Frontier Bioorganization Forum 2017:

Dynamical ordering and integrated functions of biomolecular systems

Symposium Program

2017

April 24 - 26

College of Science,
National Chiao Tung University

Institute of Biological Chemistry,
Academia Sinica

JSPS Grant-in-Aid for Scientific Research on Innovative Areas
"Dynamical ordering of biomolecular systems for creation of integrated functions"

Venue:

Auditorium 103

Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

Frontier Bioorganization Forum 2017：

Dynamical ordering and integrated functions of biomolecular systems

April 24th (Mon.) －26th (Wed.)

Organized by

College of Science | National Chiao Tung University

Institute of Biological Chemistry | Academia Sinica

JSPS Grant-in-Aid for Scientific Research on Innovative Areas

"Dynamical ordering of biomolecular systems for creation of integrated functions"

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Frontier Bioorganization Forum 2017:

Dynamical ordering and integrated functions of biomolecular systems

(April 24th～April 26th, 2017)

Program

APR. 24 (Mon.)

08:45 – 09:15	Registration	
09:15 – 09:30	Opening ceremony	
Opening remarks	Chair: Prof. Ming-Daw Tsai	Institute of Biological Chemistry, Academia Sinica
	Chair: Prof. Y. K. Li	College of Science, National Chiao Tung University
	Prof. Koichi Kato	Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences

Session 1 09:30 – 10:30

(Chair: Prof. Hans Chun-Hung Lin (Institute of Biological Chemistry, Academia Sinica))

Time	Title of Talk	Speaker
09:30-10:00	Structural insights into dynamic orchestration of biomolecular systems	Koichi Kato
10:00-10:30	Mass spectrometry-based Protein Glycosylation Analysis – a close-up view on the sweetened protein functions	Kay-Hooi Khoo
10:30-11:00	Coffee Break	

Session 2 11:00 – 12:30

(Chair: Prof. Hironari Kamikubo (Graduate School of Materials Science, Nara Institute of Science and Technology))

Time	Title of Talk	Speaker
11:00-11:30	Native mass spectrometry: complementary to other biophysical methods	Susumu Uchiyama
11:30-12:00	Structural basis to develop potent and selective inhibitors for galectin and glucuronidase	Hans Chun-Hung Lin
12:00-12:30	Structure-Based Drug Design for Wnt/β-catenin Signaling Diseases	Weontae Lee
12:30-13:30	Lunch	

Session 3 13:30 – 15:00

(Chair: Prof. Takafumi Ueno (Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology))

Time	Title of Talk	Speaker
13:30-14:00	Biomolecular simulations in generalized ensemble	Yuko Okamoto
14:00-14:30	Reconstructing microRNA-mediated gene regulatory networks in human	Hsien-Da Huang
14:30-15:00	Theoretical study on substituent and solvent effects for nanocube formed with gear-shaped amphiphile molecules	Masanori Tachikawa
15:00-15:30	Coffee Break	

Session 4 15:30 – 17:30

(Chair: Prof. Po-Huang Liang (Institute of Biological Chemistry, Academia Sinica))

Time	Title of Talk	Speaker
15:30-16:00	Molecular Mechanism for Axon Navigation in the Brain	Naoyuki Inagaki
16:00-16:30	Biosynthesis of Streptothricin: activation, resistance and development	Tsung-Lin Li
16:30-17:00	Strain-sensitive regulation of mechanical activities of dyneins underlying oscillatory movement of sperm flagella	Chikako Shingyoji
17:00-17:30	Toward decoding complexity and logic of protein polySUMO/Ub modifications	Yane-Shih Wang
18:00-	Banquet	

APR. 25 (Tue.)

Session 5 09:00 – 10:30

(Chair: Prof. Ryota Iino (Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences))

Time	Title of Talk	Speaker
09:00-09:30	Multi-component equilibrium in biological systems explored by using continuous titration SAXS	Hironari Kamikubo
09:30-10:00	Wetting and Instability Studies of Polymer Nanomaterials in Porous Templates	Jiun-Tai Chen
10:00-10:30	Design of Protein Assemblies as Supramolecular Platforms	Takafumi Ueno
10:30-11:00	Coffee Break	

Session 6 11:00 – 12:30

(Chair: Prof. Tung-Kung Wu (Department of Biological Science &Technology, National Chiao Tung University))

Time	Title of Talk	Speaker
11:00-11:30	Investigation on domain motion of protein by neutron scattering	Masaaki Sugiyama
11:30-12:00	Folding and misfolding of topologically knotted ubiquitin C-terminal hydrolases	Shang-Te Danny Hsu
12:00-12:30	Versatile structural architectures of archaeal homolog of proteasome assembly chaperone	Maho Yagi-Utsumi
12:30-13:30	Lunch	

Session 7 13:30 – 15:00

(Chair: Prof. Ping-Chiang Lyu (Institute of Bioinformatics and Structural Biology, National Tsing Hua University)

Time	Title of Talk	Speaker
13:30-14:00	A primary ion pump: proton/sodium pumping pyrophosphatases	Yuh-Ju Sun
14:00-14:30	Protein Structure Prediction/Determination by Global Optimization	Jooyoung Lee
14:30-15:00	Structural basis of the *Klebsiella pneumoniae* PmrA response regulator in gene transcription and inhibitor development	Chinpan Chen
15:00-15:30	Coffee Break	

Session 8 15:30 – 17:30

(Chair: Prof. Wen-Sheng Chung (Department of Applied Chemistry, National Chiao Tung University))

Time	Title of Talk	Speaker
15:30-16:00	Nanocube: Ultra-Thermostble Self-Assemblies in Water	Shuichi Hiraoka
16:00-16:30	Amphiphilic nanoparticles of resveratrol–norcantharidin and its peptide analog to enhance the toxicity in *Escherichia coli* and zebrafish embryo	Chien-Chung Cheng
16:30-17:00	Control over differentiation of a metastable supramolecular assembly	Kazunori Sugiyasu
17:00-17:30	Molecular Interactions in the Formation of Self-assembled Peptide Hydrogels	Hsin-Chieh Lin
17:30-18:00	Panel Discussion	
18:30-	Invited Dinner	

APR. 26 (Wed.)

Session 9 09:00 – 10:30

(Chair: Prof. Hsiao-hua Yu (Institute of Chemistry, Academia Sinica))

Time	Title of Talk	Speaker
09:00-09:30	Chemo-mechanical coupling mechanisms of linear and rotary molecular motors revealed by high-speed single-molecule imaging analysis	Ryota Iino
09:30-10:00	Direct visualization of dynamic molecular interactions using HS-AFM	Takayuki Uchihashi
10:00-10:30	Viscosity, thermal diffusivity, and polarity of lipid bilayer membranes estimated from fast time-resolved spectroscopic measurements	Koichi Iwata
10:30-11:00	Coffee Break	

Session 10 11:00 – 12:30

(Chair: Prof. Masaaki Sugiyama (Research Reactor Institute, Kyoto University))

Time	Title of Talk	Speaker
11:00-11:30	Understanding of Self-Assembly Process at Molecular Level	Hirofumi Sato
11:30-12:00	Towards pharmacological analysis using zebrafish models of cardiovascular diseases	Ian Liau
12:00-12:30	Smart PEDOT Nanostructures for Circulating Tumor Cells Related Cancer Prognosis	Hsiao-hua Yu
12:30-13:30	Lunch	

Abstracts

Structural insights into dynamic orchestration of biomolecular systems

Koichi Kato[1,2]

[1]*Okazaki Institute for Integrative Bioscience and Institute for Molecular Science,*

National Institutes of Natural Sciences,

5-1 Higashiyama Myodaijicho, Okazaki 444-8787, Japan

[2]*Graduate School of Pharmaceutical Sciences, Nagoya City University,*

3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

Email: kkatonmr@ims.ac.jp

Living systems are characterized as dynamic processes of assembly and disassembly of various biomolecules that are self-organized, interacting with the external environment. The omics-based approaches developed in recent decades have provided comprehensive information regarding biomolecules as parts of living organisms. However, fundamental questions remain unsolved as to how these biomolecules are ordered autonomously to form flexible and robust systems (Fig.1). Biomolecules with complicated, flexible structures are self-organized through weak interactions giving rise to supramolecular complexes that adopt their own dynamic, asymmetric architectures. These processes are coupled with expression of integrated functions in the biomolecular systems.

