research Fellow (DC), Japan
1997-2004	Instructor, Institute for Protein Research, Osaka University, Japan
2002-2003	Visiting Research Fellow, Dept. Biological Sciences, Purdue University, U.S.A.
2004-2009	Associate Professor, Dept. Life Sciences, University of Tokyo, Japan
2009-	Professor, Institute for Protein Research, Osaka University

Research Interest

1. Structural studies of photosynthetic membrane protein complex and related redox proteins.
2. Crystal structure analyses of dynein motor
3. Ultra-high resolution and damage-free structural analysis of metalloproteins
4. Protein Data Bank

Selected Publications

1. *Science*, **363**, 257-260 (2019) doi: 10.1126/science.aau3613.
2. *Nature Plants*, **4**, 218-224 (2018). doi:10.1038/s41477-018-0130-0.
3. *Nature*. **484,** 345-350 (2012). doi: 10.1038/nature10955.
4. *Nature Struct. Mol. Biol.*, **18**, 638-642 (2011). doi: 10.1038/nsmb.2074.
5. *Nature.* **465**, 110-114 (2010). doi: 10.1038/nature08950.

Structural Basis for the Ferredoxin-dependent Photosynthetic Electron Transfer

Genji Kurisu

Institute for Protein Research, Osaka University, Japan
**E-mail: gkurisu@protein.osaka-u.ac.jp*

Plant-type ferredoxin (Fd) is an electron transfer protein with a [2Fe-2S] cluster, carrying one-electron to Fd dependent enzymes that are important for assimilatory and regulatory reactions in photosynthetic organisms. In chloroplasts, Fd is reduced by Photosystem I (PSI) and primarily oxidized by FdzNADP+ reductase (FNR) involved in NADP+ reduction, which is called “linear electron transfer”. Electron transfer from PSI to Fd is realized by a protein protein complex formation suitable for an efficient redox reaction. After the inter molecular electron transfer, this complex dissociates quickly, which is a reason for the high turnover of the light driven photosynthetic electron transfer reaction. Besides Fd and PSI, this transient protein-protein interaction is also realized for other Fd-dependent enzymes such as FNR. Although Fd dependent enzymes vary in molecular size and prosthetic groups, they all specifically recognize Fd and form a fully functional electron transfer complex even without any common Fd binding motif or fold. To understand the structural basis for the dynamics and efficiency of the electron transfer reaction around Fd, we have studied the electron transfer complexes such as Fd:FNR, Fd:SiR [1], and Fd:PSI [2] by X ray crystallography combined with NMR spectroscopy. In order to understand the alternative photosynthetic electron transport chain named “cyclic electron transfer”, we also studied the photosynthetic complex 1 (NDH1) using a multidisciplinary approach including Cryo-electron microscopy for structural characterization [3].

References

[1] Kim, J.Y. *et al*., Structural and mutational studies of an electron transfer complex of maize sulfite reductase and ferredoxin. *J. Biochem.,* **160**, 101-109 **(2016)**.

[2] Kubota-Kawai, H. et al., X-ray structure of an asymmetric trimeric ferredoxin-photosystem I complex. *Nature Plants*, **4**, 218-224 **(2018)**

[3] Schuller, J.M., *et al*., Structural adaptations of photosynthetic complex I enable ferredoxin-dependent electron transfer. *Science*, **363**, 257-260 **(2019)**

Young-Tae Chang, Ph.D.

Professor

Pohang University of Science and Technology (POSTECH), Chemistry

Phone: +82-54-279-2101 Email: ytchang@postech.ac.kr

Education

1991 B.S., Chemistry, POSTECH, Korea

1995 M.S., Chemistry, POSTECH, Korea

1997 Ph.D., Chemistry, POSTECH, Korea

Work Experience

Professor, 2017-present, Chemistry, POSTECH

Associate Director, 2017-present, Center for Self-Assembly and Complexity, IBS

Head, 2007-2017, Laboratory of Bioimaging Probe Development, SBIC, A*STAR

Professor, 2012-2017, Chemistry, National University of Singapore

Associate professor, 2005- 2007, NYU Chemistry

Assistant Professor, 2000-2005, NYU Chemistry

Post-Doc, 1999-2000, The Scripps Research Institute

Post-Doc, 1997-1999, UC Berkeley2005-2008

Research Interest

1. Chemical Biology and Molecular Imaging
2. Chemical Cellomics
3. Fluorescence Sensing

Selected Publications

1. Imaging inflammation using an activated macrophage probe with Slc18b1 as the activation-selective gating target, Park, S. J.; Kim, B.; Choi, S.; Balasubramaniam, S.; Lee, S. C.; Lee, J. Y.; Kim, H. S.; Kim, J. Y.; Kim, J. J.; Lee, Y. A.; Kang, N. Y.; Kim, J. S.; Chang, Y. T. *Nat. Commun.* **2019**, *10*, 1111.

Strategy for Cell Discrimination using Fluorescent Probe

Young-Tae Chang

Pohang University of Science and Technology (POSTECH), Chemistry
**E-mail: ytchang@postech.ac.kr*

Our body is composed of many, and many different kinds of cells. A cell is already a complex universe, and each cell has its own unique structure and functions. The distinction of different cells is the starting point of understanding the role of each cell in the higher complexity of cell community, i.e. an organism.

Different cells have different building blocks, such as proteins, lipids and carbohydrate structures in and out of the cell. When such molecules are unique landmarks for each cell, we call them biomarkers of the cell. If a reporting molecule can recognize the biomarkers, it can be a specific probe for the cell. The probes usually give out the signal either by fluorescence or radioactivity. We may call this kind of probe as Holding Oriented Live-cell Distinction (HOLD) probe.

Different cells also have different metabolism and import and export different molecules based on the need of the cell. There are gating proteins called transporters, and they are responsible for selective in and out of the materials through the cell membrane. If a reporting molecule is selectively uptaken by a specific cell, we may call this kind of probe as Gating Oriented Live-cell Distinction (GOLD) probe.

The conventional probe development was based on HOLD approach, and we propose a less explored new approach of GOLD, as an alternative and more systematic probe development scheme. Specific example with activated macrophage probe, CDg16 will be presented and the probe will be available to any colleague who wants to test it.

References

[1] Park, S. J.; Kim, B.; Choi, S.; Balasubramaniam, S.; Lee, S. C.; Lee, J. Y.; Kim, H. S.; Kim, J. Y.; Kim, J. J.; Lee, Y. A.; Kang, N. Y.; Kim, J. S.; Chang, Y. T., Imaging inflammation using an activated macrophage probe with Slc18b1 as the activation-selective gating target, *Nat. Commun.* (**2019**), *10*, 1111.

MAP

광주과학기술원 광주과학기술원 (GIST) 오룡관 , 건물번호 W1

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

2020

한국생체분자과학 연합학회학술대회

Symposium I & II

Jungwook Kim, Ph.D.

Assistant Professor

Gwangju Institute of Science and Technology (GIST)

Phone: +82+062-715-4622 Email: jwkim@gist.ac.kr

Education

2004 Ph.D., Chemistry, Texas A&M University, U.S.A.

1995 B.S., Chemistry, Seoul National University, Korea

Work Experience

2010-2015 Associate, Department of biochemistry, Albert Einstein College of Medicine, U.S.A.

2004-2010 Research Associate, Department of biochemistry, Albert Einstein College of Medicine, U.S.A.

Research Interest

1. X-ray protein crystallography
2. Enzymology
3. RNA metabolism

Selected Publications

1. Lee, S., Kang, J., & Kim, J. (2019). Structural and biochemical characterization of Rv0187, an O-methyltransferase from Mycobacterium tuberculosis. *Sci Rep*, **9**(1), 8059. doi:10.1038/s41598-019-44592-7.

2. Ryu, H., Grove, T. L., Almo, S. C., & Kim, J. (2018). Identification of a novel tRNA wobble uridine modifying activity in the biosynthesis of 5-methoxyuridine. *Nucleic Acids Res*, **46**(17), 9160-9169. doi:10.1093/nar/gky592.

3. Kim, J., Xiao, H., Koh, J., Wang, Y., Bonanno, J. B., Thomas, K., . . . Almo, S. C. (2015). Determinants of the CmoB carboxymethyl transferase utilized for selective tRNA wobble modification. *Nucleic Acids Res*, **43**(9), 4602-4613. doi:10.1093/nar/gkv206.

Structural basis for the activation and the activity of phosphatidylserine decarboxylase in the biosynthesis of phospholipids

Jungwook Kim

Assistant Professor, Department of Chemistry, Gwangju Institute of Science and Technology.

**E-mail: jwkim@gist.ac.kr*

Phosphatidylethanolamine (PE), the most abundant phospholipids on the Escherichia coli membrane, is synthesized from phosphatidylserine (PS), where phosphatidylserine decarboxylase (PSD) catalyzes the decarboxylation of PS. In E. coli, PSD is peripherally bound to the cytoplasmic surface of the inner membrane, and its activity is essential for the viability of the organism. The enzyme is initially translated as an inactive proenzyme, where the active form is a heterodimer composed of the alpha- and beta-subunits. Maturation of the proenzyme occurs through auto-cleavage which leads to the formation of pyruvoyl group at the N-terminal of the alpha-subunit, an essential post-translational modification for decarboxylation function. Therefore, PSD contains both protease and decarboxylase activities. Despite the biological significance of the enzyme in phospholipid biosynthesis across all domains of life and therapeutic potential for antibiotic development, no structural information is available to date. Here, we present the X-ray crystal structure of PSD from E. coli to address the molecular mechanism underlying the final step of PE synthesis pathway. Through structural and biochemical analyses, we have identified key residues involved in protease activity, membrane association, and PS recognition.

Hojung Nam, Ph.D.

Associate Professor
School of Electrical Engineering and Computer Science (EECS)
Gwangju Institute of Science and Technology (GIST)
Phone: +82-62-715-2641 Email: hjnam@gist.ac.kr

Education

2009 Ph.D. in Bio and Brain Engineering, KAIST, Daejeon, Korea
2003 M.S. in Computer Science, KAIST, Daejeon, Korea
2001 B.S. in Computer Science (Cum Laude), Sogang Univ., Seoul, Korea

Work Experience

2018-present Gwangju Institute of Science and Technology (GIST), Associate Professor
2013-2018 Gwangju Institute of Science and Technology (GIST), Assistant Professor
2009-2013 University of California, San Diego, Postdoctoral Research Associate

Research Interest

1. Bioinformatics
2. Systems Biology
3. AI-based drug discovery

Selected Publications

1. Ingoo Lee, Jongsoo Keum, Hojung Nam*, "DeepConv-DTI: Prediction of drug-target interactions via deep learning with convolution on protein sequences", PLoS Computational Biology 2019 15(6).
2. Ingoo Lee, Hojung Nam*, "Identification of drug-target interaction by a random walk with restart method on an interactome network ", BMC Bioinformatics, 2018 19(Suppl 8):208.
3. Eunyoung Kim, Hojung Nam*, "Prediction models for drug-induced hepatotoxicity by using weighted molecular fingerprints", BMC Bioinformatics, 2017, 18(Suppl 7):227.
4. Hojung Nam*, Miguel Campodonico, Aarash Bordbar, Daniel R. Hyduke, Sangwoo Kim, Bernhard O. Palsson* (*co-corresponding), "A systems approach to predict oncometabolites via context-specific genome-scale metabolic networks", PLoS Comput Biol, 10(9): e1003837.
5. Hojung Nam†, Nathan E. Lewis†, Joshua A. Lerman, DaeHee Lee, Roger L. Chang, Donghyuk Kim, Bernhard O. Palsson (†equal contribution), “Network context and selection in the evolution to enzyme specificity”, Science, 2012, 337 (6098): 1101-1104.

AI-powered drug discovery and development

Hojung Nam

School of Electrical Engineering and Computer Science
Gwangju Institute of Science and Technology (GIST), Buk-gu, Gwangju, 61005, Republic of Korea
**E-mail: hjnam@gist.ac.kr*

The process of drug discovery is a challenging process considering its required time and efforts. Traditionally, developing one promising drug requires more than 10,000 initial compounds to be selected as candidates. Then these candidates undergo pre-clinical and clinical tests to be passed for several years. In recent years, the paradigm in drug development has been re-shaped in several ways. Many studies have shown the efficiency of artificial intelligence (AI) based drug development approaches that further increase the ability to predict and model the most relevant pharmacokinetic, metabolic, and toxicity endpoints, thereby accelerating the drug discovery process. This talk covers studies about the development of AI models for hit compound discovery and toxicity prediction in early drug discovery process. The first part of the talk is about the AI model that suggests hit compounds. We employ a convolutional neural network (CNN) on target protein raw sequences to capture local residue patterns participating in drug-target interactions. With the engineered features using CNN on protein sequences, our model shows improved prediction performance than previous protein descriptor-based models as well as than the previous deep learning model. By examining the pooled convolution results, we confirm that our model can detect binding regions of proteins for DTIs. Next, we developed an AI-based cardiotoxicity prediction model for screening human ether-a-go-go-related gene (hERG) blockers. We developed a precise and interpretable hERG blocker prediction model by using deep learning with a self-attention approach. The validation result showed that the model was well-optimized and had high performance and the test set performance of the proposed model was higher than that of previous machine-learning-based models. Furthermore, we interpreted the calculated attention score vectors obtained from the proposed prediction model and demonstrated the critical structural patterns that are represented in hERG blockers.

Mi Sun Jin, Ph.D.

Assisstant Professor

Gwangju Institute of Science and Technology (GIST)

Phone: + 82-010-9901-2705 Email: misunjin@gist.ac.kr

Education

2002 B.S., Chemistry, Seogang University, Korea

2002 B.S., Chemical Engineering, Seogang University, Korea

2004 M.S., Chemistry, KAIST, Korea

2008 Ph. D., KAIST, Korea

Work Experience

2008-2009 Postdoc, KAIST, Korea

2009-2013 Postdoc, Purdue University, USA

2013-2014 Research Specialist, Purdue University, USA

Research Interest

1. X-ray Crystallography
2. Cryo-EM
3. Biochemistry

Selected Publications

1. Songwon Kim, Na Jin Kim, Semi Hong, Subin Kim, Jongmin Sung, **Mi Sun Jin** (2019), The structural basis of the low catalytic activities of the two minor β-carbonic anhydrases of the filamentous fungus Aspergillus fumigatus, *J Struct Biol,* 208(1), 61-68
2. Subin Kim, Jongmin Sung, Jungyoon Yeon, Seung Hun Choi, and **Mi Sun Jin** (2019), Crystal Structure of a Highly Thermostable α-Carbonic Anhydrase from *Persephonella marina* EX-H1, *Molecules and Cells,* 42(6), 460-469
3. Ji Won Kim, Songwon Kim, Haerim Lee, Geunyoung Cho, Sun Chang Kim, Hayyoung Lee, **Mi Sun Jin***, and Jie-Oh Lee* (2019), Application of antihelix antibodies in protein structure determination, *PNAS*, 116(36), 17786-17791
4. Jung-Ah Kang, Songwon Kim, Minji Park, Hyun-Jin Park, Jeong-Hyun Kim, Sanghyeok Park, Jeong-Ryul Hwang, Yong-Chul Kim, Yoon Jun Kim, Yuri Cho*, **Mi Sun Jin*** and Sung-Gyoo Park* (2019), Ciclopirox inhibits Hepatitis B Virus secretion by blocking capsid assembly, *Nature Communications,* 10(1), doi: 10.1038/s41467-019-10200-5 (* indicates co-corresponding authors.)

The structural basis of the low catalytic activities of the two minor β-carbonic anhydrases of the filamentous fungus *Aspergillus fumigatus*

Mi Sun Jin

Gwangju Institute of Science and Technology

**E-mail: misunjin@gist.ac.kr*

The β-carbonic anhydrases (β-CAs) are widely distributed zinc-metalloenzymes that play essential roles in growth, survival, development and virulence in fungi. The majority of filamentous ascomycetes possess multiple β-CA isoforms among which major and minor forms have been characterized. We examined the catalytic behavior of the two minor β-CAs, CafC and CafD, of *Aspergillus fumigatus*, and found that both enzymes exhibited low CO_2 hydration activities. To understand the structural basis of their low activities, we performed X-ray crystallographic and site-directed mutagenesis studies. Both enzymes exist as homodimers. Like other Type-I β-CAs, the CafC active site has an “open” conformation in which the zinc ion is tetrahedrally coordinated by three residues (C36, H88 and C91) and a water molecule. However, L25 and L78 on the rim of the catalytic entry site protrude into the active site cleft, partially occluding access to it. Single (L25G or L78G) and double mutants provided evidence that widening the entrance to the active site greatly accelerates catalytic activity. By contrast, CafD has a typical Type-II “closed” conformation in which the zinc-bound water molecule is replaced by aspartic acid (D36). The most likely explanation for this result is that an arginine that is largely conserved within the β-CA family is replaced by glycine, so that D36 cannot undergo a conformational change by forming a D-R pair that creates the space for a zinc-bound water molecule and switches the enzyme to the active form.

Gwangrog Lee, Ph.D.

Associate Professor
Gwangju Institute of Science and Technology (GIST)
Phone: + 82-062-715-3558 Email: glee@gist.ac.kr

Education

2006 Ph.D., Duke Univ. Mechanical Engineering and Material Sciences, Durham, NC, USA
2000 M.S., Sungkyunkwan Univ. Department of Mechanical Engineering, Korea
1998 B.S., Sungkyunkwan Univ. Department of Mechanical Engineering, Korea

Work Experience

02/2012 - present Associate Professor at Gwangju Institute of Science and Technology
11/2006-1/2012 Research Associate
Department of Physics & HHMI
University of Illinois at Urbana-Champaign, IL, 61801-3080
5/2006-10/2006 Research Associate
Center for Biologically Inspired Materials & Materials Systems (CBIMMS)
Duke University, Department of Mechanical Engineering and Materials Science

Research Interest

1. Single molecule Biophysics (Enzymology: helicases, polymerases, and nucleases)
2. Protein-protein and protein-nucleic acid interactions
3. Genome Maintenance: DNA damage Pathways (BER, NER, ICL repair)
4. Cell recognition & Mechanics: phagocytosis and efferocytosis
5. Cellular stress: ERAD pathway

Selected Publications

1. Hwang W, Yoo J, Lee Y, Park S, Hoang PL, Hoa Vo TM, Shin M, Jin MS, Park D and **G. Lee** "Dynamic coordination of two-metal-ions orchestrates λ-exonuclease catalysis.", ***Nature Communications,*** 9(1): 4404-, Oct, 23 2018
2. J. Yoo and **G. Lee** "Allosteric ring assembly and chemo-mechanical melting by the interaction between 5'-phosphate and λ exonuclease.", ***Nucleic Acids Research,*** 43(22): 10861-9, Dec, 15 2015
3. **G. Lee**, M. Bratkowski, F. Ding, A. Ke and T. Ha "Elastic coupling between RNA degradation and unwinding by an exoribonuclease.", ***Science,*** 336(6089):1726-1729, Jun 29 2012.
4. **G. Lee**, J. Yoo, B.J. Lesile and T. Ha " Single molecule analysis reveals three distinct phases of DNA degradation by an exonuclease.", ***Nature Chemical Biology,*** 7(6):367-374, Jun 2011
5. **G. Lee**, K. Abdi, Y. Jiang, P. Michaely, V. Bennett, and P.E. Marszalek, "Nanospring Behavior of Ankyrin repeats.", ***Nature***, 440 (7081): 246-249 March 9 2006.

Chemical friction along the minor groove of DNA facilitates enzymatic translocation of λ exonuclease via electrostatic ratchet

Jungmin Yoo[1], HyeokJin Cho[1], Jejoong Yoo[2], and Gwangrog Lee[1]

[1]School of Life Sciences, Gwangju Institute of Science and Technology
[2]Center for Self-assembly and Complexity , Institute for Basic Science
**E-mail: glee@gist.ac.kr*

DNA is a long polymeric substrate that provides specific binding sites for many proteins in cell nucleus. Both diffusion and directed translocation play an essential role for protein-DNA interactions in gene regulation processes but it is still not fully understood how specific proteins move and search their target sites in the myriad of DNA sequence repertoires. We use λ-exonuclease as a model of nucleic acid-molecular motors that processively translocates along the DNA. Here, we combined single molecule FRET and molecular dynamics (MD) simulation to examine how the dynamic interaction transmits into overall enzymatic activity. Transient coupling between λ-exonuclease and its substrate DNA significantly alters its translocation by a factor of ~30 due to chemical friction between a positive reside (ARG45) of the protein and electrostatic potential (EP) along the minor groove of DNA. Repulsive interaction gives rise to futile slippage events whereas attractive coupling between ARG45 and adenines at the minor grooves provides chemical ratcheting for unidirectional translocation, preventing diffusive backtracking by electrostatic friction. We propose an anti-friction-based ratchet for processive translocation. Our study provides new insights into not only interplay between dynamic chemophysical interaction and enzyme activity but also a role of the minor groove in regulating enzymatic activity based on DNA sequences.

Yun Hak Kim, M.D., Ph.D.

Assistant Professor

Pusan National University

Phone: +82-51-510-8091 Email: yunhak10510@pusan.ac.kr

Education

2010 B.S., Chemistry, Korea University, Korea

2016 M.D., Ph.D., School of Medicine, Pusan National University, Korea

Work Experience

2019-present Scientific Reports, Academic editor

2018-present Assistant Professor, School of Medicine, Pusan National University

2016-2018 Postdoctoral Researcher, Gene & Cell Therapy Research Center for Vessel-associated Diseases, Pusan National University

Research Interest

1. Bioinformatics
2. Glucose Transporter
3. Imaging

Selected Publications

1. Risk of metastatic pheochromocytoma and paraganglioma in SDHx mutation carriers: A systematic review and updated meta-analysis. J Med Genet. 2019
2. Development of Risk Scoring System for Patients with Papillary Thyroid Cancer. J Cell Mol Med. 2019.
3. Prognostic scoring system for osteosarcoma using Network-Regularized high-dimensional Cox regression analysis and potential therapeutic targets. J Cell Physiol. 2019.
4. SIRT1 attenuates PAF-induced MMP-2 production via down-regulation of PAF receptor expression in vascular smooth muscle cells. Vasc Pharmacol. 2015.
5. PAF enhances MMP-2 production in rat aortic VSMCs via a β-arrestin2-dependent ERK signaling pathway. J Lipid Res. 2013.

ESurv: a user-friendly web server for integrative survival analysis

Kyoungjune Pak[1], Tae Sik Goh[2], Hye Jin Heo[3], Myoung-Eun Han[3], Junho Kang[4], Eun Jung Kwon[4], Ji Wan Kang[4], Sae-Ock Oh[3,*], Yun Hak Kim[3,4,*]

[1]Department of Nuclear Medicine, Pusan National University Hospital, Republic of Korea

[2]Department of Orthopaedic Surgery, Pusan National University Hospital, Republic of Korea

[3]Department of Anatomy, School of Medicine, Pusan National University, Republic of Korea

[4]Department of Biomedical Informatics, School of Medicine, Pusan National University, Republic of Korea

*E-mail: yunhak10510@pusan.ac.kr

Although some web-based survival analysis tools have been developed, they have several limitations. To overcome these, we developed ESurv that can perform advanced survival analyses of data from The Cancer Genome Atlas (TCGA) or directly from users. Users can conduct univariate analyses and grouped variable selections using multi-omics data in TCGA or users' data. In univariate analyses, they can identify prognostic significances of single gene with survival curve (median or optimal cut-off), area under the curve (AUC) with C statistics, and receiver operating characteristics (ROC). They can obtain prognostic variable signatures based on multi-omics data by using grouped variable selections with the above results. In addition, users can make custom gene signatures for cancers with genes of interest. One of the most important functions is that users can perform all survival analyses using their own data. With advanced statistical techniques, ESurv (https://easysurv.net) overcomes all the limitations of previous web-based tools.

Jeong-Mo Choi, Ph.D.

Research Assistant Professor

Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea

E-mail: jeongmochoi@kaist.ac.kr Website: https://sites.google.com/site/jeongmochoi

Education

2011 B.S., Chemistry and Physics, KAIST, Daejeon, Republic of Korea

2015 A.M., History of Science, Harvard University, Cambridge, United States

2016 Ph.D., Chemistry, Harvard University, Cambridge, United States

Work Experience

2016-2019 Postdoctoral Associate, Washington University in St. Louis, St. Louis, United States

2019-present Research Assistant Professor, KAIST, Daejeon, Republic of Korea

Research Interest

1. Biological applications of statistical physics
2. Intrinsically disordered proteins (IDPs) and neurodegenerative diseases
3. Protein evolution and design
4. Protein-protein interactions (PPIs) and PPI networks

Selected Publications

1. Choi and Pappu, ***Journal of Chemical Theory and Computation*** **15**: 1367 (2019)
2. Choi and Pappu, ***Journal of Chemical Theory and Computation*** **15**: 1355 (2019)
3. Bai, Sargent, Choi, Pappu, and Zhang, ***Nature Communications*** **10**: 3317 (2019)
4. Wang, Choi, Holehouse, …, Pappu, Alberti, and Hyman, ***Cell*** **174**: 688 (2018)
5. Yoo, Choi, Conway, Yu, Pappu, and Needleman, ***eLife*** **7**: e36392 (2018)
6. Choi, Gilson, and Shakhnovich, ***Physical Review Letters*** **118**: 088302 (2017)
7. Bershtein,* Choi,* Bhattacharyya, Budnik, and Shakhnovich, ***Cell Reports*** **11**: 645 (2015)

The stickers and spacers framework for describing phase behavior of multivalent intrinsically disordered proteins

Jeong-Mo Choi

Natural Science Research Institute, KAIST, Daejeon, Republic of Korea

**E-mail: jeongmochoi@kaist.ac.kr*

Accurate spatial and temporal organization of cellular components is essential for viability and functionality of a cell. Eukaryotic cells have invented membrane-bound organelles, but cells also employ *membraneless organelles* for rapid and efficient operations. In the last decade, it has been shown that liquid-liquid phase separation (LLPS) of biopolymers is the underlying principle behind formation, regulation, and dissociation of membraneless organelles *in vivo*. The LLPS drivers need to bear multivalency and flexibility, and intrinsically disordered proteins (IDPs) occasionally take the role. Although the amount of experimental data has been rapidly growing, however, our theoretical understanding is far from mature.

We recently conceptualized the *stickers and spacers* framework, where IDP residues are designated into two groups: stickers, which drive chain-chain interactions, and spacers, which modulate the chain properties. Based on the framework, we developed an analytical mean-field model and applied it to the saturation concentration data of FUS family proteins to validate the utility of the model. The model predicts additive contributions from different types of sticker pairs, which explains why a dominant sticker pair type in one system can be insignificant in another system.

We also demonstrate the results from graph-based simulations, which complement the analytical model. The simulation engine incorporates the concept of a *cluster*, which is a set of stickers that belong to the same polymer or to the polymers linked by sticker bonds. Intra-cluster interactions have different entropic contributions from inter-cluster ones, which the mean-field approach only considers. Moreover, three- and four-body interactions are implemented to test the effect of higher-order interactions. The mean-field predictions have been successfully reproduced, and it was shown that intra-cluster interactions are more crucial than multi-body interactions to explain the deviations of FUS variants from the mean-field prediction.

Mookyung Cheon, Ph.D.

Senior Researcher

Korea Brain Research Institute

Phone: +82-53-980-8434 Email: mkcheon@kbri.re.kr.kr

Education

1994 B.S., Physics, Pusan National University, Korea

1996 M.S., Physics, Pusan National University, Korea

2001 Ph.D., Physics, Pusan National University, Korea

Work Experience

2003-2005 Postdoctoral Researcher, Department of Physics, Pusan National University

2005-2007 Postdoctoral Researcher, Department of Chemistry, University of Cambridge, UK

2007-2009 Postdoctoral Researcher, Department of Chemical and Biomolecular engineering, North Carolina State University, USA

2009-2016 Research Professor, Creative Research Initiative Center for Proteome Biophysics Pusan National University and DGIST

2016-present Senior Researcher, Dementia Research Group, KBRI

Research Interest

1. Development of neuroinformatics toolkit for brain images and diseases.

2. Neurodegenerative Disease and Protein Aggregation

Selected Publications

1. *Polymorphism of fibrillar structures depending on the size of assembled A ß17-42 peptides* **Scientific Reports**, 6, 38196 (2016)

2. *Uncovering Multi Loci-Ordering by Algebraic Property of Laplacian Matrix and its Fiedler Vector.* **Bioinformatics**, 32, 801 (2016)

3. *Structural Conversion of Aβ17-42 Peptides from Disordered Oligomers to U-Shape Protofilaments via Multiple Kinetic Pathways* **PLOS Computational Biology**, 11, e1004258 (2015)

4. Spontaneous Formation of Twisted Aβ16-22 Fibrils in Large-Scale Molecular-Dynamics Simulations. **Biophysical Journal** 101, 2493-2501 (2011)

5. A simple and exact Laplacian clustering of complex networking phenomena: Application to gene expression profiles. **Proceeding of The National Academy of Sciences USA** 105, 4083-4087 (2008)

6. Structural reorganisation and potential toxicity of oligomeric species formed during the assembly of amyloid fibrils. **PLOS Computational Biololgy** 3, 1727 (2007) Times cited: 127

A practical application of generative adversarial networks for RNA-seq analysis to predict the molecular progress of Alzheimer's disease

Mookyung Cheon*

Neuroinformatics Lab, Dementia Research Group, Korea Brain Research Institute (KBRI)

**E-mail: mkcheon@kbri.re.kr*

Next-generation sequencing (NGS) technology has become a powerful tool for dissecting the molecular and pathological signatures of a variety of human diseases. However, the limited availability of biological samples from different disease stages is a major hurdle in studying disease progressions and identifying early pathological changes. Deep learning techniques have recently begun to be applied to analyze NGS data and thereby predict the progression of biological processes. In this study, we applied a deep learning technique called a generative adversarial network (GAN) to predict the molecular progress of Alzheimer's disease (AD) for the first time. We successfully applied a GAN to analyze RNA-seq data from a 5xFAD mouse model of AD, which recapitulates major AD features that present as massive amyloid-β (Aβ) accumulation in the brain. The coexpression features are trained well in the network to generate specific sample profiles based on weight parameters with biological gene associations. Virtual disease progress was introduced by latent space interpolation, which has been a key method for various image conversions in GANs, to yield the transition curves of various genes with pathological changes from normal to AD state. By performing pathway analysis based on the transition curve patterns, we identified several pathological processes with progressive changes, such as inflammatory systems and synapse functions, which have previously been demonstrated to be involved in the pathogenesis of AD. Interestingly, our analysis indicates that alteration of cholesterol biosynthesis begins at a very early stage of AD, suggesting that it is the first effect to mediate the cholesterol metabolism of AD downstream of Aβ accumulation. Here, we suggest that GANs are a useful tool to study disease progression when the sample size is limited, leading to the identification of early pathological signatures.

Sun Choi, Ph.D.

Professor
College of Pharmacy and Graduate School of Pharmaceutical Sciences, Ewha Womans University
Phone: +82-10-7252-6737 Email: sunchoi@ewha.ac.kr

Education

1999 Ph.D. Medicinal Chemistry, State University of New York at Buffalo, USA
1987 B.S. College of Pharmacy, Ewha Womans University, Korea

Work Experience

2005-current 이화여자대학교 약학대학 & 대학원 약학과 조교수, 부교수, 교수
2015-2017 이화여자대학교 약학대학 약학과장
2001-2005 Molecular Modeler / Computational Chemist: Tripos, Inc., USA
1997-2001 Postdoctoral Fellow: Department of Chemistry and the Drug Discovery Program, Northwestern University, USA
2018-2019 한국단백질학회 회장 2019 한국유기합성학회 부회장
2017-current 한국생명정보학회 부회장 2020 한국분자·세포생물학회 부회장

Research Interest

1. Computer-Aided Drug Design (CADD) Methods and Applications
2. Protein-Ligand Interaction & Mechanism of Action Studies
3. Drug Discovery Research for Various Diseases in Cell Signaling
4. Biomolecular Simulations: Protein Motion and Allostery
5. Artificial Intelligence (AI) & Big Data Analysis

Selected Publications

1. "Transmembrane 4 L Six Family Member 5 Senses Arginine for mTORC1 Signaling" J. W. Jung, S. J. Y. Macalino, M. Cui, J. E. Kim, H. J. Kim, D. G. Song, S. H. Nam, S. Kim, S. Choi*, J. W. Lee*, ***Cell Metabolism*** 2019, *29(6)*, 1306-1319
2. "In Vivo Albumin Traps Photosensitizer Monomers from Self-Assembled Phthalocyanine Nanovesicles: A Facile and Switchable Theranostic Approach" X. Li, S. Yu, Y. Lee, T. Guo, N. Kwon, D. Lee, S. C. Yeom, Y. Cho, G. Kim, J.-D. Huang*, S. Choi*, K. T. Nam*, and J. Yoon*, ***J. Am. Chem. Soc.*** 2019, *141(3)*, 1366–1372 (Cover).
3. "Water-Soluble Phthalocyanines Selectively Bind to Albumin Dimers: A Green Approach Toward Enhancing Tumor-Targeted Photodynamic Therapy" X. Li, K. Jeong, Y. Lee, T. Guo, D. Lee, J. Park, N. Kwon, J.-H. Na, S. K. Hong, S.-S. Cha, J.-D. Huang*, S. Choi*, S. Kim*, J. Yoon*, ***Theranostics*** 2019, *9(22)*, 6412-6423
4. "Discovery of Conformationally Restricted Human Glutaminyl Cyclase Inhibitors as Potent Anti-Alzheimer's Agents by Structure-Based Design" V.-H. Hoang, V. T. H. Ngo, M. Cui, N. V. Manh, P.-T. Tran, J. Ann, H.-J. Ha, H. Kim, K. Choi, Y.-H. Kim, H. Chang, S. J. Y. Macalino, J. Lee, S. Choi*, J. W. Lee*, ***J. Med. Chem.*** 2019, *62(17)*, 8011-8027
5. "Recent Advances in Structure-Based Drug Design Targeting Class A G Protein-Coupled Receptors Utilizing Crystal Structures and Computational Simulations" Y. Lee, S. Basith, and S. Choi*, ***J. Med. Chem.*** 2018, *61(1)*, 1-46.
6. "Link between allosteric signal transduction and functional dynamics in a multi-subunit enzyme: *S*-adenosylhomocysteine hydrolase" Y. Lee, L. S. Jeong, S. Choi*, and C. Hyeon*, ***J. Am. Chem. Soc.*** 2011, *133(49)*, 19807-19815.

Comprehensive mechanistic studies for the structural and functional elucidation of a tetraspanin arginine sensor

Sun Choi

College of Pharmacy and Graduate School of Pharmaceutical Sciences,
Ewha Womans University, Seoul 03760, Korea
**E-mail: sunchoi@ewha.ac.kr*

Transmembrane 4 L six family member 5 (TM4SF5) is a transmembrane protein known to interact with other TM4SFs, signaling proteins, and integrins. These interactions lead to uncontrollable cell growth and proliferation, eventually resulting in critical conditions, namely fibrosis and cancer. TM4SF5 consists of 4 transmembrane (TM) helices: two extracellular loops, an intracellular loop, and N- and C-terminal cytosolic tails. The long extracellular loop (LEL) region of TM4SF5 plays a key role in its functions and interactions with molecular partners. Recently, we have identified TM4SF5 as an arginine sensor for mTORC1 activation and subsequent phosphorylation of its downstream effectors. The functions of mTORC1 are significantly affected when conserved residues in LEL region of TM4SF5 were mutated, supporting the involvement of TM4SF5 in this pathway.

First, we did homology modelling using the structure of CD81 as a template in order to investigate the interactions and the functions of TM4SF5. We predicted the binding mode of L-arginine and TSAHC using docking studies and found that both molecules can interact with conserved residues and potentially affect N-glycosylation sites in the TM4SF5 LEL region. Furthermore, we applied Molecular Dynamics (MD) simulations for the apo wild-type and mutant (W124A and Y126S) structures, figuring out the importance of these residues in structural stability of TM4SF5. Protein-protein docking of TM4SF5 with mTORC1, MD simulation of ensuing complexes, and trajectory and network analysis were sequentially done. The analysis allowed us to distinguish the importance of these residues to TM4SF5 structural integrity and function in the mTORC1 pathway.

MEMO

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

2020

한국생체분자과학 연합학회학술대회

Symposium III & IV

Hee-Jung Choi, Ph.D

Associate Professor

Seoul National Unversity

Phone: + 02 880 6605 Email: choihj@snu.ac.kr

Education

1991 B.S., Honours. Chemistry, Seoul National University, Korea.

1993 M.S., Chemistry (Biochemistry), Seoul National University, Korea.

1997 Ph.D., Chemistry (Biochemistry & Structural biology), Seuol National University, Korea.

Work Experience

1997-2000 Postdoctoral fellow, KRIBB, Korea

2000-2012 Research Associate, Department of Structural Biology, Stanford University

2012-2016 Assistant Professor, School of Biological Sciences, Seoul National University

2016-now Associate Professor, School of Biological Sciences, Seoul National University

Research Interest

1. Structural and biochemical studies on Wnt signaling
2. Structural and functional studies on G-protein coupled receptors (GPCRs)
3. Structures of cellular adhesion proteins

Selected Publications

1. Bang I., Kim H.R., Beaven A.H., Kim J., Ko S.B., Lee G.R., Lee H., Im W., Seok C., Chung K.Y.*, **Choi H.-J.*** (2018) Biophysical and functional characterization of Norrin signaling through Frizzled4. *Proc. Natl. Acad. Sci. USA*. **115**, 8787-8792. (***corresponding author)**
2. Shao X., Kang H., Loveless T., Lee G.R., Seok C., Weis W.I., **Choi H.-J.***, & Hardin J.* (2017) Cell–cell adhesion in metazoans relies on evolutionarily conserved features of the α-catenin•β-catenin–binding interface. *J. Biol. Chem.*,**292**, 16477-490. (***corresponding author)**
3. Kang H., Bang I., Jin K.S., Lee B., Lee J., Shao X., Heier J., Kwiatowski A.V., Nelson W.J., Hardin J., Weis W.I. & **Choi H.-J.*** (2017) Structural and functional characterization of *Caenorhabditis elegans* α-catenin reveals constitutive binding to β-catenin and F-actin. *J. Biol. Chem.*,**292**, 7077-7086. (***corresponding author)**
4. Yoo Y. et al., (2017) GABBR2 Mutations Determine Phenotype in Rett Syndrome and Epileptic Encephalopathy. *ANN. NEUROL.* **82**, 466–478.
5. **Choi H.-J.***, Loveless T., Lynch A., Bang I., Hardin J. & Weis W.I.* (2015) A conserved phosphorylation switch controls the interaction between cadherin and β-catenin *in vitro* and *in vivo*. *Dev. Cell* **33**, 82-93.(***corresponding author)**
6. Rasmussen S.G. F.*, **Choi H.-J.***, Fung J.J.*, et al. (2011) Structure of a nanobody-stabilized active state of β(2) adrenoceptor. *Nature*, **469**, 175-180. (*** co-first authors)**

Biophysical and functional characterization of Frizzled4-mediated Norrin signaling

Injin Bang[1], Hee Ryung Kim[2], Andrew H. Beaven[3], Jinuk Kim[1], Seung-Bum Ko[1], Gyu Rie Lee[4], Wonpil Im[5], Chaok Seok[4], Ka Young Chung[2], and Hee-Jung Choi[1*]

[1]Department of Biological Sciences, Seoul National University, Korea, [2]School of Pharmacy, Sungkyunkwan University, Korea, [3]Department of Chemistry, The University of Kansas, USA, [4]Department of Chemistry, Seoul National University, Korea, [5]Department of Biological Sciences, Lehigh University, USA

**E-mail: choihj@snu.ac.kr*

Wnt signaling is an evolutionarily deep-rooted pathway with pleiotropic effects on cell cycle and tissue homeostasis. Disruption of this pathway has been linked to various diseases including cancer and neurodegenerative disease. Published structures of cysteine-rich domain (CRD) of Frizzled receptor (Fzd) in complex with Wnt or Norrin have revealed much information on the molecular interaction between CRD and ligand but the question remains how that interaction with the ligand is transferred across the transmembrane domain (TMD) to the intracellular region. Here, we studied Fzd4-Norrin signaling complex to characterize the features of canonical Wnt signaling. Using computational modeling, hydrogen-deuterium exchange mass spectrometry (HDX-MS), *in vitro* binding assays, and cell-based signaling assays, we discovered that the Fzd4 linker domain, which connects the N-terminal CRD to the transmembrane domain (TMD), played an important role in specific Norrin binding and downstream signal transduction. In addition, structural dynamics study of Fzd4 associated with Norrin binding revealed conformational changes on the linker domain and the intracellular loop 3 (ICL3) region of Fzd4. Together, our results provide novel insights into Fzd4 activation in response to Norrin.

Acknowledgement

[1] This work was supported by the National Research Foundation of Korea Grant (NRF-2016R1A2B4013488 to H.-J.C.), the Republic of Korea.

Young Jun Im, Ph.D.

Professor
College of Pharmacy, Chonnam National University
Yongbongro 77, Bukgu, Gwangju, 61186, South Korea
Phone: +82-62-530-2943 Email: imyoungjun@jnu.ac.kr

Education

1998: Dept. of Manufacturing Pharmacy, Chonnam National University, B.S.

2000: Dept. of Life Science, Gwangju Institute of Science & Technology, M.S.

2005: Dept. of Life Science, Gwangju Institute of Science & Technology, Ph.D.

Work Experience

2010 - Present: Assistant/Associate/Full Professor. College of Pharmacy

2010: Postdoc. NIDDK, National Institutes of Health

2006: Postdoc. Gwangju Institute of Science & Technology

2005: Predoctoral visiting fellow, NIDDK, National Institutes of Health

Research Interest

1. Structural mechanism of proteins in antibiotic resistance and in the pathogenesis of fungal infections
2. Structural and biochemical studies on lipid sensing and transport mechanisms by lipid-binding or transfer proteins
3. Structural analysis of target-inhibitor complex for the structure based drug design

Selected Publications

1. Tong J, Tan L, Chun C, Im YJ. Structural basis of human ORP1-Rab7 interaction for the late-endosome and lysosome targeting. *PLoS One*. 2019 Feb 5;14(2):e0211724.
2. Tong J, Manik MK, Im YJ, Structural basis of sterol recognition and non-vesicluar transport by lipid transfer proteins anchored at membrane contact sites. *PNAS*. 2018 Jan 30;115(5):E856-E865.
3. Lee MA, Tan L, Yang H, Im YG, Im YJ. Structures of PPARg complexed with lobeglitazone and pioglitazone reveal key determinants for the recognition of antidiabetic drugs. *Sci Rep*. 2017 Dec 4;7(1):16837.
4. Manik MK, Yang H, Tong J, Im YJ. Structure of yeast OSBP-related protein Osh1 reveals key determinants for lipid transport and protein targeting at the nucleus-vacuole junction. *Structure*. 2017 Apr 4;25(4):617-629.
5. Tong J, Manik KM, Yang H, Im YJ. Structural insights into nonvesicular lipid transport by the oxysterol binding protein homologue family. *BBA Molecular and Cell Biology of Lipids*. 2016 Aug;1861(8 Pt B):928-39.
6. Yang H, Tong J, Lee CW, Ha S, Eom SH, Im YJ. Structural mechanism of ergosterol regulation by fungal sterol transcription factor Upc2. *Nature Communications*. 2015 Feb 6; 6:6129

Structural Mechanism of Intracellular Sterol Distribution by Lipid Transfer Proteins

Young Jun Im

College of Pharmacy, Chonnam National University, Gwangju 61186, Republic of Korea
**E-mail: youngjun.im@jnu.ac.kr*

Most lipids including cholesterol are synthesized in the endoplasmic reticulum (ER) and redistributed to other organellar membranes by multiple pathways. Due to the hydrophobic nature of membrane sterols, their free diffusion through cytoplasm is very slow and sterols are mainly distributed by soluble lipid transfer proteins (LTPs). ORP (Oxysterol-binding protein-related protein), StART (steroidogenic acute regulatory protein-related transfer protein), and LAMs (LTPs anchored at membrane contact sites) are known to perform non-vesicular sterol transport. My group has focused on the structural and biochemical studies on these LTPs. We determined the key domain structures of representative LTP homologs and characterized the lipid transport mechanisms by sterol binding and transfer assays and analyzed the protein-protein interaction critical for intracellular protein localization. I am going to present the structural and functional aspects regarding the ligand specificity and protein targeting and explain how they are optimized for the transport role at the membrane contacts sites.

Hanseong Kim, Ph.D.

