July 6 (Sat)		July 7 (Sun)		July 8 (Mon)		July 9 (Tue)	
		9:20-9:30	Opening Remark Jooyoung Lee				
		9:30-11:00	Todd L. Lowary Shih-Hsiung Wu Saeko Yanaka	9:00-10:30	Po-Huang Liang Sun Choi Art Choi	9:00-10:30	Guang-CHao Chen Tsung-Lin Li Ming-Daw Tsai
		11:00-11:30	Break	10:30-11:00	Break	10:30-11:00	Break
		11:30-12:30	Steven Gross Susumu Uchiyama	11:00-12:30	Motohiro Nishida Chaok Seok Takahiro Kosugi	11:00-12:30	Yangmee Kim Shigetoshi Aono Weontae Lee
		12:30-14:00	Lunch	12:30-13:30	Lunch	12:30-13:30	Lunch
		14:00-15:30	Chien-chih Yang Nobuyuki Shiina Rita Pei-Yeh Chen	13:30-15:00	Koichi Kato Hyeonjoo Ahn Takayuki Uchihashi		
		15:30-16:00	Break	15:00-15:30	Break		
17:00 ~	Registration Reception	16:00-18:00	Makoto Tominaga Chii-Shen Yang Maho Yagi-Utsumi Wonpil Im	15:30-17:00	Shin-ichi Higashijima Yasuhiro Go In-Ho Lee		

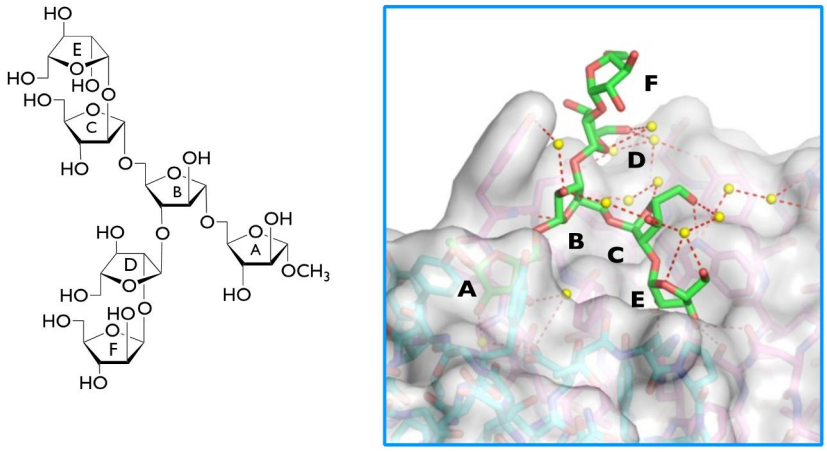
Recognition of Mycobacterial Lipoarabinomannan by Monoclonal Antibodies

Todd L. Lowary

Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

Email: tlowary@ualberta.ca

As part of their cell wall, mycobacteria produce the polysaccharide lipoarabinomannan (LAM), an immunomodulatory molecule composed of arabinofuranose and mannopyranose residues. Individuals infected with mycobacteria, including the human pathogen *Mycobacterium tuberculosis*, produce high titres of antibodies against this glycan. The structural motifs in LAM that are immunodominant have been poorly defined. In this presentation, investigations detailing the interaction of LAM with monoclonal antibodies will be described.



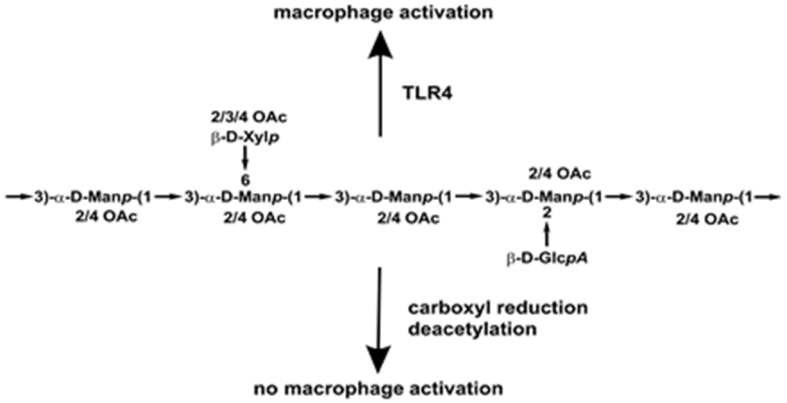
Hexasaccharide fragment of mycobacterial LAM (left) and structure of this compound in complex with the CS-35 monoclonal antibody (right).

Polysaccharides from Edible Mushrooms Possess Immunostimulatory Activity through Toll-like Receptor 4

Shih-Hsiung Wu

Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan
Email: shwu@gate.sinica.edu.tw

Auricularia auricula-judae (AAJ, wood ear mushroom) is well known for its culinary, nutritional, and medicinal values. The main dietary components present in AAJ are carbohydrates and proteins, and α-and β-glucans have been reported to be the major polysaccharides found in wood ear mushroom. Other heteropolysaccharides with varying compositions of glucose, galactose, mannose, xylose, rhamnose, arabinose, and hexauronic acid were also reported. Many studies explored the myriad of biological activities exerted by AAJ polysaccharides, including anticancer, anticoagulant, antioxidant, hypolipidemic, hypoglycemic, and immunomodulatory activities, etc. Although the high potential of AAJ polysaccharides as an immunoceutical is well recognized, a rigorous and systematic study based on the structure-activity relationship related to the immunomodulatory mechanism of AAJ polysaccharides is not yet to be reported. The absence of such important evidence will minimize the potential benefits from these polysaccharides as a nutraceutical4c. We comprehensively report the structure, immunostimulatory activity, and receptor interactions of cold water soluble glucuranoxylomannan from AAJ mushroom. The chemical structure and immuno-enhancing activity of cold-water-soluble glucuronoxylomannan (AAPS) from wood ear mushroom, *Auricularia auricula-judae*, were explored. Toll-like receptor 4 (TLR4) was identified to be the sole receptor involved in AAPS-induced macrophage activation to secrete pro-inflammatory cytokines. The essentiality of both carboxylic acid moiety and O-acetylation modification of AAPS in TLR4 binding to exert *in-vitro* immunostimulatory activity was further discussed.



Reference

N. Perera, F-L. Yang, J. Chern, H-W. Chiu, C-Y. Hsieh, L-H. Li, Y-L. Zhang*, K-F. Hua*, **S-H. Wu*** (2018)
Chem. Comm. 54, 6995-6998.

Dynamic Views of the Fc Portion of Immunoglobulin G Provided by Experimental and Computational Observations

Saeko Yanaka [1,2,3], Rina Yogo [1,2], Rintaro Inoue [5], Masaaki Sugiyama [5], Satoru G. Itoh [1,4], Hisashi Okumura [1,4], Yohei Miyanoiri [6], Hirokazu Yagi [2], Tadashi Satoh [2], Takumi Yamaguchi [2,7] and Koichi Kato [1,2,3]

[1] *Exploratory Research Center on Life and Living Systems (ExCELLS) and Institute for Molecular Science (IMS), National Institutes of Natural Sciences, Okazaki, Aichi 444-8787, Japan*
[2] *Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Aichi, 467-8603, Japan*
[3] *Department of Functional Molecular Science, SOKENDAI, Okazaki, Aichi 444-8787, Japan*
[4] *Department of Structural Molecular Science, SOKENDAI, Okazaki, Aichi 444-8585, Japan*
[5] *Institute for Integrated Radiation and Nuclear Science, Kyoto University, Kumatori, Osaka 590-0494, Japan*
[6] *Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan;*
[7] *School of Materials Science, Japan Advanced Institute of Science and Technology (JAIST), Nomi, Ishikawa 923-1292, Japan*

E-mail: saeko-yanaka@ims.ac.jp

The Fc portion of immunoglobulin G (IgG) is a horseshoe-shaped homodimer, which interacts with various effector proteins, including Fcγ receptors (FcγRs). These interactions are critically dependent on the pair of N-glycans packed between the two C_H2 domains. Fucosylation of these N-glycans negatively affects human IgG1-FcγRIIIa interaction. The IgG1-Fc crystal structures mostly exhibit asymmetric quaternary conformations with divergent orientations of C_H2 with respect to C_H3. We aimed to provide dynamic views of IgG1-Fc by performing long-timescale molecular dynamics (MD) simulations, which were experimentally validated by small-angle X-ray scattering and nuclear magnetic resonance spectroscopy.