To address these issues, we conduct multidisciplinary approaches based on detailed analyses of dynamic structures and interactions of biomolecules at atomic level, in conjunction with the methodologies of molecular and cellular biology along with synthetic and computational techniques.

In this presentation, I will present our insights gained through the activities in our project "Dynamical ordering of biomolecular systems for creation of integrated functions".

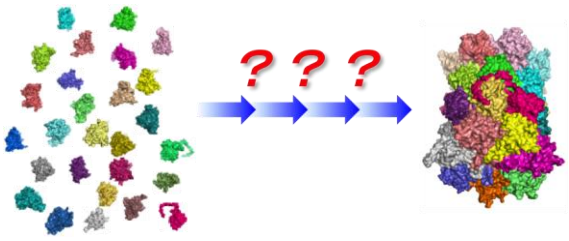

Fig.1
Formation of supramolecular machinery through dynamic assembly and disassembly of biomolecules.

Mass spectrometry-based Protein Glycosylation Analysis – a close-up view on the sweetened protein functions

Kay-Hooi Khoo

Institute of Biological Chemistry, Academia Sinica, Taiwan.

Email: kkhoo@gate.sinica.edu.tw

A majority of the key events taking place at the cell surface, including those involving the important classes of receptors, are either directly or indirectly mediated by glycosylation. Site-specific glycosylation has significant impact on the local protein conformation, stability and binding site accessibility. It thus affects and attenuates receptor-ligand binding, protein-protein interactions, dimerization, and other bimolecular ordering. Glycomics or analysis of released glycans only informs the range of glycan heterogeneity that are expressed ensemble. Important as it is from glycobiology perspectives, a close-up view on how a perturbed glycosylation event may dictate the outcome of signaling network can only be investigated at the level of protein site-specific glycosylation. The same glycan structure may be of no functional consequence when carried on one site as a bystander but will act as key determinant when attached to another site near or at the center of interactions. Over the last decades, advances in mass spectrometry has enabled increasingly meaningful glycoproteomic venture but such global undertakings often lacks in specific details and misses the key glycoforms from key protein sites. We propose instead to develop workflows that allow in depth analysis of specific target glycoproteins. How different mass spectrometry techniques currently available can be utilized judiciously and in concert will be demonstrated in this presentation using specific case examples. These include complementary modes of fragmentation that can be acquired in parallel or in product ion dependent manner, the high resolution accurate mass capability at not only MS1 but also MS2/MS3 level, the data independent acquisition mode at increasing speed and sensitivity, and the requisite informatics solutions. Despite notable success and advances, current impasse in glycopeptide sequencing and identifications, particularly when both N- and multiple O-glycosylation are present on a single peptide, still awaits more technical breakthrough before its becoming a routine application.

Native mass spectrometry: complementary to other biophysical methods

Susumu Uchiyama

Department of biotechnology, Graduate School of Engineering, Osaka University

Okazaki Institute for Integral Biosciences, NINS

Email: suchi@bio.eng.osaka-u.ac.jp

Inter-molecular interactions according to the differences of Gibbs free energy (d*G*) among each state. Physico-chemical parameters, stoichiometry and dissociation constant (k_D), are directly connected to d*G* thus determination of stoichiometry and k_D of an interaction is essential for understanding the interaction from energetical point of view. Several biophysical methods that enable us to monitor dissociation-association equilibrium of a protein-protein or protein-ligand interactions are now available, however most of the method requires stoichiometric model of the interaction to determine the correct k_D. We have been employed native mass spectrometry (native MS) to clarify stoichiometry of interactions unambiguously. The information on stoichiometry of the interaction is incorporated in the analysis of protein-protein and protein-ligand interactions monitored by other biophysical methods including analytical ultracentrifugation (AUC) and small angle x-ray scattering (SAXS). We first studied antibody-antigen interactions by native MS and AUC, where two methods consistently showed that an antibody (Ab) to hapten binds to its antigen (Ag, hapten conjugated BSA) dominantly in 1:2 stoichiometry ($AbAg_2$), although surface plasmon resonance (SPR) method showed that the interaction is 1:1 stoichiometry. We then applied the combination of native MS and AUC to investigate the assembly state of a nucleosome chaperon, NAP-1. Native MS showed NAP-1 is assembled into dimer and the dimer is further assembled to higher oligomers, such as tetramer, hexamer, and octamer. In the subsequent study, these assembly process was employed as a prerequisite condition for the numerical analysis of sedimentation velocity AUC. The analysis successfully identified the population of even-numbered oligomers with quantitative information. In this presentation I will introduce how native MS provide precise stoichiometry of an inter-molecular interactions that can be used for the determination of k_D and/or hydrodynamic shape of each proteins by other biophysical approaches.

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Structural basis to develop potent and selective inhibitors for galectin and glucuronidase

Hans Chun-Hung Lin
Institute of Biological Chemistry, Academia Sinica, Taipei, TAIWAN

Human galectins are promising targets for cancer immunotherapeutic and fibrotic disease-related drugs. We report herein the binding interactions of three thio-digalactosides (TDGs) including TDG itself, TD139 (3,3'-deoxy-3,3'-bis-(4-[*m*-fluorophenyl]-1H-1,2,3-triazol-1-yl)-thio-digalactoside, recently approved for the treatment of idiopathic pulmonary fibrosis), and TAZTDG (3-deoxy-3-(4-[*m*-fluorophenyl]-1H-1,2,3-triazol-1-yl)-thio-digalactoside) with human galectins-1, -3 and -7 as assessed by X-ray crystallography, isothermal titration calorimetry and NMR spectroscopy. Five binding subsites (A–E) make up the carbohydrate-recognition domains of these galectins. We identified novel interactions between an arginine within subsite E of the galectins and an arene group in the ligands. In addition to the interactions contributed by the galactosyl sugar residues bound at subsites C and D, the fluorophenyl group of TAZTDG preferentially bound to subsite B in galectin-3, whereas the same group favored binding at subsite E in galectins-1 and -7. The characterised dual binding modes demonstrate how binding potency, reported as decreased K_d values of the TDG inhibitors from μM to nM, is improved and also offer insights to development of selective inhibitors for individual galectins.

Additionally we will also discuss the development of inhibitors for targeting bacterial β-glucuronidases. Xenobiotics are subjected to attachment of β-linked glucuronic acid in the liver, to increase the extent of their excretion. However, the action is abolished due to the interference of gut bacterial β-glucuronidases. It is thus important to develop inhibitors to distinguish between human and bacterial enzymes.

Structure-Based Drug Design for Wnt/β-catenin Signaling Diseases

Weontae Lee

Department of Biochemistry, College of Life Science & Biotechnology, Yonsei University, Seoul 120-749, Korea
Email : wtlee@yonsei.ac.kr

Wnt/b-catenin pathway is aberrantly activated in most human colorectal cancers (CRCs) and many proteins interact with other molecules cooperatively in tumor promotion. Destabilization of both b-catenin and Ras signaling via targeting axin is a potential therapeutic strategy for treatment of CRC and other type cancers activated Wnt/b-catenin and Ras pathways. We identified axin as a direct target through *in vitro* binding studies, and uncovered details of the interaction between a novel drug compound and regulators of the G-protein signaling (RGS) domain of axin using nuclear magnetic resonance (NMR) experiments. Other example is targeting for Dishevelled (Dvl)/CXXC5 interaction in regulation of the Wnt/β-catenin pathway in osteoblast differentiation. We previously identified that CXXC5 is a negative feedback regulator of the Wnt/b-catenin pathway via its interaction with Dvl and suggested the Dvl–CXXC5 interaction as a potential target for anabolic therapy of osteoporosis. Nuclear magnetic resonance (NMR) and X-ray crystallographic analysis confirmed interaction between Dvl PDZ domain and a representative of the screened compounds. In conclusion, small-molecule inhibitors to block regulation of Wnt/b-catenin signaling are potential candidates for the development of both cancer and anti-osteoporosis drugs.