Research Professor
Pukyong National University (PKNU)
Phone: + 82 51-629-5595 Email: hanseonk@pukyong.ac.kr

Education

1992 B.A., Agricultual Chemistry, Korea University, Korea
2000 M.A., Fermentation Chemistry and Biochemistry, Korea University, Korea
2005 M.S., Biophysics, University of California, Davis, United States
2012 Ph.D., Chemistry, Arizona State University, United States

Work Experience

2018- Pukyong National University, Department of Chemistry (Dr. Minseonk Kwak)
2012-2018 University of Michigan, Department of Biological Chemistry (Dr. Uhn-Soo Cho)

Research Interest

1. Structural Biology
2. Enzymology
3. Epigenetics

Selected Publications

1. Kim *et al*. MMOD-induced strucutural changes of hydroxylase in soluble methane monooxygenase. (2019) *Science Advances*.
2. Kim *et al.*, Janus-faced Sestrin2 controls ROS and mTOR signaling through two separate functional domains. (2015) *Nature Communications*.
3. Landry *et al.* A catalytic trisulfide in human sulfide quinone oxidoreductase catalyzes coenzyme A persulfide synthesis and inhibits butyrate oxidation. (2019) *Cell Chemical Biology*.
4. Kim *et al.*, A hinge migration mechanism unlocks the evolution of green-to-red photoconversion in GFP-like proteins. (2015) *Structure*
5. Park *et al.*, Cryo-EM structure of the human MLL1 core complex bound to the nucleosome. (2019) *Nature Communications.*

A Catalytic Trisulfide in Human Sulfide Quinone Oxidoreductase Catalyzes Coenzyme A Persulfide Synthesis and Inhibits Butyrate Oxidation

Hanseong Kim

Pukyong National University
**E-mail: hanseonk@pukyong.ac.kr*

Mitochondrial sulfide quinone oxidoreductase (SQR) catalyzes the oxidation of H2S to glutathione persulfide with concomitant reduction of CoQ_{10}. We report herein that the promiscuous activity of human SQR supported the conversion of CoA to CoA-SSH (CoA-persulfide), a potent inhibitor of butyryl-CoA dehydrogenase, and revealed a molecular link between sulfide and butyrate metabolism, which are known to interact. Three different CoQ_1-bound crystal structures furnished insights into how diverse substrates access human SQR, and provided snapshots of the reaction coordinate. Unexpectedly, the active site cysteines in SQR are configured in a bridging trisulfide at the start and end of the catalytic cycle, and the presence of sulfane sulfur was confirmed biochemically. Importantly, our study leads to a mechanistic proposal for human SQR in which sulfide addition to the trisulfide cofactor eliminates ^{201}Cys-SSH, forming an intense charge-transfer complex with flavin adenine dinucleotide, and ^{379}Cys-SSH, which transfers sulfur to an external acceptor.

References

[1] Landry, A.P., Moon, S., Kim, H., Yadav, P.K., Guha, A., Cho, U.-S., Banerjee, R. A Catalytic Trisulfide in Human Sulfide Quinone Oxidoreductase Catalyzes Coenzyme A Persulfide Synthesis and Inhibits Butyrate Oxidation. *Cell Chemical Biology* (**2019)**.

Ka Young Chung, Ph.D.

Associate Professor
Shungkyunkwan University
Phone: +82-31-290-7787 Email: kychung2@skku.edu

Education

2001 B.Pharm., College of Pharmacy, Seoul National University, Korea
2003 M.Pharm., College of Pharmacy, Seoul National University, Korea
2008 Ph.D., Molecular and Cellular Pharmacology, University of Wisconsin-Madison, USA

Work Experience

2008-2011 Postdoc, Molecular and Cellular Physiology, Stanford University, USA
2012-2016 Assistant Professor, Sungkyunkwan University, Korea
2016-present Associate Professor, Sungkyunkwan University, Korea

Research Interest

1. Structural mechanism of GPCR signaling
2. Structural mechanism of Arrestin signaling
3. Conformational dynamics of singliang proteins

Selected Publications

1. Du Y, Duc NM, Rasmussen SGF, ... Lodowski DT*, Kobilka BK*, and **Chung KY***. (2019) Assembly of a GPCR-G protein complex. ***Cell*** 177(5):1232-1242e11 *Corresponding authors
2. Par JY, Qu C, ... Sun J*, and **Chung KY***. (2019) Structural mechanism of the arrestin-3/JNK3 interaction. ***Structure*** 27(7):1162-1170e3 *Corresponding authors
3. Bang I, Kim HR, ... **Chung KY***, Choi H-J*. (2018) Biophysical and functional characterization of Norrin signaling through Frizzled4. **PNAS** 115(35):8787-8792 *Corresponding authors
4. Park JY, Yun Y, and **Chung KY*.** (2017) Conformations of JNK3a splice variants analysis by hydrogen/deuteirum exchange mass spectrometry. ***Journal of Structural Biology*** 197(3):271-278.
5. Lee SY, Giladi M, ... **Chung KY***, and Khananshvili D*. (2016) Stucture-dynamic basis of splicing dependent regulation in tissue-specific variants of the sodium-calcium exchanger (NCX1). ***FASEB Journal*** 30(3):1356-1366.

Time-resolved Conformational Analysis during GPCR-Gs Coupling

Yang Du[1,†], Nguyen Minh Duc[2,†], Søren G.F. Rasmussen[3,†], Daniel Hilger[1], Xavier Kubiak[3], Liwen Wang[4], Jennifer Bohon[4,5], Hee Ryung Kim[2], Marcin Wegrecki[3], Awuri Asuru[4], Kyung Min Jeong[2], Jeongmi Lee[2], Mark R. Chance[4,5], David T. Lodowski[4,#], Brian K. Kobilka[1,#], and Ka Young Chung[2,#,*]

[1]Molecular and Cellular Physiology, School of Medicine, Stanford University, USA
[2]School of Pharmacy, Sungkyunkwan University, Republic of Korea
[3]Department of Neuroscience, University of Copenhagen, Denmark
[4]Department of Nutrition, Center for Proteomics and Bioinformatics, School of Medicine, Case Western Reserve University, USA
[5]Case Center for Synchrotron Biosciences, Brookhaven National Laboratory, USA
[†]Equally contributed
[#]Correspondence
**E-mail: kychung2@skku.edu*

Protein-protein interactions and conformational changes of a signaling protein are major mechanisms of cellular signal transduction. To understand the precise signaling mechanism, studies have investigated the structural mechanism of signaling proteins using various biochemical and/or biophysical techniques such as X-ray crystallography, nuclear magnetic resonance (NMR), electron microscopy, and electron paramagnetic resonance. In addition to these techniques, surface labeling mass spectrometry has been successfully used for conformational analysis of signaling proteins. Exposed or flexible regions have higher labeling rates and buried or ordered regions have lower labeling rates. Although surface labeling mass spectrometry does not provide 3D structural information, it analyzes dynamic protein conformations that are difficult to be analyzed with other techniques. GPCR signal transduction involves extensive protein-protein interactions and conformational changes of related signaling proteins. In this seminar, I will discuss the conformational mechanisms of GPCR signaling analyzed by surface labeling mass spectrometry.

Hyun-Ho Lim, Ph.D.

Principal Researcher

Korea Brain Research Institute (KBRI)

Phone: +82.53.980.8330 Email: hhlim@kbri.re.kr

Education

2005 Ph.D. Life Science (Molecular Neurobiology), GIST, Korea
1999 M.S., Life Science (Neurobiochemistry), GIST, Korea
1995 B.S., Agricultural Biology, Seoul Nat'l University, Korea

Work Experience

2013-present Principal Investigator, KBRI
2007-2013 Postdoctoral Fellow, Brandeis University / HHMI
2005-2007 Postdoctoral Fellow, GIST

Research Interest

1. Structural and functional studies on the membrane proteins involved in the neuro-glia interactions
2. Structure-function relationship of chloride channels and transporters

Selected Publications

1. Park KW, Lee BC, and **Lim HH**. Mutation of external glutamate residue reveals a new intermediate transport state and anion binding site in a CLC Cl^-/H^+ antiporter. ***Proc. Natl. Acad. Sci. USA***, 2019. (*in press*)
2. **Lim HH**, Stockbridge RB, and Miller C. Fluoride-dependent interruption of the transport cycle of a CLC Cl^-/H^+ antiporter. ***Nat. Chem. Biol.* 9(11)**:721-725, 2013.
3. **Lim HH**, Shane T and Miller C. Intracellular proton access in a Cl^-/H^+ antiporter. ***PLoS Biology***, 10(12): e1001441, 2012. (*Recommended by* Faculty of 1000)
4. Stockbridge RB, **Lim HH**, Otten R, Williams C, Shane T, Weinberg Z and Miller C. Fluoride resistance and transport by riboswitch-controlled CLC antiporters. ***Proc. Natl. Acad. Sci. USA***, **109**:15289-15294, 2012.
5. **Lim HH** and Miller C. It takes two to transport, or is it one? ***Nat. Struct. Mol. Biol*. 19**:129-130, 2012. (*corresponding author*)

Coupled ion-transport mechanism in a CLC-type Cl^-/H^+ antiporter

Hyun-Ho Lim

Research Group for the Neuro-vascular Unit, Korea Brain Research Institute (KBRI), Daegu, Republic of Korea, 41068

**E-mail: hhlim@kbri.re.kr*

The CLC family proteins are involved in a variety of physiological processes to control cellular chloride concentration. Two distinct classes of CLC proteins, Cl^- channels and Cl^-/H^+ antiporters, have been functionally and structurally investigated over the last several decades. Previous studies have suggested that the conformational heterogeneity of the critical glutamate residue, Glu_{ex}, could explain the transport cycle of CLC-type Cl^-/H^+ antiporters. However, the presence of multiple conformations (*Up*, *Middle*, and *Down*) of the Glu_{ex} has been suggested from combined structural snapshots of two different CLC antiporters: CLC-ec1 from *Escherichia coli* and cmCLC from a thermophilic red alga, *Cyanidioschyzon merolae*. Thus, we aimed to investigate further the heterogeneity of Glu_{ex}-conformations in CLC-ec1, the most deeply studied CLC antiporter, at both functional and structural levels. Here, we show that the crystal structures of the Glu_{ex} mutant, E148D and wild-type CLC-ec1 with varying anion concentrations suggest a new structural intermediate, the *"Mid-low"* conformation. We also found that an extra anion can be located above the external Cl^--binding site in the E148D mutant when the anion concentration is high. Moreover, we observed that a carboxylate in solution can occupy either the external or central Cl^--binding site in the ungated E148A mutant using an anomalously detectable short carboxylic acid, bromoacetate. These results lend credibility to the idea that the Glu_{ex} can take at least three distinct conformational states during the transport cycle of a single CLC antiporter.

Acknowledgement

[1] This work was partly supported by the KBRI Basic Research Program funded by the Ministry of Science and ICT, the Republic of Korea (19-BR-01-02) and NRF Brain Research Program funded by the Ministry of Science and ICT, the Republic of Korea (2017M3C7A 1048086).

Byoung-Cheol Lee, Ph.D.

Senior Researcher

Korea Brain Research Institute

Phone: + 82-53-980-8338 Email: lbc0523@kbri.re.kr

Education

2005 B.S., Department of Bioinformatics, Soongsil University, Korea

2007 M.S., School of Life Sciences, Gwangju Institute of Science and Technology (GIST), Korea

2013 Ph.D., School of Life Sciences, GIST, Korea

Work Experience

2013-2014 Post-Doctoral Researcher, School of Life Sciences, GIST, Korea

2014-2018 Post-Doctoral Researcher, Department of Anesthesiology, Weill Cornell Medicine, USA

Research Interest

1. Structural and functional studies on the brain specific TMEM16 scramblase
2. Understanding of synaptic pruning by TMEM16 scramblase in brain
3. Modulation of Ion channel activity by the change of lipid distribution induced by TMEM16 scramblase

Selected Publications

1. Khelashvili G, Falzone ME , Cheng X, **Lee BC**, Accardi A, Weinstein H. Dynamic modulation of the lipid translocation groove generates a conductive ion channel in Ca^{2+}-bound nhTMEM16. ***Nat Commun***. 2019 (*in press*)
2. Falzone ME, Rheinberger J, **Lee BC**, Peyear T, Sasset L, Raczkowski AM, Eng ET, Di Lorenzo A, Andersen OS, Nimigean CM, Accardi A. Structural basis of Ca^{2+}-dependent activation and lipid transport by a TMEM16 scramblase. ***Elife***. 2019 Jan 16;8. pii: e43229. doi: 10.7554/eLife.43229
3. **Lee BC**, Kelashvili G, Falzone M, Menon AK, Weinstein H, Accardi A. Gating mechanism of the extracellular entry to the lipid pathway in a TMEM16 scramblase. ***Nat Commun***. 2018 Aug 14;9(1):3251
4. Malvezzi M, Andra KK, Pandey K, **Lee BC**, Falzone M, Brown A, Iqbal R, Menon AK, Accardi A. Out of the groove transport of lipids by TMEM16 and GPCR scramblases. ***Proc Natl Acad Sci.*** 2018 Jul 24;115(30):E7033-E7042
5. **Lee BC**, Menon AK, Accardi A. The nhTMEM16 Scramblase Is Also a Nonselective Ion Channel. ***Biophys J.*** 2016 Nov 1;111(9):1919-1924.

Ion and lipid transport via TMEM16 scramblases

Byoung-Cheol Lee

Research Group for the Neurovascular Unit, Korea Brain Research Institute, Daegu, Republic of Korea

**E-mail: lbc0523@kbri.re.kr*

Members of the TMEM16/ANO family of membrane proteins are Ca^{2+}-activated phospholipid scramblases and/or Cl- channels. A membrane-exposed hydrophilic groove in these proteins serves as a shared translocation pathway for ions and lipids. However, the mechanism by which lipids gain access to and permeate through the groove remains poorly understood. Here, we confirmed that fungal TMEM16 homologue, nhTMEM16 also has an ion channel activity by testing the effect of GFP-tagging and lipid composition on ion and lipid transport. Also, we combined quantitative scrambling assays and molecular dynamic simulations to identify the key steps regulating lipid movement through the groove. Lipid scrambling is limited by two constrictions defined by evolutionarily conserved charged and polar residues, one extracellular and the other near the membrane mid-point. The region between these constrictions is inaccessible to lipids and water molecules, suggesting that the groove is in a non-conductive conformation. A sequence of lipid-triggered reorganizations of interactions between these residues and the permeating lipids propagates from the extracellular entryway to the central constriction, allowing the groove to open and coordinate the headgroups of transiting lipids. Furthermore, by comparison of lipid scrambling and ion transporting activity, we concluded that ion and lipids shares their pathway in the hydrophilic groove.

KyeongJin Kang, Ph.D.

Associate Professor

Sungkyunkwan University School of Medicine

Phone: +8231-299-6085 Email: kangk@skku.edu

Education

1997 B.S., Genetic Engineering, Korea University, Korea

1999 M.S., Graduate School of Life Science and Biotechnology, Korea University, Korea

2006 PhD., Medical Sciences, Faculty of Medicine, The University of Calgary, Canada

Work Experience

2006-2012 Postdoctoral fellow, Dept of Biology, Brandeis Univ.

2012-present Assistant/Associate professor, Dept of Anatomy and Cell Biology, Sungkyunkwan Uni. School of Medicine

Research Interest

1. Chemical nociception and its implications in organisms.
2. Sensory processing via neuronal communications
3. Natural variation of sensory mechanisms

Selected Publications

1. Du, E.J., Ahn, T.J., Sung, H., Jo, H., Kim, H.-W., Kim, S.-T., and Kang, K. (2019). Analysis of phototoxin taste closely correlates nucleophilicity to type 1 phototoxicity. Proc. Natl. Acad. Sci. 116(24):12013-12018.
2. Lee, M.J., Sung, H.Y., Jo, H., Kim, H.-W., Choi, M.S., Kwon, J.Y., and Kang, K. (2017). Ionotropic Receptor 76b Is Required for Gustatory Aversion to Excessive Na+ in Drosophila. Mol. Cells 40 (10), 787-795.
3. Du, E.J., Ahn, T.J., Wen, X., Seo, D.-W., Na, D.L., Kwon, J.Y., Choi, M., Kim, H.-W., Cho, H., and Kang, K. (2016). Nucleophile sensitivity of Drosophila TRPA1 underlies light-induced feeding deterrence. elife 5, e18425.
4. Du, E.J., Ahn, T.J., Lee, J.H., Park, J.-H., Park, S.H., Kang, T.M., Cho, H., Kim, T.J., Kim, H.-W., et al. … Kang, K. (2016). TrpA1 Regulates Defecation of Food-Borne Pathogens under the Control of the Duox Pathway. PLoS Genet. *12*, e1005773.
5. Kang, K. (2016). Exceptionally high thermal sensitivity of rattlesnake TRPA1 correlates with peak current amplitude. Biochim. Biophys. Acta 1858, 318–325.

Analysis of Phototoxin Taste Closely Correlates Nucleophilicity to Type-I Phototoxicity

KyeongJin Kang

Department of Anatomy and Cell Biology, School of Medicine, Sungkyunkwan University, Suwon 16419, Republic of Korea

**E-mail: kangk@skku.edu*

Pigments often inflict tissue-damaging and pro-aging toxicity upon light illumination by generating free radicals and reactive oxygen species (ROS). However, the molecular mechanism by which organisms sense phototoxic pigments is unknown. Here, we discover that Transient Receptor Potential Ankyrin 1-A isoform (TRPA1(A)), previously shown to serve as a receptor for free radicals and ROS induced by photochemical reactions, enables *Drosophila melanogaster* to aphotically sense phototoxic pigments for feeding deterrence. Thus, TRPA1(A) detects both cause (phototoxins) and effect (free radicals and ROS) of photochemical reactions. A group of pigment molecules not only activate TRPA1(A) in darkness, but also generate free radicals upon light illumination. Such aphotic detection of phototoxins harboring the type-I (radical-generating) photochemical potential requires the nucleophile-sensing ability of TRPA1. Besides, agTRPA1(A) from malaria-transmitting mosquitoes *Anopheles gambiae* heterologously produces larger current responses to phototoxins than Drosophila TRPA1(A), similar to their disparate nucleophile responsiveness. Along with TRPA1(A)-stimulating capabilities, type-I phototoxins exhibit relatively strong photo-absorbance and low energy gaps between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). However, TRPA1(A) activation is more highly concordant to type-I phototoxicity than those photochemical parameters. Collectively, nucleophile sensitivity of TRPA1(A) allows flies to taste potential phototoxins for feeding deterrence, preventing post-ingestive photo-injury. Conversely, pigments need to bear high nucleophilicity (electron-donating propensity) to act as type-I phototoxins, which is consistent with the fact that transferring photo-excited electrons from phototoxins to other molecules causes free radicals. Thus, identification of a novel sensory mechanism in Drosophila reveals a property fundamental to type-I phototoxins.

Acknowledgement

[1] This work was supported by the National Research Foundation of Korea (NRF-2015R1D1A1A01057288, NRF-2017R1A4A1015534 and NRF-2018R1A2B6003321) funded by the Korean Government.

Gyu-Sang Hong, Ph.D.

Senior Researcher

Neuroscience department of Brain Science Institute, KIST

Phone: +8229586986 (Office) Email: gshong@kist.er.kr

Education

2016 Ph.D. Medicine and Biopharmaceutical Sciences, Seoul National University, Korea

2008 B.S. Biochemistry, Kookmin University, Korea

Work Experience

2018-Present Senior Researcher, Brain Science Institute, Korea Institute of Science and Technology (KIST)

2016-2018 Postdoctoral Research Fellow, Brain Science Institute, Korea Institute of Science and Technology (KIST)

2016 Postdoctoral Researcher, Seoul National University

Research Interest

1. Molecular Mechanism and Biophysics of Ion Channels
2. Sensory Perception Pathways such as Touch and Pain
3. The Physiological Function of Ion Channels in the Brain (Memory and Neurogenesis)

Selected Publications

1. Hong GS*, Lee SH*, Lee BJ, Jang Y, Jung J, Kim IB, Oh U. ANO1/TMEM16A Regulates Process Maturation in Radial Glial Cells in the Developing Brain. Proc Natl Acad Sci U S A. 31 May 2019.

2. Gyu-Sang Hong, Byeongjun Lee and Uhtaek Oh. Evidence for Mechanosensitive Channel Activity of Tentonin 3/TMEM150C. Neuron, Volume 94, Issue 2, p271–273.e2, 19 April 2017.

3. HONG GS*, Lee B*, Wee J, Chun H, Kim H, Jung J, Cha JY, Riew TR, Kim GH, Kim IB, Oh U. Tentonin 3/TMEM150c Confers Distinct Mechanosensitive Currents in Dorsal-Root Ganglion Neurons with Proprioceptive Function. Neuron, 91, p107-118. June 16, 2016.

Cytoskeleton dependent activation of Tentonin 3/TMEM150C, a novel mechanosensitive channel

Gyu-Sang Hong, PhD.

Neuroscience department of Brain Science Institute, KIST, Republic of Korea

**E-mail: gshong@kist.re.kr*

Tentonin 3/TMEM150C (TTN3) has been recently discovered as a novel mechanosensitive (MS) channel. It is activated by mechanical stimuli followed by slow inactivation in the heterologous system, distinct from Piezo1 or 2 that has a rapid inactivation. However, the activation mechanism of TTN3 has been challenged. Because TTN3 fails to show MS currents in *Piezo1*-deficient HEK (HEK-P1KO) cells, TTN3 is considered to be a regulatory protein controlling Piezo1 activity. To address this issue, we hypothesized that mechanosensitivity of TTN3 depends on the cytoskeleton integrity of the cell. Here, we found that loss of TTN3 MS current in HEK-P1KO is highly correlated with the focal adhesion (FA) complex. When the F-actin assembly is strengthened after jasplakinolide-treatment, mechanical stimuli robustly evoked MS currents in *Ttn3*-transfected HEK-P1KO cells. HEK-P1KO cells showed dramatic loss of FA proteins and knockdown of FA proteins in HEK cells also reduced the TTN3-dependent MS currents. Thus, we conclude that mechanosensitivity of TTN3 is dependent on the cytoskeleton integrity. This finding supports the idea that TTN3 is an essential component of the slowly inactivating MS channel complex.

References

[1] Gyu-Sang Hong, Byeongjun Lee and Uhtaek Oh. Evidence for Mechanosensitive Channel Activity of Tentonin 3/TMEM150C. Neuron, Volume 94, Issue 2, p271–273.e2, 19 April 2017.

[2] HONG GS*, Lee B*, Wee J, Chun H, Kim H, Jung J, Cha JY, Riew TR, Kim GH, Kim IB, Oh U. Tentonin 3/TMEM150c Confers Distinct Mechanosensitive Currents in Dorsal-Root Ganglion Neurons with Proprioceptive Function. Neuron, 91, p107-118. June 16, 2016.

MEMO

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

2020

한국생체분자과학 연합학회학술대회

Symposium V & VI

Jae-Sung Woo, Ph.D.

Associate Professor
Korea University
Phone: 02-3290-3406 Email: jaesungwoo@korea.ac.kr

Education

2002 B.S., Life Science, POSTECH, Korea
2007 Ph.D., Structural Biology, POSTECH, Korea

Work Experience

2007-2009 Postdoctoral fellow, POSTECH, Korea
2009-2013 Postdoctoral fellow, ETH Zurich, Switzerland
2013-2017 Research Fellow, Institute for Basic Science
2017- Associate Professor, Korea University

Research Interest

1. Structures and functional mechanisms of cell junction proteins and RNA-binding proteins
2. Development of the expression system for structural biology

Selected Publications

1. Kwon SC, Baek SC, Choi Y-G, Yang J, Lee Y, Woo J-S§, Kim VN§ (2018) Molecular Basis for the Single-Nucleotide Precision of Primary microRNA Processing Molecular Cell 1–14
2. Kwon SC, Nguyen TA, Choi YG, Jo MH, Hohng S, Kim VN§, Woo J-S§ (2016) Structure of Human DROSHA Cell 164(1–2):81–90
3. Nguyen TA, Jo MH, Choi Y-G, Park J, Kwon SC, Hohng S, Kim VN§, Woo J-S§ (2015) Functional Anatomy of the Human Microprocessor Cell 161(6):1374–1387
4. Woo J-S†, Zeltina A†, Goetz B a, Locher KP (2012) X-ray structure of the Yersinia pestis heme transporter HmuUV. Nature Structural & Molecular Biology 19(12):1310–5
5. Woo J-S†, Lim J-H†, Shin H-C, Suh M-K, Ku B, Lee K-H, Joo K, Robinson H, Lee J, Park S-Y, Ha N-C, Oh B-H (2009) Structural Studies of a Bacterial Condensin Complex Reveal ATP-Dependent Disruption of Intersubunit Interactions Cell 136(1)

Cryo-EM Structure of Human Cx31.3/GJC3 Hemichannel

Hyuk-Joon Lee[1,†], Hyeongseop Jeong[1,2,†], Bumhan Ryu[1], Jaekyung Hyun[2,3], Jae-Sung Woo[1,*]

[1]Department of Life Sciences, Korea University, Seoul 02841, Korea

[2]Korea Basic Science Institute, Chungcheongbuk-do 28119, South Korea

[3]Molecular Cryo-electron Microscopy Unit, Okinawa Institute of Science and Technology, Okinawa 904-0496, Japan

**E-mail: jaesungwoo@korea.ac.kr*

Connexin family proteins assemble into hexameric channels called hemichannels/connexons in the cell membrane, and two hemichannels from adjacent cells dock together to form a gap junction intercellular channel. Although the permeability of both half and full channels is known to be finely regulated by various environmental factors including divalent ions, the underlying molecular mechanisms remain unknown. Here we determine the cryo-EM structures of human Cx31.3/GJC3 hemichannel, its Ca2+-bound state, and the ATP-impermeable R15G mutant, at 2.3, 2.5, and 2.6 Å resolutions, respectively. We find that N-terminal helices (NTHs) of the hemichannel are collocated at the cytoplasmic entrance forming ~8 Å diameter pore, suggesting that these structures represent closed conformation refractory against ATP transport. Ca^{2+}-binding in a deep tunnel between transmembrane and extracellular regions interrupts water-mediated interconnexin interactions and inhibits reorganization of the salt-bridge network possibly required for interconnexon docking and channel opening. Our study provides a structural basis for understanding NTH-mediated and Ca^{2+}-dependent closing mechanism of connexin channels.

Je-Kyung Ryu, Ph.D.

Postdoctoral researcher
Technische Universiteit of Delft (TU Delft)
Phone: +31-638703809 Email: workrjk@gmail.com

Education

2006, **KAIST**, B.S.in Mathematics (Double major : Physics)

2014, **KAIST,** Ph.D. in Physics

Work Experience

2016 ~ Present, Postdoctoral Research Fellow, TU Delft, Delft, The Netherlands, (PI: Cees Dekker).

2014 ~ 2015, Postdoctoral Research Fellow, KAIST, (PI : Tae-Young Yoon).

2012, Visiting program, Max Plank Institute Biochemistry for Biophysical Chemistry, Göttingen, Germany (PI : Reinhard Jahn).

2007 ~ 2008, Visiting Scholar, Howard Hughes Medical Institute, University of Illinois, Urbana, Illinois, USA (PI : Taekjip Ha).

Research Interest

1. High-Speed Atomic Force Microscopy (HS-AFM)
2. SMC proteins for chromosome orgnization
3. Phase separation
4. Single-molecule biophysics

Selected Publications

1. **Je-Kyung Ryu**, Duyoung Min, Sang-Hyun Rah, Soo Jin Kim, Yongsoo Park, Haesoo Kim, Changbong Hyeon, Ho Min Kim, Reinhard Jahn & Tae-Young Yoon "Spring-loaded unraveling of a single SNARE complex by NSF in one round of ATP turnover", ***Science***, **347**(6229)**,** 1485-1489 (2015).
2. **Je-Kyung Ryu**, Soo Jin Kim, Sang-Hyun Rah, Ji In Kang, Hi Eun Jung, Heung-Kyu Lee, Jie-Oh Lee, Beom Seok Park, Tae-Young Yoon, and Ho Min Kim, "Reconstruction of LPS transfer cascade reveals structural determinants within LBP, CD14 and TLR4-MD2 for efficient LPS recognition and transfer", ***Immunity***, **46**(1), 38-50 (2017).
3. Yongsoo Park* and **Je-Kyung Ryu***, "Models of synaptotagmin-1 to trigger Ca2+-dependent vesicle fusion", ***FEBS Letters***, (2018). * Correspondence authors.
4. **Je-Kyung Ryu**, Allard Katan, Eli van der Sluis, Shveta Bisht, Thomas Wisse, Indra Shaltiel, Jaco van der Torre, Wayne Yang, Ralph de Groot, Christian Haering, and Cees Dekker, "AFM imaging of Condensin architecture for DNA loop extrusion", *Molecular Cell*, **(2019).** *In preparation.*
5. **Je-Kyung Ryu**, Minamino Masashi , Eugene Kim, Jaco van der Torre, Allard Katan, Ralph de Groot, Mart Last, Frank Uhlmann, and Cees Dekker, "DNA bridging by cohesin phase separates for liquid droplet formation", *Science* **(2019).** *In preparation.*

AFM imaging of open and collapsed states of yeast condensin suggest a scrunching model for DNA loop extrusion

Je-Kyung Ryu,[1] Christian Haering,[2] and Cees Dekker[1, 3]

[1]*Department of Bionanoscience, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, Netherlands.*

[2]*Cell Biology and Biophysics Unit, Structural and Computational Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.*

**E-mail: J.Ryu@tudelft.nl*

Structural Maintenance of Chromosome (SMC) protein complexes are the key organizers of the spatiotemporal structure of chromosomes. The condensin SMC complex, which compacts DNA in mitosis, was recently shown to be a molecular motor that extrudes large loops of DNA. The mechanism of this unique motor, which takes large steps along DNA at low ATP consumption, remains elusive however. Here, we use atomic force microscopy to visualize the structure of yeast condensin and condensin-DNA complexes. Condensin is found to exhibit mainly open 'O' shapes and collapsed 'B' shapes, and it toggles dynamically between these two states over time. Condensin binds double-stranded DNA via a heat domain and, surprisingly, also via the hinge domain. For DNA loops, we observe a single condensin complex at the stem each loop, where the neck size of the DNA loop correlates with the width of the condensin complex. Our results suggest that condensin extrudes DNA by a cyclic fast switching of its conformation between the O and B shapes, consistent with a scrunching model.

Acknowledgement

This project has received funding from the European Union's Horizon 2020 research and in novation programme under the Marie Sklodowska-Curie grant agreement No 753002.

Suk-youl Park, Ph.D.

Senior Research Scientist
Beamline Research Division, Pohang Accelerator Laboratory, POSTECH, Korea
Phone: +82-54-279-1523 Email: navypsy@postech.ac.kr

Education

2006 B.S., Chemistry, Chonnam National University, Gwangju, Korea
2008 M.S. Chemistry, Chonnam National University, Gwangju, Korea
2012 Ph.D. Chemistry, Chonnam National University, Gwangju, Korea

Work Experience

2012-2013 Postdoctoral Fellow, Advanced Institutes of Convergence Technology, Seoul National University (SNU), Suwon, Korea.
2013-2015 Postdoctoral Fellow, Laboratory of Metabolism, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, Maryland, USA
2015-Present Senior Research Scientist, Pohang Accelerator Laboratory (PAL), POSTECH, Korea

Research Interest

1. Serial millisecond crystallography using synchrotron beam.
2. Histone deacetylase and SMRT/NCoR complex.
3. Centrosome biogenesis and functions.

Selected Publications

1. Park, S.Y. and Nam, K.H. Sample delivery using viscous media, a syringe and a syringe pump for serial crystallography. *J. Synchrotron Rad.* 26, 1815-1819 (2019).
2. Park, S.Y. et al., Structural basis of the specific interaction of SMRT corepressor with histone deacetylase 4. *Nucleic Acids Res.* 46(22), 11776-11788 (2018).
3. Cross, M. et al., Trehalose 6-phosphate phosphatases of Pseudomonas aeruginosa. *FASEB J.* 32(10), 5470-5482 (2018).
4. Park, S.Y. et al., Molecular basis for unidirectional scaffold switching of human Plk4 in centriole biogenesis. *Nat. Struct. Mol. Biol.* 21(8), 696-703 (2015).
5. Park, S.Y. et al., Structural characterization of a modification subunit of a putative type I restriction enzyme from Vibrio vulnificus YJ016. *Acta Crystallogr. Sect. D Biol. Crystallogr.* D68, 1570-1577 (2012).

BL11C: a High-flux Micro-MX beamline for micron-sized protein crystals at PAL

Suk-Youl Park

Pohang Accelerator Laboratory, POSTECH
**E-mail: navypsy@postech.ac.kr*

BL-11C is an in-vacuum undulator based microfocus beamline dedicated for macromolecular crystallography at the Pohang Accelerator Laboratory, and has been opened to users in June 2017. The beamline is energy tunable in the range of 5.0 - 20 keV to support conventional single anomalous dispersion and also multi-wavelength anomalous dispersion experiments against a wide range of heavy metals. At the standard working energy of 12.659 keV, the monochromatized beam is focused to 4.1 (V) × 8.5 (H) μm^2 FWHM at the sample position, and the measured photon flux is 1.3×10^{12} photons/sec. The experimental station is equipped with the PILATUS 6M detector, micro-diffractometer (MD2S) incorporating a multi-axis goniometer, and a robotic sample exchanger (CATS) with a dewar capacity of 90 samples. This beamline is suitable for structural determination of weakly diffracting crystals such as biomaterials including protein, nucleic acids and their complexes. Moreover, as a new technique, serial crystallography using synchrotron beam is also possible from micron sized inorganic and organic chemical crystals as well as micron sized protein crystals. This presentation will introduce the current beamline characteristics, future plans, and some recent scientific highlights.

References

[1] Park, S.Y., Ha, S.C., Kim, Y.G. The Protein Crystallography Beamlines at the Pohang Light Source II. *Biodesign.* 5 (1), 30-34. (**2017**).
[2] Park, S.Y. and Nam, K.H. Sample delivery using viscous media, a syringe and a syringe pump for serial crystallography. *J. Synchrotron Rad.* 26, 1815-1819 (**2019**).

Kyung hyun Kim, Ph.D.

Professor
Korea Unversity Sejong Campus
Phone: + 02 3290 3444 Email: khkim@korea.ac.kr

Education

1982 B.S., Honours. Seoul National University, Korea. Agricultural Chemistry
1985 M.S., Seoul National University, Korea. Agricultural Chemistry (Biophysics)
1990 Ph.D., Iowa State University, Ames, U.S.A. (Biophysics)

Work Experience

1991 Postdoctoral Research Associate, Purdue University, West Lafayette, U.S.A
1995 Assistant Professor of Biophysics, Department of Biotechnology, Korea University
1998 Associate Professor of Biophysics, Graduate School of Biotechnology, Korea University
2003 Professor of Biophysics, Department of Biotechnology & Bioinformatics, Korea University

Research Interest

1. Structure-based vaccine discovery
2. Universal influenza vaccine development
3. pH-mediated protein dynamics

Selected Publications

1. Ji-Hye Lee et al., Insight into the interaction between RNA polymerase and VPg for norovirus replication. Frontiers Microbiol (2018) 9, 1466 (1-15).
2. Dan Bi Lee et al. Supersaturation controlled microcrystallization and visualization analysis for serial femtosecond crystallography. Sci. Rep. (2018) 8, 2541.
3. Kang Rok Han et al. Nucleotide triphosphatase and RNA chaperone activities of murine norovirus NS3 J. Gen. Virol. (2018) 99, 1482-1493.
4. Jong Hyeon Seok et al. Conformational modulation of influenza virus hemagglutinin: characterization and in vivo efficacy of monomeric form. Sci. Rep. (2017) 7, 7540.
5. Sella Kim et al. Structural basis of novel iron-uptake route and reaction intermediates in ferritins from Gram-negative bacteria. J. Mol. Biol. (2016) 428, 5007-5018.

Time-resolved pH-induced protein dynamics using serial femtosecond crystallography

Ji-Hye Lee[1], Dan Bi Lee[1], Jeong Suk An[1], Jong Hyeon Seok[1], Jeong-Won Nam[1,†], Jungkweon Choi[2,3], Donghyeok Kim[2,3], Tianxiang Yang[1], Han Byul Jung[1], Hye Jin Kwon[1], Jaehyun Park[4], Intae Eom[4], Minseok Kim[4], Sang Jae Lee[4], Sehan Park[4], Ki Hyun Nam[4,†], Jangwoo Kim[4], Gisu Park[4], Hyotcherl Ihee[2,3], Mi Sook Chung[5], Nadia Zatsepin[6], and Kyung Hyun Kim[1,*]

[1]Department of Biotechnology & Bioinformatics, Korea University, Sejong 30019, [2]Center for Nanomaterials and Chemical Reactions, Institute for Basic Science, Daejeon 34141, [3]Department of Chemistry and KI for the BioCentury, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, [4]Pohang Accelerator Laboratory, Pohang, Gyeongbuk 37673, [5]Department of Food and Nutrition, Duksung Women's University, Seoul 01369, Korea, [6]Department of Physics, Arizona State University, Tempe, Arizona 85281, USA

**E-mail: khkim@korea.ac.kr*

Although protein structure and function are highly dependent on pH, pH-induced structural dynamics has been elusive. While time-resolved (TR) structural studies can track protein conformation dynamics, their applications have been mostly limited to light-responsive proteins. Here, we report a successful pH-jump TR-serial femtosecond crystallography (TR-SFX) on NowGFP, a green-emitting fluorescent protein, using *ortho*-nitrobenzaldehyde as a caged molecule. NowGFP shows spectral characteristics caused by the laser-triggered pH-jump in solution and microcrystals. The structures determined by TR-SFX reveal pH-induced conformational changes, involving the movement of Lys61 away from the chromophore, shown in an alternative conformation at 100 ns, along with the introduction of water molecules to an open state of the β7–β10 channel at 1 μs. Considering that most biological macromolecules are not light-responsive but pH-dependent, this new framework of pH-jump TR-SFX has a great potential for addressing critical gaps for dynamics studies of many important biological processes.

Acknowledgement

[1] We acknowledge technical support from the staffs at the LCLS CXI beamline, PAL-XFEL NCI beamline, and PLS-II 11C and would like to thank Dr. Joon-Yeon Kim at Iowa State University for his helpful comments on the manuscript. This work was supported by grants from the strategy (NRF-2016R1E1A1A01942558, K.H.K.), SGER (NRF-2018R1D1A1A02049225, J-H.L.) and BK21 Plus programs from the National Research Foundation (NRF) funded by the Korean government, by Korea Drug Development Fund funded by Ministry of Science and ICT, Ministry of Trade, Industry, and Energy, and Ministry of Health and Welfare (KDDF-201712-14, K.H.K.), by the Ministry of Science and ICT of Korea (2018-2nd-NCI-001, I.E.), and by Institute for Basic Science (IBS-R004, H.I).

Yongwon Jung, Ph.D.

Associate Professor
Korea Advanced Institute of Science and Technology (KAIST)
Phone: + 82-42-350-2817 Email: ywjung@kaist.ac.kr

Education

1998 B.S., Chemistry, KAIST, Korea
2000 M.S., Biochemistry, KAIST, Korea
2005 Ph.D., Biological Chemistry, MIT, USA

Work Experience

2005-2012 Senior Scientist, BioNanotechnology Research Center, KRIBB, Korea
2012-2016 Assistant Professor, Department of Chemistry, KAIST, Korea
2016-present Associate Professor, Department of Chemistry, KAIST, Korea

Research Interest

1. Supramolecular protein assembly
2. Biomolecular multivalent interactions
3. Biomolecular phase separation (condensation)

Selected Publications

1. Song D and Jung Y, "Homo-molecular Fluorescence Complementation for Direct Visualization of Receptor Oligomerization" *Angew. Chem.* 2019, 58, 2045
2. Yoon HR, Choi H, Choi YA, et. al. and Jung Y, "Fabrication of Oligomeric Avidin Scaffolds for Valency-Controlled Surface Display of Functional Ligands" *Angew. Chem.* 2018, 57, 12410
3. Ahn B, Lee SG, Yoon HR, et.al. Kim H and Jung Y, "Four-fold Channel-Nicked Human Ferritin Nanocages for Active Drug Loading and pH-Responsive Drug Release" *Angew. Chem.* 2018, 57, 2909
4. Lee JM, Hwang A, et. al. Kim B, Kang T, and Jung Y, "Multivalent Structure-Specific RNA Binder with Extremely Stable Target Binding but Reduced Interaction to Nonspecific RNAs" *Angew. Chem.* 2017, 56, 15998
5. Lee JM, et. al. and Jung Y, "A Rhizavidin Monomer with Nearly Multimeric Avidin Proteins-Like Binding Stability Against Biotin Conjugates" *Angew. Chem.* 2016, 55, 3393
6. Kim YE, et. al. and Jung Y, "Green fluorescence protein nanopolygons as monodisperse supramolecular assemblies of functional proteins with defined valency" *Nat. Commun.* 2015, 6, 7134

Supramolecular Biochemistry: engineering artificial protein assemblies

Yongwon Jung

Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Korea

**E-mail: ywjung@kiast.ac.kr*

Nature utilizes a wide range of sophisticated biomolecular assemblies to perform diverse cellular processes in a highly precise and synergistic manner. Proteins are major building blocks for these precisely organized assemblies by virtue of their abundant structures and functions. A myriad of supramolecular protein assemblies exist in nature, where their highly diverse structures include linear, circular, tubular, and cage forms. Accordingly, engineered, artificial protein assemblies have also attracted considerable interest as new biomaterials with the potential to offer dynamic functionalities and molecular level structural accuracy.

One of major research goals in our group is to create precise nano-assemblies of functional proteins with both defined structures and a controlled number of protein building blocks. With these protein assemblies, we aim to conduct or control highly precise but dynamic behaviors of supramolecular bio-assemblies. Here, I will introduce several our newly developed artificial protein assemblies with defined valency and structures. In particular, our strategies to fabricate a series of fluorescent protein based assemblies with diverse shapes and functions will be discussed. In addition, examples of a few bio-analytical tools that utilize these protein assemblies will also be briefly presented.

Kyubong Jo, Ph.D.

Professor
Sogang University
Phone: +82 2 705-8881 Email: jokyubong@sogang.ac.kr

Education

1993 B.S., Chemistry Education, Seoul National University, Korea
1995 M.S., Chemistry, Seoul National University, Korea
2006 Ph.D., Chemistry, University of Wisconsin-Madison, Korea

Work Experience

2006-2008 University of Illinois at Urbana-Champaign
1995-2001 Samsung Advanced Institute of Technology

Research Interest

1. Single-molecule DNA Mapping
2. DNA-Protein Interaction
3. Sequence-specific Dye

Selected Publications

1. *Nucleic Acids Research*, 2018, 46 (18), e108
2. *Small*, 2017, 13, 160192
3. *Nucleic Acids Research*, 2016,44(1),e6
4. *Chemical Communications*, 2013,49(42),4740
5. *PNAS*, 2007, 104, 2673

Visualization of A/T Specific Sequence on a Large DNA Molecule

Kyubong Jo

Sogang University
**E-mail: jokyubong@sogang.ac.kr*

Large DNA molecules are a promising platform for in vitro single-molecule biochemical ana lysis to investigate molecular events by fluorescence microscopic visualization. For many stu dies, intercalating fluorescent dyes (YOYO-1) have been a primary DNA staining reagent, b ut they often cause photo-induced DNA breakage as well as structural deformation. As a so lution, we developed several approaches to stain DNA molecules such as fluorophore-linked, sequence-specific DNA binding polyamide, and fluorescent-protein DNA-binding peptides or proteins (FP-DBP). These new developments overcome the limitation of DNA staining by in tercalating dye such as structural deformation as well as photo-induced DNA damage. Furth ermore, their staining is reversible, which is advantageous for monitoring biochemical reacti ons in a microfluidic device. In this presentation, I'd like to present how to identify large DNA based on its staining pattern and how to visualize biochemical reactions on a large D NA molecule, such as digestion and folding.

References

[1] Single-molecule DNA visualization using AT-specific red and non-specific green DNA-bi nding fluorescent proteins, ***Analyst***, 2019, 144 (3), 921-927
[2] TAMRA-Polypyrrole for A/T Sequence Visualization on DNA Molecules, ***Nucleic Acids Research***, 2018, 46 (18), e108
[3] Visualization of surface-tethered large DNA molecules with a fluorescent protein DNA b inding peptide, ***Journal of Visualized Experiments***, 2016, e54141
[4] Investigation of Various Fluorescent Protein-DNA Binding Peptides for Effectively Visual izing Large DNA Molecules, ***RSC Advances***, 2016, 6, 46291-46298
[5] DNA Binding Fluorescent Proteins for the Direct Visualization of Large DNA Molecules ***Nucleic Acids Research***, 2016,44(1), e6

Acknowledgement

[1] This work was supported by the Korea Research Foundation Grant (code KRF-2008-331-E00457 and 2009-0076543), the Republic of Korea.

Sebyung Kang, Ph.D.