Our simulation results indicated that the dynamic conformational ensembles of Fc encompass most of the previously reported crystal structures determined in both free and complex forms, although the major Fc conformers in solution exhibited almost symmetric, stouter quaternary structures, unlike the crystal structures. Furthermore, the MD simulations suggested that the N-glycans restrict the motional freedom of C_H2 and endow quaternary-structure plasticity through multiple intramolecular interaction networks. Moreover, the fucosylation of these N-glycans restricts the conformational freedom of the proximal tyrosine residue of functional importance, thereby precluding its interaction with FcγRIIIa. The dynamic views of Fc will provide opportunities to control the IgG interactions for developing therapeutic antibodies.

How to histones kill bacteria? Discovery of surprising synergy!

Steven Gross

Abstract: in the 1940s, histones were first reported as anti-microbial agents. With subsequent establishment of their critical role in DNA compaction, coupled with their primary localization in the nucleus, their potential role as anti-bacterial agents was dismissed as an artifact and forgotten. However, intriguing findings keep pointing to them as playing a role in innate immunity, and they have now been established to contribute critically to the function of NETs (Neutrophil extracellular traps). I will report on our work furthering their potential. In particular, I will discuss how they are positioned in the cytosol to allow them to kill bacteria, and also how they function synergistically with anti-microbial peptides to function. The talk will both highlight this interesting area, but also point to places where modeling is needed to provide a more mechanistic understanding of the process.

Modern analytical ultracentrifugation for quantitative studies on intermolecular interactions

Susumu Uchiyama

Department of Biotechnology, Graduate School of Engineering, Osaka University

Extrapolatory Research Center for Life and Living Systems (ExCELLS), National Institute of Natural Sciences

Intermolecular interactions in biological systems are typically accompanied by Gibbs free energy changes ranging from 20 kJ/mol to 50 kJ/mol which corresponds to dissociation constant (K_D) of mM and nM levels, respectively. Modern analytical ultracentrifugation (AUC) is one of the most powerful methods to estimate quantitative parameters related to intermolecular interactions. Very weak reversible interactions, typically K_D of mM can be evaluated quantitatively as a parameter, secondary virial coefficient (B_{22}), by AUC sedimentation equilibrium. Strong interactions such as K_D in nM order can be now examined by AUC sedimentation velocity with fluorescent signal detector. Complexed system where several species are simultaneously generated due to multistep interactions can be analyzed by incorporating stoichiometric information from native mass spectrometry into numerical analysis of AUC data. In this symposium basics and applications of modern AUC will be introduced.

References

[1] Matsui T, et al., Sci Rep. 2019, 9:2330. [2] Uchihashi T, et al., *Nature Comm.* 2018, 9, 2147. [3] Krayukhina, E., et al., *mAbs* 2017, *9*, 664-679. [4] Sugiyama, et al., *Sci. Rep.* 2016, *6*, 35567. [5] Zhang, Z., et al., *Immunity* 2016, *45*, 737-748. [6] Inoue, R., et al., *Sci. Rep.* 2016, *6*, 29208. [7] Ohto, U., et al., *Nature* 2016, 534, 566-569. [8] Kabe, et al., *Nature Comm.* 2016, *7*, 11030. [9] Ishii, K., et al., *Sci. Rep.* 2015, *5*, 18167. [10] Ohto, U., et al., *Nature* 2015, 520, 702-705. [11] Fujita, D., et al., *Nature Comm.* 2012, *3*, 1093. [12] Noda, M., et al., *Biochem. J.* 2011, *436*, 101-112. [13] Danjo H., et al., *JACS* 2010, *132*, 15556-15558. [14] Nogi T., et al., *Nature* 2010, *467*, 1123-1127.

The implications of structural characterization of ICE1 on its functions

Chien-chih Yang

Department of Biochemical Science and Technology, National Taiwan University, Taipei, Taiwan

Email: ccyang@ntu.edu.tw

We previously identified ICE1 as an interacting protein of a membrane protein HHP1. ICE1 encodes a bHLH type transcription factor, which is first identified in its roles in the cold signaling pathways. In addition to cold responses, ICE1 has been reported to participate numerous physiological functions including stomatal development, endosperm development as well as jasmonate signaling in plants. We reported that ICE1 participates in ABA-mediated signaling and sugar responses. Many of these physiological functions are mediated through the protein-protein interaction between ICE1 and a diverse array of proteins, such as SPCH, Mute, FAMA and ZOU. This raised the question of how a single protein can play so many physiological roles. Analysis of the primary structure of ICE1 indicated that it contains intrinsically disordered regions. The capability that ICE1 can receive and integrate signals from different environmental and growth signals is likely to be related to its disordered regions.

Liquid- and solid-like RNA granule formation and its implications for neuronal functions

Nobuyuki Shiina[1,2]

[1]*Exploratory Research Center on Life and Living Systems / National Institute for Basic Biology, National Institutes of Natural Sciences,*
38 Nishigonaka Myodaijicho, Okazaki 444-8585, Japan
[2] *Department of Basic Biology, SOKENDAI,*
38 Nishigonaka Myodaijicho, Okazaki 444-8585, Japan
Email: nshiina@nibb.ac.jp

RNA granules are membrane-less assemblies of RNA-protein complexes formed by liquid-liquid phase separation. Recently, RNA granules have been shown to contain solid-like cores rather than just liquid droplets. However, it remained unclear how these different substructures are formed, whether they are assembled by different scaffolds, and whether different RNA granule scaffold proteins induce the formation of different substructures. We found that RNA granule scaffold proteins, when expressed separately in cultured fibroblasts, induce the formation of either liquid-type or solid-type granules. When co-expressed in the same cells, the liquid-type and solid-type scaffold proteins were combined into the same granules and formed different substructures of liquid-type shells and solid-type cores, respectively (Fig. 1). When combined with liquid-type scaffold proteins, solid-type scaffold proteins increased their mobility and reduced their dose-dependent translation inhibition in the granules. RNG105 (also known as caprin1) was a liquid-type scaffold protein, which had a strong activity to enhance the dynamics of solid-type scaffold proteins and reduce their ability to inhibit translation in granules (Fig. 2). This activity of RNG105 suggested that in RNG105 knockout neurons, RNA granule cores could be very immobile and translation activity in the granules could be reduced. These changes in RNA granule dynamics and translation activity might underlie reduced dendritic localization of mRNAs in RNG105 knockout neurons and severe defects in long-term memory formation in RNG105 conditional knockout mice.

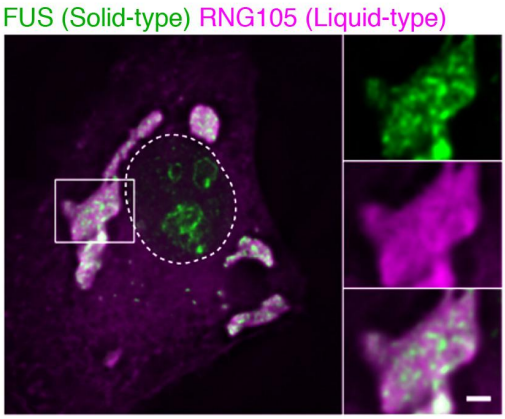


Fig.1 Formation of solid- and liquid-type RNA granule substructures in cultured cells by different scaffold proteins.