Biomolecular simulations in generalized ensemble

Yuko Okamoto

Department of Physics, Nagoya University

Nagoya, Aichi 464-8602, Japan

Email: okamoto@tb.phys.nagoya-u.ac.jp

Conventional Monte Carlo and molecular dynamics simulations are greatly hampered by the multiple-minima problem, where the simulations tend to get trapped in some of astronomically large number of local-minimum energy states. In order to overcome this difficulty, we have been advocating the uses of generalized-ensemble algorithms which are based on non-Boltzmann weight factors (for reviews, see, e.g., Refs. [1-4] and for our recent algorithm developments and their applications, see, e.g., Refs. [5-13]). With these algorithms we can explore a wide range of the conformational space. The advantage of generalized-ensemble algorithms such as multicanonical algorithm and replica-exchange method (or, parallel tempering) lies in the fact that from only one simulation run, one can obtain various thermodynamic quantities as functions of temperature and other physical parameters by the reweighting techniques. In this talk, I will present the latest results of various applications of generalized-ensemble algorithms to classical and quantum molecular simulations.

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Reconstructing microRNA-mediated gene regulatory networks in human

Hsien-Da Huang

Institute of Bioinformatics and Systems Biology,

Department of Biological Science and Technology, National Chiao Tung University

Email: bryan@mail.nctu.edu.tw

MicroRNAs (miRNAs), i.e. small non-coding RNA molecules (~22 nt), can bind to one or more target sites on a gene transcript to negatively regulate protein expression, subsequently controlling many cellular mechanisms. We have developed several international recognized databases (i.e., miRNAMap, miRStart and miRTarBase) and computational tools (i.e., miRTar, RNALogo, RNAMST and RegRNA) as the basis for investigating the miRNAs' roles in biological systems. In particular, our miRTarBase is an updated and the most comprehensive collection of miRNA–target interactions (MTIs) with experimental supports. It provides an essential information resource to elucidating miRNA functions under different conditions and in different species.

High-throughput sequencing technology enables a revolutionary advance to identifying miRNA-target interactions, transcription factor (TF)-miRNA regulations, and subsequently to reconstructing TF-miRNA-target regulatory networks. Chromatin immunoprecipitation sequencing (ChIP-seq) can be utilized for transcriptional regulation of miRNAs. High-throughput experiments (CLIP-seq, PAR-CLIP-seq and CLASH-seq) are powerful methods for identifying miRNA-target interactions. In this talk, I will give a brief introduction to these methods. The roles of miR-122 in liver cancer will be introduced as an illustrative example.

Theoretical study on substituent and solvent effects for nanocube formed with gear-shaped amphiphile molecules

Takako Mashiko[1], Shuichi Hiraoka[2], Umpei Nagashima[2], and Masanori Tachikawa[1]

[1]Graduate School of NanoBioScience, Yokohama City University,
22-2 Seto, Kanazawa-ku, Yokohama-shi, Kanagawa 236-0027, Japan
[2]Graduate School of Arts and Sciences, The University of Tokyo,
3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan
E-mail: tachi@yokohama-cu.ac.jp

Recently, Hiraoka *et al.* have synthesized a gear-shaped amphiphile molecule **1**, as shown in Figure 1. Molecules **1**s are self-assembled into a cubic-shaped hexameric structure, nanocube ($\mathbf{1}_6$), in 25% aqueous methanol [1, 2]. Another gear-shaped molecule (**2**), in which three methyl groups of **1** are replaced with hydrogen atoms, does not form the nanocube ($\mathbf{2}_6$). Hiraoka *et al.* further found that **1** is not self-assembled in pure methanol. However, the roles of the substituent effect and the solvent effect are not fully understood at microscopic molecular level, yet. The purpose of this work is, thus, to elucidate the stability of these hexameric capsules $\mathbf{1}_6$ and $\mathbf{2}_6$ in water, 25% aqueous methanol, and methanol solvents, with the aid of molecular orbital and molecular dynamics approaces.

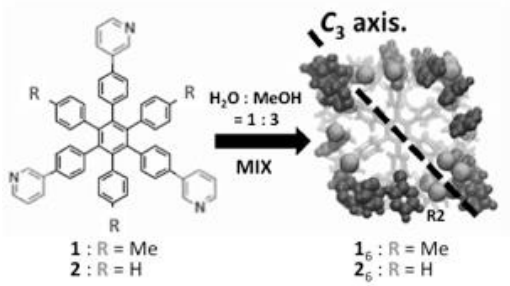

Figure 1 Chemical formulae of gear-shaped amphiphile molecules, **1** (R = CH_3) and **2** (R = H).

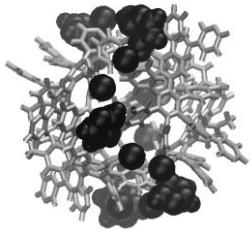

Figure 2 Black atoms are the CH-π chain in nanocube $\mathbf{1}_6$.

In all trajectories with all solvents, all the structures of nanocube $\mathbf{1}_6$ are maintained. On the other hand, parts of the structures of $\mathbf{2}_6$ are collapsed in water and 25% aqueous methanol solvents. In methanol solvent, the $\mathbf{2}_6$ for all trajectories are collapsed. The number of collapsed trajectories of $\mathbf{2}_6$ is increased, as the number of methanol solvent molecules is increased. We focus on the nanocube structure of the π-π stacking between pyridines and CH-π interactions between the methyl group and pyridine. The CH-π chain among pyridyl groups and CH_3 groups can be constructed on the $\mathbf{1}_6$ as shown in Figure 2, because the nanocube structure can be stable by van der Waals force. We also found that methanol molecules in 25% aqueous methanol solvent behave as amphiphilic ones, while the CH-π chain in nanocube is broken by methanol molecules.

References:

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Molecular Mechanism for Axon Navigation in the Brain

Naoyuki Inagaki

Graduate School of Biological Sciences, Nara Institute of Science and Technology,
Takayama-cho 8916-5, Ikoma 630-0192, JAPAN
Email: ninagaki@bs.naist.jp

Neurons extend a long process, axons, to the right destinations and form complicated networks in the brain; all our brain activities depend on the neuronal network. Axonal outgrowth can be navigated by extracellular chemical cues such as soluble chemicals (chemotaxis) and substrate–bound chemicals (haptotaxis); they are called axon guidance molecules. Actin filaments (F-actins) polymerize at the leading edge of axon (growth cone) and depolymerize proximally; this, with myosin II activity, induces retrograde flow of F-actins. It has been proposed that the forces underlying axon outgrowth and guidance may be regulated by the modulation of coupling efficiency between F-actin flow and the extracellular substrate via linker "clutch" molecules. However, how cell signaling controls the coupling efficiency remains unknown.

We recently identified a protein shootin1, and are analyzing the molecular mechanisms for axon outgrowth and guidance, using fluorescent live-cell imaging, traction force microscopy and molecular loss-of-function manipulation. We show that shootin1 and F-actin binding protein cortactin function as clutch molecules that couples F-actin retrograde flow and the substrate at axonal growth cones to promote axon outgrowth.

A soluble axon guidance molecule netrin-1 positively regulates forces at axonal growth cones via Pak1-mediated shootin1 phosphorylation. This phosphorylation enhanced the interaction between shootin1 and F-actin retrograde flow through cortactin and the interaction between shootin1 and a cell adhesion molecule L1-CAM, thereby promoting force generation and axon outgrowth. These results suggest that shootin1 is located at a critical interface, transducing a signal of netrin-1 into the forces for axonal haptotaxis.