Associate Professor
Department of Biological Sciences, School of Life Sciences
Ulsan National Institute of Science and Technology (UNIST)
Phone: +82-52-217-5325 Email: sabsab7@unist.ac.kr

Education

2001/09-2006/12: Ph.D. Biochemistry & Molecular Genetics, Univ. of Alabama at Birmingham, USA
1998/03-2000/02: MS. Microbial Engineering, Konkuk Univ. South Korea
1991/03-1998/02: BS. Microbial Engineering, Konkuk Univ. South Korea

Work Experience

2010/03-Present: Assistant & Associate Professor, School of Life Sciences, UNIST, South Korea
2018/06-present: Director, Center for Cell to Cell Communication in Cancers (University-Centered Labs)
2013/09-Present: Team leader – UNIST BK21^{+} Biomolecular Network Research Team
2017/02-2018/01: Visiting scholar, Department of Chemistry, Indiana University, USA
2007/09-2010/01: Post-Doc, Chemistry & Biochemistry, Montana State University, USA
2007/01-2007/08: Post-Doc, Microbiology, Univ. of Alabama at Birmingham, USA

Research Interest

1. Nanobiotechnology & Protein Engineering
2. Protein Nanoparticle-based Drug Delivery and Diagnostic Probe Systems
3. Biosensors & Bioimagings

Selected Publications

1. Oh, J. Y, Kim, H., Palanikumar, L., Go, E. M., Jana, B., Park, S. A., Kim, H. Y., Kim, K., Seo, J. K., Kwak,S.K., Kim,C.,* Kang, S.,* and Ryu, J-H.* Cloaking Nanoparticles with Protein Corona Shield for Targeted Drug Delivery. ***Nat. Comm.***, 2018, 9, 4548.
2. Choi, H., Choi, B., Kim, H., Kim, G. J., Kim, H., Jung, H. S., and Kang, S.* Fabrication of Nano-reaction Clusters with Dual-functionalized Protein Cage Nano-building Blocks. ***Small***, 2018, 14, 1801488.
3. Choi, B., Moon, H., Hong, S. J., Shin, C. S., Do, Y., Ryu, S.* and Kang, S.* Effective Delivery of Antigen-Encapsulin Nanoparticle Fusions to Dendritic Cells Leads to Antigen-Specific Cytotoxic T cell Activation and Tumor Rejection. ***ACS Nano***, 2016, 10, 7339-7350.
4. Min, J., Song, E. K., Kim, H., Kim, K. T., Park, T. J.* and Kang, S.* A Recombinant Secondary Antibody Mimic as a Target-specific Signal Amplifier and an Antibody Immobilizer in Immunoassays. ***Sci. Rep.*** 2016, 6, 24159.
5. Lee, J., Song, E., Bae, Y., Min, J., Rhee, H.-W., Park, T. J., Kim, M.,* and Kang, S.* An Enhanced Ascorbate Peroxidase 2/Antibody-Binding Domain Fusion Protein (APEX2-ABD) as a Recombinant Target-Specific Signal Amplifier. ***Chem. Comm.*** 2015, 51, 10945-10948.
6. Kang, H.J., Kang, Y.J., Lee, Y.M., Shin, H., Chung, S.J.,* and Kang, S.* Developing an Antibody-binding Protein Cage as a Molecular Recognition Drug Modular Nanoplatform. ***Biomaterials***, 2012, 33, 5423-5430

Development of Recombinant Secondary Antibody Mimics as Signal Amplifiers in Immunoassays

Sebyung Kang, Ph.D

Department of Biological Sciences, School of Life Sciences
Ulsan National Institute of Science and Technology (UNIST), Ulsan, 44919, Korea
**E-mail: sabsab7@unist.ac.kr*

Immunoassay refers to a biochemical analysis that measures the presence and/or concentration of specific target molecules by using antibodies which are principally based on strong and selective interactions, and thus is widely used techniques in biomedical tests such as disease diagnoses, virus or pathogen detection, and pregnancy test. In general, the immunoassay utilizes two different types of antibodies, primary and secondary antibodies, to satisfy both specificity and sensitivity. Primary antibody selectively recognizes and tightly binds to its target analytes to give sample specificity, whereas secondary antibody binds to the Fc region of target analyte-bound primary antibodies to amplify signals. To amplify signals, a conventional secondary antibody is covalently linked with active enzymes such as horseradish peroxidase (HRP) that convert multiple copies of inactive substrates into signal-generating active forms, leading to the efficient detection of low-abundant target analytes. Although enzyme-linked secondary antibodies have been widely used in immunoassays, antibodies themselves should be obtained from animals and further modified chemically in a lengthy and complicated ways.

To circumvent these issues, we have developed a couple of recombinant secondary antibody mimics, produced them at large quantity using bacterial overexpression system and simple purification methods, and demonstrated their outstanding signal amplifying capability regardless of target molecules. Each recombinant secondary antibody mimic would be selected for its own purpose and applied to various types of target analytes in combination with a variety of target-specific primary antibodies, effectively minimizing the use of animals as well as reducing cost and time for production.

Acknowledgement

[1] This work was supported by the Korea Research Foundation Grant (NRF-2016R1D1A1B03932580 and 2019R1A2C2002749), the Republic of Korea.

Dae-Ro Ahn, Ph.D.

Principal research scientist
Korea Institute of Science and Technology (KIST)
Professor
KIST School, University of Science and Technology (UST)
Phone: +8229586645 Email: drahn@kist.re.kr

Education

1998 B.S., Chemistry, Seoul National University, Korea
2000 M.S., Chemistry, Seoul National University, Korea
2003 Ph.D., Chemistry and Biochemistry, University of Bern, Switzerland

Work Experience

2005-2006 Post-doc, Chemistry, University of Florida, USA
2003-2008 Research scientist, KIST, Korea
2008-2014 Senior research scientist, KIST, Korea
2014-present Principal research scientist, KIST, Korea

Research Interest

1. Biomedical applications of DNA nanostructures
2. Engineering of guide RNAs for CRISPR system
3. Directed evolution of functional nucleic acids and proteins

Selected Publications

1. Kim, K.-R. et al., Highly tumor-specific DNA nanostructures discovered by in vivo screening of a nucleic acid cage library and their applications in tumor-targeted drug delivery, *Biomaterials*, **2019**, *195*, 1-12.
2. Kim, K.-R. et al., Streptavidin-mirror DNA tetrahedron hybrid as a platform for intracellular and tumor delivery of enzymes, *J. Control. Release*, **2018**, 280, 1-10.
3. Kim, K.-R. et al., Self-assembled mirror DNA nanostructures for tumor-specific delivery of anticancer drugs, *J. Control. Release*, **2016**, 243, 121-131.
4. Kim, K.-R. et al., Utilizing the bioorthogonal base-pairing system of L-DNA to design ideal DNA nanocarriers for enhanced delivery of nucleic acid cargos, *Chem. Sci.,* **2014**, 5, 1533-1537.
5. Kim, K.-R. et al., Sentinel lymph node imaging by a fluorescently labeled DNA tetrahedron, *Biomaterials,* **2013**, 34, 5226-5235

A library-based approach to develop tumor-targeted drug carriers

Dae-Ro Ahn*

Korea Institute of Science and Technology (KIST)
**E-mail: drahn@kist.re.kr*

Enormous efforts have been made to harness nanoparticles showing extravasation around tumors for tumor-targeted drug carriers. Owing to the complexity of in vivo environments, however, it is very difficult to rationally design a nanoconstruct showing high tumor specificity. Here, we show an approach to develop tumor-specific drug carriers by screening a library of self-assembled nucleic acid cages in vivo. After preparation of a library of 16 nucleic acid cages by combining the sugar backbone and the shape of cages, we screened the biodistribution of the cages intravenously injected into tumor-bearing mice, to discover the cages with high tumor-specificity. This tumor specificity was found to be closely related with serum stability, cancer cell uptake efficiency, and macrophage evasion rate. We further utilized the cages showing high tumor specificity as carriers for the delivery of not only a cytotoxic small molecule drug but also a macromolecular apoptotic protein exclusively into the tumor tissue to induce tumor-specific damage. The results demonstrate that our library-based strategy to discover tumor-targeted carriers can be an efficient way to develop anti-cancer nanomedicines with tumor specificity and enhanced potency.

References

[1] Kim, K.-R. et al., Highly tumor-specific DNA nanostructures discovered by in vivo screening of a nucleic acid cage library and their applications in tumor-targeted drug delivery, *Biomaterials* (**2019)**.

Acknowledgement

[1] This work was supported by the National Research Foundation of Korea (2014M3C1A3054141 and 2017M2A2A7A02019610).

MEMO

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

2020

한국생체분자과학 연합학회학술대회

Luncheon seminar

Atsushi Nakagawa, Dr. Sci.

Professor

Institute for Protein Research, Osaka University

Phone: +81-6-6879-4313 Email: atsushi@protein.osaka-u.ac.jp

Education

1985 B.S., Faculty of Science, Nagoya University, Japan

1986 M. Sci., Graduate School of Science, Osaka University, Japan

1989 Dr. Sci., Osaka University

Work Experience

1986-1995 Research Associate, Photon Factory, KEK, Japan

1995-1999 Associate Professor, Graduate School of Science, Hokkaido University, Japan

1999-2003 Associate Professor, Institute for Protein Research, Osaka University, Japan

2003-present Professor, Institute for Protein Research, Osaka University, Japan

2018-present Director, Institute for Protein Research, Osaka University, Japan

Research Interest

1. Structural Biology
2. Synchrotron Radiation

Selected Publications

1. Takeshita, K., Sakata, S., Yamashita, E., Fujiwara, Y., Kawanabe, A., Kurokawa, T., Okochi, Y., Matsuda, M., Narita, H., Okamura, Y., Nakagawa, A., *Nat. Struct. Mol. Biol.*, **21**, 352-357 (2014)
2. Sasanuma H., Tawaramoto M.S., Lao J.P., Hosaka H., Sanda E., Suzuki M., Yamashita E., Hunter N., Shinohara M., Nakagawa A., Shinohara A., *Nat. Commun*,, **4**, 1676 (2013)
3. Takeshita K., Suetake I., Yamashita E., Suga M., Narita H., Nakagawa A., Tajima S., *Proc. Natl. Acad. Sci. USA*, **108**, 9055-9059, 2011.
4. Nakagawa A., Miyazaki, N., Taka, J., Naitow, H., Ogawa, A., Fujimoto, Z., Mizuno, H., Higashi, T., Watanabe, Y., Omura, T., Cheng, R.H., Tsukihara, T., *Structure*, **11**, 1227-1238 (2003)
5. Kondo T., Yoshida, K., Nakagawa, A., Kawai, T., Tamura, H., Goto, T., *Nature*, **358**, 515-518 (1992)

OneDep: Unified System for Deposition, Biocuration, and Validation of Macromolecular Structures

Atsushi Nakagawa

Institute for Protein Research, Osaka University, Japan

**E-mail: atsushi@protein.osaka-u.ac.jp*

The Protein Data Bank (PDB) is the single global repository for experimentally-determined three-dimensional structures of biological macromolecules. The Worldwide Protein Data Bank (wwPDB) is the international collaboration that manages the PDB archive according to the FAIR principles. A common tool for deposition, validation, and biocuration of structures of biological macromolecules, OneDep, was recently developed by the wwPDB partners [Fig.1]. All data deposited to the PDB undergo critical review by wwPDB biocurators. In this presentation, I outline the importance of biocuration for structural biology data, and describe wwPDB biocuration processes and the role of expert biocurators in maintaining a high-quality, open access archive. Structure data coming into the PDB are examined for self-consistency, standardized using controlled vocabularies and value boundaries, cross-referenced with other biological data resources, and validated for scientific/technical accuracy.

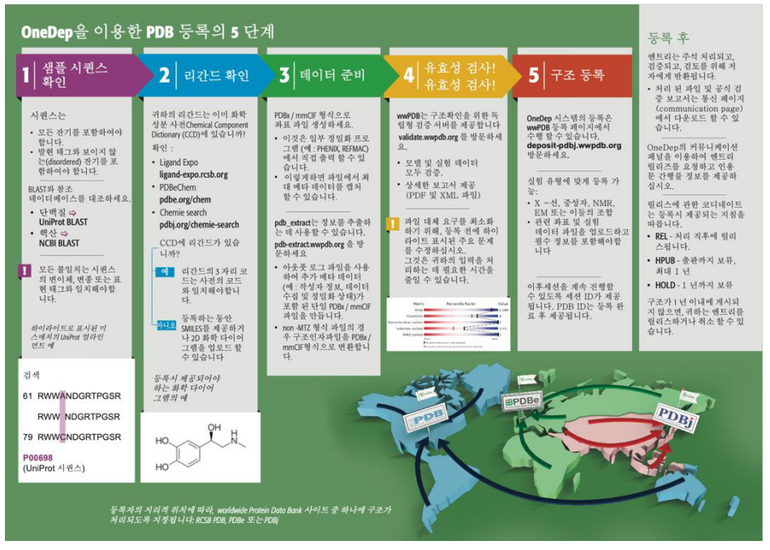


Fig.1. Five Easy Steps to PDB Deposition with OneDep

Nobutoshi Ito, Ph.D.

Professor

Medical Research Institute, Tokyo Medical and Dental University (TMDU)

Phone: +81-4-5803-4594 Email: ito.str@tmd.ac.jp

Education

1986 B. Eng., Faculty of Engineering, University of Tokyo, Japan

1988 M. Eng., Graduate School of Engineering, University of Tokyo, Japan

1991 Ph.D., Department of Biochemistry and Molecular Biology, University of Leeds, UK

Work Experience

1988-1992 Postdoctoral Fellow, Dept of Biochemistry & Molecular Biology, University of Leeds, UK

1992-1994 Scientific Staff, MRC Laboratory of Molecular Biology, Cambridge, UK

1994-1996 Postdoctoral Fellow, Dept of Biochemistry & Molecular Biology, University of Leeds, UK

1996-2001 Senior Research Fellow, Biomolecular Engineering Research Institute, Osaka, Japan

2001-2003 Scientific Staff, Japan Science and Technology Corporation (PDBj), Japan

2003-present Professor, Medical Research Institute, Tokyo Medical and Dental University, Japan

Research Interest

1. Structural Biology
2. Protein Folding

Selected Publications

1. Akatsu C, Shinagawa K, Numoto N, Liu Z, Ucar AK, Aslam M, Phoon S, Adachi T, Furukawa K, Ito N, and Tsubata T. *J. Exp. Med.,* **213,** 2691-2706 (2016)

2. Masuno H, Ikura T, Morizono D, Orita I, Yamada S, Shimizu M, and Ito N. *J. Lipid Res.,* **54,** 2206-2213 (2013).

3. Westbrook J, Ito N, Nakamura H, Herick K, Berman HM; *Bioinformatics,* **21,** 988-992 (2005)

4. Oubridge C, Ito N, Evans PR, Teo CH and Nagai K. *Nature* **372,** 432-438 (1994).

5. Ito N, Phillips SEV, Stevens C, Ogel ZB, McPherson MJ, Keen JN, Yadav KD, and Knowles PF. *Nature* **350,** 87-90 (1991).

Mandatory submission of PDBx/mmCIF format files for crystallographic depositions to the Protein Data Bank (PDB)

Nobutoshi Ito

Medical Research Institute, Tokyo Medical and Dental University, Japan

**E-mail: ito.str@tmd.ac.jp*

The Protein Data Bank (PDB) is the single global archive of experimentally determined three-dimensional structure data of biological macromolecules. The continuing growth in the numbers, size and complexity of macromolecular structures in the PDB archive, coupled with the rapid growth of evolving experimental methods such as 3D cryo-electron microscopy has made the traditional PDB format (‘legacy PDB format’) inadequate for fully representing these data. This format was based on a punched-card format that became obsolete long ago. In my presentation, I describe the changes necessary to address the challenges coming from the extraordinary success of structural biologists. Since 2003, the PDB has been managed by the Worldwide Protein Data Bank (wwPDB; https://www.wwpdb.org/), an international partnership including Protein Data Bank Japan (PDBj) that collaboratively oversees deposition, validation, biocuration and open-access dis-semination of 3D macromolecular structure data, adhering to the FAIR principles of Findability, Accessibility, Interoperability and Reusability. In 2007, the master file format for the PDB archive was officially changed to PDB Exchange/Macromolecular Crystallographic Information File (PDBx/mmCIF), supported by the PDBx/mmCIF data dictionary, to address new challenges in structure archiving. Later, in 2012, the wwPDB terminated its support of the legacy PDB file format and froze its further development. From 1 July 2019, PDBx/mmCIF became the only format allowed for deposition of the atomic coordinates for PDB structures resulting from macromolecular crystallography, including X-ray, neutron, fiber and electron diffraction methods, via OneDep [1]. The issues related to PDBx/mmCIF mandatory policy will be introduced at the Seminar.

References

[1] Adams, P.D., *et al*., Announcing mandatory submission of PDBx/mmCIF format files for crystallographic depositions to the Protein Data Bank (PDB), *Acta Cryst.* D75, 451-454, **(2019)**

Sumin Lee, Ph.D.

Chief Scientist

Tomocube Inc.

Phone: +82-42-863-1100 Email: slee@tomocube.com

Education

2007 B.S. Life Sciences, POSTECH, Korea

2013 Ph.D. Molecular and Life Sciences, POSTECH, Korea

Work Experience

2013-2014 Postdoctoral Fellow, POSTECH, Korea

2014-2017 Senior Research Scientist, National Forensic Service, Korea

2017-present Chief Scientist, Tomocube Inc, Korea

Research Interest

1. Label-free imaging of live-cell dynamics
2. Quantitative cell biology using 3D Holotomography
3. Protein quantification using 3D Holotomography

Selected Publications

1. Jiang et al. Reconstruction of bovine spermatozoa substances distribution and morphological differences between Holstein and Korean native cattle using three-dimensional refractive index tomography (2019) *Scientific Reports* 9, 8774.

2. Yoon et al. Label-Free Identification of Lymphocyte Subtypes Using Three-Dimensional Quantitative Phase
Imaging and Machine Learning. (2018) *Journal of Visualized Experiments* 141, e58305.

3. Kim et al. Combining Three-Dimensional Quantitative Phase Imaging and Fluorescence Microscopy for the Study of Cell Pathophysiology (Review). (2018) *Yale Journal of Biology and Medicine.* 91(3):267-277

Holotomography techniques for non-invasive label-free 3d imaging of live cells and materials

Sumin Lee[1*] and YongKeun Park[1,2]

1. *Tomocube Inc.*
2. *Department of Physics, Korea Advanced Institute of Science and Technology (KAIST)*

**E-mail: slee@tomocube.com*

Holotomography (HT) provides label-free and real-time three-dimensional (3D) imaging capability. HT uses laser interferometry to measure 3-D refractive index (RI) distribution. Thus, 3D images of live cells without any molecular labeling, such as dye staining or DNA transfection, can be obtained with high spatial resolution (resolved to 110 nm). Furthermore, HT images can be analyzed to provide quantitative information - cell volume, dry mass and protein concentration - of a single cell.

In this seminar, we will discuss numerous applications, including imaging of the live samples, or materials such as protein aggregates, microparticles that have been enabled by HT's unprecedented correlative and quantitative bioimaging capabilities. We will also introduce representative studies which integrate artificial intelligent (AI) technologies for elucidating the characteristics based on the HT images and their quantitative information of individual cells.

References

[1] Park et al., Quantitative phase imaging in biomedicine, ***Nature Photonics*** 12, 578–589 (2018)

[2] Shin et al., Active illumination using a digital micromirror device for quantitative phase imaging, ***Optics Letters*** 40, 5407 (2015).

[3] Jung et al., Label-free non-invasive quantitative measurement of lipid contents in individual microalgal cells using refractive index tomography, ***Scientific Reports*** 8, 6524 (2018)

[4] Lee et al., DeepIS: deep learning framework for three-dimensional label-free tracking of immunological synapses, ***BioRxiv*** preprint (2019)

MEMO

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

2020

한국생체분자과학 연합학회학술대회

Symposium VII & VIII

Dongwon Yoo, Ph.D.

Assistant Professor
Seoul National University
Phone: +82-2-880-1898 Email: dwyoo@snu.ac.kr

Education

2004 **Ph.D.**, Chemical and Biological Engineering, Seoul National University, Korea
2000 **M.S.**, Chemical Technology, Seoul National University, Korea
1998 **B.S.**, Chemical Technology, Seoul National University, Korea

Work Experience

2018-present **Assistant Professor**, School of Chemical and Biological Engineering, Seoul National University, Korea
2016-2018 **Assistant Professor**, Yonsei Institute for Basic Science, Yonsei University, Korea
2011-2015 **Research Professor**, Center for Evolutionary Nanoparticles, Yonsei University, Korea
2005-2010 **Postdoctoral Associate & Staff Research Associate**, Department of Chemistry, University of California Los Angeles, USA
2004-2005 **Research Scientist**, Engineering Research Institute, Seoul National University, Korea

Research Interest

1. Photocatalysis for organic reactions
2. Molecular Imaging and sensing
3. Therapeutics and drug delivery

Selected Publications

1. Choi, J.-s.; Kim, S.; **Yoo, D.**; Shin, T.-H.; Kim, H.; Gomes, M. D.; Kim, S. H.; Pines, A.; Cheon, J., "Distance-dependent magnetic resonance tuning as a versatile MRI sensing platform for biological targets" ***Nature Materials*** vol. 16, p. 537, (2017)
2. Jeong, S.[+]; **Yoo, D.**[+]; Ahn, M.; Miró, P.; Heine, T.; Cheon, J. "Tandem intercalation strategy for single-layer nanosheets as an effective alternative to conventional exfoliation processes" ***Nature Communications*** vol. 6, p. 5763, (2015), [+]equally contributed
3. **Yoo, D.**; Kim, M.; Jeong, S.; Han, J.; Cheon, J., "Chemical synthetic strategy for single-layer transition-metal chalcogenides" ***Journal of the American Chemical Society*** vol. 136, p. 14670, (2014)
4. **Yoo, D.**; Jeong, H.; Noh, S.-H.; Lee, J.-H.; Cheon, J., "Magnetically triggered dual functional nanoparticles for resistance-free apoptotic hyperthermia" ***Angewandte Chemie International Edition*** vol. 52, p. 13047, (2013)
5. **Yoo, D.**; Jeong, H.; Preihs, C.; Choi, J.-s.; Shin, T.-H.; Sessler, J. L.; Cheon, J., "Double-effector nanoparticles: A synergistic approach to apoptotic hyperthermia" ***Angewandte Chemie International Edition*** vol. 51, p. 12482, (2012)

Nanomaterials for Bioimaging and Therapeutics

Dongwon Yoo

School of Chemical and Biological Engineering
Seoul National University, Seoul, Korea
**E-mail: dwyoo@snu.ac.kr*

Nanomaterials have a great potential to be applied for biomedical applications, with a variety of functionalities for diagnosis and therapy. Because nanomaterials can possess entire units composed with each module for imaging/sensing and for therapeutic purposes, nanomaterials can be designed as a versatile tool for probing and manipulating biological functions.

I will present that the nanomaterials can provide (i) understanding of physiological conditions in living creatures and (ii) enhancement of possibilities for *in vivo* applications in a wide range of therapeutic medicines. More specifically, their unique and potential utilization in magnetic resonance imaging (MRI)-based molecular sensing (e.g., pH, enzyme, and pO_2) and death signal modulation in cancer will be discussed.

MRI is a high-resolution and clinically important imaging modality, which offers anatomical information, enabling diagnosis and prognosis of disease. A considerable effort has been investigated for the development of responsive MRI contrast agents capable of specifically indicating their local microenvironment such as pH, enzymatic activities, or redox status. In the presence of biological target, a switch in MRI contrast is triggered by the change of the relaxation efficiency which is derived from the variation of rotational dynamics or exchange rate of coordinated water, etc. I'll present a new design principle of modulation of the relaxation efficiency. It can serve as a novel sensing principle to augment the exploration of a wide range of biological systems.

In addition, in therapeutic viewpoint, multidrug resistance (MDR) is a leading cause of failure in current chemotherapy treatment and constitutes a formidable challenge in therapeutics. I'll demonstrate that a nanoscale apoptosis trigger, which consists of a nanomaterial and chemodrug (e.g., doxorubicin), can completely remove MDR cancer cells in both *in vitro* and *in vivo* systems.

Jiwon Seo, Ph.D.

Associate Professor

Gwangju Institute of Science and Technology (GIST)

Phone: +82-62-715-3628 Email: jseo@gist.ac.kr

Education

1999 B.S., Chemical Technology, Seoul National University, Korea

2006 Ph.D. Chemistry, Northwestern University, USA

Work Experience

2006-2008 Visiting Scientist, Lawrence Berkeley National Laboratory, USA

2007-2010 Postdoctoral Fellow, Bioengineering, Stanford University, USA

2010-2016 Assistant & Associate Professor, GIST college, GIST, Korea

2016-present Associate Professor, Dept. of Chemistry, GIST, Korea

Research Interest

1. Bioorganic & Medicinal Chemistry, Biomimetic Chemistry, Peptide & Peptidomimetics
2. Peptoids, Macrocyclic peptides, Metallopeptides
3. Antimicrobial, Mitochondria-targeting, Antiviral, Catalysis

Selected Publications

1. Nam, H. Y.; Hong, J.-A.; Choi, J.; Shin, S.; Cho, S. K.; Seo, J.*; Lee, J. * Mitochondria targeting peptoids. *Bioconjugate Chem.* **2018**, *29*, 1669-1676.

2. Lee, J.; Kang, D.; Choi, J.; Huang, W.; Wadman, M.; Barron, A. E.*; Seo, J.* Effect of side chain hydrophobicity and cationic charge on antimicrobial activity and cytotoxicity of helical peptoids. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 170-173.

3. Yang, W.; Kang, B.; Voelz, V. A. *; Seo, J.* Control of porphyrin interactions via structural changes of peptoid scaffold. *Org. Biomol. Chem.* **2017**, *15*, 9670-9679.

Engineering natural peptides for discovery of novel anti-infectives

Jiwon Seo

Department of Chemistry, Gwangju Institute of Science and Technology, 123 Cheomdan-gwagiro, Buk-gu, Gwangju 61005, Republic of Korea

**E-mail: jseo@gist.ac.kr*

Peptoids are a unique class of peptidomimetics based on oligo-*N*-substituted glycine backbone. As a biomimetic material, the properties of peptoids lie in-between natural biopolymers and non-natural synthetic polymers, exhibiting key features that are unique in natural proteins such as programmability at the monomer level and sequence specificity. The structural similarity to polypeptides has been responsible for the impressive diversity of conformation and biological activity observed for peptoids; however, non-natural *N*-substituents render them higher stability than that of natural counterpart (i.e., protease-stable). Given the unique structural properties, the potential applications of peptoid are widely open in various fields including biomedicine and material sciences. In this presentation our ongoing efforts to mimic natural peptides or proteins using peptoids and to discover novel anti-infective agents will be introduced. One of the most actively investigated field in the peptoid community is the mimicry of natural antimicrobial peptides and cell-penetrating peptides. Recent discovery of amphipathic peptoids in our lab with potent antimicrobial activity including multi-drug resistant (MDR) pathogens and enhanced selectivity will be presented. With sequence and helicity modulation of the amphipathic peptoids, subcellular organ targeting vehicle with excellent efficiency was identified, and their biomedical applications will be introduced. Finally, synthesis and structural investigation of macrocyclic peptides based on natural peptides with potent anti-infective activity will be presented.

Acknowledgement

This work was supported by the National Research Foundation of Korea (NRF-2018R1A2B6007535) and by the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI16C1074).

Kyungmoo Yea, Ph.D.

Assistant Professor

Daegu Gyeongbuk Institute of Science and Technology (DGIST)

Phone: + 053-785-1760 Email: ykm31@dgist.ac.kr

Education

2004, Mar – 2008, Jul **Ph.D.** in Life Science POSTECH, Pohang, Republic of Korea

2002, Mar – 2004, Feb **M.S.** in Life Science POSTECH, Pohang, Republic of Korea

1994, Mar – 2000, Aug **B.S.** in Life Science Korea University, Seoul, Korea

Work Experience

2017, Dec- Present: **Assistant Professor**, New Biology department, DGIST, Korea.

2015, Sep-2017, Nov: **Assistant Professor**, Cell and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, US.

2013, Aug-2015, Aug: **Research Associate Professor**, Shanghai Institute for Advanced Immunochemical Studies in ShanghaiTech University, Shanghai, China.

2012, Feb-2013, Jul: **Senior Scientist**, Scripps Korea Antibody Institute, South Korea.

2009, July-2012, Jan: **Postdoctoral Associate**, Sanford-Burnham Medical Research Institute, Orlando, US

Research Interest

1. Development therapeutic agonist antibodies
2. Engineering of surface proteins on immune cells and exosomes

Selected Publications

1. Zha Z, Bucher F, Nejatfard A, Zheng T, Zhang H, Yea K* and Lerner RA*. Interferon-γ is a master checkpoint regulator of cytokine-induced differentiation. *Proc. Natl. Acad. Sci.USA*, 2017; July 31, 2017, doi:10.1073/pnas.1706915114, *equally contributed
2. Bucher F, Zhang D, Aguilar E, Sakimoto S, Aguilar S, Rosenfeld M, Zha Z, Zhang H, Friedlander M and Yea K. Antibody-mediated inhibition of Tspan12 ameliorates vasoproliferative retinopathy through suppression of β-catenin signaling. *Circulation*. 2017 Mar 29.
3. Yea K, Zhang H, Xie J, Jones TM, Lin C, Francesconi W, Berton F, Fallahi M, Sauer K, and Lerner RA. Agonist antibody that induces human malignant cells to kill one another. *Proc. Natl. Acad. Sci.USA,* 2015 Nov 10 *(Featured on the cover)*
4. Yea K, Zhang H, Xie J, Terri TM, Yang G, Song BD, Lerner RA. Converting stem cells to dendritic cells by agonist antibodies from unbiased morphogenic selections. *Proc Natl Acad Sci U S A.* 2013 Aug 26. 2013 Sep 10;110(37):14966-71
5. Bucher F, Lee J, Shin S, Kim MM, Oh YS, Ha S, Zhang H, Yea K. Interleukin-5 suppresses Vascular Endothelial Growth Factor-induced angiogenesis through STAT5 signaling. *Cytokine.* 2018 Jun 16.

Translational application of infectious libraries of human antibodies

Kyungmoo Yea

DGIST, New Biology

**E-mail: ykm31@dgist.ac.kr*

We are interested in studying cellular communication and developing therapeutics by infectious libraries of human antibodies. Recently, we selected antibodies that affect intriguing biological processes using infectious antibody libraries. **Firstly,** we have developed an anti-Tspan12 antibody to reduce vaso-proliferative retinopathy using human combinatorial antibody libraries. Anti-angiogenic biologicals represent an important concept for the treatment of vaso-proliferative diseases. However, the need for continued treatment, the presence of non-responders and the risk of long-term side-effects limits the success of existing therapeutic agents. In this study, we found that Tspan12/β-catenin signaling is critical for the progression of vaso-proliferative disease. The newly developed anti-Tspan12 antibody has therapeutic effects in vaso-proliferative retinopathy enhancing the potency of existing anti-VEGF agents. **Secondly,** we reported on an agonist antibody against thrombopoietin receptor that induces malignant acute myeloid leukemia cells into the differentiation of potent cytotoxic killer cells. In this study, we suggested the possibility of agonist antibodies to change the differentiation state of cancer cells into those that attack and kill other members of the malignant clone from their origination. The cell that does the killing is also quite novel in that it has both natural killer cell and dendritic cell markers. It may, in fact, be a chimeric cell suggesting that antibodies such as these could open a whole new frontier in cancer therapy.

Woo-Jae Chung, Ph.D.

Associate Professor

Sungkyunkwan University

Phone: +82-31-299-4859 Email: wjchung@skku.edu

Education

1998 B.S., Department of Chemical Technology, Seoul National University, Korea

2000 M.S. Department of Chemical Technology, Seoul National University, Korea

2005 Ph.D. School of Chemical & Biological Engineering, Seoul National University, Korea

Work Experience

2007-2010 Postdoctoral fellow, University of California, Berkeley, US

2010-2012 Project scientist, Lawrence Berkeley National Lab., US

2013-present Assistant & Associate professor, Department of Integrative Biotechnology, SKKU

Research Interest

1. Colorimetric biomimetic sensor development
2. Hydrogel engineering for tissue regeneration
3. Antiviral agents for influenza

Selected Publications

1. H.-E. Jin, J. Jang, J. Chung, H. J. Lee, E. Wang, S.-W. Lee, & W.-J. Chung, Biomimetic Self-Templated Hierarchical Structures of Collagen-Like Peptide Amphiphiles, *Nano Lett*. **15**, 7138-7145 (2015).

2. D.-Y. Lee, H. Lee, Y. Kim, S. Y. Yoo, G. Kim, W.-J. Chung, Phage as versatile nanoink for printing 3-D cell-laden scaffolds, *Acta Biomater*. **29**, 112-124 (2016).

3. M. S. Gong, G. Oh, H.-S Jang, B. Y. Lee, W.-J. Chung, Hierarchically structured peptide nanofibers for colorimetric detection of gaseous aldehydes, *Sensors Actuators B; Chemical.*, **282**, 868-875 (2019).

Multivalent Sialyl Ligands and Their Use in Influenza A Virus Entry Inhibitors

Woo-Jae Chung*

Department of Integrative Biotechnology, Sungkyunkwan University, Suwon 16419, Korea

**E-mail: wjchung@skku.edu*

The influenza virus is one of the leading causes of acute respiratory diseases in humans. So far, clinically approved anti-influenza therapeutics have been targeting two surface proteins including viral M2 ion channel or neuraminidase (NA) that is responsible for uncoating in the host cell or budding of the virus, respectively. However, due to the emergence of viral strains resistant to such drugs, different anti-influenza strategies, targeting other viral proteins such as hemagglutinin (HA), nucleoprotein, and RNA polymerase involved in virus life cycle are strongly needed. Among those promising strategies, the entry blocking approach targeting conserved domains of HA, is one of the central interests. In order to enter cells, the influenza viruses employ their surface protein trimeric HA that specifically bind to various sialic acid (SA) receptors displayed on the host cells. Therefore, mimicking the host cell surface, nanostructured scaffolds that present multivalent sialic acid have gained enormous attention with significantly enhanced avidity by up to several order of magnitude. Here, we demonstrate our efforts on biomimetic multivalent sialyl ligands development and their use in influenza A virus entry blockers targeting the hemagglutinin.

Acknowledgement

[1] This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MEST) (No. 2019R1A2C1006856).

Chan Hyuk Kim, Ph.D.

Assistant Professor

Korea Advanced Institute of Science and Technology (KAIST)

Phone: +82-42-350-2621 Email: kimchanhyuk@kaist.ac.kr

Education

1997 ~ 2003 B.S., Chemistry, Seoul National University, Korea

2004 ~ 2008 Ph. D., Chemistry, Seoul National University, Korea

Work Experience

2009-2012 Postdoctoral Associate, The Scripps Research Institute (TSRI)

2012~2016 Principal Investigator, California Institute for Biomedical Research (Calibr)

2016~present Assistant Professor, Dept. of Biological Sciences, KAIST

Research Interest

1. Synthetic Immunology
2. Next-generation antibody engineering
3. Immune cell engineering

Selected Publications

1. Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. Rodgers DT, Mazagova M, Hampton EN, Cao Y, Ramadoss NS, Hardy IR, Schulman A, Du J, Wang F, Singer O, Ma J, Nunez V, Shen J, Woods AK, Wright TM, Schultz PG*, Kim CH*, Young TS*. Proc Natl Acad Sci U S A. 2016 Jan 26;113(4):E459-68.
2. Versatile strategy for controlling the specificity and activity of engineered T cells. Ma JS, Kim JY, Kazane SA, Choi SH, Yun HY, Kim MS, Rodgers DT, Pugh HM, Singer O, Sun SB, Fonslow BR, Kochenderfer JN, Wright TM, Schultz PG*, Young TS*, Kim CH*, Cao Y. Proc Natl Acad Sci U S A. 2016 Jan 26;113(4):E450-8.
3. Multiformat T-cell-engaging bispecific antibodies targeting human breast cancers. Cao Y, Axup JY, Ma JS, Wang RE, Choi S, Tardif V, Lim RK, Pugh HM, Lawson BR, Welzel G, Kazane SA, Sun Y, Tian F, Srinagesh S, Javahishvili T, Schultz PG*, Kim CH*. Angew. Chem. Int. Ed. Engl. 2015 Jun 8;54(24):7022-7.
4. Redirection of genetically engineered CAR-T cells using bifunctional small molecules. Kim MS, Ma JS, Yun H, Cao Y, Kim JY, Chi V, Wang D, Woods A, Sherwood L, Caballero D, Gonzalez J, Schultz PG*, Young TS*, Kim CH*. J. Am. Chem. Soc. 2015 Mar 4;137(8):2832-5.
5. Bispecific small molecule-antibody conjugate targeting prostate cancer. Kim CH*, Axup JY, Lawson BR, Yun H, Tardif V, Choi SH, Zhou Q, Dubrovska A, Biroc SL, Marsden R, Pinstaff J, Smider VV, Schultz PG*.Proc. Natl. Acad. Sci. U S A. 2013 Oct 29;110(44):17796-801

Towards next-generation T cell therapy for Cancer

Chan Hyuk Kim, Ph.D.

Dept. of Biological Sciences, KAIST

**E-mail: kimchanhyuk@kaist.ac.kr*

Due to its ability to recognize antigens in an MHC-independent manner, T cells that are engineered to express chimeric antigen receptor (CAR) can be a viable option for the tumors with low mutational burden. Indeed, the second-generation CD19-targeting CAR-T cells with an engineered signaling domain demonstrated unprecedented anti-leukemic responses in patients with refractory B-cell cancer, and became the first approved gene therapy for cancer last year. In light of their clinical success, there has been an explosion of interest in CAR-T cells for cancer immunotherapy. However, the inability to control the activity of this potent live drug has resulted in severe treatment related toxicities and the constraint in targeting more than one antigen have limited its general application. Furthermore, various immune-suppressive mechanisms present in the tumor microenvironment often severely limits the anti-tumor activity of CAR-T cells against solid tumors, posing a major obstacle for more widespread application of this innovative therapy. In this talk, I will discuss our recent research efforts focusing on addressing these limitations of current CAR-T therapy.

Seongjun Park, Ph.D.

Assistant Professor
Department of Bio and Brain Engineering
Korea Advanced Institute of Science and Technology (KAIST)
Phone: +82-010-2587-8971 Email: spark19@kaist.ac.kr

Education

2006-2013 B.S., Mechanical and Aerospace Engineering, Seoul National University, Republic of Korea
2013-2015 M.S., Mechanical Engineering, Massachusetts Institute of Technology (MIT), United States
2015-2018 Ph.D., Electrical Engineering and Computer Science, Massachusetts Institute of Technology (MIT), United States

Work Experience

2018-2019 Postdoctoral Associate, Research Laboratory of Electronics (RLE), Massachusetts Institute of Technology (MIT), United States
2019- Assistant Professor, Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Republic of Korea

Research Interest

1. Fiber-based Optical Bio and Neural Interfaces
2. Nanomaterials for Brain Engineering
3. Neural Tissue Engineering

Selected Publications

1. **S. Park**, Y. Guo, X. Jia, H. K. Choe, B. Grena, J. Kang, J. Park, C. Lu, A. Canales, R. Chen, Y. S. Yim, G. Choi, Y. Fink, and P. Anikeeva, "One-step Optogenetics with Multifunctional Flexible Polymer Fibers", **Nature Neuroscience**, 20 (4), 612-619, 2017.
2. C. Lu*, **S. Park***, T. J. Richner, A. Derry, I. Brown, C. Hou, S. Rao, J. Kang, C. T. Moritz, Y. Fink, and P. Anikeeva, "Flexible and stretchable nanowire-coated fibers for optoelectronic probing of spinal cord circuits", **Science Advances**, 3 (3), e1600955, 2017. (*equally contributed)
3. **S. Park**, R. A. Koppes, U. P. Froriep, X. Jia, A. K. H. Achyuta, B. L. McLaughlin, and P. Anikeeva, "Optogenetic Control of Nerve Growth", **Scientific Reports**, 5, 9669, 2015.
4. **S. Park**, G. Loke, Y. Fink, and P. Anikeeva, "Flexible Fiber-Based Optoelectronics for Neural Interfaces", **Chemical Society Reviews**, 48, 1826-1852, 2019.
5. A. Canales*, **S. Park***, A. Killas*, and P. Anikeeva, "Multifunctional Fibers as Tools for Neuroscience and Neuroengineering", Accounts of Chemical Research, 51 (4), 829-838, 2018. (*equally contributed)

Multifunctional Neural Interfaces Technologies

Seongjun Park

Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST)
**E-mail:* spark19@kaist.ac.kr

Neurological disorders affect up to a billion people worldwide, and their socioeconomic burden is projected to increase as the population ages. However, our ability to understand and to treat neural disorders is currently limited by the lack of tools capable of interfacing with the brain over extended periods of time. This is hypothesized to stem from the mismatch in mechanical and chemical properties between the neural probes and the neural tissues, which leads to foreign body response and functional device failure due to tissue scarring in the probe vicinity. To address the challenge, we developed fiber-based bioelectronic devices integrating diverse modalities within a single platform using thermal drawing process (TDP). All-polymer or hydrogel integrated probes with optical, electrical, and fluidic capabilities were developed all within the 100-200 μm diameter, which allowed one-step surgery to the mouse brain and spinal cord for optogenetic experiments. This probe also addressed the challenge of biocompatibility and enabled the recording isolated action potentials for 3 months. In addition, we applied TPD to produce biocompatible polymer-based neural scaffold with various geometries (round, rectangular, micro-grooved) and dimensions between 50-200 μm. This allowed for investigation of the enhancement of neurite growth as a function of fiber parameters. We found that the topographical features and the narrow channels generally led to enhanced growth. This presentation illustrate a variety of applications of multifunctional fiber-based devices in neuroscience and neural engineering, which anticipated to enable basic studies of the nervous system and future treatment of neurological disorders.

References

[1] S. Park et al., One-step Optogenetics with Multifunctional Flexible Polymer Fibers. *Nature Neuroscience* (**2017)**.

[2] C. Lu*, S. Park* et al., Flexible and Stretchable nanowire-coated fibers for optoelectronic probing of spinal cord circuits. *Science Advances* (**2017)**.

Daseuli Yu, Ph.D student.

Department of Biological Sciences,
Korea Advanced Institute of Science and Technology (KAIST)
Phone: +10-6250-6624 Email: sureeyu@kaist.ac.kr

Education

03/2013 - Present Ph.D. Student Department of Biological Sciences, KAIST, Daejeon, Korea
03/2009 - 02/2013 B.S. Department of Microbiology & Molecular Biology, Chungnam National University, Daejeon, Korea

Research Interest

Optogenetics, Bio-imaging, Bio-technology, Antibody engineering, Cancer therapy

Awards

2017 **Lab note contest Award**, Department of Biological Sciences, KAIST (Supported by Samsung Bioepis)
2019 **Samsung Humantech Award**, Samsung
2019 **KSMCB Young Investigator Research Award**, Korean Society for molecular and Cellular Biology

Selected Publications

1. **Daseuli Yu**, Hansol Lee, Jongryul Hong, Hyunjin Jung, YoungJu Jo, Byung-Ha Oh, Byung Ouk Park* & Won Do Heo*. Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins. ***Nature Methods.*** 2019 Oct 14. DOI: 10.1038/s41592-019-0592-7

2. Hyunjin Jung, Seong-Wook Kim, Minsoo Kim, Jongryul Hong, Ji Hye Kim, Sungsoo Kim, Yunju Lee, **Daseuli Yu**, Jihoon Kim, Doyeon Woo, Hee-Sup Shin, Byung Ouk Park* & Won Do Heo*. Noninvasive optical activation of Flp recombinase forgenetic manipulation in deep mouse brain regions. ***Nature Communications.*** 2019 Jan 18. DOI: 10.1038/s41467-018-08282-8.

Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins

Daseuli Yu

Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea
**E-mail: sureeyu@kaist.ac.kr*

Intracellular antibodies, such as nanobodies and single-chain variable fragments (scFv), have become powerful tools for imaging, modulating, and neutralizing endogenous target proteins. Although a variety of antibody engineering techniques have been developed, an optogenetic tool for activating intracellular antibody to precisely control its target protein has not been reported. Here, we describe an optogenetically activatable intracellular antibody (Optobody) consisting of split antibody fragments and blue light-mediated heterodimerization domains. Blue light stimulation activates the optobody, inducing it to capture its target protein and subsequently inhibit the target. We expanded this optobody platform by generating various optobodies from previously developed intracellular antibodies, and demonstrated that photoactivation of a gelsolin (GSN) optobody and β2 adrenergic receptor (β2AR) optobody shut down endogenous GSN activity and β2AR signaling, respectively. Applying our novel optogenetic platform to the broad pool of available intracellular antibodies will facilitate optogenetic manipulation of various endogenous proteins and may provide a basis for designing potential inducible drugs.