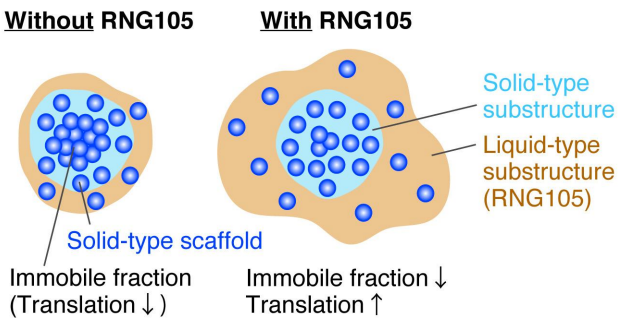


Fig.2 RNG105 increases the dynamics and decreases the translation inhibition ability of solid-type scaffold proteins.

One sequence-multiple structure: Structural conversion of Prion protein

Rita Pei-Yeh Chen

Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

Email: pyc@gate.sinica.edu.tw

Prion diseases are neurodegenerative disorder in mammals. Unlike other neurodegenerative diseases, prion diseases are highly contagious. The diseases can be transmitted not only within the same species but also across species. Most especially, the infectious material of prion diseases is a misfolded protein called PRION which is converted from a normal membrane protein called prion protein. The normal cellular prion protein called PrP^{C} is an α-helical rich globular protein. The misfolded conformer (PRION) also called as PrP^{Sc} is a β-sheet rich fibrillary polymer (called amyloid fibrils). The transmission efficiency of prion diseases depends on the sequence homology of prion protein between the host and the infectious PRION. The structure of PRION is an unsolved mystery. We used the seeding technique to explore how the prion protein sequence affects the species barrier between hamster and mouse. We conclude that residue 139 is the key residue affecting the resulting amyloid fibril structure of prion protein and hence affects the seeding efficiency.

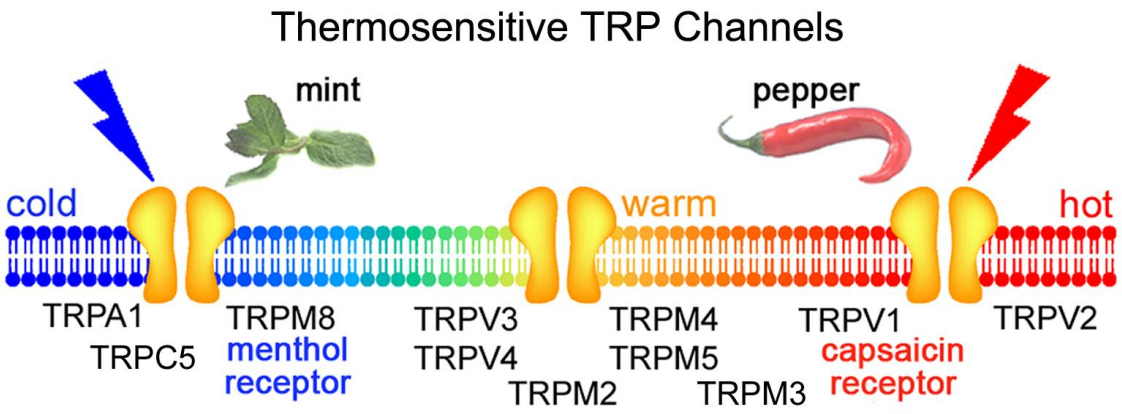
On the other hand, under non-denaturing neutral pH conditions, full-length mouse recombinant prion protein lacking the only disulfide bridge can spontaneously convert from an α-helical-dominant conformer (α-state) to a β-sheet-rich conformer (β-state), which then associates and forms β-oligomers. To reveal the molecular details of this structural conversion we introduced spin probes into different structural segments (three helices and the loop between strand 1 and helix 1), and employed a combined approach of ESR spectroscopy and protein encapsulation in nanochannels to reveal local structural changes during the α-to-β transition. Nanochannels provide an environment in which prion protein molecules are isolated from each other, but the α-to-β transition can still occur. By measuring dipolar interactions between spin probes during the transition, we showed that helix 1 and helix 3 retained their helicity, while helix 2 unfolded to form an extended structure which is the association interface of the resulting β-oligomers.

Physiological function of thermosensitive TRP channels

Makoto Tominaga[1,2]

[1]*Exploratory Research Center on Life and Living Systems / National Institute for Physiological Sciences, National Institutes of Natural Sciences,*
5-1 Higashiyama Myodaijicho, Okazaki 444-8787, Japan
[2]*The Graduate University for Advanced Studies,*
5-1 Higashiyama Myodaijicho, Okazaki 444-8787, Japan
Email: tominaga@nips.ac.jp

TRP channels are non-selective cation channels having relatively high Ca^{2+} permeability, and comprise six related protein families (TRPC, TRPV, TRPM, TRPA, TRPML, TRPP) in mammals. Among the huge TRP super family of ion channels, some have been proven to be involved in thermosensation detecting ambient temperatures from cold to hot. There are now eleven thermosensitive TRP channels (TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM3, TRPM4, TRPM5, TRPM8, TRPA1 and TRPC5) with distinct temperature thresholds for their activation. We found that some thermosensitive TRP channels make a complex with Ca^{2+}-activated chloride channel, anoctamin1 (ANO1). Interaction between TRPV4 and ANO1 is involved in water efflux in choroid plexus, salivary gland and lacrimal grand epithelial cells. And TRPV1 (TRPA1)/ANO1 interaction was found to be involved in the enhancement of nociceptive signals through further depolarization upon chloride efflux in peripheral sensory neurons. This interaction could be one of the reasons why TRP channels have high Ca^{2+} permeability. I will talk about the evolution of thermosensitive TRP channels, too.



Many faces of the microbial rhodopsins from haloarchaea

Chii-Shen Yang

Dept. of Biochemical Science & Technology, National Taiwan University, 1, Sect. 4, Roosevelt Rd. Taipei, 10617, TAIWAN

E-mail address of the corresponding author chiishen@ntu.edu.tw

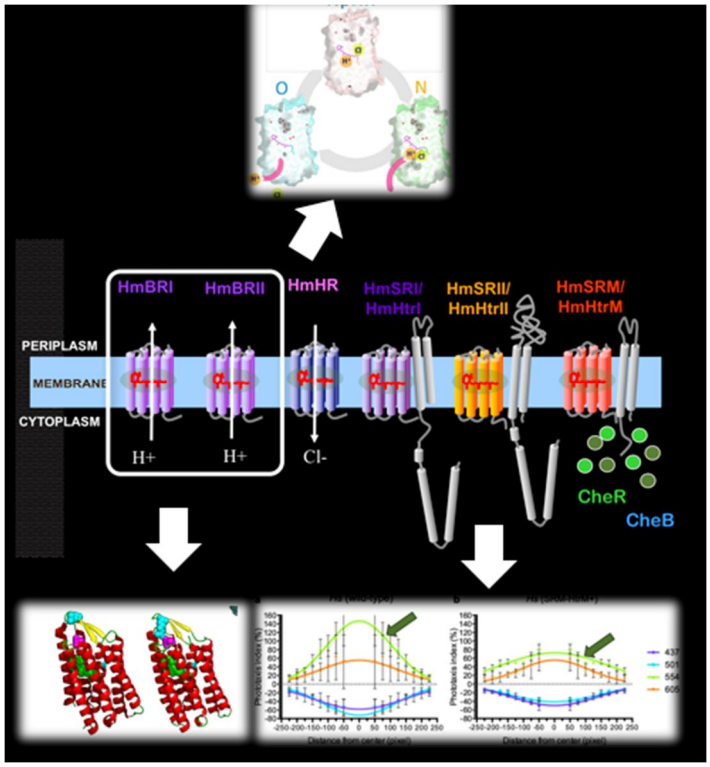
Microbial rhodopsins (M-Rho) from haloarchaea represent one of the most ancient, yet efficient protein systems that assist the survival of some life forms in the early age of Earth. Those M-Rho proteins exert their functions to harvest solar energy, regulate cellular osmolality, and mediate phototaxis responses. Here, several special characteristics and their physiological functions, or faces, of microbial rhodopsins will be discussed. **Face 1**[1]: In a six-rhodopsin system of *Haloarcula marismortui*, an archaeon from Dead Sea, a unique dual-bacteriorhodopsin (BR) system was found to be acid-tolerance as together those two BR proteins maintain the light-driven proton pumping capability under acidic condition to survive the pH 5.5 water in Dead Sea. **Face 2**[2]: In the photosensory system of *H. marismortui*, a green-sensing sensory, SRM, was identified instead of just having blue-sensing sensory rhodopsin II (SRII) and yellow-sensing sensory rhodopsin I (SRI) for photo-repellent and phototaxis, respectively. We have determined this extra SRM inhibits both photo-repellent response mediated by SRII, and photo-attractant conferred by SRIfore confers on *H. marismortui* cells to physically stay under illumination with shorter wavelength than those observed in other haloarchaea. **Face 3**[3]: Two halorhodopsins, HwHR from *Haloquadratun walsbyi* and HpHR from *Natronomonas pharaonis* feature a transient water channel in N-state to assist light-driven chloride transport, a mechanism not observed in other HRs.