We also show that the molecular machinery composed of polymerizing F-actins, L1-CAM, and clutch molecules shootin1 and cortactin is involved in axonal haptotaxis directed by substrate–bound chemical cue laminin. In this system, differential grip and slip between L1-CAM and adhesive substrates generates directional force for the haptotaxis. In contrast to the classical model for haptotaxis, this mechanism does not depend on cell signaling: L1-CAM acts as both chemo-sensor and mechano-effector for navigation

Biosynthesis of Streptothricin: activation, resistance and development

Tsung-Lin Li

Genomics Research Center, Academia Sinica, Taiwan

tlli@gate.sinica.edu.tw

Streptothricin-F (STT-F), an aminoglycoside antibiotic, consists of three components, a β-lysine homopolymer, an aminosugar D-gulosamine, and an unusual bicyclic streptolidine. The biosynthesis of STT-F has lately been elucidated to a significant extent. However, the biosynthesis of streptolidine remains a long-lasting but unresolved puzzle. A combination of genetic/biochemical/structural approaches was used to shed new light on this puzzle. The STT gene cluster was sequenced from a *Streptomyces* variant BCRC 12163, wherein two gene products OrfP and OrfR were characterized *in vitro* to be a dihydroxylase and a cyclase, respectively. Thirteen high resolution crystal structures for both enzymes in different reaction states were snapshotted to allow visualization of the catalytic process at molecular level. OrfP catalyzes an Fe^{II}-dependent double hydroxylation reaction converting L-Arg into (3*R*,4*R*)-$(OH)_2$-L-Arg via (3*S*)-OH-L-Arg; OrfR catalyzes an unusual PLP-dependent elimination/addition reaction cyclizing (3*R*,4*R*)-$(OH)_2$-L-Arg to the six-membered (4*R*)-OH capreomycidine. The biosynthetic mystery finally comes to light as the latter product was isotopically labeled and found incorporation into STT-F in a feeding experiment. Two genes *orfM* and *orfE* in the STT gene cluster code for a deacetylase and an acetyltransferase, respectively. OrfM was characterized *in vitro* and *in vivo* capable of freeing the amino group from N-acetyl streptothrisamine, thus enabling the subsequent β-lysination to bring the antimicrobial to its full play. OrfE was characterized to be a resistance gene, whereby the antimicrobial is switched to its full silence by acetylation. Of this information, we were in a position to create an array of new analogs, some of which show not only enhanced antimicrobial activity but immune to drug-resistance enzymes.

Strain-sensitive regulation of mechanical activities of dyneins underlying oscillatory movement of sperm flagella

Chikako Shingyoji

Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Hongo Tokyo, Japan

Email: chikako@bs.s.u-tokyo.ac.jp

The movement of eukaryotic flagella and cilia is characterized by oscillatory beating produced by the spatial control of sliding between adjacent doublet microtubules along the flagellar and ciliary axoneme. Dynein arms generate force for microtubule sliding. Our goal is to understand the emergence of coordinated activities of dyneins, which are the basis of flagellar oscillation. The mechanism that regulates dynein function to induce flagellar oscillation involves molecular and cellular levels of regulation, including the ATP-driven oscillation of the force produced by individual dynein molecules, coordinated sliding activities of the dynein molecules arrayed along a microtubule, the switching regulation of the pattern of active sliding around the axonemal axis, and the initiation of flagellar beating coupled with the mechanical force of bending. There is ample evidence that mechanical conditions influence the oscillation. How does dynein behave under various mechanical signals? We found that directionality of force generation by a single molecule dynein reversed after an application of mechanical strain. Bidirectional force generation was observed both in dyneins still attached on the doublet and in dynein arms isolated from sea urchin sperm flagellar axonemes. For the oscillatory force generation, however, intact state of dynein still attached on the doublet seems to be necessary. The strain-dependent modification of sliding activity was also induced in systems where ensemble of dynein molecules cooperates. As a transient response upon an application of the mechanical signal of bending, the array of dyneins on the doublet showed a change in directionality of the interacting microtubule from a forward to a backward movement. Experiments by using isolated dynein arms suggested that the ensemble of dynein molecule are capable of changing its mode under mechanical signal of bending of the interacting microtubule: dyneins showed stoppage of movement, a decrease in sliding speed and reversal of moving direction. Dyneins appear to operate in a self-regulatory feedback system, capable of acting as an actuator, a mechanical sensor and a mechanical processor, thus enabling the coordination of sliding activities of multiple dynein molecules arrayed along a microtubule, which in turn is the basis of overall flagellar oscillation.

Toward decoding complexity and logic of protein polySUMO/Ub modifications

Yane-Shih Wang

Institute of Biological Chemistry, Academia Sinica, Nankang, Teipei 115 Taiwan
E-mail: yaneshihwang@gate.sinica.edu.tw

Protein ubiquitylation and sumoylation are dynamic multifaceted protein post-translational modification (PTM) involved in nearly all aspects of eukaryotic systems. With initial lysine ubiquitylation or sumoylation, further diverse lysine modification and poly-ubiquitin/SUMO chain linkage generate a complex signal network with distinct biological consequences, so called "ubiquitin code". Using expanding genetic code technology, we develop a protein synthetic methodology to produce site-specific and homogeneous sumoylated protein of interest by pyrrolysyl-tRNA synthetase•$tRNA^{Pyl}$ pair and following protein modification and chemical ligation by using Michael addition reaction and click chemistry. Non-canonical amino acid (ncAA) incorporation was used to develop a detection method of environmental sensitive fluorescent functional groups of ncAA for detecting specific protein polyhetero-ubiquitin/SUMO modifications in normal and cancer cells. This ncAA incorporation approach is developed for the synthesis of protein with defined PTM mimics and understand the diversity and localization of protein polyhetero-ubiquitin/SUMO modifications. The intrinsic simplicity of these methods will accelerate our understanding of biochemistry and biology of protein ubiquitylation or sumoylation.

Keywords: Protein SUMOylation and ubiquitylation, non-caonical amino acid incorporation, PylRS•$tRNA^{Pyl}$ pair engineering, protein-protein coupling chemistry

Multi-component equilibrium in biological systems explored by using continuous titration SAXS

Hironari Kamikubo

Complex Molecular Systems Lab.,

Graduate School of Material Science, Nara Institute of Science and Technology

8916-5 Takayama, Ikoma, Nara, Japan

Email: kamikubo@ms.naist.jp

Various protein molecules concert with each other to express various biological functions. Because these multicomponent biological molecules weakly interact with each other, they can undergo regulatory dissociation and association upon inducing biological stimuli. In order to understand biological systems, we must, at first, aim to identify every possible unstable complex involved in the given multicomponent system, and then quantitatively analyze the interactions of these complexed molecules. However, because of the complexity, it is generally difficult to apply conventional analytical methods to analyze such multi-component equilibrium systems. We have developed a new analytical method that would enable us to perform structure and interaction analyses on multi-component equilibrium systems.

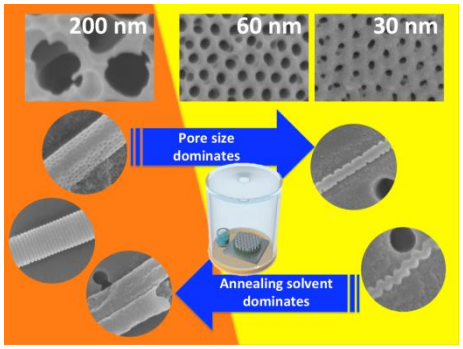
Here, we will present the progress and development of the microfluidics-based, auto sampling equipment designed for continuous titration SAXS. Using this newly designed equipment, we can automatically collect numerous scattering profiles while altering the molar ratios of each component involved in the multi-component equilibrium; thus, enabling us to determine the system's free energy landscape of the multi-component equilibrium. Very recently, we successfully installed the sampling system into the beam line at the Photon Factory, Tsukuba, Japan, and performed titration-SAXS measurements on a signal transduction system containing Photoactive Yellow Protein (PBP) and it interaction partner (PBP). The SEC chromatography shows various complexes with different size exist in a mixed solution containing PYP and PBP. The complexity prevent us from understanding the solution structure of each component. The continuous titration SAXS measurements allow us to extract scattering curves of at least three complexes and their concentration dependence. Here, I would like to introduce the potential applications of this sampling system applied for continuous titration SAXS method in addition to the recent results we have obtained.