References

Daseuli Yu, Hansol Lee, Jongryul Hong, Hyunjin Jung, YoungJu Jo, Byung-Ha Oh, Byung Ouk Park* & Won Do Heo*. Optogenetic activation of intracellular antibodies for direct modulation of endogenous proteins. *Nature Methods*. 2019 Oct 14. DOI: 10.1038/s41592-019-0592-7

Ji Won Kim, Ph.D.

Pohang University of Science and Technology (POSTECH)

Phone: +82-54-279-2323 Email: mokona5@postech.ac.kr

Education

2005 B.S., Sogang University, Department of life science
2007 M.S., KAIST, Department of Chemistry
2015 Ph.D, KAIST, Department of Chemistry

Work Experience

2015-2019 Postdoc, KAIST, Department of Chemistry
2019 ~ present Postdoc, POSTECH, Department of Life Sciences

Research Interest

1. Structural study of membrane protein
2. Binder protein modeling and design

Selected Publications

1. Ji Won Kim, Songwong Kim, Haerim Lee, Geunyoung Cho, Sun Chang Kim, Hayyoung Lee, Mi Sun Jin, and Jie-Oh Lee (2019), Application of antihelix antibodies in protein structure determination, PNAS, 2019. 8. doi: 10.1073/pnas.1910080116.

2. **Ji Won Kim**, Subin Kim, Songwon Kim, Hearim Lee, Jie-Oh Lee, Mi Sun Jin (2017), Structural insights into the elevator-like mechanism of the sodium/citrate symporter CitS, Scientific Reports, 7(1) 2548

3. Jin Hong Kim, Dong Hyun Song, Suk-Jun Youn, **Ji Won Kim**, Geunyoung Cho, Sun Chang Kim, Hayyoung Lee, Mi Sun Jin and Jie-Oh Lee (2016), Crystal structure of mono- and bi-specific diabodies and reduction of their structural flexibility by introduction of disulfide bridges at the Fv interface, Scientific Reports, 486(2) 470- 475

Application of anti-helix antibodies in protein structure determination

Ji Won Kim

Pohang University of Science and Technology (POSTECH)

**E-mail: mokona5@postech.ac.kr*

Antibodies are indispensable tools in protein engineering and structural biology. Antibodies suitable for structural studies should recognize the three dimensional conformations of target proteins. Generating such antibodies and characterizing their complexes with antigens take a significant amount of time and effort. Here, we show that we can expand the application of well-characterized antibodies by “transplanting” the epitopes they recognize to proteins with completely different structures and sequences. We demonstrate that these antibodies can be made to bind to a variety of unrelated “off-target” proteins by modifying amino acids in the pre-existing alpha helices of such proteins. Using X-ray crystallography, we determined the structures of the engineered protein-antibody complexes. All the antibodies bound to the epitope-transplanted proteins forming accurately predictable structures. Furthermore, we showed that binding of these anti-helix antibodies to the engineered target proteins can modulate their catalytic activities by trapping them in selected functional states.

References

[1] Zahnd C*, et al.* Directed in vitro evolution and crystallographic analysis of a peptide-binding single chain antibody fragment (scFv) with low picomolar affinity. *J Biol Chem* (2004)

[2] Schiefner A*, et al.* A disulfide-free single-domain V(L) intrabody with blocking activity towards huntingtin reveals a novel mode of epitope recognition. *Journal of molecular biology* (2011)

[3] Haffke M, et al. Structures of the nucleotide-binding domain
of the human ABCB6 transporter and its complexes with nucleotides. *Acta Crystallogr D Biol Crystallogr* (2010)

[4] Dawson RJ & Locher KP, Structure of a bacterial multidrug ABC transporter. *Nature* (2006)

Soobin Lee

Integrated M.S.-Ph.D. course

Pohang University of Science and Technology (POSTECH)

Phone: +82-10-4038-8373 Email: thelastlight@postech.ac.kr

Education

2016 B.S., Chemistry, POSTECH, Korea

2016~ Integrated M.S.-Ph.D. course, Chemistry, POSTECH, Korea

Research Interest

1. Chemical biology
2. Combinatorial chemistry

A Small-Molecule Identified via Forward Chemical Genetics Selectively Kills Cancer Cells

Soobin Lee, Hyun-Suk Lim*

Department of Chemistry, Pohang University of Science and Technology, Pohang 790-784, Korea

**E-mail: thelastlight@postech.ac.kr*

Forward chemical genetics is a powerful tool to explore the biological systems for chemists. In this strategy, compounds that induce a desired phenotypic change can be identified from screening a chemical library. Since it requires no hypotheses regarding the molecular basis of the phenotype in question, any genes or proteins that support this phenomenon are analysed afterwards. The unbiased nature of the inquiry can lead to novel target proteins that could serve as novel drug targets or biomarkers, while discovering compounds that modulate those target proteins.

Herein, I will discuss (1) the discovery of a small-molecule p27 activator that selectively kill leukemia cells, (2) identification of the target protein associated with the compound which may lead to the malignant behaviour of leukemia, and (3) subsequent biological studies. This molecule could be developed as a novel class of anti-leukemia agent, as well as a molecular tool that can provide valuable information about the role of the target protein in leukemia.

References

[1] Wen-Li, Z. *et. al.* Inhibition of the ecto-beta subunit of F_1F_O-ATPase inhibits proliferation and induces apoptosis in acute myeloid leukemia cell lines. *J. Exp. Clin. Cancer Res.* (**2012**).

[2] Federica, T. and Antonio, G. Systematic review of plasma-membrane ecto-ATP synthase: A new player in health and disease. *Exp. Mol. Pathol.* (**2018**).

Acknowledgement

[1] This work was supported by the National Research Foundation of Korea.

[2] This work was done in-collaboration with Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF) and Korea Institute of Science and Technology (KIST).

Sukyeong Heo

Ewha Womans University

Phone: +82-2-3277-4937 (office) Email: hcjm123@gmail.com

Education

03/2014 – Present **Ph.D. Candidate** Life Science, Ewha Womans University, Seoul, Korea

03/2010 – 02/2014 **B.S.** Life Science, Ewha Womans University, Seoul, Korea

Research Interest

1. Development of imaging technologies including imaging sensor and analytical tools.
2. Imaging and analysis of cancer cell in 3D culture system.
3. Redox-mediated regulation of cell signaling network.

Awards

2015 - present **Global Ph.D. Fellowship,** National Research Foundation of Korea

2018 **Best Presentation Award**, Ewha-JWU-Ochanomizu Joint Symposium

Publications

Sujin Park, Jung Mi Lim, Seon Hwa Park, Suree Kim, **Sukyeong Heo**, Tamas Balla, Woojin Jeong, Sue Goo Rhee*, Dongmin Kang*. Inactivation of the PtdIns(4)P phosphatase Sac1 at the Golgi by H_2O_2 produced via Ca^{2+}-dependent Duox in EGF-stimulated cells. **Free Radical Biology and Medicine.** (2019) 131:40-49

The Effects of Tumor Suppressor INPP4B Oxidation on Actin Polymerization in Glioma Cells.

Sukyeong Heo[1], Haeseung Lee[2], Wankyu Kim[2], Dongmin Kang[1]

[1] Department of Life Sceince, Ewha Womans University 52, Ewhayeodae-gil, Seodaemun-gu, Seoul 03760, Republic of Korea

[2] Research Center for Systems Biology, Department of Life Sciences, Ewha Womans University52, Ewhayeodae-gil, Seodaemun-gu, Seoul 03760, Republic of Korea

**E-mail:hcjm123@gmail.com*

The effect of intracellular hydrogen peroxide (H_2O_2) molecules on phosphoinositide signaling, which is implicated in numerous cellular processes is unclear. The tumor suppressor gene PTEN removes the 3-phosphate of PtdIns(3,4,5)P_3 to produce PtdIns(4,5)P_2. Previous researches showed that PTP family PTEN is oxidized and inactivated by H_2O_2. Similar to PTEN, INPP4B which converts PtdIns(3,4)P_2 to PtdIns(3)P contains a"CX_5R" catalytic domain characteristic of dual specificity phosphatase (DuSP). In this study, we investigated whether INPP4B is controlled by H_2O_2 under extracellular stimulation in PTEN-deficient U87MG glioma cells. Our results revealed that PDGF stimulated H_2O_2 induced the inactivation of INPP4B by oxidizing of the cysteine residue; however, serine mutation of catalytic cysteine of INPP4B (C842S) was resistant to oxidation. H_2O_2-dependent oxidation of INPP4B increased the level of PtdIns(3,4)P_2 around the plasma membrane and thereby promoting the recruitment of F-actin, Lpd, and Arp2/3 complex. Thus, oxidative inactivation of INPP4B implicated in actin polymerization at the leading edge of lamellipodia and cell migration. In addition, Low expression levels of INPP4B was associated with poor prognosis of patients with GBMs and LGGs, suggesting that the INPP4B functions as a tumor suppressor in gliomas.

Acknowledgement

This work was supported by by NRF(National Research Foundation of Korea) Grant funded by the Korean Government(NRF-2015-Fostering Core Leaders of the Future Basic Science Program/Global Ph.D. Fellowship Program)

Hee Ryung Kim, Ph.D.

Postdoc

Sungkyunkwan University (SKKU)

Phone: +82-31-299-4376 Email: heeryung.jan.kim@gmail.com

Education

2008 - 2012 B.S., Cell Biology & Genetics, University of British Columbia, Canada

2014 - 2019 Ph.D., Pharmacy, Sungkyunkwan University, Korea

Work Experience

2019 - Present Postdoc, Sungkyunkwan University, Korea

Research Interest

1. Conformational dynamics of GPCR-G protein and GPCR-arrestin coupling
2. G protein coupling selectivity

Selected Publications

1. Du Y, Duc NM, Rasmussen SGF, Hilger D, Kubiak X, Wang L, Bohon J, **Kim HR**, Wegrecki M, Asuru A, Jeong KM, Lee JM, Chance MR, Lodowski DT, Kobilka BK, Chung KY. Assembly of a GPCR-G protein complex. ***Cell.*** 2019:177(5):1232-1242.e11.
2. Bang I*, **Kim HR***, Beaven AH, Kima J, Ko SB, Lee GR, Lee H, Im W, Seok C, Chung KY, Choi H-J. Biophysical and functional characterization of Norrin signaling through Frizzled4. ***Proceedings of the National Academy of Science USA.*** 2018:115(35):8787-8792. (*First co-author)
3. Liu H, **Kim HR**, Deepak RNVK, Wan L, Chung KY, Fan H, Wei Z, and Zhang C. Orthosteric and allosteric action of the C5a receptor antagonists. ***Nature Structural and Molecular Biology.*** 2018:25(6):472-481.
4. **Kim HR**, Duc NM, and Chung KY. Comprehensive analysis of non-synonymous natural variants of G protein-coupled receptors. ***Biomolecules & Therapeutics.*** 2018:26(2):101-108.

Conformational Analysis of Gi/o α Subunits in GPCR Coupling

Hee Ryung Kim[1,#], Jun Xu[2,#], Shoji Maeda[3], Nguyen Minh Duc[1], Yang Du[4,*], and Ka Young Chung[1,*]

[1]School of Pharmacy, Sungkyunkwan University, Suwon, Korea

[2]Beijing Advanced Innovation Center for Structural Biology, School of Medicine, Tsinghua University, Beijing, China

[3]Department of Molecular and Cellular Physiology, School of Medicine, Stanford University, California, USA

[4]School of Life and Health Sciences, Kobilka Institute of Innovative Drug Discovery, Chinese University of Hong Kong, Guangdong, China

 # These authors contributed equally

**E-mail: kychung2@skku.edu*

G protein-coupled receptors (GPCRs) are membrane proteins that are responsible for transmitting extracellular stimulus to complex intracellular signaling. As GPCRs are extremely important for normal physiology, there has been a great interest in determination of the mechanism of GPCR-G protein coupling. Even though, recent breakthroughs determined the structures of numerous GPCR-G protein complexes including GPCR-Gs and GPCR-Gi/o complexes, the precise molecular mechanism of G protein selectivity is still unknown. Here, we performed Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS) and mutation studies on model GPCR-Gi/o complexes, M2R-Gi3, M2R-GoA, β2AR-Gi3, and β2AR-GoA to determine the difference in the molecular mechanism between Gs and Gi/o coupling. These structural and functional studies on GPCR-Gi/o coupling revealed that the wavy hook of Gαi/o α5 is not as critical as we previously studied in GPCR-Gs coupling. We found that α5 helix near C-terminus of Gαi/o in assistant with the wavy hook has a crucial effect on receptor-mediated GDP release. In addition, αN sequence of Gαi/o and αN-β1–ICL2 interaction between Gαi/o α subunits and receptors may not be as critical as compared to Gs coupling.

Minhee Kang, Ph.D.

Research Professor

Korea University

Phone: +82)044-806-1643 Email: mh_kang@korea.ac.kr

Education

2019 Ph.D., Chemistry, The Ohio State University, USA

2012 B.S., Chemistry, Indiana University of Pennsylvania, USA

Work Experience

2019-present Research professor, Korea University, Korea

2012-2019 Graduate Teaching Assistant, The Ohio State University, Department of Chemistry, USA

Research Interest

1. Protein structural biology
2. Nuclear Magnetic Resonance

Selected Publications

1. Solution structure of the Nucleotide hydrolase BlsM: Implication of its Substrate Specificity

Solution Structure of the Nucleotide hydrolase BlsM: Importance of its Substrate Specificity

Minhee Kang, Kiran Doddapaneni, Zach Heppner, Vicki Wysocki and Zhengrong Wu

Korea University

**E-mail: mh_kang@korea.ac.kr*

Blasticidin S is a peptidyl nucleoside antifungal reagent in *Streptomyces griseochromogenes* which is widely used to prevent rice blast [1]. Blasticidin S can inhibit protein synthesis by binding to the P-site on the 50S ribosomal subunit. One of the major components of Blasticidin S is a cytosine base. During the synthesis of Blasticidin S, therefore, the amount of free cytosine base become the limiting factor since intracellular concentration of free cytosine is very low. BlsM, which catalyzes the irreversible hydrolysis of the N-glycosidic bond in cytidine 5'-monophosphate (CMP), is a key enzyme in the biosynthesis of Blasticidin S. BlsM belongs to hydrolase family with other two known members, MilB and RCL. MilB is responsible for the liberation of hydroxyl methyl CMP in biosynthesis of mildiomycin, and RCL, one of the most responsive genes to c-myc during tumorigenesis, specifically cleaves the glycosidic bond in dGMP. Although these hydrolases are functionally similar to the nucleoside hydrolases, sequence alignment has suggested structural and mechanistic similarity with the family of 2'-deoxyribosyltransferases (DRTases), which function to cleave N-glycosidic bond of nucleoside followed by a reverse reaction between the original sugar and a new nucleobase. BlsM, MilB and RCL differ from DRTases by lacking the transferase activity and the hydrolases specifically hydrolyze a nucleotide whereas DRTases recognize any nucleoside.

In addition to its hydrolytic activity, interestingly, BlsM has also been shown to possess a novel cytidine deaminase activity, converting cytidine and deoxycytidine to uridine and deoxyuridine. This deamination activity may be unique, as all deaminases known require metal center located in the active site of the deaminases No restricted metal center has been reported for any member of this hydrolase family.

The solution structure of BlsM was determined by multi-dimensional nuclear magnetic resonance (NMR) to gain insight into the substrate specificity and dual function. BlsM is a symmetric homodimer, and each monomer consists of a five-parallel β-sheet that is sandwiched by five α-helixes. Three of the α-helixes interact with their counterparts in the neighboring monomer, forming the dimer interface. BlsM structure shows a confined active site close to the domain interface enclosed by several conserved hydrophobic residues. This active site is smaller than that of the homologous protein RCL, and recognizes a smaller pyrimidine, cytosine. In addition, the substrate specificity of BlsM for a ribosyl-nucleotide is conferred through a key residue, Phe19. Phe19 allows both CMP and dCMP to be optimally positioned in the active site of BlsM for effective hydrolysis. Upon mutation of the phenylalanine to a tyrosine residue (F19Y), the steric clash between the –OH of the tyrosine and the 2'-hydroxyl group of CMP potentially alters the substrate orientation, and prevents the substrate from hydrolysis. However, dCMP, which lacks the 2'-hydroxyl group, appeared to be almost affected by this mutation. More interestingly, in conjunction with impaired hydrolytic activity, the F19Y mutant exhibited a pronounced deaminase activity on CMP.

References

[1] Takeuchi, S., et al., Blasticidin S, a new antibiotic. *The Journal of antibiotics*, (**1958**)

[2] Zhao, G., et al., Structure of the N-glycosidase MilB in complex with hydroxymethyl CMP reveals its Arg23 specifically recognizes the substrate and controls its entry. *Nucleic acids research*, (**2014**)

[3] Ghiorghi, Y.K., et al., The c-Myc target gene Rcl (C6orf108) encodes a novel enzyme, deoxynucleoside 5′-monophosphate N-glycosidase. *Journal of Biological Chemistry*, (**2007**)

Kyungjin Min

Ph.D. candidate
Seoul National University
Phone: +821077413165 Email: km32479@snu.ac.kr

Education

2015-present Ph.D. candidate in Chemistry, Seoul National University, Korea
2010-2012 B.S. in Chemistry, The University of Texas at Austin, TX, USA

Work Experience

2012-2013 Research Assistant, College of Pharmacy, The University of Texas at Austin, TX, USA

Research Interest

Understanding of interaction between proteins and its partners

1. G-protein coupled receptors (GPCRs) and down-signaling proteins
2. Structure determination of disease related proteins

Selected Publications

1. **Min KJ**, An DR, Yoon HJ, Rana N, Park JS, Kim J, Lee M, Hesek D, Ryu S, Kim BM, Mobashery S, Suh SW, Lee HH. Structural insights into peptidoglycan reshaping by a noncanonical peptidase for helical cell shape in intestinal pathogens. *Nat. commun.* in press
2. **Min KJ**, Yoon HJ, Park JY, Baidya M, Dwivedi H, Maharana J, Chung KY, Shukla AK, and Lee HH. Crystal structure of β-arrestin 2 in complex with an atypical chemokine receptor phosphopeptide reveals an alternative active conformation. *bioRxiv* 2019 doi: https://doi.org/10.1101/785527.
3. **Min KJ**, Yoon HJ, Jo Inseong, Ha NC, Jin KS, Kim JS, and Lee HH. Insight into the apo-structure of Cpf1 protein from Francisella novicida. *Biochem. Biophy. Res. Commun.* 2018 498(4);775-781.
4. **Min KJ**, Yoon HJ, Matsuura A, Kim YH, and Lee HH. Structural basis for recognition of L-lysine, L-ornithine, and L-2,4-diamino butyric acid by lysine cyclodeaminase. *Mol. Cells* 2018 41(4); doi: 10.14348/molcells.2018.2313.
5. Koag MC, **Min KJ**, and Lee S. Structural basis for promutagenicity of 8-halogenated guanine. *J. Biol. Chem.* 2014 289(9);6289-6298.
6. Koag MC, Cheun Y, Kou Y, Ouzon-Shubeita H, **Min KJ**, Monzingo AF, and Lee S. Synthesis and structure of 16,22-diketocholesterol bound to oxysterol-binding protein Osh4. *Steroids* 2013 78(9);938-944.

Structural and functional characterization of a peptidoglycan reshaping peptidase in intestinal pathogens

Kyungjin Min and Hyung Ho Lee

Department of Chemistry, College of Natural Sciences, Seoul National University
**E-mail: km32479@snu.ac.kr*

To maintain viability of bacteria and defining bacterial cell shapes for its virulence, assembly of peptidoglycan is crucial. In the case of *Campylobacter jejuni* and *Helicobacter pylori,* their spiral cell shape is critical for gut colonization[1]. Several peptidoglycan hydrolases of these organisms belong to the cell-shape-determining (Csd) class of proteins. However, their recognition for peptidoglycan and catalytic processes are largely unknown. We have determined eight crystal structures for a member of the Csd class of *C. jejuni*. Two of the structures are for complexes with synthetic cell-wall peptidoglycan derivatives. Mass spectrometric analysis of turnover chemistry with the peptidoglycan revealed that the protein is an enzyme with the D,D-endopeptidase and D,D-carboxypeptidase activities. Furthermore, we report structural insights into binding of a cell-wall pentapeptide and a crosslinked heptapeptide (tetra-tri peptide) to the enzyme. Our works reveal for the first time the noncanonical nature of the transformations at the core of the events that define the morphological shape for intestinal pathogens, probably useful for designing inhibitors interfering with gut colonization.

References

[1] Hermans, D. et al. Colonization factors of *Campylobacter jejuni* in the chicken gut. *Vet. Res.* **(2011)**.

Acknowledgement

[1] This study was supported by the National Research Foundation (NRF) of Korea funded by the Korean government (2015R1A5A1008958 and 2018R1A2B2008142) and from the Korea CCS R&D Center (KCRC; 2014M1A8A1049296) to H.H.L.

Seung Kon Hong, Ph.D.

Researcher

Osong Medical Innovation Foundation (KBIO)

Phone: +82-43-200-9522 Email: biyahhaha@gmail.com

Education

2006 B.S., Applied Microbiology, Seoul Women's University, Korea

2008 M.S., Molecular Biology (Structural Biology), Korea University, Korea

2015 Ph.D., Biomedical Science (Structural Biology), Korea University, Korea

Work Experience

2015-2016 Postdoctoral Fellow, Korea Institute of Science and Technology (KIST)

2016-2018 Postdoctoral Fellow, Ewha Womans University

2018-present Researcher, Osong Medical Innovation Foundation (KBIO)

Research Interest

1. Crystallographic & biochemical characterization of biological macromolecules
2. Engineering of therapeutic proteins basis on structural analysis

Selected Publications

1. Ban YH†, Song MC†, Hwang JY†, Shin HR†, Kim HJ†, **Hong SK**†, Lee NJ, Park JW, Cha SS*, Liu H*, Yoon YJ*, Complete reconstitution of the diverse pathways of gentamicin B biosynthesis. *Nature Chemical Biology*. 2019. Mar; 15(3):295-303.
2. Song JM†, **Hong SK**†, An YJ, Kang MH, Hong KH, Lee Y-H*, and Cha SS*. Genetic and structural characterization of a thermo-tolerant, cold-active, and acidic endo-β-1,4-glucanase from Antarctic springtail, *Cryptopygus antarcticus*. *Journal of Agricultural & Food Chemistry*. 2017. Mar 1; 65(8), 1630-1640.
3. **Hong SK**†, Kim KH, Song EJ and Kim EE*. Structural Basis for the Interaction between the IUS-SPRY Domain of RanBPM and DDX-4 in Germ Cell Development. *Journal of Molecular Biology.* 2016 Oct 23; 428(21), 4330-4344.†contributed equally, *corresponding author

Structural investigation of *Micromonospora echinospora* GenB1 ; relationship between productivity and substrate preference

Seung Kon Hong*, Jae-yeon Hwang, Yeo Joon Yoon and Sun-Shin Cha

Protein Research Laboratory, Department of Chemistry and Nanoscience, Ewha Womans University, Seoul, 03760, Republic of Korea.
Present Address: Protein Drug Team, New Drug Development Center, Osong Medical Innovation Foundation (KBIO)

**E-mail: biyahhaha@gmail.com*

Although gentamicin B is one of the most widely used aminoglycoside antibiotics against multi-drug resistant pathogens including mycobacteria, staphylococci, and Gram-negative bacteria, its biosynthetic pathway has not been fully elucidated. GenB1 is a PLP-dependent aminotransferase involved in the biosynthesis of gentamicin B. Interestingly, GenB1 is a promiscuous enzyme with disaccharides and trisaccharides as substrates, but it displays graded preferences toward its diverse substrates depending on substituents on the sugar backbones. In this study, we have determined three high-resolution crystal structures of *Micromonospora echinospora* GenB1 (*Me*GenB1) in complex with PLP, PLP/NM (disaccharide), and PLP/JI-20A (trisaccharide). *Me*GenB1 has a two-domain structure composed of a PLP-binding domain and a flanking domain involved in active site formation. The active site of *Me*GenB1 is composed of four subsites; P, R1, R2, and R3. P-subsite is the PLP-binding site while R1-, R2-, and R3-subsites are regions for the binding of the first sugar (ring I), the second sugar linked at C4 of ring I (ring II), and the third sugar linked at C6 of ring I (ring III) of substrates, respectively. Based on structural features of each subsite, we provide insights into how the substrate preference of *Me*GenB1 is achieved in the context of the substrate promiscuity.

References

1. Ban YH, Song MC, Hwang JY, Shin HR, Kim HJ, **Hong SK**, Lee NJ, Park JW, Cha SS, Liu H, Yoon YJ, Complete reconstitution of the diverse pathways of gentamicin B biosynthesis. *Nature Chemical Biology*. (**2019**). Mar; 15(3):295-303

Acknowledgement

[1] This work was supported by the Ministry of Oceans and Fisheries Grant (the project titled "Development of biomedical materials based on marine proteins"), the Republic of Korea.

MEMO

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

2020

한국생체분자과학 연합학회학술대회

Symposium IX & X

Jiyong Park, Ph.D.

Research Fellow
Center for Catalytic Hydrocarbon Functionalizations, IBS and
Dept. of Chemistry, Korea Advanced Institute of Science and Technology (KAIST)
Phone: +82-10-3457-0132 Email: jiyongpa@ibs.re.kr

Education

2000 B.S., Physics, Seoul National University, Korea

2008 Ph.D., Physics, Seoul National University, Korea

Work Experience

2008-2012 Post-doctoral research associate, Howard Hughes Medical Institute, UCLA

2012-2013 Post-doctoral research associate, Dept. of Chemistry and Biochemistry, UCLA

2013-2016 Associate specialist, II, Dept. of Chemistry and Biochemistry, UCLA

2017-now Research fellow, Center for Catalytic Hydrocarbon Functionalizations, IBS

Research Interest

1. Molecular dynamics (MD) and hybrid QM/MM simulations of biological molecules
2. Quantum chemical studies of catalytic C-H activation reactions
3. Electronic structures electronic dynamics of photocatalysts

Selected Publications

1. Ilhan Yavuz, Blanton N. Martin**, Jiyong Park,** and Kendall N. Houk**, 'Theoretical study on the molecular ordering, paracrystallinity and charge-transport parameters of oligomeric semi-conductors in different crystalline phases',** *Journal of the American Chemical Society*, ***137,*** 2856-2866 (2015)
2. Elizabeth L. Noey, Nidhi Tibrewal, Gonzalo Jiménez-Osés, Sílvia Osuna, **Jiyong Park,** Carly Bond, Duilio Cascio, Jack Liang, Xiyun Zhang, Gjalt Huisman, Yi Tang, and Kendall. N. Houk, '**Origins of Stereoselectivity in evolved ketoreductases**,' *Proceedings of National Academy of Science, USA*, ***112,*** E7065-7072 (2015)
3. **Jiyong Park,** Joseph McDonald, Russell Petter, and Kendall N. Houk, **'Molecular dynamics analysis of binding of kinase inhibitors to WT EGFR and the T790M mutant,'** *Journal of Chemical Theory and Computation*, ***12,*** 2066-2078 (2016)
4. **Jiyong Park**, Sunny Chun, Thomas A. Bobik, Kendall N. Houk, and Todd O. Yeates, **'Molecular Dynamics Simulations of Selective Metabolite Transport Across the Propanediol Bacterial Microcompartment Shell,'** *The Journal of Physical Chemistry B*, ***121***, 8149-8154 *(2017)*
5. Jadab Majhi, Ben W. H. Turnbull, Ho Ryu, **Jiyong Park**, Mu-Hyun Baik, and P. Andrew Evans, "**Dynamic Kinetic Resolution of Alkenyl Cyanohydrins Derived from α,β-Unsaturated Aldehydes: Stereoselective Synthesis of *E*-Tetrasubstituted Olefins**, *Journal of the American Chemical Society*, ***141,*** 11770-11774 (2019)

Advanced MD Simulations that Quantify Target-Inhibitor Interactions: Case Studies of

EGFR Kinase Inhibitors and Androgen Receptor Antagonists

Jiyong Park, Ph. D.

Center for Catalytic Hydrocarbon Functionalizations, Institute for Basic Science and
Department of Chemistry, KAIST
**E-mail: jiyongpa@ibs.re.kr*

Rapid progresses in classical molecular dynamics (MD) simulations have enabled scientists to trace structural dynamics of large biomolecular systems up to an order of microseconds. However, the predictions of selectivity and potency of drugs that are correlated with large-scale conformational changes in the target protein are beyond the capacities of conventional MD simulations. This is because the changes are associated with high activation barriers that are difficult to overcome within the time-scales of MD simulations. Herein, I will introduce advanced MD simulation methods that can accelerate otherwise slow conformational transitions and overcome the difficulties of conventional MD methods. Those advanced methods include thermodynamic integration (TI), metadynamics, and accelerated MD (aMD) simulations. Brief introductions to the methodologies will be provided, that are intended for audiences who are less experienced with the molecular simulations. The utilities of the methods will be discussed in details by introducing two worked examples: 1) influences of a drug-resistant mutations to the binding affinities and target structure selectivities of epidermal growth factor receptor (EGFR) inhibitors and 2) conformational dynamics of the ligand-binding domain of androgen receptor (AR) bound to anti-androgens, that explain the mechanism of AR antagonism.

References

[1] **Jiyong Park,** Joseph McDonald, Russell Petter, and Kendall N. Houk, **'Molecular dynamics analysis of binding of kinase inhibitors to WT EGFR and the T790M mutant,'** *Journal of Chemical Theory and Computation*, ***12,*** 2066-2078 (2016)

[2] Hyo Jin Gim, **Jiyong Park**, Michael E. Jung, and Kendall N. Houk, "**The Differences in the Conformational Dynamics of Androgen Receptors bound to Agonists and Antagonists**," *in preparation*

Jin Hae Kim, Ph.D.

Assistant Professor

Daegu Gyeongbuk Institute of Science & Technology (DGIST)

Phone: +82-53-785-1770 Email: jinhaekim@dgist.ac.kr

Education

2011 Ph.D. in Biophysics, University of Wisconsin-Madison, WI, USA

2005 B.A in Biological Sciences and Physics, Seoul National University, Seoul, Republic of Korea

Work Experience

2018 – present Assistant Professor, Department of New Biology, DGIST, Daegu, Republic of Korea

2015 - 2018 Senior Researcher, Samsung Advanced Institute of Technology, Samsung Electronics, Suwon, Republic of Korea

2014 - 2015 Postdoctoral Researcher, German Center for Neurodegenerative Diseases, c/o Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

2014 - 2015 Postdoctoral Researcher, Mitochondrial Protein Partnership, Center for Eukaryotic Structural Genomics, University of Wisconsin-Madison, WI, USA

2014 - 2015 Research Assistant, University of Wisconsin-Madison, WI, USA

Research Interest

1. Structural characterization of protein aging and pathogenesis
2. Structural elucidation of protein structures and dynamics with NMR spectroscopy
3. Biochemical/biophysical characterization of protein aggregation
4. Investigation of protein-protein/protein-ligand interactions

Selected Publications

1. Oroz J.*, Kim J.*, et al. (2017) *Nat. Struct. Mol. Biol.* 24, 407-413 (*equal contribution).
2. Kim J. et al. (2016) *Angew. Chem. Int. Ed.* 55, 16168-16171.
3. Kim J. et al. (2014) *J. Am. Chem. Soc.* 136, 11586–11589.
4. Kim J.*, Bothe J.*, et al. (2014) *J. Am. Chem. Soc.* 136, 7933-7942 (*equal contribution).
5. Kim J. et al. (2013) *J. Am. Chem. Soc.* 135, 8117-8120.

The structural features of transthyretin in its aged aggregation-prone state

Jin Hae Kim

Daegu Gyeongbuk Institute of Science & Technology (DGIST), Daegu, Republic of Korea

**E-mail: jinhaekim@dgist.ac.kr*

Proteins are intrinsically dynamic. Many proteins accommodate structurally-distinctive conformations upon being exposed to different conditions or interacting with various molecules, and understanding to these structural transitions is a key to elucidate the mechanistic details of the protein functions and activities. Notably, the native equilibrium states over diverse structural conformations of proteins are maintained with delicate balance, perturbation of which could shift the original structural equilibrium. Diseases of proteinaceous origins are often caused by this disruption of protein dynamics, and, among various factors, ‘protein aging’ is one of the major culprits that break the dynamic balance of protein structures. This is particularly true for many proteins showing amyloidogenic properties. In this talk, I will introduce advanced techniques of nuclear magnetic resonance (NMR) spectroscopy and how these techniques have been contributed to appreciate structural deformation of aged proteins. In addition, I will present our work exemplifying direct correlation of structural perturbation of a protein with its pathogenic mechanism. More specifically, I would like to discuss about 1) the structural feature of transthyretin in its monomeric amyloidogenic state, 2) the structural feature of transthyretin with its amyloidosis-suppressive mutation, and 3) the preferential binding interaction between Hsp90 and the amyloidogenic state of transthyretin.

Acknowledgement

This work was supported by the National Research Foundation (NRF-2018R1C1B6008282) and the DGIST Start-up Fund Program (2019010103), both of which were granted by the Ministry of Science and ICT, Republic of Korea.

Eun Joo Song, Ph.D.

Associate Professor

College of Pharmacy, Ewha Womans University

Phone: +82-2-3277-3373 Email: esong@ewha.ac.kr

Education

1997 B.S., Pharmacy, Ewha Womans University, Korea

1999 M.S., Pharmacy, Ewha Womans University, Korea

2003 Ph.D. Pharmacy, Ewha Womans University, Korea

Work Experience

2003-2004 Post-doc. Ewha Womans University

2004-2007 Research Scientist, Korea Institute of Science & Technology

2008-2009 Post-doc. University of California, Berkeley, USA

2007-2013 Senior Research Scientist, Korea Institute of Science & Technology

2013-2019 Principal Research Scientist, Korea Institute of Science & Technology

2019- Associate Professor, College of Pharmacy, Ewha Womans University

Research Interest

1. Functional analysis of deubiquitinating enzymes in cell division and survival
2. Understanding the functional role of ubiquitin system in cancer
3. Development of analytical methods for biomolecules

Selected Publications

1. Chae DK, Park J, Cho M, Ban E, Jang M, Yoo YS, Kim EE, Baik JH, Song EJ*. Mol. Oncology 2019
2. Das T, Kim EE*, Song EJ*. J Mol Biol. 2019 431(19):3900-3912
3. Park J, Kwon MS, Kim EE, Lee H, Song EJ*. Nature Communications 2018 Feb 15;9(1):688
4. Das T, Park JK, Park J, Kim E, Rape M, Kim EE*, Song EJ*. Nucleic Acids Res. 2017, 45(8):4866-4880
5. Park JK, Das T, Song EJ*, Kim EE*. Nucleic Acids Res. 2016 44(11):5424-37

Phosphorylation of USP15 and USP4 Regulates Localization and Spliceosomal Deubiquitination

Tanuza Das[1], Joon Kyu Park[1], Eunice EunKyeong Kim[1] and Eun Joo Song[2]

[1]Biomedical Research Institute, Korea Institute of Science and Technology, Seoul, Korea

[2]Graduate School of Pharmaceutical Sciences, College of Pharmacy, Ewha Womans University, Seoul, Korea

**E-mail: esong@ewha.ac.kr*

Deubiquitinating enzymes have key roles in diverse cellular processes whose enzymatic activities are regulated by different mechanisms including post-translational modification. In this study, we performed siRNA library screening targeting ~70 human DUBs to identify proteins required for cell cycle control. We focused on DUB candidates whose depletion lead to significant spindle checkpoint bypass in response to taxol treatment. Subsequently, we selected and analyzed the role of USP15 and USP4, depletion of which showed mitotic defect such as chromosome missegregation and multiple poles. We found that USP15 and USP4 interact with SART3 which has an important role in the translocation of USP15 and USP4 from the cytoplasm to the nucleus and serves as a targeting factor for co-localization with substrates. In addition, nuclearcytoplasmic fractionation and mass spectrometric analysis revealed that Thr149 and Thr219 of human USP15, which is conserved among different species, are phosphorylated in the cytoplasm. The phosphorylation status of USP15 at these two positions alters the interaction with its partner protein SART3, consequently leading to its nuclear localization and deubiquitinating activity toward the substrate PRP31. Collectively, our data suggest that modifications of USP15 and USP4 by phosphorylation are important for the regulation of their localization required for cellular function in the spliceosome.

Min-Kyu Kim, Ph.D.

Senior Researcher

Korea Atomic Energy Research Institute (KAERI)

Phone: +82-63-570-3212, +82-10-3766-4494 Email: mkkim@kaeri.re.kr

Education

2002 B.S., School of Biological Sciences, Seoul National University, Korea

2010 Ph.D., School of Biological Sciences, Seoul National University, Korea

Work Experience

2010.09-2015.06 Post-doc, Korea Institute of Ocean Science and Technology (KIOST)

2015.07-present Senior Researcher, Korea Atomic Energy Research Institute (KAERI)

Research Interest

1. Structure and function of proteins from extreme radiation-resistant bacteria and animals
2. Novel antibiotics target proteins
3. Molecular machines and structural proteins

Selected Publications

1. Zhang J, Zhao L, Seo HS, Jung JH, Choi JI, **Kim MK***, and Lim S* (2019) Crystal structure of the highly radiation-inducible DinB/YfiT superfamily protein DR0053 from *Deinococcus radiodurans* R1. *Biochem Biophys Res Commun.* 513(2):354-359.

2. Park SB¶, Bae DW¶, Clavio NAB¶, Zhao L, Jeong CS, Choi B, Macalino SJY, Cha HJ, Park JB, Lee JH, Nam SJ, Choi S*, **Kim MK***, and Cha SS* (2018) Structural and biochemical characterization of the curcumin-reducing activity of CurA from *Vibrio vulnificus*. *J Agric Food Chem*. 66(40):10608-10616.

3. **Kim MK¶**, An YJ¶, Na JH¶, Seol JH, Ryu JY, Lee JW, Kang LW, Chung KM, Lee JH, Moon JH, Lee JS, and Cha SS* (2017) Structural and mechanistic insights into the inhibition of class C β-lactamases through the adenylylation of the nucleophilic serine. *J Antimicrob Chemother.* 72(3):735-743.

4. **Kim MK¶**, Kim JH¶, Kim JS¶, and Kang SO* (2015) Structure of the 34 kDa F-actin-bundling protein ABP34 from *Dictyostelium discoideum*. *Acta Crystallogr D Biol Crystallogr*. 71(Pt 9):1835-1849.

5. **Kim MK**, An YJ, Song JM, Jeong CS, Kang MH, Kwon KK, Lee YH, and Cha SS* (2014). Structure-based investigation into the functional roles of the extended loop and substrate-recognition sites in an endo-β-1,4-D-mannanase from the Antarctic springtail, *Cryptopygus antarcticus*. *Proteins* 82(11):3217-3223.

Inhibitory Activity of Nucleotide-based Metabolites Against Class C β-Lactamases

Min-Kyu Kim[1], Young Jun An[2], Jung-Hyun Na[3], and Sun-Shin Cha[4*]

[1]Korea Atomic Energy Research Institute, [2]Korea Institute of Ocean Science and Technology, [3]Osong Medical Innovation Foundation, [4]Ewha Womans University Republic of Korea

**E-mail: mkkim@kaeri.re.kr, chajung@ewha.ac.kr*

β-Lactam antibiotics are the most frequently prescribed antimicrobial agents. However, their clinical application is challenged by the emergence and dissemination of bacterial resistance to these antibiotics. Although some β-lactamase inhibitors are prescribed in combination with β-lactam antibiotics to overcome this resistance, the emergence of enzymes resistant to current inhibitors necessitates the development of novel β-lactamase inhibitors. Class C β-lactamases are widely distributed among Gram-negative pathogens and are responsible for bacterial resistance to a broad spectrum of β-lactam antibiotics. Here, we investigated the inhibitory effect of nucleotides on extended-spectrum class C β-lactamases with structural, biochemical, and *in vivo* mouse model studies.

References

[1] Na JH, Lee TH, Park SB, Kim MK, Jeong BG, Chung KM, and Cha SS. *In vitro* and *in vivo* inhibitory activity of NADPH against the AmpC BER class C β-lactamase. *Front Cell Infect Microbiol*. 8:441. **(2018)**

[2] Kim MK, An YJ, Na JH, Seol JH, Ryu JY, Lee JW, Kang LW, Chung KM, Lee JH, Moon JH, Lee JS, and Cha SS. Structural and mechanistic insights into the inhibition of class C β-lactamases through the adenylylation of the nucleophilic serine. *J Antimicrob Chemother.* 72(3):735-743. **(2017)**

[3] Na JH, An YJ, and Cha SS. GMP and IMP are competitive inhibitors of CMY-10, an extended-spectrum class C β-lactamase. Antimicrob Agents Cehmother. 61(5). E00098-17. **(2017)**

Jae-Byum Chang, Ph.D.

Assistant Professor

Korea Advanced Institute of Science and Technology (KAIST)

Phone: +82-10-4098-4085 Email: jbchang03@gmail.com

Education

2008 B.S., Physics, Korea Advanced Institute of Science and Technology (KAIST), Korea

2014 Ph.D., Materials Science and Engineering, Massachusetts Institute of Technology (MIT), Korea

Work Experience

2016 – 2018 Assistant professor, Sungkyunkwan University, Korea

2014 – 2016 Postdoctoral associate, MIT Media lab, USA

Research Interest

1. Super-resolution molecular imaging
2. Brain imaging, brain disorders
3. Hydrogel, nanofabrication, nanomaterial synthesis

Selected Publications

1. Min, K., Choi, M. & Chang, J., Multiplexed expansion microscopy of the brain through fluorophore screening, Methods doi: 10.1016/j.ymeth.2019.07.017 (2019)
2. Cho, I., Seo, J. & Chang, J., Expansion microscopy, J. Microsc. doi.org/10.1111/jmi.12712 (2018)
3. Chang, J., Chen, F., Yoon, Y., Jung, E. E., Babcock, H., Kang, J. S., Asano, S., Suk, H., Pak, N., Tillberg, P. W., Wassie, A., Cai, D. & Boyden, E. S., Iterative expansion microscopy, Nat. Methods doi:10.1038/nmeth.4261 (2017)
4. Chen, F., Wassie, A. T., Cote, A. J., Sinha, A., Alon S., Asano S., Daugharthy, E. R., Chang, J., Marblestone, A., Church G. M., Raj A. & Boyden, E. S., Nanoscale imaging of RNA with expansion microscopy, Nat. Methods 13, 679-684 (2016)
5. Chang, J., Choi, H. K., Hannon, A. F., Alexander-Katz, A., Ross, C. A. & Berggren, K. K. Template rules for tile-based directed self-assembly. Nat. Commun. 5, 3305 (2014)

Expansion microscopy techniques and their applications to reveal fine molecular details

Jae-Byum Chang

Department of Materials Science and Engineering, KAIST

**E-mail: jbchang03@kaist.ac.kr*

Visualizing multiple biomolecular species with nanometer-scale precision can provide essential knowledge to understand the molecular mechanisms of biological phenomena. In 2015, a new super-resolution microscopy technique, called expansion microscopy, was reported; in this technique, higher lateral and axial resolution are achieved by physically expanding specimens via a swellable hydrogel (ExM; Science 347(6221):534-548, Nat. Biotechnol. 34(9):987-992). Briefly, target specimens, such as cultured cells or tissue slices, are embedded in a swellable hydrogel. During the embedding process, proteins of interest are chemically anchored to the hydrogel. Then, the specimen-hydrogel composite is isotropically expanded in de-ionized water. During the expansion, closely-located proteins move apart, resulting in 60-nm resolution when imaged with diffraction-limited microscopy. In this talk, we show three applications of expansion microscopy: (1) simultaneous super-resolution imaging of proteins and mRNA, (2) super-resolution imaging of actin structures of the brain, (3) super-resolution imaging of dopaminergic neurons in a PD model mouse.

Acknowledgment

[1] This research was supported by National Research Foundation of Korea (NRF-2019R1F1A1063145, NRF-2017M3C7A1043841). This work was also supported by Samsung Research Funding & Incubation Center for Future Technology (SRFC-IT1702-09).

Myunghwan Choi, Ph.D.

Associate Professor

Department of Biomedical Engineering, Sungkyunkwan University

Phone: +82-31-299-4358 Email: photomodulation@gmail.com

Education

2006 B.S., Bio and Brain Engineering, KAIST

2010 Ph.D., Bio and Brain Engineering, KAIST

Work Experience

2011-2015 Postdoc, Harvard Medical School

2015- Assistant/Associate Professor, Sungkyunkwan University

Research Interest

1. Myelin
2. Taste
3. Voltage imaging

Selected Publications

1. Jo Y, Kwon J, Kim M, Choi W, **Choi M**. Microsphere-based interferometric optical probe. *Nature Communications* 2018; in press.