Reference

[1] Structural and functional studies of a newly grouped *Haloquadratum walsbyi* bacteriorhodopsin reveal the acid-resistant light-driven proton pumping activity.
J. of Biological Chemistry 290, 29567-29577 (2015)
Hsu MF, Fu HY, Cai CJ, Yi HP, Yang CS*, Wang AH*,

[2] **The blue-green sensory rhodopsin SRM from *Haloarcula marismortui* attenuates both phototactic responses mediated by sensory rhodopsin I and II in *Halobacterium salinarum*.** *Scientific Reports* 9:5672 (2019)
Jheng-Liang Chen, Yu-Cheng Lin, Hsu-Yuan Fu, and **Chii-Shen Yang***.

[3] A unique light-driven proton transportation signal in halorhodopsin from *Natronomonas pharaonis*. ***Biophys J*** 111(12): 2600-7 (2016)
Chen XR, Huang YC, Yi HP, **Yang CS*.**



NMR characterization of conformational transition of amyloid-β on ganglioside membrane

Maho Yagi-Utsumi [1,2,3], Satoru G. Itoh [1,2], Hisashi Okumura [1,2], Katsuyuki Nishimura[2] and Koichi Kato [1,2,3]

[1] Exploratory Research Center on Life and Living Systems (ExCELLS) and [2]Institute for Molecular Science (IMS), National Institutes of Natural Sciences, Okazaki, Aichi 444-8787, Japan
[3]Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Aichi 467-8603, Japan
Email: mahoyagi@ims.ac.jp

Lipid membranes provide active platforms for dynamic interactions of a variety of biomolecules on cell surfaces, where glycolipids are involved in physiological and pathological molecular recognition events. Growing evidence has recently demonstrated that gangliosides on neuronal cell membranes can be targets for various amyloidogenic proteins that are associated with neurodegenerative disorders exemplified by amyloid β (Aβ) in Alzheimer's disease. Our previous studies have indicated that size and curvature of ganglioside clusters along with ganglioside density are the determining factors for the membrane-interactions and the subsequent conformational transition of these proteins [1]. However, less has been known about the transiently formed complexes between the ganglioside cluster and the pathogenic proteins, although such encounter complexes can be potential targets in the medicinal approaches.

To understand the effects of the interface on oligomerization at the atomic level, we performed NMR experiments and molecular dynamics simulations for an Aβ monomer in the presence and absence of the hydrophilic/hydrophobic interface. We found that the Aβ oligomer is formed easily at the interface compared to that in bulk water, because the conformational transition into β-hairpin structure is readily occurred at the interface [2].

Furthermore, we applied soli-state NMR technique to characterize the interactions of Aβ with GM1 gangliosides. Our solid-state NMR data of Aβ employing GM1 ganglioside-containing DMPC vesicles as model membrane system have elucidated the membrane-induced conformation of Aβ, giving insights into the mechanisms underlying the α-to-β conformational transition of Aβ on ganglioside clusters, which promote nucleation processes in Aβ aggregation in the membrane environments.

References

[1] M. Yagi-Utsumi, "NMR Characterization of Conformational Dynamics and Molecular Assemblies of Proteins," *Biol. Pharm. Bull.* **42**, 867-872 (2019).
[2] S.G. Itoh, M. Yagi-Utsumi, K. Kato, H. Okumura,"Effects of a Hydrophilic/Hydrophobic Interface on Amyloid-β Peptides Studied by Molecular Dynamics Simulations and NMR Experiments," *J. Phys. Chem. B*. **123**, 160-169 (2019).

S01-1

Quantitative Characterization of Protein-Lipid Interactions

Wonpil Im

Departments of Biological Sciences and Bioengineering, Lehigh University, USA.

Understanding the delicate balance of forces governing protein-lipid interactions is central to understanding membrane structure and function. These membrane constituent interactions play an essential role in determining the structure and function of membrane proteins, and protein interactions in membranes, and thus form the basis for many vital processes, including transmembrane signaling, transport of ions and small molecules, energy transduction, and cell-cell recognition. In this talk, I will present our efforts toward quantitative characterization of protein-lipid interactions based on free energy simulations.

Targeting cysteines for anti-viral and anti-cancer drug discovery

Po-Huang Liang

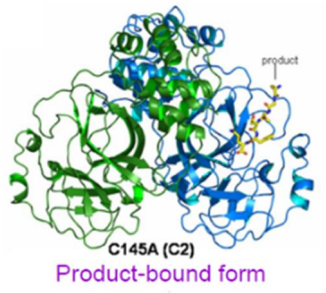
Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

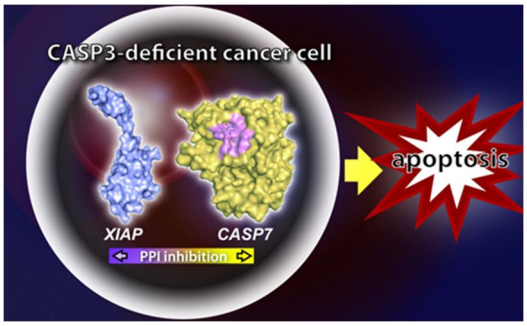
Email: phliang@gate.sinica.edu.tw

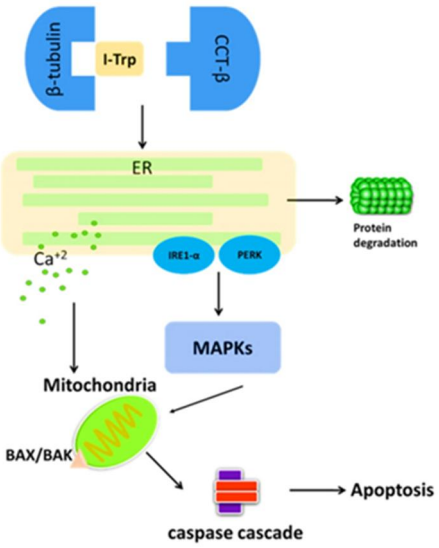
Middle East respiratory syndrome coronavirus (MERS-CoV) causes severe acute respiratory illness with fever, cough and shortness of breath. Our peptidomimetic inhibitors of enterovirus 3Cpro inhibited 3CLpro of MERS-CoV and severe acute respiratory syndrome coronavirus (SARS-CoV) with IC50 of 0.2 to 1.7 μM and good selectivity index (over 70 against MERS-CoV).[1] For some toxic Cys-alkylating agents, we identified their targets as XIAP/caspase-7 and CCT-β:β-tubulin complexes and their cancer cells-killing mechanisms.[2,3]

Reference

1. Kumar, V., Shin, J. S., Shie, J. J., Ku, K. B., Kim, C., Go, Y. Y., Huang, K. F., Kim, M.*, and Liang, P. H.* (2017) Identification and evaluation of potent Middle East respiratory syndrome coronavirus (MERS-CoV) 3CLPro inhibitors. (2017) *Antiviral Res.* **141**, 101-106.
2. Lin, Y. F. Lai, T. C., Chang, C. K., Chen, C.L., Huang, M.S., Yang, C.J., Liu, H. G., Dong, J. J., Chou, Y.A., Teng, K. H., Chen, S. H., Tian, W. T., Jan, Y. H., Hsiao, M., and Liang, P. H.* Targeting the XIAP/caspase-7 complex selectively kills caspase-3 deficient malignancies. (2013) *J. Clinical Investigation* **123**, 3861-3875.
3. Liu, Y. J., Kumar, V., Lin, Y. F., and Liang, P. H.* Disrupting CCT-β:β-tubulin selectively kills CCT-β overexpressed cancer cells through MAPKs activation. (2017) *CDDis* **8**, e3052.