Wetting and Instability Studies of Polymer Nanomaterials in Porous Templates

Jiun-Tai Chen
Department of Applied Chemistry, National Chiao Tung University, Hsinchu, Taiwan.
E-mail: jtchen@mail.nctu.edu.tw; Tel: +886-3-5731631

We study the fabrication and characterization of different polymer-related nanomaterials by wetting porous templates.[1-5] The templates we choose are anodic aluminum oxide (AAO) templates because of the regular pore distribution, high pore density, and high aspect ratio of the pores. Different nanomaterials such as amorphous carbon nanotubes, amphiphilic block copolymer nanotubes, and porous inorganic materials are fabricated by using these templates. Hierarchical polymer nanostructures are also made by wetting the porous template with polymer microspheres or electrospun polymer fibers. In addition, we study the instabilities of polymer nanomaterials under confinement within the cylindrical pores of the template, which provides a new route for generating novel polymer nanostructures.

We also develop the solvent-annealing-induced nanowetting in templates (SAINT). The morphology of hompolymer nanostructures can be controlled, depending on whether the swollen polymers are in the complete or partial wetting regimes. Polystyrene-block-polydimethylsiloxane (PS-b-PDMS) block copolymer nanostructures are also fabricated using the SAINT method. At larger pore sizes, the morphology of the nanostructures is mainly affected by the annealing solvent. At smaller pore sizes, however, the morphology is mainly controlled by the confinement effect (pore sizes).



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Design of Protein Assemblies as Supramolecular Platforms

Takafumi Ueno

Department of Life Science and Technology, Tokyo Institute of Technology,
Yokohama 2268501, Japan
Email: tueno@bio.titech.ac.jp

Protein assemblies have recently become known as potential molecular scaffolds for applications in materials science and bio-nanotechnology. Efforts to design protein assemblies for construction of protein-based hybrid materials with metal ions, metal complexes, nanomaterials and proteins now represent a growing field with a common aim of providing novel functions and mimicking natural functions. However, the important roles of protein assemblies in coordination and biosupramolecular chemistry have not been systematically investigated and characterized. We focus on our recent progress in rational design of protein assemblies using bioinorganic and bioorganic chemistry for (1) exploration of unnatural reactions, (2) construction of functional protein architectures, and (3) in vivo applications.
In this symposim, I will introduce our current progress on design of protein assemblies. I hope that any overlaps of our interests to initiate any collaborative projects.

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Investigation on domain motion of protein by neutron scattering

Masaaki Sugiyama

Research Reactor Institute, Kyoto University,

Kumatori, Osaka 590-0494 Japan

Email: sugiyama@rri.kyoto-u.ac.jp

Slow domain motions of protein (10^0 - 10^3 nsec) is deeply related with its function. These motions are mainly investigated with computer simulations because it is difficult to observe these coherent motions with the high energy resolution experimentally. In other words, it is one of significant issues to measure the domain motion experimentally and confirm the simulation results.

One approach is to find and analyze the footprint of the domain motions in the static measurement, namely MD-SAXS (Molecular Dynamics – Small-Angle X-ray Scattering). This approach basically stands the following concept: if the protein has the domain motions, the SAXS profile should be expressed by the ensemble average of the profiles corresponding to all configurations in the trajectories of the motions. Therefore, through the SAXS results, the result of MD simulation is examined and then the protein motion could be revealed. This complementary method has succeeded and is in progress. However, the experimental potion, SAXS, is not the "direct" measurement of the domain motion. Therefore, the more direct measurement of the domain motion is really demanded.

Neutron spin echo (NSE), which was developed by F. Mezei in 1970s, is an inelastic coherent neutron scattering spectroscopy. NSE gives us the intermediate scattering function, F(Q,t) which is suitable to analyze the slow relaxations and the diffusions. In the recent developments of neutron intensity and control techniques of magnetic field, NSE can measure the Q-dependency of diffusion coefficient in 0.01-0.5 $Å^{-1}$ with the high resolution. The difficulty of NSE is to derive the correct Q-dependency of diffusion coefficient from NSE spectrum and then analyze it. We are overcoming these difficulties by utilizing with molecular dynamics calculation. Here, I will present our recent NSE result.

Folding and misfolding of topologically knotted ubiquitin C-terminal hydrolases

Shang-Te Danny Hsu

Institute of Biological Chemistry, Academia Sinica, Taiwan

Ubiquitin C-terminal hydrolases (UCHs) are papain-like cysteine proteases that form a subfamily of the much larger superfamily of deubiqutinases (DUBs).[1] There are four UCHs in the human genome, namely UCH-L1, -L3, -L5 (also known as UCH37) and BAP1 (BRCA1-associated protein 1). While UCH-L1 and -L3 are single-domain proteins, UCH-L5 and BAP1 have an additional UCH37-like domain (ULD) at their C-termini, which are involved in autoinhibition and protein-protein interactions. Despite their sequence divergence, UCHs share a common 5_2 Gordian knotted topology, with five projected crossings in their backbone topologies. The question of how a single polypeptide chain manages to thread through itself to attain such an intricate knotted topology without the aid of auxiliary factors, i.e., molecular chaperones, has emerged to become a major challenge to biophysicists. The implications of UCHs in various diseases further make them important biomedical targets. In this talk, I shall discuss our recent efforts in delineating the folding mechanisms of the neuron-specific, highly abundant UCH-L1, and how a Parkinson's disease associated mutation, I93M, affects its folding stability and kinetics.[2,3] Next, I shall discuss our recent finding that the proteasome-associated UCH-L5 exhibits exceedingly slow unfolding kinetics that may be beneficial for the tug of war between DUBs and UPS for maintaining protein homeostasis.[4] Finally, I shall discuss our efforts in studying the impacts of cancer-associated mutations on the folding and function of BAP1. Our findings indicate that some of these mutations could lead to long-range structural perturbations within the UCH domain that lead to increased aggregation propensity and amyloidogenesis.

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Versatile structural architectures of archaeal homolog of proteasome assembly chaperone

Maho Yagi-Utsumi[1,2,3], Arunima Sikdar[1,2], Tadashi Satoh[3] and Koichi Kato[1,2,3]

[1]Okazaki Institute for Integrative Bioscience and Institute for Molecular Science,
National Institutes of Natural Sciences,
5-1 Higashiyama Myodaijicho, Okazaki 444-8787, Japan
[2]SOKENDAI,
Shonan Village, Hayama, Kanagawa 240-0193 Japan
[3]Graduate School of Pharmaceutical Sciences, Nagoya City University,
3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan
Email: mahoyagi@ims.ac.jp

The naturally evolved proteins are highly sophisticated in structures with diverse properties. Most often these elements can interact with the external environment to form supramolecular complex architectures. To interpret the biological significance of those molecular assemblies in living systems, it is important to characterize their structural architectures and dynamics in detail.

The archaeal homologs of proteasome assembly chaperones PbaA and PbaB are examples of those biomolecules which exhibits various structural architectures regarding its flexible C-terminal segments. We attempted to characterize the structural features of PbaA and PbaB from *Pyrococcus furiosus* by an integrative structural analysis using X-ray crystallography, high-speed atomic force microscopy, native mass spectrometry, electron microscopy, and solution scattering. Our results revealed that the archaeal homologs of assembly chaperones PbaA and PbaB are different from the eukaryotic counterparts in terms of their oligomeric states and biological functions.

Moreover, distinct structural architecture of PbaA suggests its intriguing structural mechanism associated with an as yet undiscovered function. In fact, a structural genomics report identified a putative binding protein PF0014 which makes complex with PbaA. Thus, we attempted to perform structural characterization of the complex formed between PbaA and PF0014. The three-dimensional structure of PbaA-PF0014 complex shows that its molecular construction resembles a classical Greek "tholos" where the PF0014 proteins bound to the PbaA homopentamers mimic the columns of this unique architecture through intermolecular interactions.

Our findings thus revealed a unique, multiple structural architectures of PbaA, suggesting its intriguing unique functions, which can provide new insights into the structural design underlying the dynamic ordering of biomolecules with internal complexities for promoting higher functions.