2. Kwon J, Kim M, Park H, Kang B, Jo Y, Kim JH, James O, Yun SH, Kim SG, Suh M, **Choi M**. Label-free nanoscale optical metrology on myelinated axons in vivo. *Nature Communications* 2017; 8(1): 1832.

3. **Choi M**, Humar M, Kim S, Yun SH. Step-index optical fiber made of biocompatible hydrogels. *Advanced Materials* 2015; 27:4081-4086.

4. **Choi M**, Choi JW, Kim S, Nizamoglu S, Hahn SK, Yun SH. Light-guiding hydrogels for cell-based sensing and optogenetic synthesis in vivo. *Nature Photonics* 2013; 7 (12): 987-994

5. **Choi M**, Ku T, Chung K, Yoon J, Choi C. Minimally invasive molecular delivery into the brain using optical modulation of vascular permeability. *PNAS* 2011;108 (22):9256-9261

Comprehensive functional screening of taste sensation in vivo

Myunghwan Choi

Department of Biomedical Engineering, Sungkyunkwan University

**E-mail: photomodulaiton@gmail.com*

The initial event in taste sensation is mediated by taste cells on the tongue that translate ingested chemicals into cellular signals. Current understanding on this cellular level taste encoding process has relied on ex vivo model systems that cannot fully recapitulate natural cellular microenvironment in vivo. To resolve this methodological limitation, we invented a microfluidics-on-a-tongue imaging chamber that has integrated multichannel microfluidics for auto-controlled tastant delivery. Using this system, we screened over 100 fungiform taste cells to the five basic taste qualities, and obtained comprehensive functional maps. We revealed that taste cells are composed of 70% of single-tuned and 30% of dual-tuned cells, and also discovered a novel population of dual-tuned taste cells encoding a positive valence. We believe that our novel screening platform will pave a way for the deeper understanding of taste coding logic.

Jinseop S. Kim, Ph.D.

Assistant Professor

Sungkyunkwan University

Phone: +82-31-290-7014 Email: jinseopskim@skku.edu

Education

2000 B.S., Physics, Seoul National University, Korea

2005 M.S., Physics, Seoul National University, Korea

2010 Ph.D., Physics, Seoul National University, Korea

Work Experience

2010-2010 Visiting Postdoctoral Researcher, Department of Brain and Cognitive Science, SNU, Korea

2010-2014 Postdoctoral Associate/Fellow, Department of Brain and Cognitive Science, MIT, USA

2014-2015 Postdoctoral Associate, Princeton Neuroscience Institute, Princeton University, USA

2015-2019 Principal Researcher, Korea Brain Research Institute, Korea

2019-present Assistant Professor, Department of Biological Sciences, Sungkyunkwan University, Korea

Research Interest

1. Connectomics, structure and function of brain cells and neural circuits
2. Computational neuroscience, neural computation of microcircuits
3. Biophysical basis of brain functions including perception, cognition, and motion

Selected Publications

1. Bae†, Mu†, Kim†, Turner†, Seung*, et al., Cell 173 (5), 1293–1306.e9 (2018). (†equal contribution)
2. Greene†, Kim†, Seung*, et al., Cell Reports 14 (8), 1892–1900 (2016). (†equal contribution)
3. Kim†, Greene†, Zlateski, Lee, Seung*, et al., Nature 509, 331–336 (2014). (†equal contribution)
4. Kim and Kaiser*, Philos. Trans. R. Soc. Lond. B 369, 20130529 (2014).

Anatomy and connectivity of the cerebellum revealed by electron microscope images

Jinseop S. Kim

Department of Biological Sciences, Sungkyunkwan University, Suwon 16419, Korea

**E-mail: jinseopskim@skku.edu*

The connectomic analysis of 3D electron microscope images provides both ultrafine anatomy of individual neurons and complete connectivity of the circuits in a sample. Here I present the quantitative anatomy and connectivity of cerebellar neurons in a mouse using computational analyses. Contrary to the conventional knowledge that the cerebellar molecular layer is regularly organized and repeats simple structure, we find multifaceted variabilities and exquisite organization. We also discover various connectivity among neuronal cell types, which we hypothesize is critical to the physiology and function of the cerebellum. The discoveries call upon new experiments to confirm the hypotheses.

Sung Hyun Kim, Ph.D.

Associate Professor

Kyung Hee University, School of Medicine

Phone: + 82-2-961-0286 Email: sunghyunkim@khu.ac.kr

Education

2007 Ph.D., Gwangju institute of science and technology (GIST), Korea

2002 M.S., Gwangju institute of science and technology (GIST), Korea

2000 B.S., Genetic Engineering, SungKyunKwan University, Korea

Work Experience

2013-Present Assistant/Associate Professor., Kyung Hee University, Korea

2011-2013 Instructor., Weill Cornell Medical College, New York, USA

2007-2011 Postdoctoral fellow., Weill Cornell Medical College, New York, USA

Research Interest

1. High Fidelity Optical Imaging
2. Synapse physiology
3. Membrane trafficking

Selected Publications

1. Kyung JW, Kim J-M, Cha S-H, Chung K-H, Choi D-J, Jou I, Song WK, Joe E-H, Kim SH*, Park SM*. DJ-1 deficiency impairs synaptic vesicle endocytosis and reavailability at nerve terminals. *PNAS* 2018 Feb 13;115(7):1629-1634.
2. Baek SH*, Park SJ*, Jeong JI*, Kim SH*, et al. Inhibition of Drp1 Ameliorates Synaptic Depression, Aβ Deposition, and Cognitive Impairment in an Alzheimer's Disease Model. *J Neurosci*. 2017 May 17;37(20):5099-5110.
3. Lee T-S, Lee J-Y, Kyung JW, Yang Y, Park SJ, Lee S, Pavlovic I, Kong B, Jho YS, Jessen HJ, Kweon D-H, Shin Y-K, Kim SH*, Yoon T-Y*, Kim S*. Inositol pyrophosphates inhibit synaptotagmin-dependent exocytosis. *PNAS* 2016 Jul 19;113(29):8314-9
4. Kyung JW, Zhang P, Cho IH, Lee S, Song WK, Ryan TA, Hoppa MB*, Kim SH*. Adaptor Protein 2 (AP-2) complex is essential for functional axogenesis in neurons. *Sci Rep* 2017 Jan 31;7:41620
5. Kyung JW, Kim D-H, Song WK, Kim SH. Epsin1 modulates synaptic vesicle retrieval capacity at CNS synapses. *Sci Rep* 2016 Aug 25;6:31997

Distinct property of presynaptic physiology between excitatory and inhibitory neurons

Sung Hyun Kim

Department of Physiology, School of Medicine, Kyung Hee University

**E-mail: sunghyunkim@khu.ac.kr*

Proper brain function requires a balance between excitatory and inhibitory neuronal activity. This balance, which is disrupted in various neural disorders, ultimately depends on the functional properties of both excitatory and inhibitory neurons; however, how the physiological properties of presynaptic terminals are controlled in these neurons is largely unknown. In this study, we generated pHluorin-conjugated, synaptic vesicle-specific tracers that are preferentially expressed in excitatory or inhibitory nerve terminals. We found that synaptic vesicle recycling is ~1.8-fold slower in inhibitory nerve terminals than excitatory nerve terminals, resulting in reduced efficacy of synaptic transmission in inhibitory presynaptic terminals during repetitive activities. Interestingly, this relative difference in trafficking efficiency is mediated by synaptic vesicle protein 2A (SV2A), which is more highly expressed in inhibitory synapses and differentially controls sorting of synaptic protein, synaptotagmin I. These findings indicate that SV2A coordinates distinct properties of synaptic vesicle recycling between excitatory and inhibitory synapses.

Acknowledgement

This work was supported by the Korea Research Foundation Grant (code KRF-2017M3C7A1048268), the Republic of Korea.

MEMO

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

2020

한국생체분자과학 연합학회학술대회

Poster Session

[STR-1]

Crystal Structure of IlvC, a Ketol-Acid Reductoisomerase from *Streptococcus pneumoniae*

Gyuhee Kim[1], Donghyuk Shin[1,3], Sumin Lee[2], Jaesook Yun[2] and Sangho Lee[1,*]

[1]Department of Biological Sciences, [2]Department of Chemistry, Sungkyunkwan University, 2066, Seobu-ro, Suwon, 16419, Republic of Korea

[3]Max Plank Institute of Biophysics, Frankfurt am Main, 60438, Germany

**E-mail: sangholee@skku.edu*

Biosynthesis of branched-chain amino acids (BCAAs), including isoleucine, leucine and valine, is required for survival and virulence of a bacterial pathogen such as *Streptococcus pneumoniae*. IlvC, a ketol-acid reductoisomerase (E.C. 1.1.1.86) with NADP(H) and Mg^{2+} as cofactors from the pathogenic *Streptococcus pneumoniae* (SpIlvC), catalyzes the second step in the BCAA biosynthetic pathway. To elucidate the structural basis for the IlvC-mediated reaction, we determined the crystal structure of SpIlvC at 1.69 Å resolution. The crystal structure of SpIlvC contains an asymmetric dimer in which one subunit is in apo-form and the other in NADP(H) and Mg^{2+}-bound form. Crystallographic analysis combined with an activity assay and small-angle X-ray scattering (SAXS) suggested that SpIlvC retains dimeric arrangement in solution and that D83 in the NADP(H) binding site and E195 in the Mg^{2+} binding site are the most critical in the catalytic activity of SpIlvC. Crystal structures of SpIlvC mutants (R49E, D83G, D191G and E195S) revealed local conformational changes only in the NADP(H) binding site. The NADP(H) binding site mutants have rotameric change as His-31, Lys-52, Phe-54 and His 135. Taken together, our results establish the molecular mechanism for understanding functions of SpIlvC in pneumococcal growth and virulence.

References

[1] Gyuhee Kim and Sangho Lee, Crystallization and preliminary crystallographic analysis of IlvC, a ketol-acid reductoisomerase, from *Streptococcus pneumoniae* D39. *Biodesign* (2017).

[2] Gyuhee Kim, Donghyuk Shin, Sumin Lee, Jaesook Yun and Sangho Lee, Crystal Structure of IlvC, a Ketol-Acid Reductoisomerase, from *Streptococcus pneumoniae. Crystals* (2019).

[STR-2]

Molecular details of the VxGΦL motif of PP2Cs from *Oryza sativa* involved in regulation of ABA responsiveness

Seungsu Han[1], Ji-young Lee[2], Yeongmok Lee[1], Tae-Houn Kim[2] and Sangho Lee[1,*]

[1]Department of Biological Sciences, Sungkyunkwan University, Suwon 16419, Korea

[2]Department of Prepharm·*Med/Health Functional Biomaterials, Duksung Women's University, Seoul 01369, Korea*

**E-mail: sangholee@skku.edu*

Plants always face abiotic stresses to suppress their growth and reproduction. The abscisic acid (ABA) signaling is highly involved in balancing tolerance to abiotic stresses and plant growth. An ABA receptor (PYL/RCAR) and a protein phosphatase (PP2C), a co-receptor, form a complex upon binding to ABA. Previously we reported that new motif of PP2C in plant, the VxGΦL motif, is critical in the interaction of PP2Cs with PYL/RCARs. Here we surveyed the second and fourth positions of the VxGΦL motif by combination of biochemical, structural and physiological analyses. We found that the fourth position of the VxGΦL motif, highly conserved to small hydrophobic residues, was a key determinant of the OsPP2C50:OsPYL/RCAR interactions across subfamilies. Large hydrophobic or any hydrophilic residues in the fourth position abrogated ABA responsiveness. Analysis of crystal structures of OsPP2C50 mutants in complex with ABA and OsPYL/RCAR3, along with energy calculation of the complexes, uncovered that a bulky hydrophobic residue in the fourth position of the VxGΦL motif pushed away side chains of nearby residues, conferring side-chain rotameric energy stress. Hydrophilic residues in this position imposed solvation energy stress to the PP2C:PYL/RCAR complex. Germination and gene expression analyses corroborated that OsPP2C50 AS and AK mutants modulated ABA responsiveness in *Arabidopsis*. Our results suggest that ABA responsiveness could be fine-tuned by the fourth position of the VxGΦL motif on PP2Cs.

[STR-3]

Effects of a hydrophobic residue in pyrabactin recognition by a rice ABA receptor

Seungsu Han[1], Yeongmok Lee[1], Eun Joo Park[2], Myung Ki Min[3], Yongsang Lee[3], Tae-Houn Kim[2], Beom-Gi Kim[3] and Sangho Lee[1,*]

[1] Department of Biological Sciences, Sungkyunkwan University, Suwon 16419, Korea
[2] Department of Biotechnology, Duksung Women's University, Seoul 01369, Korea
[3] Gene Engineering Division, Department of Agricultural Biotechnology, National Institute of Agricultural Sciences, Rural Development Administration, Jeonju 55365, Korea
**E-mail: sangholee@skku.edu*

Pyrabactin is a synthetic mimicry of abscisic acid (ABA), a phytohormone modulating abiotic stress responses. Previous researches in *Arabidopsis* revealed that functionality of pyrabactin depends on polymorphism of specific residue and subfamily-specific manner of ABA receptors. However, *Oryza sativa* ABA receptors showed unexpected responses toward pyrabactin in our preliminary study. To identify structural determinants for pyrabactin recognition, we determined the molecular structure of OsPYL/RCAR3:pyrabactin:OsPP2C50 complex by X-ray crystallography. Pyrabactin is located in the ligand binding pocket of OsPYL/RCAR3 as an unobserved conformation in Arabidopsis researches. Phe125, unconserved residue in binding pocket appears to be the culprit for the differential conformation of pyrabactin. Especially, this phenylalanine is found in *Oryza* and many other monocot crops, but not in *Arabidopsis*. Although the gate closure essential for complex formation is preserved in the presence of pyrabactin, Phe125 apparently restricts accessibility of pyrabactin, leading to decreased affinity for OsPYL/RCAR3 in phosphatase assay. However, Phe125 does not affect conformation and accessibility of ABA. Yeast two-hybrid, germination and gene transcription analyses in rice also support that pyrabactin induces a weak effect on ABA signaling pathway. Taken together, our results suggest that phenylalanine substitution of OsPYL/RCARs subfamily I may be one of considerations for ABA synthetic agonist development

[STR-4]

Structural basis of small RNA hydrolysis by oligoribonuclease (*Cps*ORN) from *Colwellia psychrerythraea* strain 34H

Chang Woo Lee[1*], Chang-Sook Jeong[1,2], Jisub Hwang[1,2], Sun-Shin Cha[3], & Jun Hyuck Lee[1,2]

[1]Unit of Research for Practical Application, Korea Polar Research Institute, Incheon, 21990, Republic of Korea. [2]Department of Polar Sciences, University of Science and Technology, Incheon, 21990, Republic of Korea.
[3]Department of Chemistry & Nanoscience, Ewha Woman's University, Seoul, 03760, Republic of Korea.
**E-mail: justay@kopri.re.kr*

Cells regulate their intracellular mRNA levels by using specific ribonucleases. Oligoribonuclease (ORN) is a 3′.5′ exoribonuclease for small RNA molecules, important in RNA degradation and re-utilisation. However, there is no structural information on the ligand-binding form of ORNs. In this study, the crystal structures of oligoribonuclease from Colwellia psychrerythraea strain 34H (CpsORN) were determined in four different forms: unliganded-structure, thymidine 5′-monophosphate p-nitrophenyl ester (pNP-TMP)-bound, two separated uridine-bound, and two linked uridine (U-U)-bound forms. The crystal structures show that CpsORN is a tight dimer, with two separated active sites and one divalent metal cation ion in each active site. These structures represent several snapshots of the enzymatic reaction process, which allowed us to suggest a possible one-metal-dependent reaction mechanism for CpsORN. Moreover, the biochemical data support our suggested mechanism and identified the key residues responsible for enzymatic catalysis of CpsORN.

References

Lee, C. W. et al. Structural basis of small RNA hydrolysis by oligoribonuclease (CpsORN) from Colwellia psychrerythraea strain 34H. Scientific reports 9, 2649, doi:10.1038/s41598-019-39641-0 (2019).

[STR-5]

Unique unfoldase/aggregase activity of a molecular chaperone that dysregulates a translational elongation factor

Mi-Seong Kim, Chae-Yeon Wang, Eun-Jae Jeon, Kibin Lee, Hyung-Sik Won*

Department of Biotechnology, Konkuk University, Chungju, Chungbuk 27478, Korea
**E-mail: wonhs@kku.ac.kr*

The various chaperone activities of heat shock proteins contribute to ensuring cellular proteostasis. The prokaryotic molecular chaperone, Hsp33, was originally discovered as a heat-inducible, but post-translationally regulated chaperone that is activated upon oxidation [1]. Here, we demonstrate the non-canonical unfoldase activity as an inherent functionality of Hsp33 [2]. The holding-inactive, reduced form of Hsp33 (^{R}Hsp33) strongly bound to the translational elongation factor, EF-Tu, and catalyzed the EF-Tu aggregation via evoking its aberrant folding, resulting in its susceptibility to proteolytic degradation by Lon. This interaction was found to be critically mediated by the redox-switch domain of ^{R}Hsp33 and the guanine nucleotide-binding domain of EF-Tu. The ^{R}Hsp33-induced *in vivo* aggregation of EF-Tu was evident in a Lon-deficient strain and inhibited cell growth. Unlike wild-type *Escherichia coli*, the strain lacking both Hsp33 and Lon showed a non-reduced level of EF-Tu and diminished capability of counteracting heat shock. These findings suggest that the unique unfoldase/aggregase activity of Hsp33 potentially involved in protein turnover confers a cellular survival advantage under heat-stressed conditions.

References

[1] Lee, Y.-S. *et al*., Semi-empirical structure determination of *Escherichia coli* Hsp33 and identification of dynamic regulatory elements for the activation process. *J. Mol. Biol.* (**2015)**.

[2] Jo, K.-S. *et al*., Unique unfoldase/aggregase activity of a molecular chaperone Hsp33 in its holding-inactive state. *J. Mol. Biol.* (**2019)**.

Acknowledgement

This work was supported by the Korea Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (no. 2019R1A2C1004883).

[STR-6]

Identification of the Pyruvate dehydrogenase kinase 2 structure complexed with inhibitors targeting the ATP-binding and lipoyl-binding pocket

Jihoon Kang and Jungwook Kim

Department of Chemistry, Gwangju Institute of Science and Technology (GIST), Gwangju, 61005, Korea
**E-mail: kangjihoon@gist.ac.kr, jwkim@gist.ac.kr*

The pyruvate dehydrogenase kinase (PDK) inactivates the human pyruvate dehydrogenase complex (PDC) by phosphorylation. The PDC regulates mitochondrial metabolism as a gatekeeper by converting the pyruvate to CO2 and acetyl-CoA. The high expression level of PDK leads the excessively inactivated PDC, triggering the metabolic disorders. To treat the diseases, inhibitors of PDK have been developed, although they need to be optimized for efficacy and specificity. Here, we present the crystal structures of PDK2 complexes with GM10030, GM67520, and compound 8c, respectively. The GM10030 and the GM67520 have the core, 4-phenyl-5-(5-chlororesocinyl)isoxazole-3-carboxamido, that primarily contributes to binding the ATP pocket. Notably, GM67520 occurs the conformational change of ATP lid that may increase the binding affinity. The compound 8c, a lypoyl-binding pocket targeting inhibitor, is newly introduced to have an anthraquinone moiety that is sandwiched with two phenylalanines. We suggest our structural study is helpful to optimize inhibitors and drug discoveries.

[STR-7]

Crystal structure of phosphatidylserine decarboxylase reveals molecular mechanism of auto-cleavage and decarboxylation

Gyuhyeok Cho and Jungwook Kim

Department of Chemistry, Gwangju Institute of Science and Technology (GIST), Gwangju, 61005, Korea

**E-mail: gyuhyeokcho@gist.ac.kr, jwkim@gist.ac.kr*

Phosphatidylethanolamine (PE) is a major phospholipid constituting cellular membrane in many bacteria. Phosphatidylserine decarboxylase (PSD), an essential peripheral membrane protein in many Gram-negative pathogenic bacteria, is an enzyme catalyzing the last step of PE biosynthesis. The PSD is enzymatically auto-cleaved into an N-terminal beta chain and C-terminal alpha chain leaving an essential pyruvoyl prosthetic group on its N-terminus of the alpha chain. Here, we report the first crystal structure of PSD from *E. coli* (EcPSD) in the processed apo state. EcPSD forms a dimer. Each protomer contains N-terminal hydrophobic three alpha helices surrounding the active site pyruvoyl residues. Detergents binding mode and electrostatic distribution propose that the helices anchor the PSD to the membrane. Site-directed mutagenesis identifies that His144-Ser254 dyad is essential for the auto-cleavage. Substrate phosphatidylserine (PS) binding model suggests that the His144 may interact with the carboxylate of the substrate stabilizing Schiff base intermediate. This structure gives insights into the auto-cleavage and PS decarboxylation mechanism of PSD.

[STR-8]

Cryo-EM study a small protein using bispecific diabody

Sang-Soo Lee Su-Bin Kim, Jun-Gyou Park,Mi-Sun Jin

School of life science, Gwangju Institute of Science and Technology, Gwangju 61005, Republic Of Korea

E-mail: dltkdtnqqwe@gist.ac.kr

Recent advances in cryo-EM technology have enabled the determination of protein structures at close to atomic resolution. However, application of this method is still difficult for proteins below ~100 kDa due to lower contrast. Additionally, the structural flexibility of the proteins is a major obstacle to successful EM analysis. In this research, we aim to develop novel “bispecific diabodies” as tools for structural studies of small and/or flexible proteins. Diabodies are bivalent and bispecific antibody fragments, which consist of two heavy chain variable domains (VHs) and two light chain variable domains (VLs) in a single polypeptide, and so can simultaneously bind two different proteins. For EM applications, diabodies can serve as rigid linkers connecting small targets and large helper proteins as fiducial markers, allowing easy detection by improving contrast because of the increased size of the whole protein complex. We confirmed the stable formation of “target-diabody-helper protein” complexes by both size-exclusion chromatography and negative staining EM. We plan to expand the use of this technique to eukaryotic membrane proteins, one of the most challenging types in structural biology.

[STR-9]

Structure of the MICU1-MICU2 Heterodimer Provides Insights into the Gatekeeping Threshold Shift

Jongseo Park[1,2*], Youngjin Lee[1,2,4], Taein Park[2,3], Jung Youn Kang[1,2], Sang A Mun[1,2], Minwoo Jin[1,2], Jihyeong Yang[1,2] and Soo Hyun Eom[1,2,3†]

[1]School of Life Sciences, [2]Steitz Center for Structural Biology, [3]Department of Chemistry, Gwangju Institute of Science and Technology (GIST), Buk-gu, Gwangju 61005, Republic of Korea, [4]Infection and Immunity Research Laboratory, Metabolic Regulation Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejoun 34141, Republic of Korea

**E-mail: jongseopark@gist.ac.kr*

Mitochondrial calcium uptake 1 and 2 (MICU1 and MICU2) mediates mitochondrial Ca^{2+} influx via mitochondrial calcium uniporter (MCU). Its molecular action for Ca^{2+} uptake is tightly controlled by the MICU1 and MICU2 heterodimer, which are Ca^{2+} sensing proteins that act as gatekeepers at low [Ca^{2+}] or facilitators at high [Ca^{2+}]. However, the mechanism underlying the regulation of Ca^{2+} gatekeeping threshold for mitochondrial Ca^{2+} uptake through MCU by the MICU1-MICU2 heterodimer remains unclear. In this study, we determined the crystal structure of the apo-form of the human MICU1-MICU2 heterodimer that functions as the MCU gatekeeper. MICU1 and MICU2 assemble in the face-to-face heterodimer with salt bridges and methionine knobs, stabilizing the heterodimer in an apo-state. Structural analysis suggests how the heterodimer sets a higher Ca^{2+} threshold than the MICU1 homodimer. The structure of the heterodimer in the apo-state provides a framework for understanding the gatekeeping role of MICU1-MICU2 heterodimer.

Acknowledgement

This work was supported by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea(NRF-2017R1A2B2001878) and GIST Research Institute(GRI) grant funded by the GIST in 2019.

[STR-10]

Structural basis of Wnt signaling inhibition by sclerostin

Jinuk Kim[1*], Wonhee Han[2], Injin Bang[1], Hyunsik Lee[1], Jin-Kwan Han[2], Hee-Jung Choi[1]

[1]Department of Biological Sciences, Seoul National University, Seoul 08826, Republic of Korea

[2]Department of Life Sciences, Pohang University of Science and Technology, Pohang 37673, Gyeongbuk, Korea

**E-mail: kimjinuk12@snu.ac.kr*

LRP6 is an essential coreceptor in the β-catenin dependent Wnt signaling. The ectodomain of LRP6 provides the binding sites for not only Wnts, but also Wnt inhibitors, such as sclerostin (SOST), a key negative regulator of Wnt signaling in osteocytes. Here, we present the crystal structure of LRP6 E1E2-SOST complex, identifying the novel second binding site of SOST on the LRP6 E2 domain. This novel interaction mediated by the C-terminal tail of SOST was confirmed by in vitro binding assays and in vivo study using Xenopus embryos. Functional significance of this C-tail interaction is further demonstrated by the inhibition of Wnt2 and Wnt9B signaling, which is achieved only with full-length SOST, but not C-tail truncation mutant. Our results provide insight into the effective and selective inhibitory mechanism of SOST on Wnt signaling activated by various Wnt subtypes.

Acknowledgement

This work was supported by the National Research Foundation of Korea NRF-2016R1A2B4013488 and NRF-2019M3E5D6063903 (to H.-J.C.) funded by the Korean government. We thank beamline 11C staff at Pohang Accelerator Laboratory (PAL, Korea) for help with X-ray diffraction data collection.

[STR-11]

Structural insights of arginine glycosylation in pathogenic effector proteins

Jun Bae Park[1]*, Youngki Yoo[1], Myung Kyung Choi[1], Ui Jin Kim[1] and Hyun-Soo Cho[1]

1 Department of Systems Biology, College of Life Science and Biotechnology, Yonsei University, Seoul 03722, Korea

**E-mail: hscho8@gmail.com*

Glycosylation is one of the protein modifications and plays a key role in protein stability, function, signaling regulation and even cancer. NleB and SseK are bacterial effector proteins and possess glycosyltransferase activity even though they have different substrate preference. NleB/SseKs transfer the GlcNAc sugar to an arginine residue of host proteins, leading to reduced NF-κB-dependent responses. By combining X-ray crystallography, NMR, molecular dynamics, enzyme kinetics assay and in vivo experiments, we demonstrated that a conserved HEN (His-Glu-Asn) motif in the active site plays a key role in enzyme catalysis and virulence. The lid-domain regulates the opening and closing of the active site and the HLH domain determines the substrate specificity. Our findings provide evidence for the enzymatic mechanism by which arginine can be glycosylated by SseK/NleB enzymes.

References

[1] Jun Bae Park, Young Hun Kim, Youngki Yoo, Juyeon Kim, Sung-Hoon Jun, Jin Won Cho, Samir El Qaidi, Samuel Walpole, Serena Monaco, Ana A. García-García, Miaomiao Wu, Michael P. Hays, Ramon Hurtado-Guerrero, Jesus Angulo, Philip R. Hardwidge, Jeon-Soo Shin and Hyun-Soo Cho "Structural basis for arginine glycosylation of host substrates by bacterial effector proteins" Nature Communications **(2018)**

[2] Jun Bae Park, Youngki Yoo and Hyun-Soo Cho "Structural insights showing how arginine is able to be glycosylated by pathogenic effector proteins" BMB Reports **(2018)**

[STR-12]

Structural basis of human ORP1-Rab7 interaction for the late-endosome and lysosome targeting

Lingchen Tan, Young Jun Im*

College of Pharmacy, Chonnam National University, Gwangju, Republic of Korea

**E-mail: imyoungjun@jnu.ac.kr*

Oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) constitute a family of lipid transfer proteins conserved in eukaryotes. ORP1 transports cholesterol at the interface between the late endosomes/lysosomes (LELs) and the endoplasmic reticulum (ER). ORP1 is targeted to the endosomal membranes by forming a tripartite complex with the LE GTPase Rab7 and its effector RILP (Rab7-interacting lysosomal protein). Here, we determined the crystal structure of human ORP1 ANK domain in complex with the GTP-bound form of Rab7. ORP1 ANK binds to the helix α3 of Rab7 located away from the switching regions, which makes the interaction independent of the nucleotide-binding state of Rab7. Thus, the effector-interacting switch regions of Rab7 are accessible for RILP binding, allowing formation of the ORP1-Rab7-RILP complex. ORP1 ANK binds to Rab7 and the Rab7-RILP complex with similar micro-molar affinities, which is consistent with the independence binding of ORP1 and RILP to Rab7. The structural model of the ORP1-Rab7-RILP complex correlates with the recruitment of ORP1 at the LEL-ER interface and the role in lipid transport and regulation.

[STR-13]

Expression, Purification, and Structural Studies on a Microglial Membrane Protein, TREM2

Authors: **Yeon-Woo Park[1], Hyun Jung Kim[2], Hee Soon Choi[1], and Hyun-Ho Lim[1]**

*[1] Neurovascular unit research group, [2] Neurodegenerative disease research group, Korea Brain Research Institute (**KBRI**), 61 Chemdan-ro, Dong-gu, Daegu, 41062, Republic of Korea.*

E-mail: parkyeonwoo@kbri.re.kr

Triggering receptor expressed on myeloid cells 2 (TREM2) is an innate immune receptor for which expression is restricted to the surface of microglia in the brain. TREM2 consists of an extracellular domain (ECD) with one V-type Ig-like domain, transmembrane (TM) domain, and a cytoplasmic domain. The extracellular membrane of TREM2 recognizes amyloid, apoptotic cells, and lipid molecules, activates the signaling process through the transmembrane adapter protein TYROBP/DAP12 to help phagocytosis pathogens and cellular debris. Genetic mutations in humans are known to cause the abnormal TREM2 function increases the overall inflammatory response and accelerate the accumulation of amyloid-beta (Aβ) and p-tau. So, these mutations increase the risk of neurodegenerative diseases such as Alzheimer's disease, frontotemporal dementia, Parkinson's disease, and amyotrophic lateral sclerosis.

However, the intrinsic ligands of TREM2 and the mechanism of Aβ phagocytosis are poorly understood. In this study, we have been trying to solve the molecular structures of full-length wild-type (WT) and R47H mutant human TREM2 (hTREM2) with antibody fragments. We over-expressed and purified hTREM, R47H mutant and soluble TREM2 (sTREM2) from baculovirus infected insect cells and generated monoclonal antibodies used hTREM2 protein fragments. Also, we are trying to identify intrinsic ligands of hTREM2 from WT or AD-patient's CSF samples. Through this study, we will provide critical information for understanding the physiological and pathological role of TREM2.

References

[1] Bouchon, A. *et al., J. Immunol.* **(2000)**.
[2] Takegahara, N. *et al., Nat. Cell Biol.* **(2006)**.
[1] Daniel L. Kober *et al.*, *Journal of Molecular Biology* **(2017)**.
[2] Yeh *et al.*, *Trends Mol. Med.* **(2017)**.
[3] Ying jun Zhao *et al.*, *Neuron* **(2018)**.
[4] Tyler K. Ulland *et al.*, *Nature Reviews: Neurology* **(2018)**.
[5] Cody M. Wolfe *et al.*, *Int.J.Mol.Sci.* **(2019)**.

Acknowledgement

This research was supported by KBRI basic program through Korea Brain Research Institute funded by Ministry of Science and ICT (**19-BR-01-02**, Neurovascular Unit group) and National Dementia Research and Development Project funded by Ministry of Health and Welfare (**H18C1254**)

[STR-14]

Expression and purification of recombinant Ovastacin from mouse

Dae-hyun Kim[1] and Beom Seok Park[1,2]

[1] Department of Senior Healthcare, BK21 Plus Program, Graduate School, Eulji University, Daejeon, 34824, Korea
[2] Department of Biomedical Laboratory Science, Eulji University, Seongnam-si, Gyeonggi-do, 13135, Korea
**E-mail: bspark74@eulji.ac.kr*

The zona pellucida (ZP), which surrounds the mammalian oocyte, functions in various aspects of fertilization. The ZP consists of three or four glycoproteins, specially, mouse ZP is composed of three glycoproteins (ZP1, ZP2, and ZP3), of which ZP2 is proteolytically cleaved after gamete fusion to prevent polyspermy. Based on the cleavage site of ZP2, ovastacin was selected as a candidate protease. Ovastacin is a pioneer component of mouse cortical granules and plays a definitive role in the postfertilization block to sperm binding that ensures monospermic fertilization and successful development.

Thus, this study will be analyzed the biochemical characterization of Ovastacin, a key metallo-endoprotease in the production of cleaved ZP2 in native zonae pellucidae, and be determined protein structure. The gene cloning and protein expression of Ovastacin was performed and was purified protein by Nickel affinity chromatography, ion exchange chromatography, size exclusion chromatography, and was performed screening of protein crystallization of Ovastacin.

References

[1] Naoto Yonezawa, Posttranslational Modifications of Zona Pellucida Proteins, Advances in Experimental Medicine and Biology (2014)
[2] Anna D. Burkart, Bo Xiong, Boris Baibakov, Maria Jiménez-Movilla, and Jurrien Dean, Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy, The journal of cell biology (2011)

Acknowledgement

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2015R1C1A1A01054639 to Beom Seok Park)

[STR-15]

Analysis of Conformational Dynamics of Chimeric Arrestin Mutants by Hydrogen-Deuterium Exchange Mass Spectrometry

Kiae Kim[1], Arun K. Shukla[2], Ka Young Chung[1,*]

[1]*School of Pharmacy, Sungkyunkwan University, 2066 Seobu-ro, Jangan-gu, Suwon-si, Gyeonggi-do, Republic of Korea, 16419*
[2]*Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, India, 208016*

**E-mail: kimkiae95@skku.edu, kychung2@skku.edu*

G protein-coupled receptor (GPCR) is a large family of membrane proteins in the human genome and one of the targets of drugs. Upon activation of GPCRs by agonists, arrestins interact with the intracellular loop 3 (ICL3) or the C-tail of GPCRs phosphorylated by G protein-coupled receptor kinases (GRK). Arrestins have various roles which consist of desensitization and internalization of GPCRs as well as scaffolding downstream signaling proteins. Arrestins are divided into four subtypes: arrestin-1, arrestin-2 (β-arrestin-1), arrestin-3 (β-arrestin-2), arrestin-4. Surprisingly, these subtypes share a great structural similarity, but the mechanism of their differential functions between subtypes is still unanswered. Thus, we constructed the chimeric mutants, swap2 and swap5 by swapping β-arrestin-1 and β-arrestin-2 to elucidate the correlation between structure and intrinsic conformational dynamics by hydrogen deuterium exchange mass spectrometry (HDX-MS).

References

[1] Eshan Ghosh, et al. Conformational sensors and domain swapping reveal structural and functional differences between β-arrestin isoforms. *Cell reports* (**2019**).
[2] Wanchao Yin, et al. A complex structure of arrestin-2 bound to a G protein-coupled receptor. *Cell research* (**2019**).
[3] Yanyong Kang, et al. Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser. *Nature* (**2015**).

Acknowledgement

[1] This research was supported by the BK21Plus Program.

[STR-16]

Conformational factors that regulate GDP-binding in Gi3 alpha subunit

Donghoon Ahn[†], Dong Hee Ham[†], and Ka Young Chung*
*[†]Equally contributed, *Corresponding author*

School of Pharmacy, Sungkyunkwan University, 2066 Seobu-ro, Jangan-gu, Suwon-si, Gyeonggi-do, Republic of Korea, 16419
**E-mail: jnsd5102@gmail.com, geg94@naver.com, kychung2@skku.edu*

G Protein alpha subunit plays an important role in intracellular signaling pathway. Especially, Ras-like domain of the alpha subunit has a function of nucleotide exchange. We opt for some conserved residues on Ras-like domain and make mutations on these sites. The selected sites are in α5 Helix, β6 strand, αG Helix, α1 Helix, and ploop known as significant regions for GDP/GTP binding. We expect that the nucleotide binding site will become unstable, as a result of the mutation to delete the function of critical residues. The unstable sites would make GDP swap quickly.

The Site directed mutagenesis switching to alanine is performed to eliminate the functional side chains. We execute hydrogen/deuterium exchange mass spectrometry (HDX-MS) to see the change of structural dynamics, and BODIPY Fluorescence microplate Assay to observe functional difference as time passed. The research support to figure out structural dynamic and nucleotide exchange by the region associated with nucleotide binding.

References

[1] Mark A. Wall et al. The structure of the G protein heterotrimer Giα1β1γ2 *Cell* **(1995).**
[2] Ali I. Kaya et al. A Conserved Phenylalanine as a Relay between the α5 Helix and the GDP Binding Region of Heterotrimeric G_i Protein α Subunit. *The Journal of Biological Chemistry* **(2014).**
[3] Ali I. Kaya et al. A Conserved Hydrophobic Core in Gαi1 Regulates G Protein Activation and Release from Activated Receptor. *The Journal of Biological Chemistry* **(2016).**

Acknowledgement

[1] This research was supported by the BK21Plus Program, the Republic of Korea.

[STR-17]

Scaffolding Mechanism of β-arrestin 1 for ERK Signaling Cascade

Min Woo Yun, Ji Young Park, Ka Young Chung*

School of Pharmacy, Sungkyunkwan University, 2066 Seobu-ro, Jangan-gu, Suwon, 16419, South Korea

**E-mail: 93ymw@hanmail.net, kychung2@skku.edu*

In G protein-coupled receptors (GPCR) signaling, arrestins play an important role as regulator, and mediate G protein-independent signaling. Among the arrestins, Arrestin 2 and 3 (termed the β-arrestin 1 and 2), which is a nonvisual arrestin, are expressed ubiquitously, unlike Arrestin 1 and 4. Arrestin-mediated GPCR signaling occurs mainly by scaffolding mitogen-activated protein kinases (MAPKs). Currently, there are many studies on arrestins and MAPKs, but these scaffolding mechanism is not yet known. To find out the scaffolding mechanism between β-arrestin 1 with C-Raf and MEK1, I analyzed the interaction site by used the β-arrestin 1 R169E mutant, which is a pre-activation. The main experimental method used in this experiment is hydrogen/deuterium exchange mass spectrometry (HDX-MS), and tryptophan-induced fluorescence quenching. In conclusion, C-Raf appears to interact in both the basal and pre-activated states of β-arrestin, and MEK1 appears to interact in only the pre-activated state of β-arrestin. Furthermore, MEK1 appears to contact β-arrestin only in the presence of ATP. These results could lead to further studies that selectively regulate arrestin-mediated GPCR signaling, it can be used as drug target by dealing with intracellular phenomena.

[STR-18]

The Structural Dynamics and the Nucleotide Binding Studies of the Gαs with Natural Variants within the α-Helical Domain

Ye Jin Jeong and Ka Young Chung*

School of Pharmacy, Sungkyunkwan University, 2066 Seobu-ro, Jangan-gu, Suwon, 16419, South Korea

**E-mail: yenij@skku.edu, kychung2@skku.edu*

Heterotrimeric G proteins (consisting of α, β and γ subtunits) are the major downstream signaling molecule of GPCR. Gα subunit consists of a Ras-like domain and a helical domain. The Ras-like domain is involved in GDP/GTP binding and GTPase activity and is an important site for binding to Gβγ dimers, GPCRs, and effector proteins. Therefore, there have been extensive studies to understand the structural and functional mechanisms the Ras-like domain while the helical domain has been neglected. However, recent studies suggest that the helical domain undergoes extensive movement upon GDP release from G protein. Mutations in the helical domain of the Gα subunit have also been observed in naturally occurring diseases. Here, we hypothesized that the helical domain also has a crucial role on G protein signaling. We expressed and purified five naturally-occurring mutants within the helical domain of the Gα subunit. To understand the structural dynamics caused by these mutants, we analyzed the structures using a hydrogen/deuterium exchange mass spectrometry (HDX-MS). We monitored impact on the binding and dissociation of GDP or GTP in these mutants using by GTP labeled with environment sensitive dye of BODIPY. This study will help to understand the role of the helical domain of the Gα subunit.

References

[1] Viktoriya Syrovatkina, Kamela O. Alegre, Raja Dey, and Xin-Yun Huang, Regulation, Signaling, and Physiological Functions of G-Proteins, *Journal of Molecular Biology*, Volume 428, Issue 19 **(2016)**.
[2] H. G. Dohlman, and J. C. Jones, Signal activation and inactivation by the Gα helical domain: A long-neglected partner in G protein signaling. *Sci. Signal.* 5, re2 **(2012)**.
[3] Dyke P. McEwen, Kyle R. Gee, Hee C. Kang, and Richard R. Neubig, Fluorescent BODIPY-GTP Analogs: Real-Time Measurement of Nucleotide Binding to G Proteins, *Analytical Biochemistry*, Volume 291, Issue 1, **(2001)**.

Acknowledgement

[1] This research was supported by the BK21Plus Program.

[STR-19]

Backbone assignments of the Forkhead box protein O4 (FOXO4) transactivation domain (TAD) and its interaction with forkhead domain (FHD)

Jinwoo Kim, Dabin Ahn and Chin-Ju Park*

Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Korea

**E-mail: cjpark@gist.ac.kr*

Forkhead box protein O4 (FOXO4) is a transcription factor and member of the human forkhead box class O (FOXO) subfamily that regulates cell apoptosis. It is one of the intrinsic disordered proteins (IDPs) like many transcription factors and has no secondary or tertiary structures except for the forkhead domain (FHD). Here, we provide structural information of transactivation domain (TAD), intrinsic disordered region (IDR) of FOXO4, through backbone assignments using triple resonance experiments by NMR spectroscopy. Also, we identified intramolecular interaction between FHD and TAD at atomic level through ^{1}H-^{15}N HSQC experiments and isothermal titration calorimetry (ITC) analysis. Our work contributes to understand the structural information of FOXO4 and to elucidate the cell senescence pathway associated with it.

[STR-20]

S92 phosphorylation induces structural changes in the N-terminus domain of human mitochondrial calcium uniporter

Jihyeong Yang[1,2], Youngjin Lee[1,2,4†*], Jongseo Park[1,2†], Choon Kee Min[1,3], Jung Youn Kang[1,2], Tae Gyun Kim[1,2,§], Takenori Yamamoto[5,6], Do Han Kim[1,3], and Soo Hyun Eom[1,2*]

[1]School of Life Sciences, [2]Steitz Center for Structural Biology, [3]Systems Biology Research Center, Gwangju Institute of Science and Technology (GIST), Buk-gu, Gwangju 61005, Republic of Korea. [4]Infection and Immunity Research Laboratory, Metabolic Regulation Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, Republic of Korea. [5]Institute for Genome Research, Tokushima University, Kuramotocho-3, Tokushima 770-8503, Japan. [6]Faculty of Pharmaceutical Sciences, Tokushima University, Shomachi-1, Tokushima 770-8505, Japan

E-mail: wlgud0208@gist.ac.kr

The mitochondrial calcium uniporter (MCU) plays essential roles in mitochondrial calcium homeostasis and regulates cellular functions, such as energy synthesis, cell growth, and development. Thus, MCU activity is tightly controlled by its regulators as well as post-translational modification, including phosphorylation by protein kinases such as proline-rich tyrosine kinase 2 (Pyk2) and AMP-activated protein kinase (AMPK). In our *in vitro* kinase assay, the MCU N-terminal domain (NTD) was phosphorylated by protein kinase C isoforms ($PKC_{\beta II}$, PKC_{δ}, and PKC_{ε}) localized in the mitochondrial matrix. In addition, the conserved S92 was phosphorylated by the PKC isoforms. To reveal the structural effect of MCU S92 phosphorylation (S92p), we determined crystal structures of the MCU NTD of S92E, an S92p mimic, and D119A mutants at a resolution of 2.50 Å and 2.85 Å, respectively. We observed conformational changes of the conserved loop2-loop4 (L2-L4 loops) in MCU NTD_{S92E} and NTD_{D119A} due to the breakage of the S92-D119 hydrogen bond. Although the S92E mutation does not affect uniporter formation and mitochondrial calcium uniporter regulator 1 (MCUR1) interaction, we suggest that the phosphorylation of S92 might induce conformational changes at the L2-L4 loops, and disrupt dimerization of two MCU-EMRE tetramers due to changes in electrostatic charge.

Acknowledgement

This work was supported by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea(NRF-2017R1A2B2001878).