Computational mechanistic elucidation of a tetraspanin arginine sensor via docking and molecular dynamics simulation

Sun Choi

College of Pharmacy and Graduate School of Pharmaceutical Sciences,
Ewha Womans University, Seoul 03760, Republic of Korea

Transmembrane 4 L six family member 5 (TM4SF5) contains 4 transmembrane (TM) helices, two extracellular loops (short (SEL) and long (LEL)), an intracellular loop, and N- and C-terminal cytosolic tails. The LEL region contains conserved residues and N-glycosylation sites, making it vital to its function and interactions with molecular partners (e.g. TM4SFs, signaling proteins, and integrins), which can lead to fibrosis and tumorigenic processes. Recently, we have identified TM4SF5 as an arginine sensor for mTORC1 activation. Mutations of conserved residues in the LEL region were found to significantly affect mTORC1 function supporting the involvement of TM4SF5 in this pathway.

To explore TM4SF5 structure and function, we first established a homology model using CD81 as template. We implemented docking studies for binding mode prediction of L-arginine and TSAHC and observed that both molecules can interact with conserved residues and potentially affect N-glycosylation sites in the TM4SF5 LEL region. We also performed molecular dynamics (MD) simulations for the apo wild-type and mutant (W124A and Y126S) TM4SF5 structures. Protein-protein docking with mTORC1 and MD simulation of the resulting complexes were also carried out. Trajectory and network analysis distinguished the contributions of W124 and Y126 to TM4SF5 structural integrity and function in the mTORC1 pathway.

Development and applications of Water-Pharmacophore

By running molecular dynamics simulations and tracking the movement of waters in a binding site of a target protein, one can extract useful information for drug design. This idea has been exploited extensively since the publication of PNAS article by Tom Young et al. in 2007. One of the resulting works has been implemented as a commercial product named WaterMap. Our team has been working on a a method that can generate pharmacophore models from the information about binding sites produced by a similar algorithm. The developed method lends itself readily to structure-based drug design (SBDD) processes. In this talk, I will describe the development of what we call “Water-Pharmacophore” method and its potential applications to a drug design platform involving artificial intelligence.

Cardiac plasticity regulated by protein-protein interaction (PPI)

Motohiro Nishida[1,2]

[1]Division of Cardiocirculatory Signaling, Exploratory Research Center on Life and Living Systems (National Institute for Physiological Sciences), National Institutes of Natural Sciences, Okazaki, Aichi 444-8787,

[2]Department of Translational Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582,

Protein-protein interaction (PPI) has been attracted attention as a new drug target, since this strategy is expected to reduce the risk of adverse effect caused by chronic drug treatment. Our group investigates the molecular mechanism underlying regulation of cardiac plasticity, and has found that the pathology-dependent formation of PPI induced by chemical/physical stresses play key roles in the development of cardiac tissue remodeling. In addition, disruption of pathology-dependent PPI was found to improve chronic heart failure in mice. I will introduce two pathology-dependent PPIs and discuss their therapeutic insights on metabolic diseases.

1. TRPC3-Nox2 complex in muscle atrophy

Our laboratory studies on the common mechanism underlying reduction of myocardial flexibility due to hemodynamic loading and unloading. We used doxorubicin (DOX), a highly effective anticancer agent but induces myocardial atrophy, for investigating molecular mechanism of myocardial atrophy. We found that transient receptor potential canonical 3 (TRPC3) protein, a major component of diacylglycerol-activated cation channel, participate in the development of myocardial atrophy in mice. DOX increased production of reactive oxygen species (ROS) in rodent cardiomyocytes through hypoxic stress-mediated upregulation of NADPH oxidase 2 (Nox2), which formed a stable complex with TRPC3. Inhibition of TRPC3-Nox2 complex prevent physico-chemical stress-induced cardiac stiffness including interstitial fibrosis and myocardial atrophy in mice.

2. Drp1-filamin complex in myocardial early senescence

Disorders of mitochondrial dynamics is recognized as a key determinant of myocardial vulnerability after MI. We identified filamin A, an actin binding protein, as a new guanine nucleotide exchange factor of dynamin-related protein 1 (Drp1). Filamin activated Drp1 in coordination with Actin, and this filamin-actin-Drp1 complex mediated hypoxia-induced mitochondrial fission and senescence in cardiomyocytes. Moreover, we found that cilnidipine, known as a dihydropyridine-derivative voltage-dependent L/N-type Ca^{2+} channel blocker, inhibited mitochondrial hyperfission by suppressing Drp1-filamin complex formation. Administration of cilnidipine to mice ameliorated cardiac senescence and heart failure after MI. These data demonstrate that Drp1 association with filamin and the actin cytoskeleton underlies cardiac fragility after MI, and represents a potential repurposing of cilnidipine, as well as a starting point for innovative drug development.

Prediction of protein structure and interaction by physics and informatics

Chaok Seok

Department of Chemistry, Seoul National University, Seoul 08826, Republic of Korea

Protein structure prediction problem has challenged theoretical and computational physical scientists since the first protein structure was published in 1958. There have been steady progresses in protein structure prediction since then, but major contributions to the progress came from informatics-based approaches rather than from physics-based approaches. Recently, DeepMind's AlphaFold made a further contribution by introducing deep learning to extract structural information from the large sequence database. Meaningful contribution of physics-based approaches began to be made only in 2012 in the field of structure refinement. However, structural improvements that can be achieved by refinement with current physics-based approaches are very limited due to both energy and sampling problems. To overcome this limitation, we are taking an approach that combines physics and informatics, including deep learning. We take similar approaches to predict interactions of proteins with other proteins or small ligands including short peptides and oligosaccharides. Our goal is to develop protein structure modeling techniques that can provide useful predictions even in the absence of available information, although currently available experimental data would play important roles in developing such techniques. Such modeling methods would be very useful for applications to a wide range of biomedical research and drug discovery.

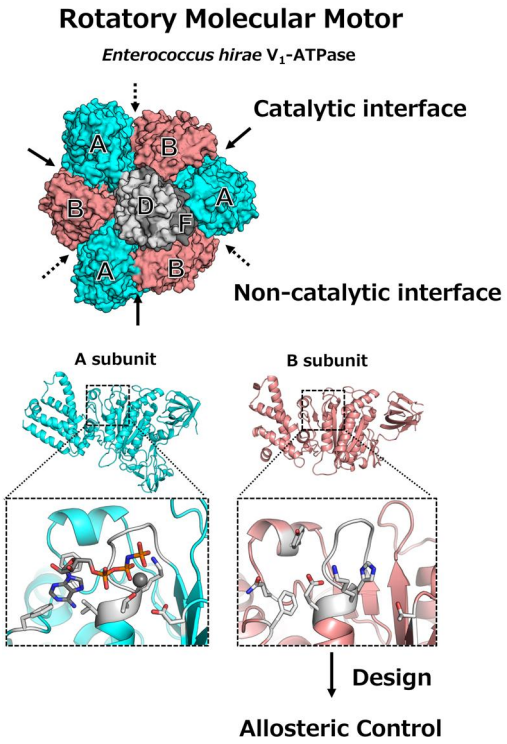
Design of Allosteric Site to Regulate Rotary Molecular Motor V_1-ATPase

Takahiro Kosugi

Exploratory Research Center on Life and Living Systems / Institute for Molecular Science,
National Institutes of Natural Sciences,
38 Nishigo-Naka Myodaijicho, Okazaki 444-8585, Japan
Email: takahirokosugi@ims.ac.jp

There are many proteins which work as complex states in cell. Many of such complex-forming proteins exert functions by cooperative works between their constituent proteins. Artificially regulating of the concerted function leads to be a better understanding the mechanism, controlling the function and creating novel proteins with a concerted function. One of the approaches to regulate a cooperative function is designing an allosteric site. Here, we attempted to design an allosteric site in protein complex and regulate the concerted function. Recently, many computational methods for protein design have been developed and many artificial functionalized proteins, for example enzymes and small molecule binding proteins, have been reported. In this work, such computational protein design methods were used to create a new binding site at an allosteric candidate site. As the allosteric candidate site, we focused to a site which is a separated site from function sites and has similar structure with function sites in protein complex.