A primary ion pump: proton/sodium pumping pyrophosphatases

Yuh-Ju Sun

Institute of Bioinformatics and Structural Biology, College of Life Science, National Tsing Hua University, Hsinchu 30013,Taiwan.
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Membrane bound pyrophosphatases (M-PPase) possess the enzymatic function to catalyse the hydrolysis of pyrophosphate (PPi) and show the ion pumping activity to transport ion across the membranes. M-PPase was isolated as a homodimer and each monomer contains 16 transmembrane helices. M-PPase from plant, *Vigna radiate* (*Vr*PPase), function as a proton pump. However, M-PPase from bacterial, *Thermotoga maritima* (*Tm*PPase), act as a sodium pump. The overall crystal structures of M-PPase folded in a rosette manner with two concentric walls, inner and outer walls. From M-PPase structures of various catalytic states, a complete catalytic cycle for M-PPases was proposed. The helical movements are found to involve in the closure and open of the substrate-binding pocket and to rearrange the key residues and leads to ion pumping. The structural and functional information provide the basis for understanding a unique ion translocation pathway and to identify the ion selection among various M-PPases. *Tm*PPase and *Vr*PPase suggest a conserved coupling mechanism for M-PPase.

Protein Structure Prediction/Determination by Global Optimization

Jooyoung Lee

School of Computational Sciences, Korea Institute for Advanced Study

85 Hoegiro, Dongdaemun-gu, Seoul 02455, Korea

E-mail: jlee@kias.re.kr; Web-page: http://lee.kias.re.kr

First, I will discuss our recent progresses on the protein structure prediction using the methodology of global optimization as illustrated in CASP11&12 competitions held in 2014 and 2016. We will demonstrate that this method can be applied to difficult MR (molecular replacement) targets to determine X-ray crystallography structures of proteins and protein complexes, which could not be solved using conventional MR methods. We will also discuss the potential application of our method to the high throughput NMR protein structure determination including large proteins (over 20 kDa) and membrane proteins.

Structural basis of the *Klebsiella pneumoniae* PmrA response regulator in gene transcription and inhibitor development

Chinpan Chen

Institute of Biomedical Sciences, Academia Sinica,
Taipei, Taiwan
Email: bmchinp@ibms.sinica.edu.tw

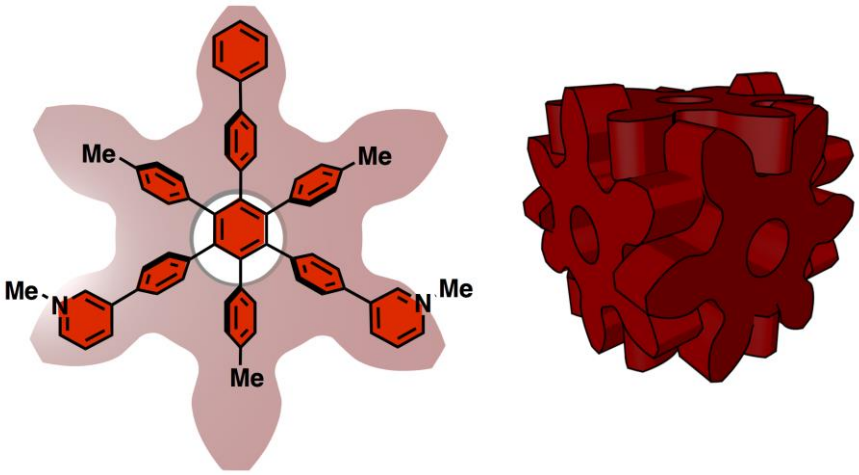
The emergence of resistance to multiple antimicrobial agents in pathogenic bacteria, such as *Klebsiella pneumoniae*, is a significant global public health threat and causes considerable patient mortality and morbidity. In bacteria, two-component signal-transduction system is the most predominant signaling scheme to sense and respond to environmental changes for survival and proliferation. For example, in the PmrA/PmrB two-component system, PmrA, an OmpR/PhoB family response regulator (RR), manages genes for polymyxin B resistance. It is known that phosphorylation of OmpR/PhoB RR induces the formation of a symmetric dimer in the N-terminal receive domain (REC), promoting 2 C-terminal DNA-binding domains (DBDs) to recognize the promoter DNA to elicit adaptive responses. Previously, we reported the 3.2-Å -resolution crystal structure of the BeF_3^--activated PmrA-DNA complex as well as its dynamics in solution by NMR. X-ray structure showed that both N- and C-domains in the upstream protomer have strong interactions mainly by H-bonds. However, NMR studies showed that 2 domains tumble separately and a REC-DBD interaction is transiently populated in solution. Reporter gene assay of PmrA variants with altered interface residues suggested that the interface is not crucial in supporting gene transcription. Based on PmrA-DNA complex structure, we have tried to develop highly potency inhibitors against PmrA-DNA complex formation. In addition, to further gain insights into gene transcription of PmrA-activated genes in the transcription initiation step, we designed a sigma4 chimera protein of sigma70 factor by fusing it with the short β-flap tip helix of RNAP through an artificial linker to improve its solubility and stability to perform a variety of biophysical studies. Solution structure of the sigma4 chimera protein was solved, which adopts a similar conformation as that within RNAPH. The sigma4 chimera protein is shown to recognize the consensus -35 element DNA by NMR and fluorescence polarization. The interactions among the sigma4 chimera protein, the PmrA box promoter DNA and the BeF_3^--activated PmrA are currently under investigation, which along with PmrA-DNA complex and inhibitor design will be discussed in this presentation.

Nanocube: Ultra-Thermostble Self-Assemblies in Water

Shuichi Hiraoka

Department of Basic Science, The University of Tokyo,
Komaba 153-8902 Japan
Email: chiraoka@mail.ecc.u-tokyo.ac.jp

Although many proteins can be seen to be denatured by heating, proteins found in hyperthermophilic organisms, which thrive at high temperature near and above 100 ℃, have extremely high thermal stability, which is quite fascinating from the perspective of understanding how nature evolves and establishing design principle of new materials. What are surprising in such hyperthermophilic proteins is that these proteins consist of usual natural amino acids and that their structure is maintained by molecular interactions, which are much weaker than covalent chemical bonds. Although more than three decades have past since the discovery of hyperthermophilic microorganisms, how such proteins stabilize their structure at extremely high temperature has remained to be elusive. Here we present ultra-thermostable self-assemblies, *Nanocube*, composed of perfectly artificial gear-shaped amphiiles (GSAs), which are robust at above 100 ℃ in water. Like hyperthermophilic proteins, molecular interactions among GSAs of the nanocubes are hydrophobic, electrostatic, and van der Waals interactions. Several point mutations of the GSA, which alter electrostatic interaction and/or contact surface area of the GSAs in the nanocube, enabled us to obtain a nanocube whose decomposition temperature (T_d) is 130 ℃. Furthermore, filling a 1 nm-sized vacant inner space of the nanocube with hydrophobic molecules increased the T_d over 150 ℃, which is higher than that of the highest-ever hyperthermophilic protein, *Ph*CutA1, 148.5 ℃.



Amphiphilic nanoparticles of resveratrol–norcantharidin and its peptide analog to enhance the toxicity in *Escherichia coli* and zebrafish embryo

Chien-Chung Cheng

Department of Applied Chemistry, National Chia-Yi University,

Chia-Yi City, 60004, Taiwan

Email: cccheng@mail.ncyu.edu.tw

The introduction of liposomes using nature products provides an alternative pathway in medical treatment. Liposomes are biocompatible, possess the permeability needed for drug uptake, and have a particle size suitable for the encapsulation of drugs. Currently, our lab is to develop novel functional liposome using the target drugs (anticancer, antibiotic, and antioxidants) to link with a hydrophobic group to increase the bioavailability. These modified compounds are capable of self-assembling to be functional liposomes in water to fulfil their biological behaviors. For instance, resveratrol is known to be an effective compound in anti-inflammation, cardiovascular protection, anti-aging, anti-oxidation, and tumor suppressions. A mono-substituted resveratrol derivative, resveratrol-modified stearate (RMS), was synthesized by selectively linking stearic acid to resveratrol in order to increase the stability and bioavailability. During the preparation of functional liposomes, the self-assembly of RMS spontaneously induced a series of structural transformations that converted liposomes to metastable helical structures, wire-like linear structures, and inert spherical nanoparticles confirmed by transmission electron microscope (TEM). For an increasing lipophilic property, the bicyclic compound norcantharidin (NCTD) was used instead of stearic acid. For an increasing hydrophilic property, cell penetration peptide, KTTKS, was introduced into the amphiphilic molecule. NCTD retains its potential antitumor activity and apoptotic properties, and exhibits reduced inflammation and nephrotoxicity compared with cantharidin. A peptide of KTTKS is known to be capable of stimulate the regeneration of collagen in skin. Direct coupling of a hydrophobic and a hydrophilic natural product via an ester/amide bond produced an amphiphilic adduct that self-assembled functional liposomes. These liposomes showed high toxicity in *E. coli* and zebrafish embryos compared with their monomer. This result provides a novel insight pathway to explore the traditional Chinese medicines, which often contain a significant amount of flavonoids and polyphenol analogs. The biological activity of compounds of traditional Chinese medicines may arise from the formation of liposomes, nanoparticles, or supramolecules to facilitate the cell uptake.