[STR-21]

Structural and functional studies of a sensor histidine kinase from *Staphylococcus aureus* and its implications in ligand recognition

Truc Kim*, Changsuk Oh, and Kyeong Kyu Kim

Department of Molecular Cell Biology, Institute for Antimicrobial Resistance and Therapeutics, Sungkyunkwan University School of Medicine, Suwon 16419, Republic of Korea

**E-mail: kdttruc83@gmail.com*

The two-component signal transduction system (TCS) is the fundamental procedure by which the bacteria adapt to the changes of the surrounding environment. Among TCSs present in *Staphylococcus aureus*, a life-threatening infectious agent, the SA1441/SA1442 TCS is considered as a global regulator of virulence factors. In this system, SA1441 plays the role of a sensor histidine kinase (HK), while SA1442 functions as a response regulator. Even though SA1441/SA1442 TCS has been long known to respond to the low-oxygen condition, the underlying molecular mechanism, especially the ligand, of this system is still the biggest question to be addressed. In the current study, we have successfully solved the crystal structure of the extracellular domain of SA1441 at a 1.73-Å resolution using the multi-wavelength anomalous diffraction method. The atomic model revealed a common fold of PDC (PhoQ-DcuS-CitA) domain, suggesting that this extracellular domain might function as a sensory module. Structural comparison, subsequent biochemical, and mutational analyses showed that a compound named B06AB01 interacted with SA1441, triggered SA1441 dimerization, and induced the phosphatase activity of SA1441. The availability of the SA1441 crystal structure along with its putative ligand information will not only shed light on its signal transduction mechanism but also provide a template for the development of novel antibacterial drugs against *S. aureus* infections.

Acknowledgment

This work was supported by a grant from the National Research Foundation of Korea to Truc Kim (2017R1D1A1B03031067).

[STR-22]

Structural analysis of Ca^{2+}-binding proteins from *Schistosoma mansoni*

Ji-Ho Lee[1*], Bo Keun Kim[1*], Junga Park[1*], Kwang Yeon Hwang[1]†, and Sulhee Kim[1]†

[1] Department of Biosystems & Biotechnology, College of Life Sciences & Biotechnology, Korea University, Seoul 136-701, Republic of Korea

**E-mail: chahong@korea.ac.kr, sulhee@korea.ac.kr*

Schistosomiasis (or bilharzia) is the most common cause of death from a parasitic disease after malaria [1,2]. The disease can be treated effectively with the drug praziquantel (PZQ) [3]. The tegumental allergen-like (TAL) proteins from *Schistosoma mansoni* (SmTAL) are part of a family of calcium binding proteins found only in parasitic flatworms. These proteins have attracted interest as potential drug or vaccine targets, yet comparatively little is known about their biochemistry. For elucidating the exact mechanism of drug-interaction with tegument associated proteins from parasites (TALs), here, we had grown the suitable crystals of TALs from *Schistosoma mansoni*. The crystals were diffracted at 2.1 Å and 2.5 Å at synchrotron radiation, respectively. The spacegroup of smTAL1 and smTAL2 are hexagonal system ($P6_4$ and $P6_422$), respectively. They consist of two domains, calmodulin-like (EF hand) and dynein-like (DLC domain). We will discuss the difference between smTAL1 and smTAL2.

References

[1] P. Steinmann, J. Keiser, R. Bos, M. Tanner, J. Utzinger, Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk, Lancet Infect. Dis. 6 (2006)

[2] J. Utzinger, S.L. Becker, S. Knopp, J. Blum, A.L. Neumayr, J. Keiser, C.F. Hatz, Neglected tropical diseases: diagnosis, clinical management, treatment and control, Swiss Med. Wkly. 142 (2012)

[3] D. Cioli, L. Pica-Mattoccia, A. Basso, A. Guidi, Schistosomiasis control: praziquantel forever? Mol. Biochem. Parasitol. 195 (2014)

[STR-23]

Structural analysis of Butyryl-CoA:Acetate CoA-transferase from *Faecalibacterium prausnitzii* A2-165

Bo Keun Kim[1], Jung-A Park[1], Ji-Ho Lee[1], Sulhee Kim[1] and Kwang Yeon Hwang[1*]

[1] *Department of Biosystems & Biotechnology, College of Life Science and Biotechnology, Korea university, Anam-dong-5, Seongbuk-gu, Seoul, 136-701, Republic of Korea*

[*] *E-mail: chahong@korea.ac.kr*

Atopic dermatitis, an inflammatory skin disease, is driven by T-helper cell type 2 inflammation. A balance between a specific gut microbe subspecies can affect AD and butyrate, a metabolic product of their gut microbe, is one of the most important substances. [1] Butyryl CoA:acetate CoA-transferase, a key enzyme in a butyrate produce, could be an interesting target for AD study. Basically, butyryl CoA:acetate CoA-transferase belongs to the family Ⅰ CoA transferases, which conserved a catalytic glutamate residue with CoA thioester intermediate. [2] In this study, we solved the crystal structures of butyryl CoA:acetate CoA-transferase (B:ACoT) and complexed with Coenzyme A (B:ACoT-CoA). Crystals diffracted at 1.5 Å (B:ACoT) and 1.7 Å (B:ACoT-CoA), respectively. The crystals belong to C2 space group, with the unit cell parameters of a = 102 Å, b = 118 Å, c = 95 Å and $\alpha = \gamma = 90^{\circ}$, $\beta = 117^{\circ}$. Vm of the crystal is 2.54 $Å^3$/Da and solvent content is about 51%. Also, we found that Glu245 is important to bind to CoA and consists of active site. The binding site of B:ACoT-CoA is clearly identified and biochemical analysis is still ongoing.

References

[1] Song H, Kim H S. *Faecalibacterium prausnitzii* subspecies-level dysbiosis in the human gut microbiome underlying atopic dermatitis. *Journal of Allergy and Clinical immunology* **(2016)**.

[2] Vanderwinkel E, Furmanski P. Growth of *Escherichia coli* on fatty acids: Requirement for coenzyme A transferase activity. *Biochemical and biophysical research communications* **(1968)**

[STR-24]

Conformational dynamics and functional implications of phosphorylated β-arrestins

Hyunook Kang[1,3*], Han-Sol Yang[2,3], Ah Young Ki[2], Seung-Bum Ko[1], Kwon Woo Kim[2], Chang Yong Shim[2], Kiae Kim[2], Ka Young Chung[2], Hee-Jung Choi[1],

[1]*Department of Biological Sciences, Seoul National University, Seoul, 08826, Republic of Korea*
[2]*School of Pharmacy, Sungkyunkwan University, Suwon, 16419, Republic of Korea*
[3]*These authors contributed equally.*
**E-mail: hyunook2006@snu.ac.kr*

Arrestins were first discovered as proteins that desensitize and/or internalize G protein-coupled receptors by interacting with the phosphorylated C-terminal tail of the receptor. Since then, a few studies have suggested that arrestins themselves can be phosphorylated, and proposed that the phosphorylation status of arrestins modulates their cellular functions. However, the effects of phosphorylation on arrestin structure have not been studied. Here, we investigated the conformational changes of β-arrestin-1 and -2 upon incorporation of phospho-mimetic mutations into known phosphorylation sites (i.e. S412D for β-arrestin-1 and S14D, T276D, S14D/T276D, S361D, T383D, and S361D/T383D for β-arrestin-2) by using hydrogen/deuterium exchange mass spectrometry (HDX-MS). HDX-MS analysis suggested that β-arrestin-2 T276D and S14D/T276D may have a similar conformation to the pre-active states, which was confirmed by biochemical and cellular studies. The S13D/T275D β-arrestin-1 mutant was shown to be pre-activated, and structural details were investigated by X-ray crystallography.

Reference

Kang H. *et al.*, Conformational dynamics and functional implications of phosphorylated β-arrestins. *Structure, accepted*.

Acknowledgement

This work was supported by grants from the National Research Foundation of Korea funded by the Korean government (NRF-2018R1A2B6001554 and NRF-2012R1A5A2A28671860 to K.Y.C. and NRF-2016R1A2B4013488 to H.-J. C.). We thank beamline 5C staff at Pohang Accelerator Laboratory (PAL) for help with X-ray diffraction data collection.

[STR-25]

Structure and function of HOCl-defensing flavoprotein RclA from *Escherichia coli*

Yeongjin Baek[1*], Jinsook Ahn[1], Inseong Jo[1], Seokho Hong[1], and Nam-Chul Ha[1]

[1]Department of Agricultural Biotechnology, Center for Food Safety and Toxicology, Center for Food and Bioconvergence, and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea

E-mail: yj.baek@snu.ac.kr

The immune system generates hypochlorous acid (HOCl) to kill microorganisms via oxidative burst, and the toxicity of HOCl is amplified in the phagosome by importing Cu^{2+}. In *Escherichia coli* and *Salmonella*, the transcriptional regulator RclR recognizes HOCl stress and induces the RclA, B, and C proteins to defend against stress. However, the structure and biochemical roles of Rcl proteins remain to be elucidated. In this study, we first examined the role of the flavoprotein disulfide reductase (FDR) RclA in the survival of *Salmonella* in macrophage phagosomes. To understand the molecular mechanism of RclA, we determined the crystal structure of RclA from *E. coli*. The homodimeric RclA was similar to typical FDRs, exhibiting two conserved cysteine residues near the flavin ring of the cofactor flavin adenine dinucleotide (FAD). We observed that Cu^{2+} accelerated the oxidation of NADH by RclA, leading to a lowering of the oxygen level in the solution. Mutation of the conserved cysteine residues lowered the specificity to Cu^{2+} or substantially increased the production of superoxide anion in the absence of Cu^{2+} compared to the RclA wild type. The lowered oxygen level by RclA would contribute to the inhibition of oxidative bursts in phagosomes. Our study provides the molecular basis for how bacteria can survive HOCl stress in our immune systems.

References

[1] Argyrou, Argyrides, and John S. Blanchard. "Flavoprotein disulfide reductases: advances in chemistry and function." *Progress in nucleic acid research and molecular biology* 78 **(2004)**: 89-142.

[2] Parker, Benjamin W., et al. "The RclR protein is a reactive chlorine-specific transcription factor in Escherichia coli." *Journal of Biological Chemistry* 288.45 **(2013)**: 32574-32584.

[STR-26]

Cleavage-dependent activation of ATP-dependent protease HslUV from Staphylococcus aureus

Soyeon Jeong[1,*], and Nam-Chul Ha[1]

[1] Research Institute for Agriculture and Life Sciences, Center for Food and Bioconvergence, Center for Food Safety and Toxicology, Seoul National University, Seoul 08826, Republic of Korea

E-mail: syjeong0711@snu.ac.kr

HslUV is a bacterial heat shock protein complex consisting of the AAA+ ATPase component HslU and the protease component HslV. HslV is a threonine protease employing the N-terminal Thr residue in the mature protein as the essential catalytic residue. To date, HslUV from Gram-negative bacteria has been extensively studied. However, it remains to be elucidated in the action and activation mechanism of HslUV from Gram-positive bacteria, which has the N-terminal additional sequence before the catalytic Thr residue. In this study, we determined the crystal structures of HslV and HslU from the Gram-positive bacteria Staphylococcus aureus, with and without HslU in the crystallization conditions. The structural comparison showed an asymmetric to symmetric transition by HslU in the hexameric forms. More importantly, the N-terminal additional sequence was cleaved in the presence of HslU and ATP, exposing the Thr9 residue at the N-terminus and activating the protease activity. Following the biochemical study further demonstrated that HslV uses the N-terminal Thr residue for the catalysis by interaction with the symmetric HslU hexamer in the presence of ATP. Since eukaryotic proteasome has a similar N-terminal additional sequence, our results will help understand the common molecular mechanism for the activation of proteasomes.

References

[1] M. Rohrwild, O. Coux, H.–C. Huang, R.P. Moerschell, S.J. Yoo, J.H. Seol, C.H. Chung, and A.L. Goldberg. HslV-HslU: A novel ATP-dependent protease complex in Escherichia coli related to the eukaryotic proteasome. *Proc. Natl. Acad. Sci. USA* **(1996)**.

[2] Kang, M. S., Lim, B. K., Seong, I. S., Seol, J. H., Tanahashi, N., Tanaka, K., & Chung, C. H. The ATP-dependent CodWX (HslVU) protease in Bacillus subtilis is an N-terminal serine protease. *The EMBO Journal* **(2001)**.

[STR-27]

Cryo-electron microscopy for structural analysis of protein complex

Hyunbum Jeon[1,2], Ji Young Mun[1]

[1]Neural Circuits Group, Korea Brain Research Institute, Daegu, 41068, Republic of Korea.
[2]Department of Brain and Cognitive Sciences, Daegu Gyeongbuk Institute of Science and Technology, Daegu 42988, Republic of Korea

E-mail: hbjeon@kbri.re.kr

The nucleosomal subunit organization of chromatin provides a multitude of functions. Nucleosomes elicit an initial ~7-fold linear compaction of genomic DNA. Nucleosomes protect the genome from DNA damaging agents and provide a lattice onto which a myriad of epigenetic signals are deposited. Moreover, vast strings of nucleosomes provide a framework for assembly of the chromatin fiber and higher-order chromatin structures. Post translational modifications of histone tails have been demonstrated to influence numerous biological developments, as well as disease onset and progression. Histone post-translational modifications, one of the key events that regulate gene activation, seem to play a prominent role in the epigenetic mechanism of neurodegenerative diseases.

Single particle analysis using by cryo-electron microscopy is a powerful technique for structure determination of isolated protein complexes at near atomic resolution. Early electron microscopy and X-ray crystallographic analysis of nucleosomes revealed that DNA is wrapped around the core histones in left-handed superhelical turns, forming a disk-shaped particle. Here, we have used the recombination protein-DNA complex of the nucleosome and single particle analysis to determine the structure of a core histones and double strand DNA. Total of 712 micrographs were collected on Titan Krios Cryo-EM using Falcon III detector. Frames were aligned and bad frames were removed. 177,210 Particles were auto-picked using the RELION 3.0. The particles were sorted by 2D and 3D classification, from which the 84,284 particles were selected for high-resolution. The refined nucleosome structure from 23,345 particles has a final resolution of 3.9Å and the density map exhibits structural features consistent with the estimated resolution.

References

[1] EY Chau et al., 3.9Å structure of the nucleosome core particle determined by phase-plate cryo-EM. *Nucleic Acids Res* (2016)

Acknowledgement

[1] Cryo-EM imaging was carried out in electron microscopy research center, Korea Basic Science Institute.

[2] This research was supported by KBRI basic research program through Korea Brain Research Institute funded by Ministry of Science and ICT (19-BR-01-08, Neural Circuits Group).

[STR-28]

Structural analysis of the manganese transport regulator MntR from *Bacillus halodurans*

Myeong Yeon Lee, Dong Won Lee, Hyun Kyu Joo, Kang Hwa Jeong, and Jae Young Lee*

Department of Life Science, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do, 10326, Republic of Korea

E-mail: auddus456@dongguk.edu

The manganese transport regulator (MntR) is a metal-ion activated transcriptional repressor of manganese transporter genes to maintain manganese ion homeostasis. MntR, a member of the diphtheria toxin repressor (DtxR) family of metalloregulators, selectively responds to Mn^{2+} and Cd^{2+} over Fe^{2+}, Co^{2+} and Zn^{2+}. The DtxR/MntR family members are well conserved transcriptional repressors that regulate the expression of metal ion uptake genes by sensing the metal ion concentration. MntR functions as a homo-dimer with one metal ion binding site per subunit. Each MntR subunit contains two domains: an N-terminal DNA binding domain, and a C-terminal dimerization domain. However, it lacks the C-terminal SH3-like domain of DtxR/IdeR. The metal ion binding site of MntR is located at the interface of the two domains, whereas the DtxR/IdeR subunit contains two metal ion binding sites, the primary and ancillary sites, separated by 9 Å. In this paper, we reported the crystal structures of the apo and Mn^{2+}-bound forms of MntR from *Bacillus halodurans*, and analyze the structural basis of the metal ion binding site. The crystal structure of the Mn^{2+}-bound form is almost identical to the apo form of MntR. In the Mn^{2+}-bound structure, one subunit contains a binuclear cluster of manganese ions, the A and C sites, but the other subunit forms a mononuclear complex. Structural data about MntR from *B. halodurans* supports the previous hypothesizes about manganese-specific activation mechanism of MntR homologues.

References

[1] McGuire AM, Cuthbert BJ, Ma Z, Grauer-Gray KD, Brunjes Brophy M, Spear KA, et al. Roles of the A and C sites in the manganese-specific activation of MntR. Biochemistry **(2013)**.

[2] Helmann JD. Specificity of metal sensing: Iron and manganese homeostasis in bacillus subtilis. Journal of Biological Chemistry **(2014)**.

[STR-29]

Integrative structural modeling of FOXL2/XRCC6 complex regulating DSB repair in NHEJ system

Yuri Choi and Hyung Ho Lee*

Department of Chemistry, College of Natural Sciences, Seoul National University

**E-mail: hyungholee@snu.ac.kr*

Non-homologous end joining (NHEJ) is one of two major pathways for DNA double-strand-break (DSB) repair, where a ligase is involved to connect the cleaved ends, and does not require template strands. In NHEJ system, Ku70 (XRCC6) and Ku80 (XRCC5) form the Ku complex for initiation of the NHEJ repair system and binds to the broken DNA. However, the upstream signaling mechanism of the Ku complex was unknown. When DSB stress is present, FOXL2 is no longer bound to the Ku proteins and XRCC5 and XRCC6 forms a heterodimer to initiate the repair system. Forkhead (FH) domain of FOXL2 was shown to be the key binding region, and acetylation of K124 residue plays a critical role in its binding to XRCC5 and XRCC6. In this study, we focused on the architecture of the XRCC6-FOXL2 complex. Through bio-layer interferometry assay, we found that XRCC6 showed better binding affinity with acetylation-mimic form of FOXL2 FH domain. SEC-MALS results suggested that both FOXL2 and XRCC6 exist in monomers in solution, and the FOXL2-XRCC6 complex exists as a 1:1 heterodimer. We also used XL-MS to find out the binding interface between them, and performed protein-protein docking study using HADDOCK. Based on these results, we suggest an integrative structural model of the FOXL2-XRCC6 complex.

References

[1] Thacker, J., Zdzienicka, M.Z., The mammalian XRCC genes: their roles in DNA repair and genetic stability, *DNA Repair* **(2003)**

[STR-30]

Structural characterization of a membrane tethering protein Uso1

Yoonyoung Heo, and Hyung Ho Lee*

Department of Chemistry, College of Natural Sciences, Seoul National University

**E-mail: hyungholee@snu.ac.kr*

Membrane tethering is important for targeting transport vesicles to the correct membrane compartment. In intracellular trafficking from ER to Golgi, it is mediated by a diverse array of tethering proteins including Ypt1/Rab family GTPases and coiled-coil proteins that can link intracellular membranes together. One of the well-known tethering proteins from ER to Golgi is Uso1 (yeast homolog of p115). Previous molecular and biochemical studies suggest that Uso1 interacts with GTPase Ypt1, which could promote docking of vesicles to the correct target membrane, but physically blocks docking to the wrong target. To get structural insights into the role of Uso1, we determined several crystal structures of Uso1. The Uso1 consists of right-handed α-solenoid composed of helices and loops. By comparing crystal structures of Uso1 and p115, we observed different domain orientation of Uso1 compared to that of p115. Moreover, an elongated loop and additional helix was newly identified in Uso1 structures, which were not found in p115 structure. From this observation, we suggest that these regions might be responsible for structural characteristics of Uso1 compared to other Uso1 homologues.

References

[1] Striegl, H., Roske, Y., Kümmel, D., Heinemann, U., Unusual Armadillo Fold in the Human General Vesicular Transport Factor p115. *PLoS ONE* **(2009)**.

[STR-31]

Crystal structure of a cupin-family protein YaiE and its purine/pyrimidine nucleoside phosphorylase activity monitored by NMR

Sung-Hee Lee[1], Hyung-Sik Won[2], Ji-Hun Kim[1,*]

[1]*College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 28160, Korea*
[2]*Department of Biotechnology, Konkuk University, Chungju, Chungbuk 27478, Korea*
**E-mail: nmrjhkim@chungbuk.ac.kr*

The structurally related cupin-family proteins in bacteria and plants are recognized as a group of ubiquitous proteins that share a characteristic β-barrel fold [1]. However, the cupin-family proteins are extremely diverse in their primary sequences and in molecular functions, and also include a huge number of functionally uncharacterized proteins. Among them, an *Escherichia coli* cupin protein, YaiE, has been recently suggested to be involved in a novel activity of purine/pyrimidine nucleoside phosphorylase (PPNP) [2]. In the present study, crystal structure of YaiE verified that the protein adopted a monomeric β-barrel that represents the smallest unit of cupin fold composed of 10 β-strands and a 3_{10}-helix. Subsequently, the PPNP activity of YaiE was validated in solution by one-dimensional nuclear magnetic resonance (NMR) spectroscopy monitoring the phosphorolysis reaction of adenosine. Finally, backbone NMR assignments of YaiE were conducted by three-dimensional NMR experiments using the [^{13}C/^{15}N]-enriched protein samples [3]. The assigned chemical shifts identified its secondary structure in solution consistent with that shown in crystal structure. In addition, two-dimensional NMR analysis with nucleoside titration revealed that T36, F37, and S38 residues in the β-strand 4 constitute a central binding site for both purine and pyrimidine nucleosides. Collectively, these results provide a structural identification of YaiE as the first example of a cupin-family protein having PPNP activity. We expect that our progressing investigation on the atomic structure of YaiE in complex with nucleosides and/or phosphates would eventually elucidate the structural basis of nucleoside phosphorolysis reaction that is still elusive for known PPNP enzymes.

References

[1] Sim, D.-W. *et al*., Structural identification of the lipopolysaccharide-binding capability of a cupin-family protein from *Helicobacter pylori*. *FEBS Lett.* (**2016**).
[2] Sévin, D.C. *et al*., Nontargeted *in vitro* metabolomics for high-throughput identification of novel enzymes in *Escherichia coli*. *Nat. Methods.* (**2017**).
[3] Lee, S.-H. *et al*., Backbone NMR assignments and secondary structure determination of a cupin-family protein YaiE from *Escherichia coli*. *J. Kor. Magn. Reson. Soc.* (**2017**).

[STR-32]

Structural studies of the niacin-responsive transcriptional repressor NiaR from *Bacillus halodurans*

Dong Won Lee, Myeong Yeon Lee, Young Woo Park, Kang Hwa Jeong, and Jae Young Lee*

Department of Life Science, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do, 10326, Republic of Korea

**E-mail: dygks1229@dongguk.edu*

A NiaR was first studied in *Bacillus subtilis* and defined as a niacin-responsive transcriptional repressor. When the niacin (nicotinic acid), a precursor of nicotinamide adenine dinucleotide (NAD) in salvage pathway, is present, the NiaR binds to its target DNA in the presence of niacin and represses the expression of genes involved in NAD synthesis. NAD is essential in various cellular metabolic activities as cofactors found in all organisms. The NAD^+ synthesis processes are slightly different due to variation of their genes involved in NAD^+ synthesis depending on the species of living organisms. However, they have common metabolic processes, *de novo* and salvage pathways, which are elaborately regulated by niacin-responsive repressor, NiaR, for NAD^+ synthesis. Here, we determined the crystal structures of NiaR from *Bacillus halodurans* at 2.0 Å resolution in apo form and 1.8 Å in niacin bound form. Particularly, the niacin bound NiaR structure was first determined. The NiaR has an N-terminal HTH (Helix-Turn-Helix) domain which binds to DNA and a C-terminal dimerization/substrate binding domain, consisting of 6 β-stands, 6 α-helices. We identified the substrate binding site through the niacin-bound structure of the NiaR and showed the structural differences from the apo form.

References

[1] Afzal, M., Kuipers, O. P., and Shafeeq, S. D, Niacin-mediated Gene Expression and Role of NiaR as a Transcriptional Repressor of niaX, nadC, and pnuC in *Streptococcus pneumoniae*. Front Cell Infect Microbiol **(2017)**.
[2] Johnson,M.D.L.,Echlin,H.,Dao,T.H.,andRosch,J.W. Characterization of NAD salvage pathways and their role in virulence in Streptococcus pneumoniae. Microbiology. **(2015)**.
[3] Rodionov, D. A., et al. Transcriptional regulation of NAD metabolism in bacteria: genomic reconstruction of NiaR (YrxA) regulon. Nucleic Acids Res **(2008)**.

[STR-33]

Crystal structure of carbohydrate esterase SmAcE1 from *Sinorhizobium meliloti*

Changsuk Oh[1, *], Truc Kim[1], T. Doohun Kim[2, §], Kyeong Kyu Kim[1, §]

[1] Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, 16419, Republic of Korea

[2] Department of Chemistry, College of Natural Science, Sookmyung Women's University, Seoul, 04310, Republic of Korea

**E-mail: csoh@skku.edu*

Biofuel has been developed as an alternative energy source of fossil fuel for global demand to environmental protection. After first generation using edible crops, the second generation of biofuel using biomass from plants was emerged. The process from biomass to biofuel requests pre-treatment and process such as deacetylation and monomerization using heat, pH regulation, oxidation or biocatalysts. Bacterial carbohydrate esterases are one of promising sources for biocatalysts to remove acetyl group from plant biomass for further process in industrial field. With the potency of industrial application, we identify the structure of the carbohydrate esterase, SmAcE1 from *Sinorhizobium meliloti*. The crystal structure of SmAcE1 was determined at 2.05 Å resolution, and revealed that it belonged to an α/β hydrolase fold in GDSL superfamily. It formed a hexameric structure by dimer of trimer with supporting of size exclusion chromatography analysis. Catalytic triad (Ser15, His195 and Asp192) and oxyanion hole-forming SGNH (Ser15, Gly57, Asn97 and His195) were conserved in its tertiary structure. In docking analysis using acetylated carbohydrate, the charged residues in the active site played a crucial role in recognizing the polar groups in the acetylated sugars. The models from crystal structure and docking are expected to be applied to enhancement of its selectivity and activity by further structure-based engineering.

References

[1] Oh, C. *et al*. Identification and crystallographic analysis of a new carbohydrate acetylesterase (SmAcE1) from *Sinorhizobium meliloti*. *Crystals* (**2018)**.

[STR-34]

Identification of DCLK1-Specific Inhibitor Candidates: High-Throughput Screening and Validation

Hyo Jin Lim[1,2] and Hyoun Sook Kim[1,2]

[1]Division of Precision Medicine, National Cancer Center, Goyang 10408, Korea, [2]Department of Cancer Biomedical Science, The national cancer center graduate school of cancer science and policy (GCSP), Goyang 10408, Korea

**E-mail: 91543@ncc.re.kr, hskim@ncc.re.kr*

Doublecortin-like kinase 1 (DCLK1) is a serine/threonine kinase that belongs to the family of microtubule-associated proteins, known as one of the top 15 putative driver genes for gastric cancer. Most of cancer-related mutations within the kinase domain impair its function, affecting cell growth, cell differentiation, and deregulation of tubulin polymerization. Therefore, the direct inhibition of DCLK1 has been considered as a good strategy for cancer drug discovery. This study aims to find inhibitor candidates specific for DCLK1 and validate them with several methods. Using fluorescence-based protein thermal shift assay, we primarily evaluated 2,104 compounds of clinical inhibitor library, thereby identifying twelve hits that thermally stabilized DCLK1. To validate the twelve candidates, the kinase inhibitory activity of them was measured using the homogeneous time resolved fluorescence assay. After verifying more reliable candidates, molecular docking was performed to predict the binding affinity and the interaction mode between DCLK1 and inhibitor candidates. We believe that the results and further study could serve as the foundation for the DCLK1-specific inhibitor development.

References

[1] Patel O *et al*. Biochemical and Structural Insights into Doublecortin-like Kinase Domain 1. *Structure* (**2016)**.

[2] Chandrakesan P *et al.* DCLK1 facilitates intestinal tumor growth via enhancing pluripotency and epithelial mesenchymal transition. *Oncotarget* (**2014)**.

Acknowledgement

[1] This work was supported by National Cancer Center, the Republic of Korea.

[CHE-1]

Ubiquitin-dependent proteasomal degradation of AMPK gamma subunit by Cereblon inhibits AMPK activity

Seung-Joo Yang, Seung-Je Jeon, Thang Van Nguyen, Hyemin Han, Junhyung Jung, Chul-Seung Park*, and Kwang Min Lee*

Gwangju Institute of Science and Technology
**E-mail:ysj@gist.ac.kr*

Cereblon (CRBN), a substrate receptor for Cullin-ring E3 ubiquitin ligase (CRL), is a major target protein of immunomodulatory drugs. An earlier study demonstrated that CRBN directly interacts with the catalytic γ subunit of AMP-activated protein kinase (AMPK), a master regulator of energy homeostasis, down-regulating the enzymatic activity of AMPK. However, it is not clear how CRBN modulates AMPK activity. To investigate the mechanism of CRBN-dependent AMPK inhibition, we measured protein levels of each AMPK subunit in brains, livers, lungs, hearts, spleens, skeletal muscles, testes, kidneys, and embryonic fibroblasts from wild-type and *Crbn*$^{-/-}$ mice. Protein levels and stability of the regulatory AMPK γ subunit were increased in *Crbn*$^{-/-}$ mice. Increased stability of AMPK γ in *Crbn*$^{-/-}$ MEFs was dramatically reduced by exogenous expression of *Crbn*. In wild-type MEFs, the proteasomal inhibitor MG132 blocked degradation of AMPK γ. We also found that CRL4^{CRBN} directly ubiquitinated AMPK γ. Taken together, these findings suggest that CRL4^{CRBN} regulates AMPK through ubiquitin-dependent proteasomal degradation of AMPK γ.

References

[1] Author, A.B. Title. *Journals* (**2011**).

Acknowledgement

[1] This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education.

[CHE-2]

RecQ C-terminal domain of Human Bloom Syndrome protein binds and destabilizes the various G-quadruplex DNA topologies

Sungjin Lee and Chin-Ju Park

Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju, 61005, Republic of Korea
**E-mail: cjpark@gist.ac.kr*

Bloom syndrome protein (BLM) is one of five human RecQ helicases that plays an essential role in DNA metabolism. It recognizes the G-quadruplex (G4) DNA via its RecQ C-terminal (RQC) domain with high specificity. G4s are non-canonical DNA structures that are found in G-rich sequences such as telomeres and promotor sites. Although several studies have revealed the detailed mechanisms of duplex DNA binding and G4 unwinding process of BLM, how BLM RQC initially recognizes G4 structure is not elucidated. Here, we investigated the interaction between BLM RQC and the G4 DNAs from the c-Myc promoter and hTelo sequence by NMR spectroscopy. c-Myc and hTelo sequences form the parallel and the (3+1) anti-parallel G4, respectively. The β-wing region of BLM RQC has significant chemical shift perturbations for both c-Myc and hTelo G4 titrations and experiences intermediate time scale exchanges upon G4 binding. Our CD data indicate that RQC can destabilize the parallel G4 while cannot destabilize the anti-parallel G4. We suggest that BLM RQC binds to G4s by using the β-wing, and it has a different destabilization ability upon G4 topologies.

[CHE-3]

Development of Protein Probes for Direct Visualization of Receptor Oligomerization in Living Cells

Daesun Song and Yongwon Jung*

Department of Chemistry, KAIST, Daejeon, Korea

E-mail : daesun61@kaist.ac.kr

Cell surface receptor oligomerization is a key part of complex regulation processes for many receptors. Since these receptors have been by far the most important drug targets, receptor oligomerization is recognized as an attractive target process for a wide range of diseases. At present, however, simple but reliable (and thus high-throughput) visualization methods for receptor oligomerization are still lacking.

Herein, we developed the first single-construct complementation method, Homo-molecular Fluorescence Complementation (Homo-FC), which allows for a direct visualization of oligomerization of diverse surface receptors with high S/N ratios by simple expression of a single receptor-probe construct.[1] The Homo-FC probe was developed by optimized flopped fusion of split fragments of superfolder GFP and subsequent surface charge engineering. The developed Homo-FC probe consistently provided extremely low background self-complementation signals as well as high oligomerization-dependent complementation signals for many different receptors with a simple same sensor design (receptor-Homo-FC probe fusion). Oligomerization of diverse receptors from a model transmembrane domain to natural receptors such as GPCR, EGFR, and even cytosolic DAI were all reliably monitored. Importantly, high S/N ratio was not affected by expression level variations of labeled target receptors. These results represent many ideal features of Homo-FC for reliable and robust ligand/drug screening against various types of receptor oligomerization. By specifically screening ligands against receptor oligomers, allosteric drug candidates that manipulate different regions or processes of target receptors can be discovered. Furthermore, many multimerization proteins including fibrous proteins such as alpha-synuclein and tau can also be studied.

References

[1] Song D, Jung Y. *Angew Chem Int Ed Engl.* **2019**, 58(7) : 2045-2049

Acknowledgement

[1] This work is supported by the Samsung Science & Technology Foundation (SSTF-BA1501-09). D.S. is supported by a TJ Park Science Fellowship of the POSCO TJ Park Foundation.

[CHE-4]

The membrane targeting of MLC1 regulates cellular morphology and motility

Junmo Hwang[1], Hung M. Vu[2], Min-Sik Kim[2], and Hyun-Ho Lim[1,3]

[1]Research Group for the Neurovascular Unit, Korea Brain Research Institute (KBRI), 41062 Daegu, Republic of Korea; [2]Department of New Biology, Daegu Gyeongbuk Institute of Science & Technology (DGIST); [3]Department of Brain & Cognitive Sciences, Daegu Gyeongbuk Institute of Science & Technology (DGIST), 42988 Daegu, Republic of Korea

**E-mail :hhlim@kbri.re.kr*

Background: Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a very rare form of infantile-onset leukodystrophy. The disorder is caused primarily by mutation of *MLC1*, which leads to vacuolation of myelin and astrocytes, subcortical cysts, brain edema, and macrocephaly. Recent studies have indicated that functional interactions among MLC1, GlialCAM, and ClC-2 channels play key roles in the regulation of neuronal, glial, and vascular homeostasis. However, the physiological role of MLC1 in cellular communication remains poorly understood. Thus, in the present study, we investigated the cellular function of MLC1 and its effects on cell–cell interactions. **Methods:** MLC1-dependent cellular morphology and motility were analyzed by using confocal and live cell imaging technique. Biochemical approaches such as immunoblotting, co-immunoprecipitation, and surface biotinylation were conducted to support data. **Results:** Regulating the level of MLC1 expression drastically altered cellular morphology and motility via actin remodeling: Overexpression induced filopodia formation and suppressed motility. Interestingly, expression of patient-derived *MLC1* mutants, which were mainly trapped in the ER, did not alter morphology or motility. Knockdown of *Mlc1* induced Arp3-Cortactin interaction, lamellipodia formation, and increased the membrane ruffling of the astrocytes. These data indicate that the expression of MLC1 at the plasma membrane is critical for changes in actin dynamics through ARP2/3 complex. Thus, our results suggest that misallocation of pathogenic mutant MLC1 may disturbs the stable cell-cell communication and the homeostatic regulation of astrocytes in patients with MLC.

Acknowledgement

This work was supported by the KBRI Basic Research Program funded by the Ministry of Science and ICT of Republic of Korea (19-BR-01-02 to H.-H. L), by the NRF Brain Research Program funded by the Ministry of Science and ICT of Republic of Korea (2017M3C7A 1048086 to H.-H. L), by the Korea Health Technology R&D Project funded by the Ministry for Health and Welfare of Republic of Korea (H18C1254 to H.-H. L.), and by the NRF Brain Research Program funded by the Ministry of Science and ICT of Republic of Korea (2017M3C7A1027472 to M.-S. K.).

[CHE-5]

Regulation of Protein Phosphatases by Carbonylation

Hyunseung Cha[1], Haewon Choi[1], and Youngjun Kim[1, 2*]

[1]Department of Biomedical Chemistry and [2]Nanotechnology Research Center, Konkuk University, Chungju, Chungbuk 27478, Republic of Korea

**E-mail: ykim@kku.ac.kr*

Protein phosphorylation is a common post-translational modification that can generate new recognition motifs for protein interactions and cell locations, affect protein stability and modulate enzyme activity. PTEN is a protein expressed by the PTEN gene in the human body. Mutations in this gene are involved in the onset of many cancers. PTEN acts as a tumor suppressor gene. This phosphatase is involved in the regulation of the cell cycle, preventing the cells from growing and dividing too quickly, and acts as a target for many anticancer agents. Protein-tyrosine phosphatase non-receptor type 1, also known as protein-tyrosine phosphatase 1B(PTP1B), is encoded by the PTPN1 gene in humans. PTP1B is a negative regulator of the insulin signaling pathway and is known as a potential therapeutic agent for the treatment of type 2 diabetes. It is also associated with the development of breast cancer. Recently, the carbonylation of protein phosphatases has been investigated widely in terms of oxidation through the regulation of their activities by structural change of them. We are interested in the effect of lipid peroxides that produce from reactive oxygen species on some protein. Therefore, we tested the enzyme activities of several protein phosphatases such as PTEN, PTP1B, and CTDSP1, and studied kinetical effect on K_m and k_{cat} by treating lipid peroxides on them. We are currently trying to get the structural effect of carbonylation on them through the identification of amino acid residues affected by lipid peroxides by mass spectrometry and the computer simulation with their structures determined by previous studies. We will suggest generalized regulation model of protein phosphatases by carbonylation.

Acknowledgement

Acknowledgments: This work was supported by the Basic Science Research Program (NRF-2019R1F1A1059216) through the National Research Foundation of Korea (NRF) in Republic of Korea.

[CHE-6]

Biophysical/biochemical characterization of the human chitooligosaccharide deacetylase

Seung Jin Lee[1, 2] and Byung Il Lee[1, 2*]

[1]Division of Precision Medicine, National Cancer Center, Goyang 10408, Korea, Department of Cancer Biomedical Science, [2]The national cancer center graduate school of cancer science and policy (GCSP), Goyang 10408, Korea

**E-mail: bilee@ncc.re.kr*

The human chitooligosaccharide deacetylase (HCD), belongs to the carbohydrate esterases family 4 (CE4), is found in various organisms from bacteria to human. Putatively, the HCD catalyzes the deacetylation of acetylated carbohydrates and has been considered to play an important role in the degradation of oligosaccharides. It has been known that the HCD is involved in several inflammatory diseases including ulcerative colitis, psoriasis, and Crohn's disease. Recently, it was also revealed that the HCD is involved in pathological progress of lung cancer such as invasion and migration. Therefore, inhibition of this enzyme can be a novel strategy to prevent the progression of inflammatory diseases and lung cancer. Here, we report the biophysical and biochemical characterization of the HCD. Our analytical ultracentrifugation (AUC) results showed that HCD is a monomer in solution. Among the various acetylated carbohydrates, N-acetyl-D-galactosamine was found to be the most potent substrate candidate using fluorescence-based enzyme activity assay. Next, based on the induced coupled plasma mass spectroscopy (ICP-MS) results and enzyme assay, manganese ion is most active metal cofactor of HCD. We also found that the Asp13 of HCD was directly involved in deacetylase activity. Our results provide insight into its biological characteristics in cellular signaling pathway as well as pathological progress such as inflammatory diseases and cancer. We also performed HTS inhibitor screening of the enzyme using 1,922 compounds of FDA-approved library and 4 hits were selected as the deacetylase inhibitors. This research might open a breakthrough to application of this protein as a potential biomolecule for drug target in cancer treatment.

[CHE-7]

Interaction of replication protein A with Bloom syndrome protein and Fanconi Anemia Group J

Jinwoo Kim, Donguk Kang and Chin-Ju Park*

Department of Chemistry, Gwangju Institute of Science and Technology, Gwangju 61005, Korea

**E-mail: cjpark@gist.ac.kr*

Human DNA helicases, BLM (Bloom syndrome protein) and FANCJ (Fanconi Anemia Group J) have common interaction partner, Replication Protein A (RPA). It has been identified that interaction of BLM and FANCJ with RPA stimulate their DNA unwinding ability, but the binding surface of both proteins are not investigated. Here, we observed interactions of RPA-BLM and RPA-FANCJ by NMR spectroscopy and Fluorescence Polarization anisotropy experiments. We revealed that two acidic peptide regions of BLM specifically bind to RPA70N domain. Also, we first identified that the acidic region present at the C-terminal of FANCJ binds with RPA70N. Our analysis show that the common binding strategy underlies for maintaining RPA-helicase interactions.

References

[1] Donguk Kang et al., Interaction of replication protein A with two acidic peptides from human Bloom syndrome protein. *FEBS letter.* (**2018)**.

[2] Gyuho Yeom et al., Investigation of the core binding regions of human Werner syndrome and Fanconi anemia group J helicases on replication protein A. *Scientific Reports.* **(2019)**

[CHE-8]

Potential sites of AGE modulators; multiple approach to treat diabetes and diabetic complications

Seung Min Lee[1], Tae Hun Kim[1], Amna Parveen[1,*], Sun Yeou Kim[1,*],

[1)]College of Pharmacy, Gachon University, No. 191, Hambakmoero, Yeonsu-gu, Incheon 21936, Korea

**E-mail: amnaparvin@gmail.com, sunnykim@gachon.ac.kr*

The role of advanced glycation end products (AGEs) is not limited to diabetes and diabetes related complications; there are also multiple modulators, such as receptors for advanced glycation end products (RAGEs), High mobility group box 1 (HMGB1), glyoxylase 1, NF-kB, TNF-α, chronic unpredictable stress, reaction oxygen species, and inflammatory cytokines, that interact with AGEs signaling and control diabetes. Controlling these interacting modulators, progression of diabetes and its complication can be controlled and treated. Also, natural products rich in bioactive constituents can interact with AGEs and its modulators through various signaling cascades, thereby controlling and prevention of diabetes progression. This review provides a deeper studying series of signaling pathway, interaction of phytochemicals and AGEs-mediated targets in order to develop a multiple approach therapy to prevent, control, and treat diabetes and its related complications.

Keywords; Phytochemicals, Advanced glycation end products, reactive oxygen species, coronary artery disease, diabetes, receptor for advanced end product glycation.

[CHE-9]

Regulation of mRNA export by API5-FGF2 interaction

Seoung Min Bong[1], Taehyun Park[1] and Byung Il Lee[1,2,*]

[1]Research Institute, National Cancer Center, Goyang-si, Gyeonggi 10408, Republic of Korea
[2]Department of Cancer Biomedical Science, National Cancer Center Graduate School of Cancer Science and Policy, Goyang-si, Gyeonggi 10408, Republic of Korea

API5 (APoptosis Inhibitor 5) and nuclear FGF2 (Fibroblast Growth Factor 2) are upregulated in various human cancers and are correlated with poor prognosis. Although their physical interaction has been identified, the function of the resulting complex in cancer is unknown. Here, we determined the crystal structure of the API5–FGF2 complex and identified critical residues driving the protein interaction. This provided structural basis of nuclear localization of the FGF2 isoform lacking canonical nuclear localization signal and identified cryptic nuclear localization sequence in FGF2. The interaction between API5 and FGF2 was important for mRNA export through both the TREX and eIF4E/LRPPRC mRNA export complexes, regulating the export of bulk mRNA and specific mRNAs containing eIF4E sensitivity elements, such as c-MYC and cyclin D1. Interestingly, API5–FGF2 complex directly interacted with UAP56, a common factor of these two mRNA export complexes. Disruption of the API5–FGF2 interaction reduced cell proliferation and increased drug sensitivity. These data reveal a previously unknown function for API5 and nuclear FGF2 and suggest a new therapeutic target for regulating the expression of oncogenes at the mRNA export level.

[CHE-10]

Unusual acid- and voltage-dependency of a prokaryotic CLC, CLC-ec2 : A marginal ion channel or broken transporter?

Kunwoong Park[1,*], Jinuk Kim[2,*], Byoung-Cheol Lee[1], Soung-Hun Roh[2,§], Hyun-Ho Lim[1,§]

[1]Neurovascular Unit Research Group, Korea Brain Research Institute (KBRI), Daegu, Korea
[2]Department of Biological Science, Seoul National University, Seoul, Korea
[*]Co-first author, [§]Corresponding author

**E-mail: kunwoong@kbri.re.kr*

The CLC superfamily can be classified into Cl^- channels and Cl^-/H^+ antiporters and be found in virtually all organisms. In *E.coli*, two CLC proteins, CLC-ec1 and CLC-ec2 are both functionally important for bacteria to survive extreme acid challenge. The double knockout cannot survive at pH 2.5, but the presence of either CLC gene is enough to survive at pH 2.5.

However, we have found that two CLCs are differentially activated by external stimuli: acidification and transmembrane voltage. Intriguingly, CLC-ec2 exchanges one H^+ with varying Cl^- (4 to 10) upon the stimulation strength.

To determine molecular mechanisms, we present the cryo-EM structure of CLC-ec2 in complex with monoclonal Fab fragment at 3.9 angstrom resolution. Overall structure of CLC-ec2 is essentially identical to that of CLC-ec1. But in CLC-ec2, Gating glutamate occupies the central binding site, similar to that observed in the structure of cmCLC, a eukaryotic CLC and no Cl^- ions appear at the external binding site which is anion-binding region in canonical CLCs. Moreover, CLC-ec2 uniquely lack the highly conserved serine residue that coordinate Cl^- ion.

To reveal the operating mechanism underlying the structural and functional difference between CLC-ec1 and CLC-ec2, we are currently focusing on biochemical and mutagenesis studies.