Motor domain of rotary molecular motors V_1-ATPase has two different types of interface in hetero hexametric ring: one is catalytic interface where ATP is hydrolyzed and the other is non-catalytic interface where ATP is not hydrolyzed. This non-catalytic interface has similar structure with catalytic one, but does not have even ATP binding affinity. By designing a nucleotide binding site in the non-catalytic interface, we regulated the rotational speed of this molecular motor allosterically. Rotational speed of the designed V_1-ATPase is faster than the native one, depending on ATP binding affinity at the designed site. The solved crystal structures and single-molecule experiments revealed that the acceleration is caused by ATP bindings to the designed site inducing ADP release in the catalytic interfaces. This strategy will open up an avenue to control proteins allosterically.



Biomolecular engineering of Lewis X-containing oligosaccharides

Koichi Kato[1,2,3,4], Tatsuya Suzuki[1,2,4], Tokio Watanabe[4], Taiki Saito[2,4], Gengwei Yan[2,5], Tadashi Satoh[4], Saeko Yanaka[1,2,3,4], Hirokazu Yagi[4], and Takumi Yamaguchi[4,5]

[1] ExCELLS and [2] IMS, National Institutes of Natural Sciences, Okazaki, 444-8787, Japan

[3] School of Physical Science, SOKENDAI, Okazaki, 444-8787, Japan

[4] Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, 467-8603, Japan

[5] School of Materials Sciences, Japan Advanced Institute of Science and Technology, Nomi, 923-1292, Japan

Email: kkato@excells.orion.ac.jp

Biomolecules generally possess motional freedoms under physiologically solvated conditions. Oligosaccharides represent one of the most extreme classes of biomolecules that are characterized by conformational flexibility. Hence, oligosaccharide structures have been described as conformational ensembles. It has been supposed that lectin selects and captures specific conformational species of oligosaccharides among the ensemble, thereby promoting biological functions. Upon their interactions, the oligosaccharides may further undergo conformational deformation to fit into the binding pocket of lectin. Therefore, to gain quantitative insights into the energetics of carbohydrate recognition by lectins, detailed structural information is needed concerning their target oligosaccharides in both the precomplexed and lectin-complexed states. Moreover, such knowledge is crucial to design artificial oligosaccharides with improved functionality.

We have developed a method to elucidate the dynamic conformations of oligosaccharides in solution employing molecular dynamics simulation with a paramagnetism-assisted NMR technique. We herein attempt to apply this method to design unnatural oligosaccharides with higher affinities for a specific target lectin using an oligosaccharide containing Lewis X (Fig.1) as a model. By inspecting the conformational ensemble of the oligosaccharide in the *prebound* state, we chemically remodeled its conformational space with an increased population of conformational species that can be accommodated in the carbohydrate-binding pocket of the target lectin without steric hindrance. Our approach, complementing the lectin-bound-state optimizations, offers an alternative strategy to create high-affinity oligosaccharides by increasing populations of on-pathway metastable conformers.

We furthermore created a *cyborg* supramolecule by hybridizing a self-assembled, spherical complex with oligosaccharides containing Lewis X, which exhibited homophilic hyper-assembly in aqueous solution in a Ca^{2+}-dependent manner through specific carbohydrate–carbohydrate interactions. Moreover, we created Lewis X-carrying neoglycolipids that evoke selective apoptosis in neural stem cells. We are currently undertaking to produce Lewis X-carrying glycoproteins by recombinant techniques.

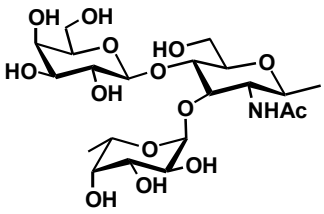


Fig.1 Lewis X

Nanoscale Dynamic Imaging of Biological Molecules with High-Speed Atomic Force Microscopy

Takayuki Uchihashi[1,2]

[1] *Department of Physics, Nagoya University,*
Furo-cho, Chikusa-ku, Nagoya 466-8602, Japan
[2]*Exploratory Research Center on Life and Living Systems / Institute for Molecular Science,*
National Institutes of Natural Sciences,
5-1 Higashiyama Myodaijicho, Okazaki 444-8787, Japan
Email: uchihast@d.phys.nagoa-u.ac.jp

Sub-second imaging on biological samples by atomic force microscopy (AFM) was first achieved in 2001 thanks to the development of several devices and control techniques. The technical advances greatly reduced the tip-sample interaction force without sacrificing the imaging rate, therefore allowing weak interactions between biological macromolecules to be studied without significant perturbation. Following the establishment of the High-Speed AFM (HS-AFM), its excellent performance has been demonstrated by imaging studies of various proteins' dynamics. Despite the youth of the technique, early investigations have not only demonstrated that HS-AFM can corroborate previously proposed models and provide intuitive views by visual evidence of indirectly described phenomena, but also that it can solve long-standing questions that had previously been difficult or impossible to be addressed by other approaches.

In this talk I will introduce recent representative results in which potential of HS-AFM is effectively demonstrated; i) membrane fission mechanism by dynamin-amphiphysin helices[1)] and ii) conformational dynamics of CRISIPR-Cas9 during DNA cleavage[2)]. In the first topic, we analyzed structural changes of dynamin-amphiphysin complexes during the membrane fission using electron microscopy (EM) and HS-AFM. Interestingly, HS-AFM analyses show that the dynamin-amphiphysin helices are rearranged to form clusters upon GTP hydrolysis and membrane constriction occurs at protein-uncoated regions flanking the clusters. We also show a novel function of amphiphysin in size control of the clusters to enhance biogenesis of endocytic vesicles. The second topic shows real-space and real-time visualization of Cas9-mediated DNA cleavage process. The HS-AFM movies show that, whereas apo-Cas9 adopts unexpected flexible conformations, Cas9–RNA forms a stable bilobed structure and interrogates target sites on the DNA by three-dimensional diffusion. Further we reveal that the Cas9 HNH nuclease domain fluctuates upon DNA binding, and subsequently adopts an active conformation, where the HNH active site is docked at the cleavage site in the target DNA.

References

1) T. Takeda, T. Kozai, H. Yang, D. Ishikuro, K. Seyama, Y. Kumagai, T. Abe, H. Yamada, T. Uchihashi, T. Ando and K. Takei, "Dynamic clustering of dynamin-amphiphysin helices regulates membrane constriction and fission coupled with GTP hydrolysis", *e-Life* **7**, e3024 (2018).

2) M. Shibata, H. Nishimasu, N. Kodera, S. Hirano, T. Ando, T. Uchihashi and O. Nureki, "Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy", *Nature Communications* **8**, article number: 1430 (2017).

