List of Abstracts

INVITED LECTURES

IL01 Shan-Ho Chou
Next Generation Resistance-Free Antibiotics Development Via Quorum Sensing Mechanism

IL02 Shang-Te Danny Hsu, Lisa D. Cabrita, John Christodoulou, Paola Fucini and Christopher M. Dobson
Structural insights into co-translational protein folding

IL03 Chwan-Deng Hsiao, Yu-Hua Lo and Kuang-Lei Tsai
Molecular interplay between the replicative hexameric Helicase DnaC with ssDNA and its Loader DnaI from Geobacillus kaustophilus

IL04 Tung-Ju Hsieh, Tien-Jui Yen, Te-Sheng Lin, Hsun-Tang Chang, Shu-Yun Huang, Chun-Hua Hsu, Lynn Farh & Nei-Li Chan
Twisting of the DNA binding surface by a β-strand-bearing proline modulates DNA gyrase activity

IL05 Roman Dronyak, Jin-Sheng Tsai, Yen-Ju Chen, Ting-Kuo Lee, Fu-Rong Chen, and Keng S. Liang
Potential Application of Coherent Electron Diffractive Technique in the Imaging of a Single Bio Molecule

IL06 Wolfgang B. Fischer
Viral proteins: how to form a pore in a lipid membrane

IL07 Che Ma
Membrane Proteins and Drug Discovery

IL08 Chi-Hsiang Lien, Wen-Shuo Kuo, Chun-Yu Lin, Keng-Chi Cho, Chia-Yuan Chang, and Shean-Jen Chen
Fabrication of 3D Plasmonic Biodevices

IL09 Hsuan-Shu Lee, Chen-Yuan Dong

Intravital imaging of dynamic liver metabolism and hepatocellular death in mice using multiphoton microscopy

IL10 Kei Yura
Prediction of Interfaces for Molecules on Protein Surfaces Based on Protein Sequences and 3D-Structures

IL11 Jung-Hsin Lin
Accommodating protein dynamics for prediction of enzyme substrates and inhibitors

IL12 Woei-Jer Chuang, Chiu-Yueh Chen, Jia-Hau Shiu, Yi-Chun Chen, Yao-Tsung Chang, Chun-Hau Huang, and Yung-Sheng Chang
Design of integrins-specific disintegrins

IL13 Chun-Lun Ni, Hoi-Ping Shi, Hui-Ming Yu, Yun-Chorng Chang, and Yun-Ru Chen
Folding Stability and Native Conformation of Amyloid-β Monomer are Important Determinants of the Nucleation Kinetics and Fibril Formation

IL14 Chin-Kun Hu
Relaxation and aggregation of biopolymers, and q-statistics

IL15 Hsuan-Yi Chen , Fu-Lai Wen, and Kwan-Tai Leung
Symmetries and the trajectory of Listeria monocytogenes

IL16 Li-Ching Hsieh, Shu-I Lin, Chun-Chieh Shih, Wen-Hsiung Li and Tzyy-Jen Chiou
Uncovering small RNA-mediated responses to stress conditions by deep sequencing-based expression analysis

IL17 Chih-Hao Chen, Hsing-Chung Lee, Qingdong Ling, Hsiao-Rong Chen, Yi-An Ko, Tsong-Shan Tsou, Sun-Chong Wang, Li-Ching Wu and H.C. Lee
Error-tracking clustering gives quantitative statistics to DNA segmentation analysis

IL18 Pei-Kuen Wei
Size-Dependent Endocytosis of Gold Nanoparticles and Its Applications

IL19 Masayuki J. Sato and Masahiro Ueda
Electrotactic signaling pathways in Dictyostelium cell

IL20 Chau-Hwang Lee
Active Waves and 3D Nanoparticle Motion on Cell Membranes Characterized by Wide-field Optical Profilometry

IL01

Next Generation Resistance-Free Antibiotics Development Via Quorum Sensing Mechanism

Shan-Ho Chou

Institute of Biochemistry, National Chung-Hsing University, Taichung 40227,Taiwan