[CHE-11]

Phosphatidylserine induced dissociation of the heterodimeric PstB2p/Pbi1p complex in yeast phosphatidylserine trafficking system

Hwa Jin Cho, Jeong-Seok Ji, and Hyung Ho Lee*

Department of Chemistry, College of Natural Sciences, Seoul National University

**E-mail: hyungholee@snu.ac.kr*

Phospholipids, essential building blocks of cellular membranes, are synthesized in the endoplasmic reticulum (ER) and distributed to the intracellular membranes. Yeast phosphatidylserine (PtdSer) is produced in the ER and transported to the mitochondria, Golgi, or vacuole, and subsequently converted to phosphatidylethanolamine by phosphatidylserine decarboxylase. The PstB2p (Sec14p homolog) and Pbi1p in yeast was shown to be involved in non-vesicular lipid transport from the ER to Golgi, however, the molecular mechanism remains unclear. In this study, we observed that PstB2p forms a homodimer, but exists as a 1:1 heterodimer in the presence of Pbi1p. When PtdSer was added to the PstB2p-Pbi1p complex, PtdSer binds to PstB2p, triggering dissociation of the PstB2p-Pbi1p complex. PstB2p in complex with PtdSer exists as a monomer in contrast to the homodimeric form in the absence of PtdSer. These studies suggest the stoichiometric model of the PstB2p-Pbi1p complex in yeast PtdSer transport system.

References

[1] Yang, H., Tong, J., Leonard, T. A., Im, Y. J., Structural determinants for phosphatidyl-inositol recognition by Sfh3 and substrate-induced dimer-monomer transition during lipid transfer cycles, *FEBS Lett* **(2013)**.

[2] Riekhof, W. R., Wu, W. I., Jones, J. L., Nikrad, M., Chan, M. M., Loewen, C. J., Voelker, D. R., An assembly of proteins and lipid domains regulates transport of phosphatidylserine to phosphatidylserine decarboxylase 2 in *Saccharomyces cerevisiae*, *J Biol Chem* **(2014)**.

[CHE-12]

The role of Ndufs4 in the muscle tissue

Jongbum Na and Won-Seok Choi*

School of Biological Sciences and Technology, College of Natural Sciences, Chonnam National University, Gwangju 500-757, Korea;

**E-mail: choiw@chonnam.ac.kr*

Mitochondrial defect has been implicated in various diseases involving the movement problems of the body. NADH dehydrogenase ubiquinone iron-sulfur protein 4 (NDUFS4) is one of the key component of mitochondrial complex I. The genetic alteration of Ndufs4 has been related with diseases caused by mitochondrial dysfunction. In this study, we investigated the effect of this protein in muscles using NDUFS4+/- and NDUFS4+/+ mice. First, we verified that mitochondrial activity was reduced in NDUFS4+/- mice. To test the effect of reduced expression of NDUFS4 on muscle tissue, we stained and compared muscle tissue in NDUFS4+/- and NDUFS4+/+ mice. The size of muscle tissue from NDUFS4+/- mice was significantly smaller than NDUFS4+/+ mice indicating the defect of muscle tissue caused from mitochondrial deficit. These results suggest that the NDUFS4 contribute to the mitochondrial function which is essential for muscle tissue structure and activity.

[CHE-13]

The role of myeloid receptor in neurodegeneration.

Bo-Ram Mun and Won-Seok Choi*

School of Biological Sciences and Technology, College of Natural Sciences, Chonnam National University, Gwangju 500-757, Korea;
**E-mail: choiw@chonnam.ac.kr*

Myeloid receptors play an important role in the function and activity of microglia. Since microglia have been involved in the pathology of neurodegenerations, the role of myeloid receptor have also been highlighted. Specially, in Alzheimer's disease, microglial activity is important to prevent the disease by promoting Aβ phagocytosis, However, the detailed molecular mechanism has not been understood regarding the function of myeloid receptor in Aβ-related pathogenesis. To test this, we investigated the behavior and Aβ-related histology in myeloid receptor TG or KO mice. We showed that learning and memory were enhanced in myeloid receptor TG mice and decreased in KO mice. The phagocytosis of Aβ was reduced in the microglia of KO mice. These data suggest that myeloid receptor is essential to prevent the defect of memory and learning in old mice by regulating Aβ phagocytosis.

[CHE-14]

Protein arginine methyltransferase-1 stimulates PARP1-induced cell death in PD model

Jin Ho Lee and Won-Seok Choi*

School of Biological Sciences and Technology, College of Natural Sciences, Chonnam National University, Gwangju 500-757, Korea;
**E-mail: choiw@chonnam.ac.kr*

Protein arginine methyltransferases (PRMTs) have been implicated for many diseases. However, their pathological role in Parkinson's disease (PD) has not been fully evaluated. In this study, we showed that MPTP treatment, which causes dopaminergic neuron death, elevated PRMT1 expression and increased the interaction between PRMT1 and PARP1 in the substantia nigra pars compacta of mice. Moreover, MPTP induced motor impairment in mice, which was attenuated in PRMT1 heterozygote (+/-) mice. In addition, MPTP-induced cell death of dopaminergic neurons was prevented in PRMT1 heterozygote (+/-) mice. In summary, PRMT1 expression and PRMT1-PARP1 interaction was increased in mouse models of PD and behavioral defect as well as dopaminergic neuronal death was prevented by PRMT1 reduction. These data suggest that increased expression and activity of PRMT1 is implicated in PARP1-mediated dopaminergic neuronal cell death, so called parthanatos, which might be implicated in the development of PD. Therefore, our study propose PRMT1 as a new factor to target for a treatment of PD.

[CHE-15]

Modulation of amyloid beta pathology in Alzheimer's disease model.

Ji hoon Jeong and Won-Seok Choi*

School of Biological Sciences and Technology, College of Natural Sciences, Chonnam National University, Gwangju 500-757, Korea;
**E-mail: choiw@chonnam.ac.kr*

Alzheimer's disease is the most common neuronal degenerative disease. It is characterized by the dysfunction of the brain including memory defect as the main symptoms. The accumulation of amyloid beta and the hyperphosphorylation of tau are typical pathological marker Alzheimer's disease. However, the exact pathology of Alzheimer's disease has not been confirmed. In the previous study, we confirmed the accumulation of amyloid beta and the defect of brain function including learning and memory in Alzheimer's disease model mouse, 5XFAD. In this study, we examined the effect of glutamate receptor antagonist to the memory deficit and amyloid beta-related mechanisms in 5XFAD mice. In the behavior tests, the antagonist attenuated the defect in learning and memory. Furthermore, it prevented amyloid beta accumulation as well as neuronal loss. These data suggest that glutamate receptor antagonist could be beneficial to prevent amyloid beta- related pathology.

[CHE-16]

DH047 and DH049 stimulates glucose uptake in 3T3L1 cells via inhibition of protein tyrosine phosphatases

Dohee Ahn[1], Sun-Young Yoon[1] and Sang J. Chung[1,2]*

[1]College of Pharmacy, Sungkyunkwan University, 2066 Seoburo Jangangu, Suwon 16419, South Korea
[2]AbTis Co. Ltd., 142-10 Saneop-ro 156beon-gil, Gwonseon-gu, Suwon, South Korea
**E-mail: sjchung@skku.edu*

Type 2 diabetes mellitus (T2DM) is a disease characterized by insulin resistance. Insulin resistance has a feature of defects in insulin signaling and several protein tyrosine phosphatases (PTPs) involved in this signaling pathway can be potential antidiabetic targets. We have screened natural compound isolated from a certain plant to identify potent inhibitors of PTPs involved in insulin resistance. Among them, DH047 and DH049 was selected as a dual-targeting inhibitor of PTP1B and SHP2. Then, we examined its IC_{50} and cooperative binding through evaluating hill coefficient *in vitro*. In 3T3L1 adipocytes, DH047 and DH049 increased GLUT4 translocation to the plasma membrane protein and stimulates glucose uptake in a concentration-dependent manner. In addition, DH047 and DH049 didn't increase the lipid components and adipogenesis in 3T3-L1 cells. Thus, these results suggest DH047 and DH049 is a potential therapeutic candidate for T2DM.

References

[1] Toni Tiganis, FEBS Journal, 2013, 280, 445-458

Acknowledgement

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (NRF-2012M3A9C4048775).

[CHE-17]

Target identification of herbal remedies related to diabetes using high-throughput screening and network analysis

Youl Lee Kim, Dohee Ahn, and Sang J. Chung*

School of Pharmacy, Sungkyunkwan University, Suwon 16419, Korea
**E-mail: sjchung@skku.edu*

Traditional oriental medicine (TOM) has been used to treat disease for thousands of years. However, the targets of TOM have been barely validated by modern analytical science because TOM has been established by qualitative and philosophical approach. To create the method which reveal the target of TOM ingredients by analytical science, we have accumulated a set of biological data based on in vitro enzyme assay with high-throughput screening (HTS) of 658 purified natural products for inhibition of human protein tyrosine phosphatase (hPTP) libraries consisting of 77 members. The resulting data were combined with the data of herbal medicine, which affords pre ready data for systemic analysis of disease, medicinal target, natural product, herbal medicine, prescription of traditional medicine for type 2 diabetes (T2D). Among 19 traditional medicine prescriptions for T2D, 16 showed targeting of T2D related PTPs and the most targeted PTP was PTPN9 (MEG2). This is the first example that a set of prescriptions for a specific disease was validated by modern analytical science. The result strongly supported that TOM has each medicinal target for specific disease. This type of analysis may pave a way to validate TOM and to discover novel medicinal targets at the same time.

Acknowledgement

[1] This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (NRF-2012M3A9C4048775)

[PHY-1]

Discovery and characterization of BK_{Ca} channel activation and bladder muscle relaxation effect of novel chemical compound against overactive bladder syndrome

Narasaem Lee, Heeji Jo, Eunyoung Lee and Chul-Seung Park*.

Gwangju Institute of Science and Technology
E-mail:skfktoa@gist.ac.kr

The large-conductance calcium-activated potassium channel(BK_{Ca} channel) is expressed on the urinary bladder smooth muscle and plays critical roles in smooth muscle relaxation. The BK_{Ca} channel is activated by intracellular Ca^{2+} ion concentration increasing and membrane depolarization. Bladder smooth muscle is relaxed by BK_{Ca} channel activation. To identify novel BK_{Ca} channel activators, chemical library is screened using a cell-based fluorescence assay and a hyperactive mutant BK_{Ca} channel. From 8,300 chemical compounds, a novel BK_{Ca} channel activator candidate is discovered. When this compound is treated to the extracellular side of cell membrane, the conductance-voltage relationship of BK_{Ca} channels shifted to more negative voltages and maximum conductance is increased. Also, bladder muscle relaxation effect of the compound was identified by *ex vivo* and *in vivo* study. These results indicate that the novel BK_{Ca} channel activator can directly activate BK_{Ca} channels and it suggest the therapeutic potentials of this new BK_{Ca} channel activator for develop anti overactive bladder medications and supplements.

References

[1] Author, A.B. Title. *Journals* (**2011**).

Acknowledgement

[1] This work was supported by grants for the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries(IPET) through Agri-Bio industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs(MAFRA)

[PHY-2]

Electrostatic ratchet model in processive DNA degradation by lambda–exonuclease

Jungmin Yoo[1]*, Hyeokjin Cho[1]*, Jejoong Yoo[2], and Gwangrog Lee[1]

[1]School of Life Science, Gwangju Institute of Science and Technology,
123 Cheomdan-gwagiro, Buk-gu, Gwangju, Republic of Korea
[2]IBS Center for Self-assembly & Complexity, 77 Cheongam-ro, Nam-gu, Pohang, Republic of Korea
E-mail: jhj@gist.ac.kr

Transient interaction through forming and breaking of physical contacts between an enzyme and its substrate greatly influence its catalytic rate and processivity but little attention has been paid to how the dynamics of the transient interaction transmit into the overall enzymatic activity. We employed lambda exonuclease as a model system and examined how the dynamics of substrate interaction alter its catalytic rate using single molecule FRET. Lambda exonuclease trimer has two arginine plugged into a DNA minor groove by electrostatic interaction and act as a translocation guiding key by sliding along the minor groove. The degree of this interaction was modified by altering the electrostatic potential (EP) of DNA minor groove by changing its sequence. Change in electrostatic interaction to the substrate alters translocation rate upto 31.7 fold, by presumably dissipating kinetic energy by molecular friction, in contrast to the belief which the degradation rate is determined by melting step of the double strand DNA. A molecular dynamics simulation of arginine residue in the minor groove reveals that its friction while sliding along minor groove was highly correlated with the minor groove EP. We constructed a DNA sequence with negative EP spikes in every 4 bp, the distance between the binding sites of the minor groove interacting arginine, using the same nucleotide content with the sequence showed slowest degradation. Although the sequence had minimal change in melting free energy, less than 0.012 kcal/mol, due to rearrangement of EP the degradation rate increased 18.8 fold. Based on these findings, we suggest the λ–exonuclease follows an anti-friction-based ratchet model, where arginine binds at low EP spot with strong friction to prevent backtracking. Our study provides new insights into the interplay between dynamic physical interaction and enzyme activity.

[PHY-3]

Unwinding mechanism of SARS coronavirus nsP13 helicase characterized by single-molecule methods

Jeongmin Yu, Hyeryeon Im and Gwangrog Lee

School of life Sciences, Gwangju Institute of Science and Technology, Gwangju, 61005, Republic of Korea
E-mail: shine4109@gist.ac.kr

SARS-CoV and MERS-CoV were epidemics in 2003 and 2015 in ASIA. SARS-CoV helicase plays critical roles in viral replication and has been proposed to be a potential target for anti-SARS-CoV therapy. Thus, the characterization of enzymatic activity might be critical for drug development. We introduce the single-molecule methods to examine the unwinding mechanism of nsP13 helicase on partial DNA duplexes as a function of the protein, ATP concentration, and tail length. Our results unravel that the tail length of the substrates governs the total amount of DNA unwound by increasing the number of proteins loaded. We also found that the relative extent of constitutive unwinding and repetitive fluctuation is defined by the enzyme oligomerization formed in the presence of ATP concentration. In general, our results identify the important cellular parameters, governing the cooperative unwinding behavior of helicase. This is a new attempt to understand the complicated behavior of unwinding motor cohorts at the single-molecule resolution.

[PHY-4]

The critical role of an aromatic ring for exolytic degradation by exonuclease III

Donghun Lee, Gwangrog Lee

GIST

E-mail: dlehdgns7536@gmail.com

DNA damage occurs as many as 1 million lesions per cell per day in human by metabolic and environmental mutagens. Base damage (one of the most frequently occurring DNA lesions) is quickly processed into an orphan nucleobase, so-called an AP (apurinic/apyrimidinic) site during Base Excision Repair (BER). AP endonuclease cleaves the DNA backbone 5' to the AP site to prime DNA repair. In E. coli, exonuclease III (exoIII) functions as an AP endonuclease as well as a 3′->5′ exonuclease. How the enzyme recognizes and incises the AP site is still poorly understood despite numerous mechanistic studies. We have focused on the processing of the AP site by ExoIII using affinity and cleavage-based assays. We performed site-directed mutagenesis on key residues at the 212 TRP and 213 PHE to unravel the functional roles of the aromatic rings during BER. By testing a serious of single-point- and swapping- mutations, we found that the activity of AP endonuclease persists if one of two aromatic residues at least is present, while a ring residue at the 213th position, if any (e.g., TRP and PHE), is indispensable for the activity of exonuclease. A careful structural examination suggests that the 213th ring-residue stabilizes the catalytically competent state with the 3' terminal base via the π-π interaction during exonucleolytic degradation. Our overall results indicate that the 213th position is essential roles in not only the recognition and cleavage of AP sites but also the degradation-processing after passing the AP site.

References

[1] K. Kaneda, "Role of the tryptophan residue in the vicinity of the catalytic center of exonuclease III family AP endonucleases: AP site recognition mechanism", Nucleic Acids Research, vol. 34, no. 5, pp. 1552-1563, 2006.

[PHY-5]

Binding mode and degradation activity of ribonuclease H on the RNA/DNA hybrid substrate by single molecule FRET

Hyun Jee Lee[p] and Gwangrog Lee[c]

School of Life Science, Gwangju Institute of Science and Technology,
123 Cheomdan-gwagiro (Oryong-dong), Buk-gu, Gwangju 61005 Republic of Korea

E-mail: lhj0346@naver.com

Ribonuclease H (RNase H), which is known as a nuclease enzyme is found in all organisms. The RNase H malfunctioning induces the autoimmune disease such as Aicardi-Goutieres syndrome (AGS). This suggests RNase H is necessary component for fundamental cellular process. The main function of RNase H is to remove RNA primers in Okazaki fragments during replication. However, how RNase H processes small RNA primer fragments in long lagging strands remains uncertain. Here, single molecule FRET was used to observe binding mode and degradation activity of RNase H on substrate, which mimics Okazaki fragments. As a result, we found that RNase H specifically binds both chimeric junction and overhang / 5' end junction. Furthermore, we discovered that during processing RNA primer, RNase H degrades RNA in a processive manner. In conclusion, the overall results proposed the model on how RNase H organizes the maturation process of Okazaki fragments.

References

1. Rychlik, M., Chon, H., Cerritelli, S., Klimek, P., Crouch, R. and Nowotny, M. (2010). Crystal Structures of RNase H2 in Complex with Nucleic Acid Reveal the Mechanism of RNA-DNA Junction Recognition and Cleavage. *Molecular Cell*, 40(4), pp.658-670.
2. Rabe, B. (2013). Aicardi–Goutières syndrome: clues from the RNase H2 knock-out mouse. *Journal of Molecular Medicine*, 91(11), pp.1235-1240.
3. SURESH, G. and PRIYAKUMAR, U. (2015). Atomistic details of the molecular recognition of DNA-RNA hybrid duplex by ribonuclease H enzyme. *Journal of Chemical Sciences*, 127(10), pp.1701-1713.

[PHY-6]

Cohesin-mediated polymer-polymer phase separation

Je-Kyung Ryu[1], Frank Uhlmann[2], and Cees Dekker[1]

[1]*Department of Bionanoscience, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, Netherlands.*

[2]*Chromosome Segregation Laboratory, The Francis Crick Institute, London, UK.*

**E-mail: workrjk@gmail.com*

Cohesin is one of the Structural Maintenance Chromosome (SMC) family which is vital for chromosome organization in every living life. Cohesin organizes the topologically associated domain (TAD) during the interphase of cell division process. However, how cohesins organize the chromosome structure is still under debate. Here, we showed that budding yeast cohesin unexpectedly phase separates to form a liquid droplet along a DNA using both time-lapse single-molecule fluorescence imaging and atomic force microscopy (AFM). First, we observed that cohesin can compact DNA with an ATP independent manner, and we found that cohesin forms a cluster formed by hundreds of cohesin complexes in the compacted spot of the DNA. Second, along the DNA, we observed two cohesin-clusters were merged, and reshaping of the clustered area, supporting a liquid droplet formation. Lastly, using AFM microscopy, we found that DNA induces cohesin clustering and this cluster size was dependent on the length of DNA, supporting polymer-polymer phase separation. Our results suggest that budding yeast cohesin forms a liquid droplet along a DNA for heterochromatin formation. This study will contribute to understanding chromosome organization how SMC protein induces phase separation.

Acknowledgment

[1] This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 753002.

[PHY-7]

A muscular hypotonia-associated STIM1 mutant at R429 induces abnormalities in intracellular Ca^{2+} movement and extracellular Ca^{2+} entry in skeletal muscle

Jun Hee Choi[1,2], Mei Huang[1,2], Changdo Hyun[1,2], Mi Ri Oh[1,2], Keon Jin Lee[1,2], Chung-Hyun Cho[3], and Eun Hui Lee[1,2,*]

[1]*Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul 06591,* [2]*Department of Biomedicine & Health Sciences, Graduate School, The Catholic University of Korea, Seoul 06591,* [3]*Department of Biomedical Sciences and Pharmacology, College of Medicine, Seoul National University, Seoul 03080, Republic of Korea*

**E-mail: ehui@catholic.ac.kr*

Stromal interaction molecule 1 (STIM1) mediates extracellular Ca^{2+} entry into the cytosol through a store-operated Ca^{2+} entry (SOCE) mechanism, which is involved in the physiological functions of various tissues, including skeletal muscle. STIM1 is also associated with skeletal muscle diseases, but its pathological mechanisms have not been well addressed. The present study focused on examining the pathological mechanism(s) of a mutant STIM1 (R429C) that causes human muscular hypotonia. R429C was expressed in mouse primary skeletal myotubes, and the properties of the skeletal myotubes were examined using single-cell Ca^{2+} imaging of myotubes and transmission electron microscopy (TEM) along with biochemical approaches. R429C did not interfere with the terminal differentiation of myoblasts to myotubes. Unlike wild-type STIM1, there was no further increase of SOCE by R429C. R429C bound to endogenous STIM1 and slowed down the initial rate of SOCE that were mediated by endogenous STIM1. Moreover, R429C increased intracellular Ca^{2+} movement in response to membrane depolarization by eliminating the attenuation on dihydropyridine receptor-ryanodine receptor (DHPR-RyR1) coupling by endogenous STIM1. The cytosolic Ca^{2+} level was also increased due to the reduction in SR Ca^{2+} level. In addition, R429C-expressing myotubes showed abnormalities in mitochondrial shape, a significant decrease in ATP levels, and the higher expression levels of mitochondrial fission-mediating proteins. Therefore, serial defects in SOCE, intracellular Ca^{2+} movement, and cytosolic Ca^{2+} level along with mitochondrial abnormalities in shape and ATP level could be a pathological mechanism of R429C for human skeletal muscular hypotonia. This study also suggests a novel clue that STIM1 in skeletal muscle could be related to mitochondria via regulating intra and extracellular Ca^{2+} movements.

[PHY-8]

Watching helical membrane proteins fold reveals a common N-to-C-terminal folding pathway

Hyun-Kyu Choi[1,2,3], Duyoung Min[4,5], Hyunook Kang[2], Min Ju Shon[2,3], Sang-Hyun Rah[1,2,3], Hak Chan Kim[2], Hawoong Jeong[1], Hee-Jung Choi[2], James U. Bowie[4], Tae-Young Yoon[2,3]

1 Department of Physics, Korea Advanced Institute of Science and Technology, Daejeon 34141, South Korea.

2 School of Biological Sciences, Seoul National University, Seoul 08826, South Korea.

3Institute for Molecular Biology and Genetics, Seoul National University, Seoul 08826, South Korea.

4 Department of Chemistry and Biochemistry, University of California–Los Angeles, Los Angeles, CA 90095, USA.

5 Department of Chemistry, Ulsan National Institute of Science and Technology, Ulsan 44919, South Korea.

E-mail: hgchoi2003@kaist.ac.kr

To understand membrane protein biogenesis, we need to explore folding within a bilayer context. Here, we describe a single-molecule force microscopy technique that monitors the folding of helical membrane proteins in vesicle and bicelle environments. After completely unfolding the protein at high force, we lower the force to initiate folding while transmembrane helices are aligned in a zigzag manner within the bilayer, thereby imposing minimal constraints on folding. We used the approach to characterize the folding pathways of the *Escherichia coli* rhomboid protease GlpG and the human β_2-adrenergic receptor. Despite their evolutionary distance, both proteins fold in a strict N-to-C-terminal fashion, accruing structures in units of helical hairpins. These common features suggest that integral helical membrane proteins have evolved to maximize their fitness with cotranslational folding.

[PHY-9]

Characterization of mutant EGFR signaling complex by single-molecule co-immunoprecipitation in combination with crosslinking

TaeGyun Kim [1,2], Tae-Young Yoon [1,2]*

1. Department of Biological Sciences, Seoul National University, Seoul, 08826, South Korea 2. Institute for Molecular Biology and Genetics, Seoul National University, Seoul 08826, South Korea

**E-mail: taegyun0806@gmail.com*

Signaling complex that controls cellular signaling pathway is mediated by a number of protein-protein interactions (PPIs). Disrupting a structural-functional integrity of the protein participating in these interaction network rewires cellular signaling pathway and leads to cancer development. Aberrant epidermal growth factor receptor (EGFR) signaling derives from the mutations amplifying its expression or kinase activity. Although therapeutic efforts to directly inhibit hyperactive EGFR have been made. mutant EGFR signaling pathway governing drug-resistance is not well understood. According to previous reports, it was suggested that PPIs in non-canonical EGFR signaling complex induced by the EGFR mutations was responsible for the resistance to the dephosphorylation action of phosphatases, resulting in dysregulated proliferation. [1] Here, we characterize mutant EGFR signaling complex by single-molecule co-immunoprecipitation(sm-CoIP) in combination with crosslinking. Because sm-CoIP can allow to quantify PPIs at single-molecule resolution and crosslinking can play a role in maintaining endogenous signaling complex, the combination between two techniques can help observe PPIs in endogenous mutant EGFR signaling complex at single-molecule level.

References

[1] Lee, H.W, et al. Profiling of protein-protein interactions via single-molecule techniques predicts the dependence of cancers on growth-factor receptors. *Nature Biomedical Engineering* **(2018)**.

[PHY-10]

Biophysical studies on exocyst-mediated assembly of the yeast SNARE complex

Chanwoo Lee, Sang-Hyun Rah, Dante Lepore, Mary Munson, Tae-Young Yoon

Seoul National University, KAIST, University of Massachusetts Medical School
E-mail: srhyhl@snu.ac.kr

The process that a cell transports the molecules out of the cell is called exocytosis. Also, the exocytosis is essential for cell growth like yeast budding. There are several experimental results about the proteins associated with exocytosis such as SNARE, SM-protein, exocyst and the others. Among them, exocyst is large protein complex composed of 8 subunits(sec3, sec5, sec6, sec8, sec10, sec15, exo70, exo84) that its subunits interact with SNAREs and GTPase. It is difficult to purify intact exocyst complex and formal research on exocyst was done with its subunit proteins. But, there is recent progress in purifying the intact exocyst and our group could purify the exocyst with cooperation with the other research group. Exocyst we purified is derived from Saccharomyces cerevisiae. So, we basically mimic intracellular part and lipid composition of the yeast and use the single-molecule fluorescence imaging and fluorescence spectroscopy to figure out the mechanism of exocytosis in yeast. Fluorescence signal is acquired by using the lipid reagent like DiD and DiI. Also, we make the 2 kinds of vesicles similar with secretory vesicle of yeast in vitro. We identified the fusion of vesicles by checking the fluorescence signal difference when exocyst and SANRE motifs are existed. In addition, using the single-molecule imaging, there is increase of docking rate between 2 kinds of vesicles when exocyst and SNARE motifs are provided. Through this result, we want to investigate the intact exocyst and mechanism of exocytosis.

References

[1] Munson, Mary, and Peter Novick. *Nature structural & molecular biology* (2006)
[2] Baker, R.W., & Hughson, F.M. *Nature Reviews Molecular Cell Biology* (2016)
[3] Heider, Margaret R., et al. *Nature structural & molecular biology* (2016)

[PHY-11]

Single molecule FRET reveals the proofreading mechanism of replicative DNA Polymerase based on the single-mismatched DNA stability.

Hoa T.M Vo[1p], Hyeokjin Cho[1], Gwangrog Lee[1]

Single Molecule and Cellular Dynamic Lab, Gwangju Institute of Science and Technology[1]

E-mail: vothiminhhoa@gist.ac.kr

Accurate DNA replication is essential for genomic stability and depends on the action of 3′–5′ exonucleases that remove misincorporated nucleotides from the newly synthesized strand. The structural studies proposed the proofreading occurs at the primer end when the protein-DNA complex for DNA synthesis is unstable, and the “melting” of primer end is required to DNA enters exonuclease active site. Here, we report a single-molecule Förster resonance energy transfer (smFRET) system that directly monitors the movement of a DNA substrate between the Pol and Exo sites of Phi29 DNA polymerase, revealing that wide –range of mismatch recognition and removal of DNA polymerase. Importantly, the Pol/Exo binding probability of different mismatch position DNA shows that the 3rd mismatch DNA increases the Exo binding complex population, thus implying the critical check-point in DNA proofreading. Therefore, the stability of single-mismatched DNA and its position play an importance role in proofreading. Hence, our works provides unique insights into the correction of misincorporated nucleotides.

[PHY-12]

Mechanism of RNA unwinding by XRN1 exonucleas

Junhyuk Rhee and Gwangrog Lee

School of Life Science, GwangjuInstitute of Science and Technology,
123 Cheomdan-gwagiro(Oryong-dong), Buk-gu, Gwangju500-712 Republic of Korea

E-mail: jjun7666@gmail.com

XRN1 is a key enzyme involving in regulation and surveillance of eukaryotic gene expression and enables multi-enzymatic activities, including exoribonuclease and helicase that unwind and degrade secondary or even high-ordered structures in mRNA. XRN catalyzes a series of reactions during messenger RNA decay as well as RNA processing. Thus, its exoribonuclease and unwinding activities are vital for the complete degradation of structured mRNA. It is,however, unclear how the unwinding activity is orchestrated by the exonuclease one. We used single-molecule fluorescence techniques to examine the mechanism of unwinding by XRN1 in real time. We found the unwinding mechanism driven by a large step size in which the enzyme utilizes a series of hydrolysis reactions to destabilize the RNA duplex that mimics secondary structures.

[PHY-13]

Contribution of a tetraspan subtype to the nociceptor sensitivity via functional enhancement of TRP channel activity

Ji Yeon Lim, Pyung Sun Cho, Minseok Kim, Haiyan Zheng, Seung-In Choi, Geunyeol Choi, Sun Wook Hwang*

Department of Biomedical Sciences and Department of Physiology, Korea University College of Medicine, Seoul 02841, Korea

**E-mail: sunhwang@korea.ac.kr*

Modulation of the peripheral nociceptor activity is an important analgesic strategy. Information on the analgesic target molecules expressed in nociceptors is currently expanding. In this regard, we tried to unveil a previously unknown target among proteins encoded by nociceptor-specific genes and to examine its analgesic utility. We analyzed parametric data from previous studies regarding gene expressions in dorsal root ganglionic (DRG) neurons, of which an important and major subset consist of nociceptors, and obtained a number of candidate genes. As a result of further multiple logical selection steps, we chose the tetraspan subfamily as the principal object of the present study. This subfamily is known to have 6 subtypes, but its roles in the somatosensory system such as nociception have not been explored so far. The contributions to pain by three of these tetraspans were evaluated by using RNA interference in DRG neurons. As a result, one subtype exhibited most significant contribution to inflammatory pain. We further examined the role of the tetraspan subtype in modulation of the activity of several known pain receptor molecules expressed in the nociceptor and also in its contribution to synaptic transmission of the nociceptors. Our data suggest that the tetraspan may tune the sensitivity of a receptor ion channel, exacerbating pain.

[PHY-14]

Antibody screening for the investigating the clustering patterns of ion channels in the cortical neurons

Euna Lee[1,2], Junmo Hwang[1], and Hyun-Ho Lim[1,2]*
[1]Lab Of Molecular Physiology and Biophysics, Korea Brain Research Institute, Cheomdan ro, Dong gu, Deagu, 41062, Republic of Korea
[2]Department of Brain and Cognitive Sciences, Daegu Gyeongbuk Institute of Science and Technology
**E-mail: hhlim@kbri.re.kr*

The neuronal cells generate the action potentials (APs) over a far wider range of frequencies, amplitude, and duration. Also, neurons can respond to small changes in input currents with significant changes in firing frequency. This diversity of neuronal APs can be attributable to the specified sets of ion channels in the neuronal cells. For example, voltage-gated calcium channels (Ca_V channels) regulate calcium influx responding to the transmembrane voltage changes. The changes in intracellular calcium concentration control the various cellular proteins and physiological processes. A calcium-activated K^+ channel, BK channel, is one of such cases to play an essential role in feedback control of calcium influx and cell excitability. A recent study revealed that $Ca_V1.3$ and BK channels are proximally clustered and functionally coupled in the cultured neurons by using super-resolution imaging and electrophysiological recording techniques. To investigate the Ca_Vs and calcium-activated ion channels' clustering patterns at the super-resolution level, we firstly screened antibodies specific to the different channels. We have examined the expression patterns of the various combination of the voltage-gated calcium channels and calcium-activated ion channels (Bestrophin-1, Ano-1, BK, and SK channels) in the hippocampal neurons. For this screening purpose, expression patterns were examined under the confocal microscope and structured illumination microscopy (SIM) imaging. From the confocal and SIM images, we found that some ion channel combinations can be assigned as "co-localization" and others as "non-colocalization". We performed to obtain quantitative results. Further analysis currently will be planning to visualize ion channels clustering patterns in the cortical pyramidal neurons by using SIM and their functional consequences by using patch-clamp recordings.

* This work was supported by the KBRI Basic Research Program funded by the Ministry of Science and ICT (19-BR-01-02 to H.-H. L) and by the NRF Brain Research Program funded by the Ministry of Science and ICT (2017M3C7A 1048086 to H.-H. L).

[PHY-15]

Kinetics of full-length PD-1:PD-L1 interaction at the single-molecule level

Gee Sung Eun, Tae-Young Yoon

School of Biological Sciences, Seoul National University
E-mail: dmswlt@gmail.com

Programmed cell death protein-1 (PD-1), one of the most well-known immune checkpoint protein interacts with its ligand PD-L1 to propagate inhibitory signal into T-cells. As membrane proteins, the extracellular domains of PD-1 and PD-L1 molecules are well studied, while the structure or structural dynamics of the proteins including the cytosolic part are yet to be discovered. Here, we utilize the full-length form of PD-1 and PD-L1, to observe the PD-1:PD-L1 protein-protein binding kinetics at the single-molecule level. With the help of single-molecule fluorescence spectroscopy, we report detailed association/dissociation kinetics of full-length PD-1:PD-L1 interaction. Full-length PD-1 molecules showed similar dissociation kinetics compared to extracellular domain-only PD-1, but faster (one-order) association kinetics compared to extracellular domain-only form. Additionally, full-length PD-1 molecules showed heterogeneous distribution of association kinetics dependent to their stoichiometry. The results demonstrate the strength of PD-1:PD-L1 interaction at the immune synapse potentially far exceeds the affinity that has been investigated with the extracellular domains of PD-1 and PD-L1.

References

[1] Cheng, X et al. Structure and interactions of the human programmed cell death 1 receptor. *Journal of Biological Chemistry* (**2013**)

[COM-1]

Functional networks study of GPCR and Gα proteins using coevolution analysis

Min Jae Seo[1], Kyeonghui Kim[2], Joongyu Heo[2] and Wookyung Yu[1]*

[1] *Department of Brain and Cognitive Sciences,*
[2] *School of Undergraduate Studies, Daegu Gyeongbuk Institute of Science and Technology (DGIST), 333 Techno jungang-daero, Daegu 42988, Korea*
**E-mail: wkyu@dgist.ac.kr*

Heterotrimeric G proteins coupling with G-protein coupled receptors (GPCR) conduct major signal transductions in diverse cell environments. Ligand binding on GPCR leads to conformational change that transduces a signal to G protein. Then G protein is dissociated into Gα and Gβ-Gγ complex to trigger various biological pathways. Even though this system is important for physiological responses and related to many diseases, it is not clearly solved whether which residues are important. In this study, we investigated Gα protein, which has direct interaction with GPCR, from the evolutionary view using statistical coupling analysis (SCA). SCA captured co-evolving residues among various species and organized them into functional subunits, so-called sectors. We rearranged the positions of these sectors to get more clearly distinguish networks and mapped on the structure of β2AR-G protein complex. Through these processes, we found five sectors that compose structure-to-functional networks and they are more clear than original SCA results on residue level. Furthermore, we applied to these methods on GPCRs (Currently on-going project), especially serotonin and dopamine receptor.

References

[1] Flock, T., Hauser, A. S., Lund, N., Gloriam, D. E., Balaji, S., & Babu, M.M. Selectivity determinants of GPCR-G-protein binding. *Nature* **(2017)**
[2] Halabi, N., Rivoire, O., Leibler, S., & Ranganathan, R. Protein sectors: evolutionary units of three-dimensional structure. *Cell* **(2009)**
[3] Alhadeff, R., Vorobyov, I., Yoon, H. W., & Warshel, A. Exploring the free-energy landscape of GPCR activation. *PNAS* **(2018)**

[COM-2]

Site-Directed Analysis of Folding Thermodynamics of WW Domain

Myung Keun Cho,[1,2,*] Song-Ho Chong,[1] Seokmin Shin,[2] and Sihyun Ham[1,*]

[1]*Department of Chemistry, The Research Institute of Natural Sciences, Sookmyung Women's University, Cheongpa-ro 47-gil 100, Yongsan-Ku, Seoul 04310, Korea*
[2]*Department of Chemistry, Seoul National University, Seoul 08826, Korea*

**E-mail: mktonycho@snu.ac.kr , sihyun@sookmyung.ac.kr*

The native structure of a protein is stabilized by a number of interactions such as main-chain hydrogen bonds and side-chain hydrophobic contacts. However, it has been challenging to determine how these interactions contribute to protein stability at an individual amino acid resolution. Here, we quantified site-specific thermodynamic stability at the molecular level to extend our understanding of the stabilizing forces in protein folding. Tens of μs-length molecular dynamics simulation study are carried out on the human Pin WW domain. Starting from the determination of its folding free energy that is comparable to the experimental value, we derive the free energy of site-specific backbone and side-chain moieties for every residue separately. The side-chain packing contributed significantly more stability than the backbone scaffold interactions from the *β*-sheet. The hydrophobic clusters formed from side-chain packing are mainly stabilized by hydrophobic interactions, incorporation of a salt-bridge to enlarge the cluster size, and the lack of hydrogen bonding of side-chain around the center of the cluster. Identification of stabilizing chemical interactions in a folding protein at various contexts will provide an insight into understanding the origin of the protein structure and into engineering a better protein.

[COM-3]

Comparison of the structural and thermodynamic characteristics between Tau43 and Aβ42

Le Thi Diem and Sihyun Ham*

Department of Chemistry, Sookmyung Women's University
Cheongpa-ro 47-gil 100, Yongsan-Ku, Seoul 04310, Korea
**E-mail: sihyun@sookmyung.ac.kr*

Recent studies on the field of Alzheimer's disease (AD) has been attempted to investigate the precise mechanisms leading to the formation of senile plaques and neurofibrillary tangles, both of those are known as two hallmark lesions of this neurodegenerative disease. Senile plaques formed by Aβ-amyloid aggregation in which Aβ42 is the dominant component of plaques seen in AD. The formation of the neurofibrillary tangles (NFT) caused by the aggregation of Tau protein in the form of paired helical filaments (PHFs), which can initiate self-assembly into the pathological PHF in vitro, is Tau43. Especially, Tau43 and Aβ42 contain critical regions of full-length Tau protein and Aβ-amyloid for the aggregation process, respectively. However, the aggregation propensity of Aβ42 is well known, whereas the Tau43 self-assembly mechanism is still far from completely understood. Therefore, we investigate the structural characteristics and the striking "aggregation-prone" of Tau43 compared to Aβ42 to acquire a greater comprehensive insight into the early-state aggregated Tau43. Herein, we performed multiple all-atom molecular dynamics simulations Tau43 in explicit water combined with solvation thermodynamic analysis. It is observed that Tau43 exhibits more aggregation propensity than Aβ42. Remarkably, assembly of Tau43 fragment containing the third repeat of tau protein is accompanied by a transformation of the hexapeptide 11VQIVYK16 into a β-structure through hydrophobic steric zipper interactions of residue V11, I13, and Y15. The knowledge of this principle governing Tau43 aggregation can be considered as Aβ42 amyloid aggregation in terms of the formation of aggregate species-rich in β-sheet structure. Thereby, our aim to identify the structural and thermodynamic characteristics of the minimal sequence of Tau43 monomer and further provision an explanation of early Tau aggregation as well as provide useful information for Alzheimer aggregation treatment for Tau and Aβ proteins.

[COM-4]

Comparative Study on the Binding Properties between Bridged α-helix Mimetic Small Molecule/MCL-1 Complexes

Haeri Im and Sihyun Ham*

Department of Chemistry, Sookmyung Women's University
Cheongpa-ro 47-gil 100, Yongsan-Ku, Seoul 04310, Korea
**E-mail: sihyun@sookmyung.ac.kr*

Anti-apoptotic protein MCL-1 promote cellular survival by trapping the critical apoptosis-inducing BCL-2 homology domain 3 (BH3) α-helix of pro-apoptotic BCL-2 family members. Cancer cells exploit this physiologic survival mechanism through anti-apoptotic protein overexpression, establishing an apoptotic blockade that secures their immortality. Inhibition of interaction between the BH3 and MCL-1 is thus significant for suppressing the growth of cancer cells. Recently, scaffolds as α-helix mimetic small molecule such as various triazine-piperazine-triazine structures have been designed for binding affinity with MCL-1. Here, we report the structural and thermodynamic characteristics for the binding complexes of the α-helix mimetic small molecules to MCL-1. We performed molecular dynamics simulations to investigate the structural properties of the complexes. The binding free energy calculations based on the integral equation theory was then executed to quantify the binding affinity for the complexes and to understand the factors responsible for the binding affinity.

[COM-5]

Free Energy Landscape Characterization of the Coupled Folding and Binding Process

Song-Ho Chong, Haeri Im and Sihyun Ham*

Department of Chemistry, Sookmyung Women's University
Cheongpa-ro 47-gil 100, Yongsan-Ku, Seoul 04310, Korea
**E-mail: sihyun@sookmyung.ac.kr*

The most fundamental aspect of the free energy landscape of proteins is that it is globally funneled such that protein folding is energetically biased. Then, what are the distinctive characteristics of the landscape of intrinsically disordered proteins, apparently lacking such energetic bias, that nevertheless fold upon binding? Here, we address this fundamental issue through the explicit characterization of the free energy landscape of the paradigmatic pKID–KIX system. This is done based on unguided, fully atomistic, explicit-water molecular dynamics simulations with an aggregated simulation time of >30 microseconds and on the computation of the free energy that defines the landscape. We find that, while the landscape of pKID before binding is considerably shallower than the one for a protein that autonomously folds, it gets progressively more funneled as the binding of pKID with KIX proceeds. This explains why pKID is disordered in a free state, and the binding of pKID with KIX is a prerequisite for pKID's folding. In addition, we observe that the key event in completing the pKID–KIX coupled folding and binding is the directed self-assembly where pKID is docked upon the KIX surface to maximize the surface electrostatic complementarity, which, in turn, require pKID to adopt the correct folded structure. This key process shows up as the free energy barrier in the pKID landscape separating the intermediate nonspecific complex state and the specific complex state. The present work not only provides a detailed molecular picture of the coupled folding and binding of pKID, but also expands the funneled landscape perspective to intrinsically disordered proteins.

[COM-6]

Molecular dynamics study for potential Abl tyrosine kinase inhibitors derived from 2-pyrazolinyl-1-carbothioamide

Beom Soo Kim[1], Sang Won Jung[2], Wookyung Yu[1,2,*]

[1]*Department of Brain and Cognitive Sciences, Daegu GyeongbukInstitute of Science and Technology (DGIST), 333 Techno jungang-daero, Daegu 42988, Korea*
[2]*Center for Supercomputing and Big Data, Daegu GyeongbukInstitute of Science and Technology (DGIST), 333 Techno jungang-daero, Daegu 42988, Korea*
**E-mail: wkyu@dgist.ac.kr*

The ABL 1 is proto-oncogene encoding non-receptor tyrosine kinase that is one of the proteins cause chronic myeloid leukemia (CML). Many Abl tyrosine kinase inhibitors were developed to treat CML, and marketed like imatinib, dasatinib, and so on. However, the issue of their side effect is still discussed. Our previous study reported that derivatives of 2-pyrazolinyl-1-carbothioamide (2PC) synthesized from chalcone showed inhibitory effects to Abl kinase and their structure and activity relationships (SAR) were also analyzed by QSAR. In this study, after predicting their molecular binding mode between 2PC and Abl kinase using in silico docking for 11 derivatives, we carried out molecular dynamics (MD) simulations to identify the dynamic behaviors of Abl kinase upon the binding of 2PC. The results of Principal Component Analysis (PCA) showed the motion of Abl kinase upon ligands binding, and relationships between protein motion and activities. In addition, the binding free energy between Abl kinase and 2PC was calculated by molecular mechanics Poisson-Boltzmann and generalized-Born surface area (MM/PBSA and MM/GBSA), and there are some correlations between binding free energy and experimental activities.

Acknowledgement

[1] We thank the DGIST supercomputing and big-data center for providing supercomputing resources.