Regulation of locomotor speed and selection of active sets of neurons by V1 neurons

Shin-ichi Higashijima

Studies in the past 15 years have implicated a number of transcription factors in the specification of different spinal interneurons. Functional analysis of a specific class of interneurons in amniotes, however, is not trivial due to enormous complexity of their spinal circuits. In contrast, zebrafish spinal circuits are much simpler with less number of distinct classes interneurons, making it more feasible to address this issue. We are investigating the morphology and functional properties of spinal neurons that express a particular transcription factor.

In this talk, I wll focus on a class of inhibitory neurons that express the transcription factor En1 (V1 neurons). We performed functional analyses of V1 neurons in larval zebrafish by selectively killing spinal V1 neurons using diphtheria toxin A (En1-DTA fish). We have revealed two functions of V1 neurons. The first is the long-proposed role of V1 neurons. In En1-DTA fish, the cycle period in swimming was prolonged. The second is completely new: V1 neurons were found to play an important role in the selection of active sets of neurons. In En1-DTA fish, slow-type motoneurons were vigorously active during strong movements.

During fast movements in vertebrates, slow motor units are thought to be deactivated due to the mechanical demands of muscle contraction, but the associated neuronal mechanisms for this have been unknown. Our study revealed the neuronal basis that accounts for the silencing of slow-component neurons during fast/strong movements.

Spatiotemporal brain transcriptome architecture and application for disease model in primates

Yasuhiro Go

[1]Exploratory Research Center on Life and Living Systems (ExCELLS) / National Institute for Physiological Sciences (NIPS), National Institutes of Natural Sciences, 38 Nishigonaka Myodaiji, Okazaki, Aichi, 444-8585, Japan
Email: yago@nips.ac.jp

Spatiotemporal transcriptome gene regulations are essential for the construction of brain structure and for proper function. Comprehensive analyses of the dynamics and the architecture of transcriptome in the both wild and diseased animal models also lead to understanding the molecular causality of the human neuropsychiatric disease. Currently we examine the spatiotemporal transcriptome dynamics using the primate brain to identify the spatiotemporal-specific modulating genes from macro-scale to single-cell level. Through this study, we aim to identify the molecular dynamics and trajectories between proper and atypical brain gene expressional networks. Additionally, we perform massive population genetic analysis for the neuropsychiatric related genes in primates to identify an individual that has a spontaneous loss-of-functional (LoF) mutation in the neuropsychiatric genes and aim to make disease primate models for the neuropsychiatric disease.

Global optimization of action using conformational space annealing

Juyong Lee[1], In-Ho Lee[2], InSuk Joung[3], Jooyoung Lee[3], and Bernard R. Brooks[4]

[1]*Department of Chemistry, Kangwon National University, Chuncheon-si, Kangwon-do 24341, Republic of Korea*
[2]*Korea Research Institute of Standards and Science, Daejeon 34113, Republic of Korea*
[3]*School of Computational Sciences, Korea Institute for Advanced Study, Seoul 02455, Republic of Korea*
[4]*Laboratory of Computational Biology, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, Maryland 20892, USA*

In computational physics, chemistry, and biology, global searching for reaction pathways is a long-standing challenge. Here we present a computational approach, Action-CSA, to find multiple reaction pathways connecting fixed initial and final states through global optimization of the Onsager–Machlup action using the conformational space annealing (CSA) method. The computer simulations are reduced into many interatomic potential and force calculations, but key information on their target conformational changes is ultimately retained, so the resulting reaction pathway can be a set of conformations. This protocol provides conceptual insight into how computer simulations can discover conformational changes based on interatomic potential energy and global optimization alone, and it paves the way for a more routine application.

Regulation of protein tyrosine phosphatases in membrane trafficking and human diseases

Guang-Chao Chen

Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan.

Protein tyrosine phosphatases (PTPs) are a group of tightly regulated enzymes that coordinate with protein tyrosine kinases to control protein phosphorylation during cell adhesion and cell migration processes. We found that Drosophila FERM and PDZ domain-containing protein tyrosine phosphatase, dPtpmeg, negatively regulates border cell migration during oogenesis. dPtpmeg and its human homolog PTPN3 targets Eps15, a scaffolding adaptor protein in EGFR endocytosis and trafficking. PTPN3 dephosphorylates Eps15 and promotes EGFR for lipid raft-mediated lysosomal degradation. Moreover, depletion of PTPN3 impairs the degradation of EGFR and enhances proliferation and tumorigenicity of lung cancer cells. PTPN3 also inhibits cell migration and cancer invasion by affecting focal complex assembly and actin cytoskeleton organization. Our results indicate multifaceted roles of PTPN3 in cellular signaling.

Evidence of diradicals involved in the yeast transketolase catalyzed keto-transferring reactions

Tsung-Lin Li

The Genomics Research Center, Academia Sinica, Taipei, Taiwan
Email: tlli@gate.sinica.edu.tw

Transketolase (TK) catalyzes a reversible transfer of a two-carbon (C_2) unit between phosphoketose donors and phosphoaldose acceptors, in which the group-transfer reaction that follows a one- or two-electron mechanism and the force that breaks the C2"–C3" bond of the phosphoketose donors remain elusive. Here, we report previously unknown intermediate states in TK (TKps) from *Pichia stipitis* by ultrahigh-resolution crystal structures, which support an unprecedented diradical mechanism for the reversible group-transfer reaction. In conjunction with MS, NMR spectroscopy, EPR and computational analyses, we proposed that the enzyme-catalyzed non-Kekulé diradical cofactor brings about the C2"–C3" bond cleavage/formation for the C_2-unit transfer reaction, for which suppression of activation energy and activation and destabilization of enzymatic intermediates are facilitated.

Reference

(1) N. S. Hsu, Y. L. Wang, K. H. Lin, C. F. Chang, S. C. Ke, S. Y. Lyu, L. J. Hsu, Y. S. Li, S. C. Chen, K. C. Wang, **T. L. Li***, 2018, "Evidence of diradicals involved in the yeast transketolase catalyzed keto-transferring reactions", *CHEMBIOCHEM*, 19(22), 2395-2402.

(2) N. S. Hsu, Y. L. Wang, K. H. Lin, C. F. Chang, S. Y. Lyu, L. J. Hsu, Y. C. Liu, C. Y. Chang, C. J. Wu, **T. L. Li***, 2018, "The mesomeric effect of thiazolium on non-Kekule diradicals in Pichia stipitis transketolase", *ANGEWANDTE CHEMIE-INTERNATIONAL EDITION*, 57(7), 1802-1807.

(3) L. J. Hsu, N. S. Hsu, Y. L. Wang, C. J. Wu, **T. L. Li***, 2016, "Structural and biochemical interrogation on transketolase from Pichia stipitis for new functionality", *PROTEIN ENGINEERING DESIGN & SELECTION*, 29(11), 513-522.

(4) S. H. Chen, D. R. Hwang, G. H. Chen, N. S. Hsu, Y. T. Wu, **T. L. Li***, C. H. Wong*, 2012, "Engineering transaldolase in Pichia stipitis to improve bioethanol production", *ACS CHEMICAL BIOLOGY*, 7(3), 481-486.

Evidence of diradicals involved in the yeast transketolase catalyzed keto-transferring reactions

Tsung-Lin Li

The Genomics Research Center, Academia Sinica, Taipei, Taiwan
Email: tlli@gate.sinica.edu.tw

Transketolase (TK) catalyzes a reversible transfer of a two-carbon (C_2) unit between phosphoketose donors and phosphoaldose acceptors, in which the group-transfer reaction that follows a one- or two-electron mechanism and the force that breaks the C2"–C3" bond of the phosphoketose donors remain elusive. Here, we report previously unknown intermediate states in TK (TKps) from *Pichia stipitis* by ultrahigh-resolution crystal structures, which support an unprecedented diradical mechanism for the reversible group-transfer reaction. In conjunction with MS, NMR spectroscopy, EPR and computational analyses, we proposed that the enzyme-catalyzed non-Kekulé diradical cofactor brings about the C2"–C3" bond cleavage/formation for the C_2-unit transfer reaction, for which suppression of activation energy and activation and destabilization of enzymatic intermediates are facilitated.