Control over differentiation of a metastable supramolecular assembly

Kazunori Sugiyasu

National Institute for Materials Science

1-2-1 Sengen, Tsukuba 305-0047, Japan

Email: SUGIYAU.Kazunori@nims.go.jp

Molecular self-assembly under kinetic control is expected to yield nanostructures that are inaccessible through the spontaneous thermodynamic process. Time-dependent evolution, which is reminiscent of biomolecular systems, may occur under such conditions and allow the synthesis of supramolecular assemblies with enhanced complexities. Recently, we have reported the time-dependent evolution of a metastable supramolecular assembly of a porphyrin derivative[1)]. In this system, two aggeregation pathways interplayed; kinetically formed nanoparticle transformed into thermodynamically stable nanofiber over time through autocatalytic process. Based on their energy landscape, we could control the lag time of the time-evolution by rational molecular design.

Herein, we will report the differentiation of a metastable supramolecular assembly. In this new system, nanoparticle of a newly designed porphyrin derivative acted as a "stem" supramolecular assembly that had the capacity of differentiation into both the nanofiber and nanosheet. Mechanistic studies unveiled the energy landscape governing unique kinetic behavior. Based on this understanding, we could control the differentiation by changing mechanical agitation and achieve both one- and two-dimensional living supramolecular polymerization using an identical porphyrin monomer[2)].

Ref. K. Sugiyasu et al. 1) *Nature Chem.* **2014**, *6*, 188; 2) *Nature Chem.* **2017**, *9*, DOI: 10.1038/nchem.2684

Molecular Interactions in the Formation of Self-assembled Peptide Hydrogels

Hsin-Chieh Lin

Department of Materials Science and Engineering, National Chiao Tung University, Hsin Chu 30010 Taiwan

Email: hclin45@nctu.edu.tw

In the past three decades, nanobiotechnology has grown explosively and evolved into a subfield of science. One approach to produce nano-sized biomaterials is to utilize the functional self-assembled hydrogels which can be formed by self-assembly among designed subunits to yield shape-persistent and highly ordered nanostructures in water. Recently, we have detailed a new series of amino-acid-based self-assembled systems and proved that controllable molecular packing can be used to promote the formation of the self-assembled nanostructures and hydrogels. From experimental and computational studies, the small functional molecular units might serve as the molecular template to adjust an appropriate geometry of the hydrogelators that enable the formation of self-assembled nanofibers and hydrogels. The physical and structural properties of peptide hydrogels were found to be dictated by the amino acid sequence of the peptide building blocks. The newly discovered hydrogel has excellent biocompatibility, thus making it a potentially useful scaffolding material for biomedical applications. These results indicate the newly discovered hydrogelators are potential biomaterials. Our results illustrate the importance of structure-hydrogelation relationship and provides new insights into the design of self-assembled nanobiomaterials.

Chemo-mechanical coupling mechanisms of linear and rotary molecular motors revealed by high-speed single-molecule imaging analysis

Ryota Iino

Okazaki Institute for Integrative Bioscience and Institute for Molecular Science,

National Institutes of Natural Sciences

5-1 Higashiyama Myodaijicho, Okazaki, Aichi 444-8787, Japan

Email: iino@ims.ac.jp

We have been developing high-speed single-molecule methods monitoring fast motions of protein molecular motors. Our methods are based on dark-field scattering imaging of gold nanoparticles and nanorods [1, 2]. As probes of single-molecule imaging, the gold nanoparticles and nanorods have advantages over the commonly used ones such as fluorescent dyes and quantum dots, because much stronger signals can be obtained without suffering from photobleaching and blinking. In this presentation, I will talk about chemo-mechanical coupling mechanisms of linear molecular motors kinesin-1 [3] and chitinase, and rotary molecular motors F_1-ATPase and V-ATPase [4-6], revealed by our high-speed single-molecule imaging analysis with nanometer localization precision and microsecond temporal resolution.

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Direct visualization of dynamic molecular interactions using HS-AFM

Takayuki Uchihashi

Department of Physics, Nagoya University,

Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8602, Japan

Email: tuchihast@gmail.com

Most of biological phenomena in a living body are elicited by cascades of a wide range of dynamic molecular processes such as conformational change, binding and dissociation, and assembly and disassembly of proteins. A fundamental appreciation for complex biological processes can be inherently reduced to understanding dynamics of a small number of molecules at each stage. Since the protein motions are usually unsynchronized and often have multimodal distributions which can not be directly evaluated by ensemble-averaged methods, a single-molecule approach is required to monitor individual molecular behaviors.

Atomic force microscopy (AFM) can acquire topographic image with high-spatial resolution down to nanometer scale under various environments and hence has opened the door for visualizing biological molecules under near physiological conditions. AFM is now routinely used to analyze structure of a wide variety of biological samples from protein and nucleic acids to living cells. Furthermore, recent technical advances of AFM regarding the imaging speed have enabled us to directly monitor dynamic processes of biological molecules, which play essential roles on biological functions. So far, the high-speed AFM (HS-AFM) has been applied to a wide variety of biological molecules such as molecular motors, a photoreceptor and so on [1-6]. The initial studies demonstrated that the HS-AFM movies corroborate models previously proposed and provide intuitive views by visual evidence at single molecular level. Very recently we further expand the application of the HS-AFM to more complex systems involving two or more different molecules and investigate dynamic molecular interaction.

In this talk, first I briefly present the fundamentals for the HS-AFM imaging and demonstrate the typical applications. Then I highlight recent imaging studies regarding molecular interactions; (a) disassembly process of a tetradecamer composed of proteasomal α7 subunit induced by α6 subunit and (b) complex structure and the dynamics of circadian-clock Kai proteins.

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Viscosity, thermal diffusivity, and polarity of lipid bilayer membranes estimated from fast time-resolved spectroscopic measurements

Koichi Iwata

Department of Chemistry, Faculty of Science, Gakushuin University

Toshima-ku, Tokyo 171-8588 Japan

Email: koichi.iwata@gakushuin.ac.jp

A large number of biochemical reactions proceed at biomembranes with membrane proteins acting as catalysts for the reactions. It is therefore important to characterize the biomembranes, or lipid bilayer membranes, as a field of chemical reactions. The biomembranes, with a thickness of only several nanometers, are assembled by phospholipids interacting with weak intermolecular forces. The two-dimensional structure of the membranes is expected to be flexible, which should affect the chemical reactions that they host. The lipid bilayer membrane have the hydrophobic portion inside and the hydrophilic portion outside at the interface with the aqueous phase. One of the most attractive questions on the biomembrane is a strong structure model of "lipid raft". The raft model assumes the presence of a stiff micro-domain, or a raft, floating in the flexible membrane. We try to examine if there is a micro-domain structure formed in the lipid bilayer membranes.