[COM-7]

Mutation Effects on FAS1 Domain 4 Based on Solubility and Structure

Donggun Kim[1),2)], Song-Ho Chong[1)], Seokmin Shin[2)]* and Sihyun Ham[1)]*

1) Department of Chemistry, Sookmyung Women's University, Hyochangwon-gil 52, Yongsan-gu, Seoul 140-742, Korea

2) Department of Chemistry, Seoul National University, Seoul 08826, Republic of Korea

**E-mail: seokmin.shin@gmail.com, sihyn@sookmyung.ac.kr*

Mutations in the Fasciclin 1 domain 4 (FAS1-4) of transforming growth factor beta-induced protein (TGFBIp) are associated with opaque extracellular deposits and corneal dystrophies (CD). The decrease in solubility upon mutation has been implicated in CD, but exact molecular mechanisms are not well understood. In this study, we perform molecular dynamics simulations followed by the solvation thermodynamic analyses of the R555W and R555Q mutants of FAS1-4 domain connected to granular corneal dystrophy type 1 and Thiel-Behnke corneal dystrophy, respectively. We find that two mutants have less affinity toward solvent relative to the wild-type protein. In R555W mutant, we observe a remarkable increase in the solvation free energy because of the structural changes near the mutation site. The mutation site W555 is buried in other hydrophobic residues and R557 makes salt bridges with E554 and D561 at the same time. In R555Q mutant, on the other hand, the increase in the solvation free energy is caused by rearrangements far from the mutation site. R558 makes salt bridges with D575, E576 and E598 separately. We thus identify the relationship between the decrease in solubility and conformational changes from a molecular perspective, which can be useful to design potential therapeutics and to block FAS1 aggregation related to corneal dystrophy.

[COM-8]

Convergence of Configurational Entropy in Protein Folding

Minwoo Kim[1,2], Song-Ho Chong[1], Seokmin Shin[2] and Sihyun Ham[1*]

1. Department of Chemistry, Sookmyung Women's University, Seoul 04312, Korea

2. Department of Chemistry, Seoul National University, Seoul 08826, Korea

**E-mail: sihyun@sookmyung.ac.kr*

The configurational entropy is an important property for understanding biological processes such as protein folding and protein-ligand binding. However, the convergence of the configurational entropy is the most challenging issue. In this study, we investigate the configurational entropy of both folded and unfolded protein states using several computational methods developed so far: Quasi-harmonic (QH) method, dihedral distribution histogramming (DIH), mutual information expansion (MIE), maximum information spinning tree (MIST) and energetic approach. Conformational sampling was conducted via micro-seconds of molecular dynamics (MD) simulations of human Pin1 WW-Domain protein structures. Fast convergence of the configurational entropy can be seen owing to the introduction of the Gaussian approach, which makes the fluctuation of the structural or energetic properties predictable during the sampling. We can expect that the fast-converging entropy calculation methods can be used for the future researches which need approximate protein folding entropy estimations.

[APP-1]

DNAJA1 as a novel CRL4^{CRBN} substrate induces resistance to stress and tau pathology

Uroos Akber[1], Seung-Joo Yang[1,2], Seung-Je Jeon[1,2], Heeji Jo[1,2], Sungsu Lim[3], Yun Kyung Kim[3], Sun Ah Park[4], Chul-Seung Park[1,2*]

[1]Laboratory of Molecular Neurobiology, School of Life Sciences, and [2]Aging Research Institute, Gwangju Institute of Science and Technology (GIST), Gwangju 61005, Republic of Korea
[3]Center for Neuromedicine, Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul 136-791, Republic of Korea
[4]Laboratory for Neurodegenerative Dementia, Department of Anatomy and Department of Neurology, Ajou University School of Medicine, Suwon 16499, Republic of Korea
[*]Correspondence: cspark@gist.ac.kr
Presenter: uroos@gist.ac.kr

Tauopathies are neurodegenerative diseases involving conformational changes in protein tau, while chaperones play neuroprotective role to avert tauopathies. Here we identified tau, and chaperones HSP70 and DJANA1 as endogenous substrates of CRBN, a substrate-recruiting subunit of cullin4-RING E3 ubiquitin ligase. We focused attention on DJANA1 (DJ2) as TAU and HSP70 are well characterized. C-terminal domain of DJ2 binds to N-terminal Lon-domain of CRBN and two Lys residues (Lys32 and Lys350) of DJ2 are specifically ubiquitinated for subsequent degradation. Knocking-out CRBN enhances chaperonic activity of DJ2. Consequently, tau enters into a dynamic folding pathway and associates with microtubules rendering cells intact and functional. Depletion of CRBN also decreases activity of tau kinases including GSK3a/b, ERK and p38. Prion-like spread of tau is prevented across the brain of Crbn-/- mice after stereotaxic-injection of okadaic acid. Our findings could have important implications for neurodegeneration as chemical knockdown of Crbn may decelerate the progression of diseases because of the likelihood of therapeutic intervention of DJ2.

Acknowledgement

We thank Dr. Kim Hyoung-Ihl and Jee-Young Park for the technical assistance during stereotaxic surgery and Dr. Woo Jin Park for providing APP Knock-in and 5XFAD mice. We are also grateful to Dr. Lee Kyung Hwa for providing human brain samples. This work was supported by a GIST Research Institute (GRI) grant funded by the GIST in 2019, and Original Technology Research Program for Brain Science (NRF-2018M3C7A1056293) of the National Research Foundation of Korea (NRF) funded by the Korean government, MSIT.

[APP-2]

Visualization of different autophagy stages by multicolor autophagy sensor

Heejung Kim[1,2], Hyunbin Kim[1,2], Jaesik Choi[3], Kyung Soo Inn[2]* and Jihye Seong[1,2,4]*

[1]Center for Diagnosis Treatment Care of Dementia, Korea Institute of Science and Technology, Korea,
[2]Department of Converging Science and Technology, Kyung Hee University, Korea,
[3]Graduate School of Artificial Intelligence, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea
[4]Division of Bio-Medical Science & Technology, KIST School, Korea University of Science and Technology, Seoul, Republic of Korea
**E-mail: jesong@kist.re.kr*

Autophagy is an essential degradation pathway by which cells digest their own cytoplasmic constituents within lysosomes. When lysosomes are fused with autophagosomes to form autolysosomes, pH of autophagosomes is acidified by lysosomes. Previous fluorescent sensor for monitoring the acidification of autophagosome contains LC3 tagged with tandem fluorescent proteins (FPs), a pH-sensitive SEP and a reference FP mKate2. Here, we improved the previous sensor to visualize different stages of autophagy, by replacing FPs with a stable blue reference BFP, a pH-sensitive green SEP, and a red RFP with intermediate pKa. Utilizing this multicolor autophagy sensor, we can calculate the pH of the autophagosome which represents different stages of autophagy process. Indeed, we monitored the autophagy flux upon the treatment of rapamycin. Furthermore, our multicolor autophagy sensor was applied to study how different forms of amyloid beta can affect the process of autophagy. Therefore, we developed a new multicolor autophagy sensor, which can visualize different stages of autophagy from early autophagsome to stages of autolysosome. Our advanced fluorescent autophagy sensors, real-time imaging in live cells, and automated vesicle analysis program will be further applied to visualize different stages of autophagy in neurodegeneration diseases.

Acknowledgement

[1] This work is supported in part by the National Research Council of Science & Technology (NST) grant by the Korea government (No. CRC-15-04-KIST) (J.S.), KIST Institutional Grant 2V06750 (J.S.).

[APP-3]

Employing SNARE accessory proteins as a drug that inhibits neurotransmitter release

Haesoo Kim, Min Ju Shon, & Tae-Young Yoon

School of Biological Sciences and Institute for Molecular Biology and Genetics, Seoul National University, Seoul 08826, South Korea
E-mail: engagewaters@gmail.com

The major players responsible for neurotransmitter release are soluble NSF-attachment protein receptor (SNARE) proteins. Molecules associating with SNARE proteins, named SNARE accessory proteins (SNAP), are a group of proteins that participate for operation of neurotransmitter release. Among SNAPs, complexin(cpx) is known to destabilize C-terminal region of SNARE complex[1]. We previously discovered the inhibition occurs via N-terminal domain of cpx[2]. We went a step further and engineered N-terminal region of cpx expecting that it can strongly inhibit SNARE complex formation to arrest neurotransmission.

Surprisingly, we observed that in PC12 cells, the designed molecule inhibited neurotransmitter emission which corresponds to decreased synaptic transmission activity.

In conclusion, we developed engineered cpx based on its functional dynamics, with decreased neurotransmitter release activity. The engineered cpx might thus be applied to construct second generation drugs that substitutes botulinum toxin.

References

[1] Giraudo, C. G., A Clamping Mechanism Involved in SNARE-Dependent Exocytosis. Science, 313(5787), 676–680. (**2006**)
[2] Shon, M. J., Kim, H., & Yoon, T. Y. , Focused clamping of a single neuronal SNARE complex by complexin under high mechanical tension. Nature Communications, 9(1), 1–12., (**2018**)

[Late-1]

De novo designed decapeptide isomers with potent antibacterial activities and anti-inflammatory potentials

Su-Jin Kim[1], Sung-Hee Lee[2], Na-Young Kim[1], Eun-Jae Jeon[1], Kibin Lee[1], Hyung-Sik Won[1,]*

[1]*Department of Biotechnology, Konkuk University, Chungju, Chungbuk 27478, Korea*
[2] *College of Pharmacy, Chungbuk National University, Cheongju, Chungbuk 28160, Korea*
**E-mail: wonhs@kku.ac.kr*

Antimicrobial peptides (AMPs), also called host defense peptides (HDPs), particularly those with amphipathic helical structures, are emerging as target molecules for therapeutic development due to their immunomodulatory properties [1]. In particular, engineered HDPs with shorter length and simpler amino acid composition can be better candidates for clinical and commercial development [2,3]. Here, we generated a series of *de novo* designed decapeptide isomers, named WALK (tryptophan-containing amphipathic helical leucine/lysine)-244 peptides, consisting of three kinds of amino acids to have the same $L_4K_4W_2$ formula. Amphipathic helical properties were conferred by using four leucines and four lysines and two tryptophan residues were positioned at the critical amphipathic interface between the hydrophilic ending side and the hydrophobic starting side. According to this specified rule, 10 model peptides were generated and their helical propensity was confirmed by circular dichroism spectroscopy. Their antimicrobial and anti-inflammatory activities were compared with those of the known 12-residue peptide agent, omiganan, which is currently under therapeutic and commercial development. All of the decapeptide isomers exhibited a stronger antibacterial activity than omiganan, against both Gram-negative (*E. coli*) and Gram-positive (*B. subtilis*) bacteria. Based on nitric oxide assay results in macrophage cells, some of the decapeptides also showed promising anti-inflammatory potentials, which were more potent than that of omiganan. These results not only suggest useful candidates for development of novel antibiotic and/or anti-inflammatory agents, but also provide an efficient strategy to design such peptides.

References

[1] Shim, D.-W. *et al*., Anti-Inflammatory action of an antimicrobial model peptide that suppresses the TRIF-dependent signaling pathway via inhibition of Toll-like receptor 4 endocytosis in lipopolysaccharide-stimulated macrophages. *PLoS One* (**2015)**.
[2] Kim, S.-J. *et al*., Structural characterization of *de novo* designed L_5K_5W model peptide isomers with potent antimicrobial and varied hemolytic activities. *Molecules* (**2013)**.
[3] Lee, S.-H. *et al*., *De novo* generation of short antimicrobial peptides with simple amino acid composition. *Regul. Pept.* (**2011)**.

Acknowledgement

This work was supported by a project (no. S2728998) for Collaborative R&D between Industry, Academy, and Research Institute, funded by Korea Ministry of SMEs and Startups in 2019.

[Late-2]

Anoctamin 1/TMEM16A in pruritoceptors mediates histamine-independent itch

Hyesu Kim[1,2], Huanjun Lu[1], Hyungsup Kim[1], Jae Hyouk Choi[1], Sungmin Pak[1], Thien-Luan Nguyen[1], Uhtaek Oh[1]

[1]Brain Science Institute, Korea Institute of Science and Technology, Seoul, Korea; [2]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea

**E-mail: ssuu63@kist.re.kr*

Itch is an unpleasant sensation that causes a desire to scratch. Antihistamines are used to treat pruritus, but many patients with chronic itch may not be sensitive to antihistamine treatment. Several molecules and their circuits for itch transmission have been identified. TRPV1 is involved in mediating histaminergic itch. Mrgpr (Mas-related G protein-coupled receptor) family genes are itch receptors for non-histaminergic pruritogens, such as chloroquine(CQ) or SLIGRL. Here we found that anoctamin 1 (ANO1/TMEM16A), a Ca^{2+} activated chloride channel, is a primary transduction channel that mediates non-histaminergic itch signals. Pruritogens were injected into the nape of the neck subcutaneously to induce itch. Ano1-deficient (Adv/ Ano1$^{fl/fl}$) mice showed a significant reduction in scratching behaviors in response to non-histaminergic pruritogens like CQ or SLIGRL injection, as well as dry-skin condition, but not to histamine injection. These pruritogens activate DRG neurons via ANO1 both in vitro and in vivo. The application of CQ and SLIGRL to cultured DRG neurons excited these neurons. However, the CQ and SLIGRL evoked excitation was markedly reduced in DRG neurons from Adv/ Ano1$^{fl/fl}$ mice or when the pruritogens were co-applied with MONNA, a selective ANO1 blocker. These results clearly suggest that ANO1 mediates histamine-independent itch signaling in pruriceptors.

[Late-3]

Focal Adhesion Dependent Activation of Mechanosensitive Channel Tentonin 3/ TMEM150C

Sungmin Pak[1,2], Gyusang Hong[1], Mi-Ock Lee[2], Uhtaek Oh[1].

[1]*Korea Institute of Science and Technology*, [2]*Seoul National University*

**E-mail: mingee992@gmail.com*

Tentonin3/TMEM150C (TTN3) is a mechanosensitive ion channel that has characteristics of slowly inactivating kinetics by mechanical stimulation. Recent studies have proposed whether TTN3 is an ion channel and its independent channel activity is validated. The opening mechanism of mechanosensitive ion channels have been suggested into two theories. One theory is regarding the activation through direct cell plasma membrane stretch, for example, the Piezo family. Another theory pertains the influence of tethering proteins near the plasma membrane activating mechanosensitive channels, for example, MEC complex in *C. elegans*. Focal Adhesion (FA) Complex is a multi-protein assembly of transmembrane proteins and intracellular proteins that provides adhesion between the cell and the extracellular matrix. We report that the FA Complex of Piezo1-Knockout (P1KO) HEK293T cells is hampered and selective knockdown of FA related proteins, Talin1, Vinculin and Integrin, diminished TTN3 mechanical current in *Ttn3*-transfected HEK293T cells. Therefore, we propose the disturbed FA Complex in P1KO HEK293T cells is responsible for the absence of TTN3 mechanical current in *ttn3*-transfected P1KO-HEK293T cells.

Acknowledgement (if necessary)

[1] This work was supported by the Korea Research Foundation Grant (code KRF-2001-331-E00457 and 2002-0076543), the Republic of Korea.

Federation
of the Korean Societies for
Biomolecular Sciences
(FKSBS)

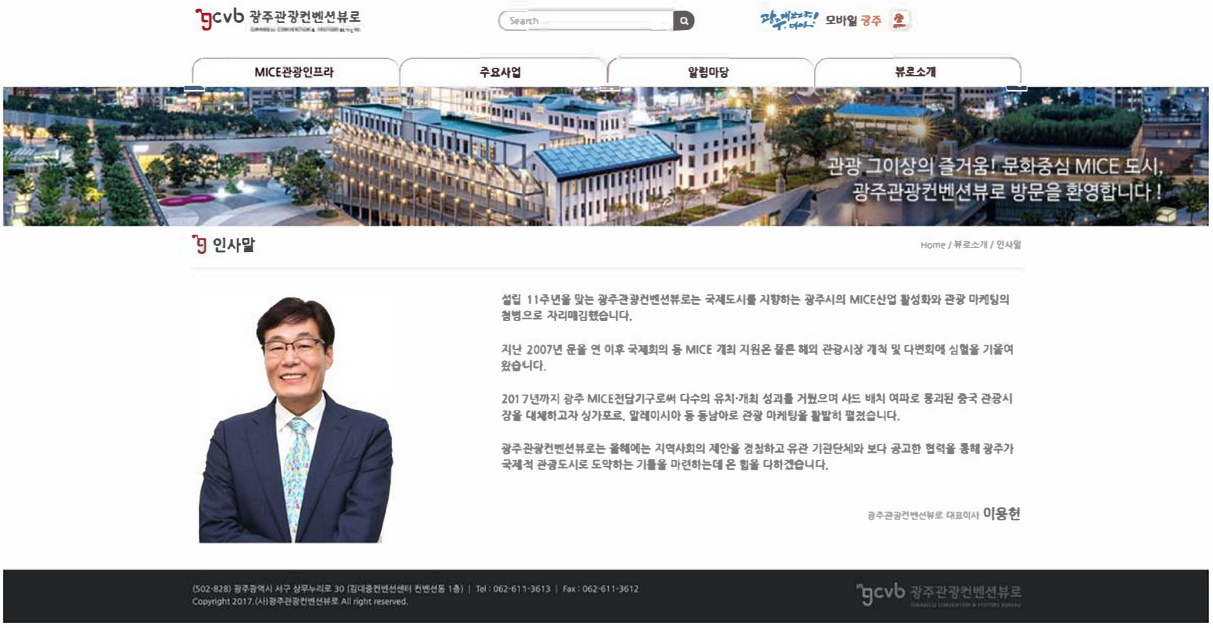
Federation
of the Korean Societies for
Biomolecular Sciences
(FKSBS)

2020

한국생체분자과학 연합학회학술대회

Sponsors

GWANGJU CVB
광주관광컨벤션뷰로



Sino Biological

High Quality !

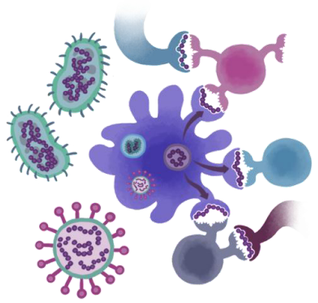
Cost-Effective!

Recombinant Protein

- >85% expressed by eukaryotic cells
- Bioactivity validated
- High purity & low endotoxin
- Carrier free
- Multiple species & various tags

cDNA clone

- Gene bank: 11,000+ molecules, 400,000+ cDNA clones
- Multiple species
- Various tags : GFPSpark®, OFPSpark®, Flag, Myc, His, HA
- Full length sequence confirmed
- 100% accurate sequence guaranteed



- **Recombinant protein, cDNA, Antibody** 등 제공
- **100% produced in-house**, no OEM products
- **고품질 / 저렴한 가격**의 제품 제공

국 내 독 점 대 리 점

LEEHYO (주)이효바이오싸이언스

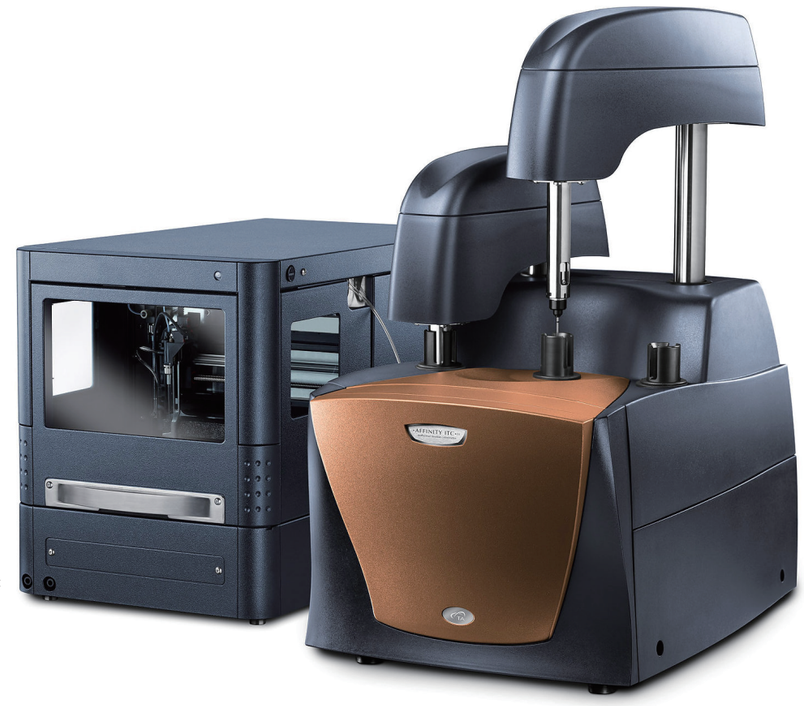
본 사 : 경기도 성남시 수정구 복정로 107 이효빌딩

Tel : (031)722-2382 Fax : (031)759-7642

광주지사 : 광주광역시 광산구 첨단내촌로 47 (월계동)

Tel : (062)973-1230 Fax : (062)973-1223

What others have ATTEMPTED, TA has PERFECTED



Affinity ITC

The Affinity ITC is a fully automated, highly sensitive, titration calorimeter for characterizing a wide variety of molecular interactions.

Features:

- **NEW** 96-well temperature-controlled liquid handling with *Intelligent Hardware Positioning* for walk-away automation and precise, reliable injections.
- **NEW** Flex Spin™ for the widest range of stirring speeds. Slow speed mixing (up to 10X lower than competitive units) for high sensitivity and to protect delicate structures.
- **NEW** AccuShot™ introduces samples at the right location for efficient mixing.
- **NEW** fully automated and user-selectable system cleaning routines eliminates run-to-run contamination.
- **NEW** Nano-Analyze software with batch processing for automated data analysis.
- The only automated ITC system with 190µl or 1ml sample cells.

FlexSpin™ | AccuShot™

TA® tainstruments.com

WYATT TECHNOLOGY 광산란기기 전문회사 와이엇 테크날러지

단백질, 접합체 나노입자 특성해석

INSUNG 인성크로마텍

1. 다각도 광산란 검출기 (MALS: Multi-Angle Light Scattering)

단백질(Proteins), 접합체(Conjugates), 그리고 나노입자(Nanoparticles)의 몰질량(Molar mass), 크기(Size)를 측정. 다각도 광산란(MALS: Multi Angle Light Scattering검출기는 배치모드(Batch Mode)나 GPC/SEC/FFF 기기에 연결하여 사용합니다.

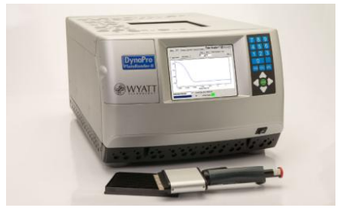
1-1) 돈 (DAWN) – 신제품 NEON 시리즈

- 18개 산란각
- On-line/Batch mode
- **Molar mass range**: 200 Da to 1000 MDa
- **Molecular size range**: 10 to 500nm, up to 1000 nm with shape-specific models
- **Molecular Size Range** (Rh, with WyattQELS DLS module or DynaPro NanoStar): Depends on the scattering angle, In flow mode, 0.5 nm – 300 nm, batch mode, 0.5 nm – 1 µm
- **Sensitivity:** 0.4 µg/mL BSA (66.4 kDa)
- Ultra-stable, high-power laser

2. 동적 광산란 검출기 (DLS: Dynamic Light Scattering)

단백질 (Proteins), 펩타이드 (Peptide), 계면활성제 마이셀(Surfactant micelles), 합성고분자 (Synthetic polymers), 나노입자 (Nanoparticles), 양자점 (Quantum dots), 백신 (Vaccines)을 최소 부피로 빠르고, 정확하게 특성해석

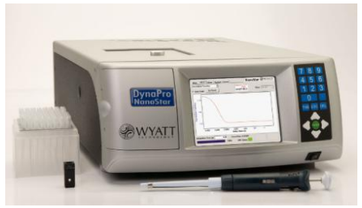
2-1) 다이나프로 나노스타 플레이트 리더 III (DynaPro Plate Reader III)



*** Application**
- Biotherapeutics
- Nanoparticle
- Polymer-encapsulated drug particles

- **Hydrodynamic radius range**: 0.5 nm to 1000 nm
- **Sensitivity** down to 0.125 mg/mL lysozyme
- **Industry standard microwell plates** of 96, 384 or 1536 wells
- **All measurements made *in situ* in the wells**
- **Temperature ramps** from 4°C to 85°C

2-2) 다이나프로 나노스타 (DynaPro NanoStar)



- **Rh** from 0.18 nm - 2500 nm
- **Temperature** from -15°C to +150°C
- **Molecular weight** from 300 Da to 1 MDa
- Separate, optimized SLS and DLS detection channels
- Auto-attenuation over five orders of magnitude

인성크로마텍㈜
서울특별시 양천구 신목로 58-9 (우: 158- 849) Tel:02-2644-1991 FAX: 02-2644-1996
Homepage: www.insung.net 인성컬럼즈: www.insungcolumns.com E-mail: is@insung.net

더 많은 가능성을 위한 도전

ProCell ProCell Therapeutics, Inc.

We Challenge, We Change

바이오 산업의 거대한 가능성을 열어 나가는
바이오헬스기업 프로셀테라퓨틱스는 MITT™ (거대분자 세포 내 전송기술)
원천기술을 기반으로 한 항암제 신약 개발과 경피투과 향장원료 개발에 주력하고 있습니다.

또한, 혁신적 기술과 창의적 연구 자세로 글로벌 시장과 경쟁하고 있으며,
3세대 MTD™ (거대 분자 전송 도메인) 개발을 통한 맞춤형 연구를
통해 다양한 분야로 진출하고 있습니다.

Contact: 82-2-6675-7202 / procellrx@procellrx.co.kr

Research & Development Services

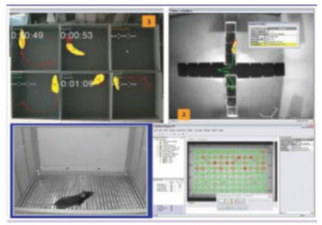
- MITT Application Service
- Process Development Service for Protein Production
- Active Ingredient Development Service for Cosmetics
- Transome (MTD Liposome) Development Service
- OEM/ODM Service for Cosmetics

Laboratory and Medical Research Products

Scitech Korea

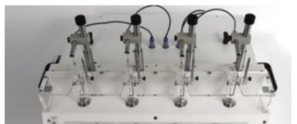
40 years Knowhow!!
300 cooperated company!!

ECG,EEG,EMG Acquisition System

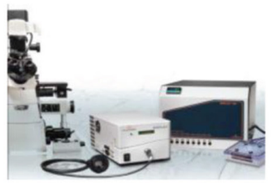


Noldus EthovisioXT Video Tracking system

Surgery Instrument

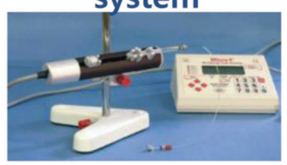


Organ&Tissue Bath



Live cell imaging system

Leaning&memory Test



Pump & Perfusion System

Animal behavior test

Sutter Manipulator

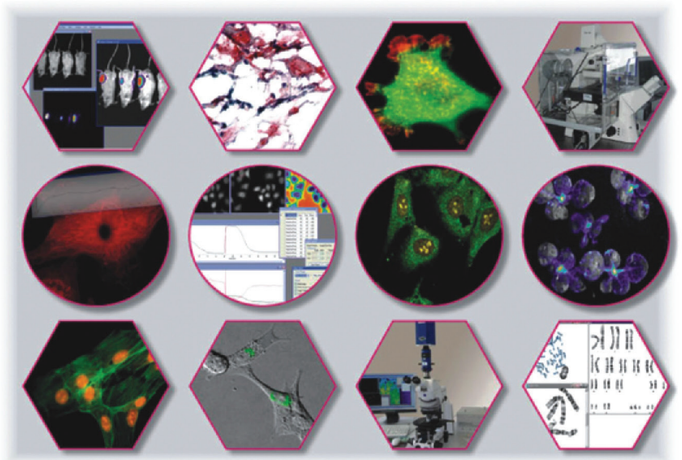
Axon Patch Clamp System

(주)싸이텍코리아 TEL : 02-986-4413~5 서울시 강북구 덕릉로40길 74, 801호

E-mail : info@scitechkorea.com Homepage : www.scitechkorea.com

High Performance Microscopy Systems

Live Cell Imaging, 6D Microscopy, Confocal Microscoy
TIRF, High-Speed Time Lapse, Calcium, Ratio Imaging,
Electrophysiology, 3D/4D Imaging, Deconvolution,
Histology, In-vivo Mouse Imaging, FRAP, Cell tracking
Ablation, Laser illumination, Cell Motiligy, Luminescence



에드사이언 ADDSCIEN

T. 02-2611-3696
E-mail. addscien@addscien.com

서울특별시 구로구 경인로 55길 58

www.addscien.com

chameleon

Next generation sample preparation for cryo-EM based on Spotiton

혁신적인 **Nanowire grid**와 **pico-liter dispensing**으로 blotting 없이 빠르고 완벽한 ice thickness의 **Cryo-EM sample preperation**

지금까지의 Cryo-EM의 bottleneck인 다량의 sample 사용, 수작업의 grid 준비, 최적의 ice thickness 형성의 어려움 등을 한번에 해결할 수 있습니다.

Grid에 sample spotting parameter를 camera로 지속적으로 monitoring 함으로써 재현성 있는 실험을 수행할 수 있으며 good quality의 grid 선택이 가능합니다.

a rapid and efficient path to quality frozen grids for cryo-EM

blot-free high speed plunging
reduce air-water interface effects and enable difficult samples

virtually no manual handling
eliminate damage and loss via precise automated grid handling

screen fewer grids at the microscope
select good grids based on visual images during freezing

easy to use even for novice users
guided workflows enables simple set up, use and cleaning

record keeping for future repeatability
capture all relevant grid parameters and images

safe storage and control of cryogens
automated drawer offers level sensing and temperature control

ttplabtech

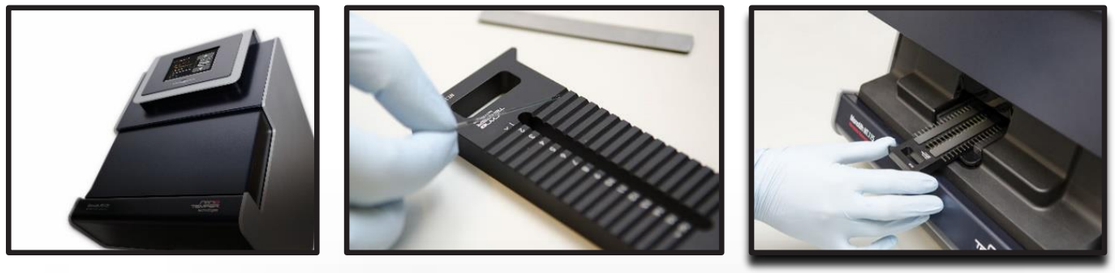
MDxK (주)엠디엑스케이
T : 02 - 578 - 8848
F : 02 - 578 - 8838
info@mdxk.co.kr
www.mdxk.co.kr

nanoTEMPER
www.nanotemper.de

Monolith NT.115 Series

Microscale Thermophoresis (MST) Technology를 이용하여 Affinity (Kd, dissociation constant)를 측정하는 새로운 방법

- **빠른 측정 시간 (약 10분)**
- Surface immobilization 없이 Affinity 측정
- 1회 교육으로 누구나 장비 & 소프트웨어 사용 가능
- 장비 관리 필요 없음
- 넓은 dynamic range : pM to mM dissociation constants
- 낮은 샘플 소비량 : **labeled target : <50nM/160µL**
  Ligand : Kd 값의 100배/20µL
- Purification Free : Cell lysate and serum 이용하여 측정 가능



- 다양한 분자간의 상호 작용 측정 가능

  - ✓ protein - protein
  - ✓ protein - DNA/RNA
  - ✓ nucleic acid - nucleic acid
  - ✓ protein - small molecule, peptides, ions
  - ✓ protein or small molecule - HMW complexes (e.g. ribosome)
  - ✓ protein or peptide - liposome/vesicle
  - ✓ ligand binding to membrane receptors

Demonstration 가능합니다. 데모 요청이나 제품에 관한 문의사항은 동영과학㈜으로 연락 주세요.

동영과학(주) DONGYOUNG
경기도 안양시 동안구 흥안대로 415 두산벤처다임 528
TEL : 031-478-4451 FAX : 031-478-3068
E-mail : product@dongyoung.co.kr
http : www.dongyoung.co.kr

ARI
Art Robbins Instruments

단백질 결정화 실험에 최적화된 시스템

Crystal Gryphon LCP

- **Nano dispenser** for soluble protein
- **LCP dispenser** for membrane protein
- **96 Syringes,** not disposable tip
- **Min. volume**

  : 100nl (nano dispenser)

  : 25nl (LCP dispenser)

General features	
Nano dispenser : 100nl ~ 100ul	8 plate position (Phoenix series)
96 syringes : 100nl ~ 100ul	2 plate position (Gryphon series)
LCP dispenser : 25nl ~ 2ul	Integrated washing system
96 syringes with flexible needle	Easy-to-use drag & drop software

절찬리 판매중

96-2 well plate

Intelli-plate **for sitting drop (1 box = 120 plates)**

- **96-2 original**
- **96-2 low volume reservoir**
- **96-2 shallow well**

(주) 이 우 과 학 교 역
IWOO Scientific Corporation
서울시 서초구 반포대로 23길 30, 5층 www.iwoo.co.kr
02-3473-2332 담당자. 정철우 과장 cwjeong@iwoo.co.kr

TAESHIN BIO SCIENCE 태신바이오사이언스
tsb@taeshinbio.co.kr 031) 928 - 4158
BLS

Latex Glove 행사

Special Event

Ultra Tex(Latex)Glove 1박스(10pack)를
주문하시면 핸드타올 1박스 또는
Glass dry Rack1개가 공짜!!

Special Event

Ultra Nitrile Glove 1박스(20pack)를
주문하시면 핸드타올 1박스 또는
Glass dry Rack1개가 공짜!!

Special Event

Ultra Nitrile Glove 2+1

PDB:단백질구조 데이터 뱅크

단백질구조 데이터 뱅크 (Protein Data Bank, PDB)는 생체 고분자의 구조 데이터를 수집 한 세계 공용의 유일한 데이터베이스 입니다. PDB는 1971 년 7 개의 구조로 부터 시작되어, 2019년 150,000 개 이상의 구조 정보를 제공하고 있습니다.

2003년 PDB를 유지하기위해 국제 조직 "wwPDB" (worldwide Protein Data Bank, wwPDB) 가 설립되었으며, 4 개의 파트너로 구성되어 있습니다: 미국의 Research Collaboratory for Structural Bioinformatics (RCSB) PDB 와 Biological Magnetic Resonance Bank (BMRB) , 유럽의 PDBe 일본 PDBj.

각 파트너는 각국 정부 기관의 연구 비용으로 운영되고 있습니다..

또한 우리는 아래와 같은 관련 바이오사이언스 데이터베이스를 관리하는 그룹들과 연합하여, 일본 바이오 사이언스 정보 연합 (Japan alliance for Bioscience Information : JBI)을 결성하였습니다다.

- 바이오 사이언스 데이터베이스 센터 (National Bioscience Database Center : NBDC)
- 라이프 사이언스 데이터베이스 센터 (Database Center for Life Science : DBCLS)
- 일본 DNA 데이터 뱅크 (DNA Data Bank of Japan : DDBJ)
- 일본 단백질구조 데이터 뱅크 (Protein Data Bank Japan : PDBj)

JBIportal
Japan alliance for Bioscience Information

NBDC · DBCLS Database Center for Life Science · DDBJ DNA Data Bank of Japan · PDBj Protein Data Bank Japan

일본의 PDBj는 다음 자금으로 지원됩니다.

- 과학기술진흥기구 바이오사이언스 데이터베이스 센터 (JST-NBDC)
- 오사카 대학교 단백질 연구소 (IPR)에 거점을 둔 공동이용 및 공동연구거점경비 (MEXT)
- 신약 개발 등을 위한 첨단기술지원기반 플랫폼 (BINDS)

NBDC · INSTITUTE for PROTEIN RESEARCH OSAKA UNIVERSITY · BINDS

Relion / Cryo-SPARC
High Performance Computing Laboratory

**Cryo-EM 연구를 위한 강력한 솔루션,
국내 유일 전문인력의 H/W제안 및
S/W 기술지원을 받아보세요!**

Cryo-EM Application Support

- Relion 3.0
- EMAN2
- Xmipp3.0
- MotionCor2
- UCSF Chimera
- Cryo-SPARC v2
- iMOSFLM
- CCP4 7.0
- Phenix
- CNS Coot
- Pymol
- Scipion V2.0
- HOLE program V2.2

Cryo-EM Application Certified Systems

- HPC Cluster
- CPU Server / GPU Workstation
- Parallel Storage / Mass storage

Cryo-EM Appliance Line-UP

Entry-Level (Cryoworks104)
1x Intel Core X-Series CPU / 4GPU Workstation
- Relion for Cryo-EM Solution

Mid-Range(Cryoworks204)
2x Intel Xeon Scalable CPU / 4GPU Workstation
- Relion for Cryo-EM Solution

High-End(Cryoserver208)
2x Intel Xeon Scalable CPU / 8GPU Server
- Relion for Cryo-EM Solution

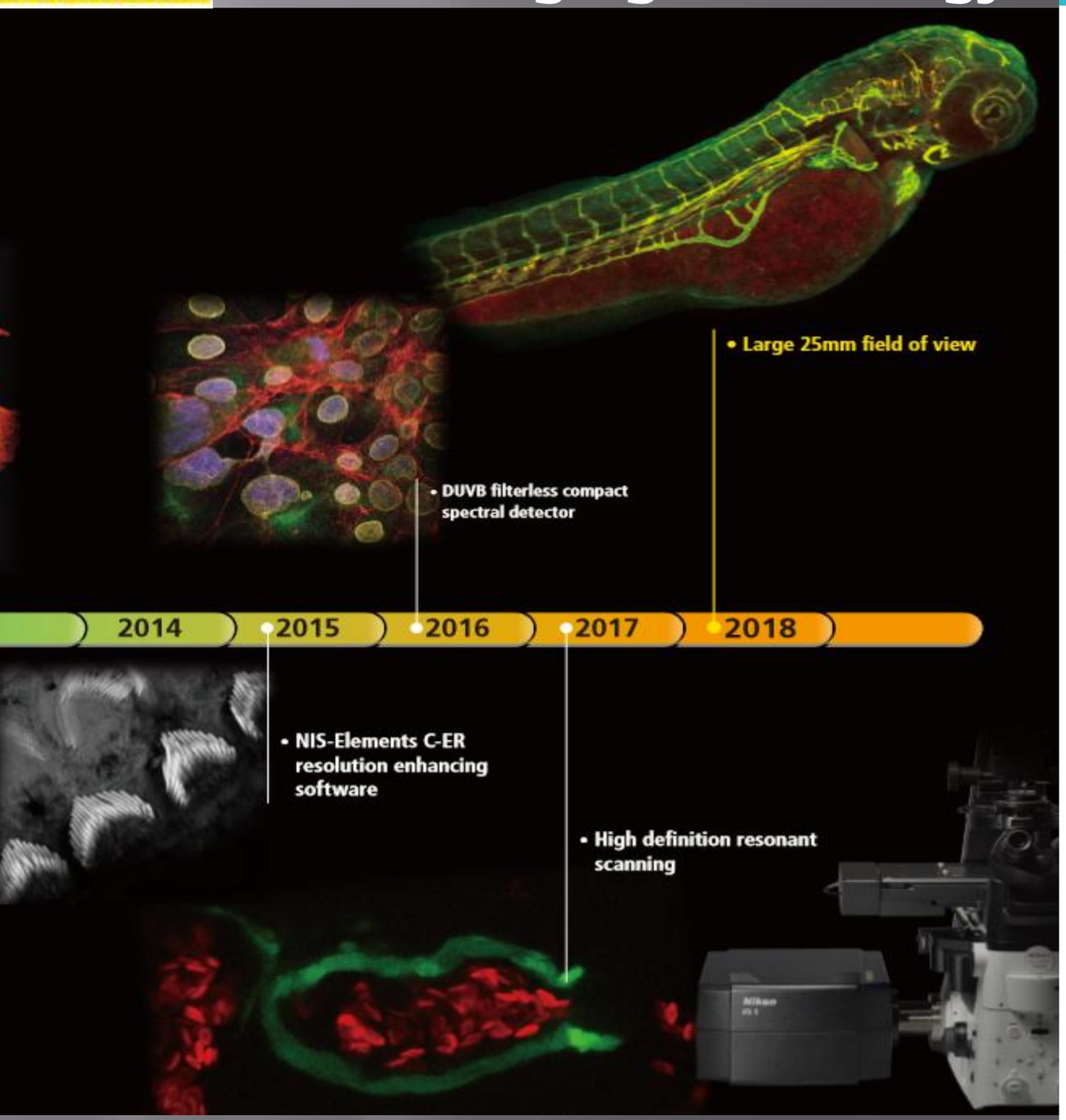
- HpcLab 고객 -

KISTI 한국과학기술정보연구원 · 서울대학교 · 고려대학교 · 한양대학교 · POSTECH · 국방과학연구소 · 연세대학교 · UNIST · 한국기초과학지원연구원 · DGIST · KAIST

(주)에이치피씨랩 | 대전 유성구 테크노4로 17 ,대덕비즈센터 B동 610호
문의전화 : 042-823-1592, 010-4458-4325 | 이메일 : yslee@hpclab.co.kr |홈페이지:www.hpclab.co.kr

HPCLAB High Performance Computing Group

MIT
Micro Imaging Technology

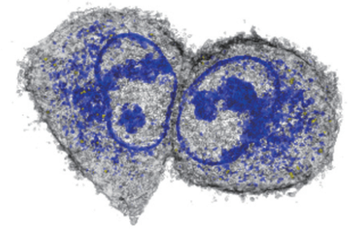


연락처: 053)811-1723
010-4516-1723
E-MAIL: pro3102@naver.com

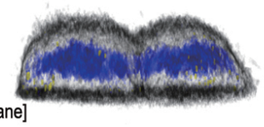
Join Our Worldwide Collaborators with

Holotomography!

3D HoloTomogram

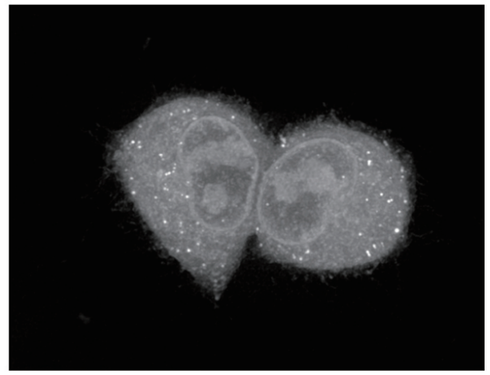


[XY Plane]



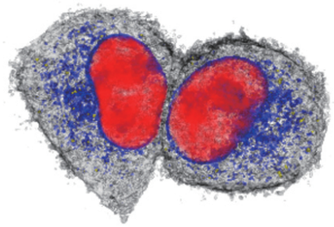
[YZ Plane]

MIP

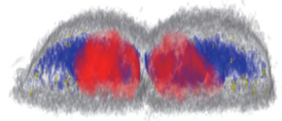


[XY Plane]

Fluorescence + 3D HoloTomogram



[XY Plane]



[YZ Plane]

Features

No Labeling Required

HT detects refractive index lilumination; No exogenous fluorescence dye needed

Ultra-fast Imaging

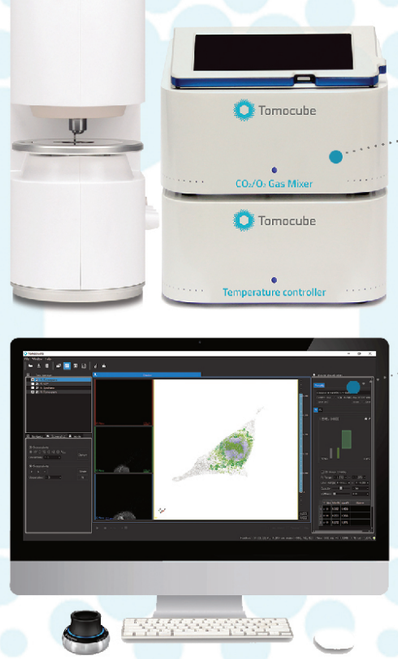
Requires only 0.4s for single 3D image; Time-lapse enabled for long-term live cell imaging

HT+FL Image Overlap

HT image correlated with fluorescent images; Increased molecular specificity

Quantitative Analysis

RI values Analyzed to understand Volume, Surface area, Dry mass, Concentration of sample



Components

1 HT-2H Microscope

3D fluorescence imaging and refractive index lilumination detection capability (HT+FL)

2 TomoChamber

Stage-off incubator providing physiological conditions (Co_2 + Temperature) for live cells

3 TomoStudio™

Software optimized for HT image operation, Visualization, Annotation, and Analysis

4 TomoDish

Glass bottom dish specifically designed for HT-2H microscope culture condition

Tomocube

www.tomocube.com
info@tomocube.com
www.facebook.com/tomocubeinc
"Tomocube, Inc"

MEMO

MEMO

Federation of the Korean Societies for Biomolecular Sciences (FKSBS)

한국생체분자과학연합학회 학술대회

한국생물물리학회
The Korean Biophysical Society