Reference

(1) N. S. Hsu, Y. L. Wang, K. H. Lin, C. F. Chang, S. C. Ke, S. Y. Lyu, L. J. Hsu, Y. S. Li, S. C. Chen, K. C. Wang, **T. L. Li***, 2018, "Evidence of diradicals involved in the yeast transketolase catalyzed keto-transferring reactions", *CHEMBIOCHEM*, 19(22), 2395-2402.

(2) N. S. Hsu, Y. L. Wang, K. H. Lin, C. F. Chang, S. Y. Lyu, L. J. Hsu, Y. C. Liu, C. Y. Chang, C. J. Wu, **T. L. Li***, 2018, "The mesomeric effect of thiazolium on non-Kekule diradicals in Pichia stipitis transketolase", *ANGEWANDTE CHEMIE-INTERNATIONAL EDITION*, 57(7), 1802-1807.

(3) L. J. Hsu, N. S. Hsu, Y. L. Wang, C. J. Wu, **T. L. Li***, 2016, "Structural and biochemical interrogation on transketolase from Pichia stipitis for new functionality", *PROTEIN ENGINEERING DESIGN & SELECTION*, 29(11), 513-522.

(4) S. H. Chen, D. R. Hwang, G. H. Chen, N. S. Hsu, Y. T. Wu, **T. L. Li***, C. H. Wong*, 2012, "Engineering transaldolase in Pichia stipitis to improve bioethanol production", *ACS CHEMICAL BIOLOGY*, 7(3), 481-486.

New Horizon in Enzymology from Cryo-EM and X-ray Free-electron Laser (XFEL)

Ming-Daw Tsai
Institute of Biological Chemistry, Academia Sinica

Abstract

Structural determination of enzymes has been an integral part of mechanistic enzymology. However, the two common approaches, X-ray crystallography and nuclear magnetic resonance (NMR), can only be obtained at specific conditions, whereas mechanistic analysis often covers a broad range of conditions. These limitations are being overcome by two emerging structural biology techniques: Cryo-EM can facilitate structural determination at multiple solution conditions with various intermediate complexes, while XFEL allows determination of multiple intermediate structures at ultrashort time intervals. In this lecture I will illustrate applications of high-resolution cryo-EM to the mechanism of ketol-acid reductoisomerase (KARI), and the emerging XFEL to the photoreduction mechanism of a DNA photolyase.

Comparison of Structures, Dynamics, and Thermostabilities of Proteins from Thermophilic, Mesophilic, and Psychrophilic Bacteria for their Thermal Adaptation

Yeongjoon Lee, Dasom Cheon, Jung Woo Park, and Yangmee Kim*

Department of Bioscience and Biotechnology, Konkuk University, Seoul 05029, Republic of Korea

Abstract: Temperature is one of the most critical factors for bacterial survival. Flexibility and rigidity in protein conformations is important for its function in wide range of temperature, playing key roles in molecular recognition, rate-limiting conformational transitions, and catalysis. Motion in protein is essential for its ability to interact with functionally different proteins or ligands. Despite dramatic differences in the thermostability of proteins in various microorganisms, proteins share high primary sequence homology and a high degree of three-dimensional structural similarity between the homologues. In this study, we will discuss the key factors in the thermostabilities, structures, and dynamics of proteins from hyperthermophilic, thermophilic, mesophilic, and psychrophilic bacteria. Molecular details of dynamics in these proteins provide insights into their thermostabilities and communication with protein partners and ligands for their functions and thermal adaptation.

Structural Characterization of HypX Responsible for CO Biosynthesis in the Maturation of [NiFe]-Hydrogenases

Shigetoshi Aono[1,2]

1. Department of Creative Research, Exploratory Research Center on Life and Living Systems (ExCELLS), National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji-cho, Okazaki 444-8787, Japan

2. Institute for Molecular Science, National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji-cho, Okazaki 444-8787, Japan

Hydrogenases are metalloenzymes that play a central role for H_2 metabolism in microorganisms. Based on the differences of metal content and the structure of the active site, they are classified into three groups: FeFe-, NiFe-, and Fe-hydrogenases containing a dinuclear Fe unit linked to a [4Fe-4S] cluster, a hetero dinuclear Ni-Fe cluster, and a mononuclear Fe center, respectively. These metal clusters in hydrogenases ligate diatomic ligands, CO for the Fe-hydrogenase, CO and CN^- for FeFe- and NiFe-hydrogenases, as terminal ligands. These CO and CN^- are biosynthesized and assembled into the metal clusters, for which several accessory and chaperone proteins are required. Though it is reported that HypX catalyzes CO biosynthesis for the maturation of NiFe-hydrogenase, the molecular mechanism of CO biosynthesis remained unknown. Here, we have determined the crystal structures of HypX with and without a substrate, based on which we elucidate the molecular mechanism of CO synthesis by HypX.

We have determined the crystal structure of HypX at a resolution of 1.8 Å. HypX consists of the N-terminal and the C-terminal domains, which are structurally homologous to the hydrolase domain of N^{10}-formyl-tetrahydrofolate dehydrogenase (FDH) and enoyl-CoA hydratase/isomerase, respectively. HypX binds CoA constitutively as a prosthetic group in the continuous cavity connecting the N- and C-terminal domains. We have also solved the structure of tetrahydrofolate (THF)-bound HypX at a resolution of 2.1 Å, in which THF is accommodated in the cavity in the N-terminal domain. Based on these crystal structures and MD simulations, we propose the molecular mechanism of CO biosynthesis by HypX as follows. The reaction starts with binding N^{10}-formyl-THF as a substrate in the N-terminal domain of HypX. The formyl-group transfer takes place from N^{10}-formyl-THF to CoA, which is a prosthetic group constitutively bound in HypX, to form formyl-CoA in the N-terminal domain. In this formyl-group transfer reaction, His74, Asp80, and Asp109 act as the catalytic triad. The resulting formyl-CoA is converted into CO and CoA by decarbonylation of the formyl group, which is catalyzed by Tyr416 and/or Glu426 in the C-terminal domain of HypX. The conformation of CoA accommodated in the continuous cavity connecting the N- and C-terminal domains interconverts between the open and the closed conformations, by which the position of the terminal group of the pantetheine moiety of CoA is changed between each active site in the N- and C-terminal domains. This work reveals not only the molecular mechanism of CO biosynthesis for the maturation of NiFe-hydrogenases but also a novel biological function of formyl-CoA as a CO precursor.

Protein Structure and Dynamics: Serial Femtosecond Crystallography(SFX)

Weontae Lee

Structural Biochemistry & Molecular Biophysics Laboratory, Department of Biochemistry, College of Life Science & Biotechnology, Yonsei University, Seoul, 120-749, Republic of Korea;
wlee@spin.yonsei.ac.kr

X-ray crystallography demonstrated its strong power to determine crystal structure of protein at cryogenic temperature. Very recently, with dramatic advances of both hardware development and experimental techniques, protein structure determination has become possible from microcrystals at room temperature using serial femtosecond crystallography (SFX) with X-ray free-electron lasers (XFELs). Non-cryogenic protein structures determined at ambient temperature may disclose significant information about protein activity together with dynamic profiles. Therefore, SFX could be a complimentary method together with NMR spectroscopy in studying protein dynamics and motion, particularly for membrane proteins and/or protein complexes. B-factor information from SFX and backbone dynamics data from NMR obtained at room temperature supported a motility change on the residual motion due to temperature effects. I will present the structure and dynamics of catalytic core domain of HIV-1 IN and membrane proteins using both NMR and SFX techniques. Although structures by NMR, X-ray and SFX were almost identical except differences in the orientation of sidechains and secondary structural regions, dynamic profiles in the active and ligand binding site are dramatically different. Our data will enhance our understanding for catalytic mechanism of enzymes and dynamics of membrane proteins by SFX.