We try to characterize lipid bilayer membranes by estimating the viscosity, thermal diffusivity or polarity in the membranes although these physical quantities usually characterize bulk materials. We estimate the viscosity, thermal diffusivity or polarity of the lipid bilayer membranes by using picosecond time-resolved fluorescence spectroscopy, picosecond time-resolved Raman spectroscopy, and femtosecond time-resolved near-infrared spectroscopy. Liposome lipid bilayer membranes with a diameter of 100 nm are prepared from a phosphatidylcholine, DLPC, DMPC, DPPC, DSPC, DOPC, or egg-PC, and used for the spectroscopic measurements.

The results of the time-resolved fluorescence measurements indicate that there are two domains, viscous and fluid, present in the liposome lipid bilayer membranes formed by a single phospholipid. The viscosity for the viscous environment estimated from the picosecond time-resolved fluorescence measurement is 30 to 290 times larger than the viscosity for the fluid environment. Presence of viscosity inhomogeneity along the lateral direction of the lipid bilayer membranes is strongly suggested from the time-resolved fluorescence measurement of a newly developed fluorescence probe.

Thermal diffusivity of the lipid bilayer membranes is estimated from picosecond time-resolved Raman spectroscopy, by using the first excited singlet (S_1) state of *trans*-stilbene as a "picosecond Raman thermometer". The thermal diffusivity values estimated for the liquid crystal phase lipid bilayer membranes are larger than the values for the gel phase lipid bilayer membranes. We think that the presence of the outer water that has a large thermal diffusivity affects the heat conduction in the thin layer of lipid bilayer membranes.

Understanding of Self-Assembly Process at Molecular Level

Hirofumi Sato

Department of Molecular Engineering and Element Strategy for Catalysis and Battery,

Kyoto University, Kyoto 615-8510 Japan

Email: hirofumi@moleng.kyoto-u.ac.jp

Order formation and its dynamics are fundamental aspects in synthesized self-assembly systems as well as in many bio-molecular systems. However, it is still challenge to characterize the process at the molecular level because of the difficulty to trace the system evolution in multiple length and time scales. In other words, the assembly is described through complicated, multi-dimensional energy surface. Theoretical and computational approaches serve unique role in clarifying the mechanism and intermediating process, which are generally difficult to be accessed by experimental method. Molecular dynamics (MD) is the most widely utilized method to describe chemical processes in condensed phase. It is, however, infeasible to simulate entire self-assembly because time scale of the process is usually much longer than its capability. An alternative is statistical mechanics for molecular liquids called RISM, which is a theory to deal with solution system consisting of polyatomic molecules. In principle, the method is equivalent to MD, but its computational demand is very small due to the algebraic nature. For instance, a closed formula of solvation free energy is available. We have been studying spatiotemporal dynamics of chemical process by utilizing integral equation theory for molecular liquids, hybrid QM/MM-type method called RISM-SCF-SEDD [1], diffusion equation at molecular level (3D-SSSV) [2], molecular dynamics and so on.

In this contribution, the following topics for self-assembly system are presented.
(1) Geometrical relationship between building block and the final product: Inspired by Professor Hiraoka's nanocube, we have developed a coarse-grained model. The six pyramidal-shape molecules assemble to form cubic structure as the global minimum and, besides that, more than 2000 different structures (local minima) were obtained by basin-hopping global optimization. We found that stability of another assembled structure (`sheet') competes with that of the cube, depending on the height of the pyramid. The topology of the entire PES was studied.
(2) Assembly path and dynamics: A computational approach utilizing master equation is proposed to treat self-assembly system. The method was applied to the nano-capsule system developed by Professor Hiraoka's group. A good agreement with the experimental observation was obtained.

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Towards pharmacological analysis using zebrafish models of cardiovascular diseases

Ian Liau

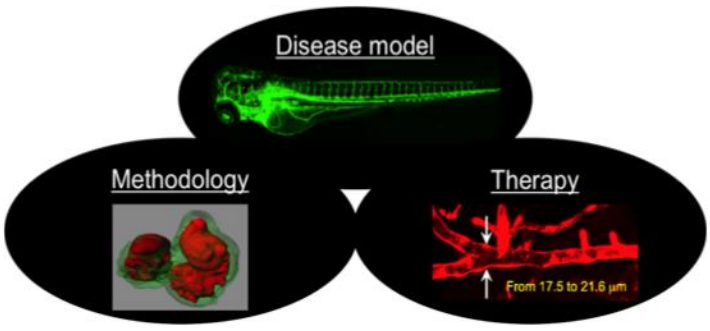
Department of Applied Chemistry and Department of Biological Science and Technology

National Chiao Tung University

Hsinchu, Taiwan

Email: ianliau@mail.nctu.edu.tw

The use of experimental animal models is an inevitable component of pharmaceutical development. Zebrafish (*Danio rerio*) has recently emerged as a popular model animal because of its numerous attractive features including convenience of drug delivery, rapid development, ease of genetic manipulation, low cost of maintenance and high degree of genetic and functional conservation to human beings. The translucency of its body at the larval stage further makes spectro-imaging interrogation of its internal organs become possible. In this presentation, I will report our recent effort on the development of zebrafish disease models and optical methods towards drug screen that target cardiovascular diseases. Acute thromboembolic diseases remain the major cause of death or disability but therapeutic options remain limited. To this end, we developed a novel zebrafish model of ischemic stroke using photochemical thrombosis; such mode is expected to facilitate the screen of effective thrombolytic drugs. With image-guided Raman spectroscopy, we delicately interrogated individual vascular lesions on the zebrafish model of hypercholesterolemia, and demonstrated the pleotropic (suppressing the accumulation and oxidation, and expediting the clearance, of vascular lipids) therapeutic efficacies of atorvastatin. Assessment of the cardiac function of zebrafish has been challenging because of the small size its heart and high beating rate. We have developed the application of pseudo-4D imaging to determine the cardiac function of larval zebrafish, and demonstrated its applications in the testing of cardio-active and toxic drugs. Through collaboration, we developed an optically elicitable polymeric nano-carrier that can be triggered to release nitric oxide (NO) and to induce vasodilation in the cerebral blood vessels of zebrafish; such strategy should help reveal the complex biological effects of NO *in vivo* and open up new clinical applications of NO. In the end, we conclude that our approach will help understand the pathophysiology of human cardiovascular diseases and facilitate therapeutic development targeting these critical diseases.



Smart PEDOT Nanostructures for Circulating Tumor Cells Related Cancer Prognosis

Hsiao-hua (Bruce) Yu

Institute of Chemistry, Academia Sinica
Taipei, Taiwan
E-mail: bruceyu@gate.sinica.edu.tw

Circulating tumor cells (CTCs) are cancer cells that shed away from either primary tumors or metastatic sites. They circulate in the peripheral blood as the cellular origin of metastases. Comparing to biopsy, the gold standard of current cancer diagnosis, CTCs offer convenient and non-invasive access to tumor cells before fatal metastasis occurs. To exploit CTCs as a new cancer "biomarker" for disease progression and guided implementation of therapy, significant research endeavors have been devoted to develop diagnostic assays capable of detecting and enumerating CTCs in cancer patients' blood. Working together with Prof. Hsian-Rong Tseng at UCLA Medical School, we investigated the application of nanomaterials for CTC capturing. It was clearly observed of a synergistic effect of capture agents (antibody) and nanostructures on the enhanced efficiency of CTC capturing. The enhanced local topographic interactions between the nanostructures and nano-scaled cellular surface components (e.g., microvilli) are analogous to the working principle of a velcro in nanoscale. Although the capturing and enumeration of CTCs provide preliminary diagnostic-relevant information, it is conceivable that the CTC-derived molecular signatures and functional readouts provide more valuable and significant insight into tumor biology during the critical window where therapeutic intervention could make a significant difference. In order to conduct molecular and functional analyses of CTCs, there is a desperate need to develop a new CTC assay that can not only capture CTCs with high efficiency, but also release CTCs with minimum contamination of the surrounding white blood cells (WBCs) and negligible disruption to CTCs' viability and functions. In this talk, I would like to talk about a new generation nanomaterial platform based on organic conductive biomatereials, PEDOT, for cell-affinity assay that is capable of not only capturing CTCs with high efficiency, but also releasing the nanosubstrate-immobilized CTCs upon the application of an external stimulus, including temperature change, electrical application, and biocompetition.

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