Drug resistance has been a serious issue causing considerable threat to human life today. A novel strategy to develop drugs that do not elicit bacterial drug resistance is thus in great need. Recently a new method to target on the virulence factors rather than killing bacteria has been developed. Quorum sensing is a bacterial signal transduction pathway that monitors bacterial population density to control pathogenicity gene expression via a novel secondary messenger molecule c-di-GMP. C-di-GMP is found to control a wide range of functions in eubacteria, yet little is known about the underlying regulatory mechanisms. In the plant pathogen *Xanthomonas campestris*, expression of sub-set of virulence genes is regulated by c-di-GMP and also by the CAP-like protein *Xc*CLP, a global regulator in the CRP/FNR superfamily. Here, we report structural and functional insights into the interplay between *Xc*CLP and c-di-GMP in regulation of gene expression. *Xc*CLP bound target promoter DNA with sub-μM affinity in the absence of any ligand. This DNA-binding capability was abrogated by c-di-GMP, which bound to *Xc*CLP with M affinity. The crystal structure of *Xc*CLP showed that the protein adopted an intrinsically active conformation for DNA binding. Alteration of residues of *Xc*CLP implicated in c-di-GMP binding through modeling studies caused a substantial reduction in binding affinity for the nucleotide and rendered DNA binding by these variant proteins insensitive to inhibition by c-di-GMP. Taken together, the current study reveals the structural mechanism behind a novel class of c-di-GMP effector protein in the CRP/FNR superfamily and indicates that *Xc*CLP regulates bacterial virulence gene expression in a manner negatively controlled by the c-di-GMP concentrations. The quorum sensing related proteins RpfF, RpfC, and RpfG, and c-di-GMP degradation enzymes thus represent potential novel targets for developing drug-resistance free antibiotics.

IL02

Structural insights into co-translational protein folding

Shang-Te Danny Hsu[1], Lisa D. Cabrita[2], John Christodoulou[2], Paola Fucini[3] and Christopher M. Dobson[1]

[1]*Department of Chemistry, University of Cambridge, Cambridge, United Kingdom*

[2]*Institute of Structural and Molecular Biology, University College London and Birkbeck College, London, United Kingdom*

[3]*Institut für Organische Chemie und Chemische Biologie, Goethe-Universität Frankfurt, Frankfurt am Main, Germany*

Correspondence e-mail address: stdh2@cam.ac.uk

Folding of polypeptide chains can take place as early as they are being synthesized by the ribosome in a co-translational manner before they are released to the hostile cellular environments. Emerging evidence indicates that such a vectorial process may differ fundamentally from those that have been extensively investigated *in vitro* in which both the amino- and carboxyl-termini of the proteins of interest are available for participating the unfolding and refolding events. In this talk, I shall present our experimental strategy that has been tailored for solution state NMR spectroscopy to enable us to probe structures and dynamics of ribosome-bound nascent chains at an apparent molecular weight of more than 2.5 megadalton (1, 2, 3) and to obtain structural insights into the process by which a growing nascent polypeptide chain assumes folded structures as it emerges from the ribosome in a way that is likely to differ from the (re)folding in isolation (3, 4). These findings may be crucial for bridging the gap between our understandings about protein folding *in vitro* and *in vivo.*

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IL03

Molecular interplay between the replicative hexameric Helicase DnaC with ssDNA and its Loader DnaI from *Geobacillus kaustophilus*

Chwan-Deng Hsiao, Yu-Hua Lo and Kuang-Lei Tsai

Institute of Molecular Biology, Academia Sinica, Taipei, 115, Taiwan

DNA helicases are motor proteins that play essential roles in DNA replication, repair, and recombination. In the replicative hexameric helicase, the fundamental reaction is the unwinding of duplex DNA. In addition, the helicase loading factors are thought to transfer the hexameric ring-shaped helicases onto the replication fork during DNA replication. However, the mechanism of how helicase transfer onto DNA under the help of helicase loading factors help remains unclear. To date, intense biochemical, genetic, and structural approaches are being pursued to gain insight into both the mechanism and role of helicase and its loader in varied biological processes. However, in different organisms have a number of different mechanisms for DNA helicase loading. Therefore, the exact function of replicative helicase and the interaction with its loader in replication initiation are uncertain. In order to delineate the interaction and possible mechanism between helicase with ssDNA and its loader, we report here the structures and biochemical characterization of the DnaC replicative helicase in conjunction with the loader DnaI from *Geobacillus kaustophilus*.

IL04

Twisting of the DNA binding surface by a β-strand-bearing proline modulates DNA gyrase activity

Tung-Ju Hsieh[1,2], Tien-Jui Yen[1], Te-Sheng Lin[1,2], Hsun-Tang Chang[2], Shu-Yun Huang[2], Chun-Hua Hsu[3], Lynn Farh[4] & Nei-Li Chan[1,2]

[1]Institute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University; [2]Institute of Biochemistry, National Chung Hsing University; [3] Department of Agricultural Chemistry, College of Bio-Resources and Agriculture, National Taiwan University; [4]Department of Chemical Biology, National Pingtung University of Education

DNA gyrase is the only topoisomerase capable of introducing (-) supercoils into relaxed DNA. The C-terminal domain of the gyrase A subunit (GyrA-CTD) and the presence of a gyrase-specific “GyrA-box” motif within this domain are essential for this unique (-) supercoiling activity by allowing gyrase to wrap DNA around itself. Here we report the crystal structure of *Xanthomonas campestris* GyrA-CTD and provide the first view of a canonical GyrA-box motif. This structure resembles the GyrA-box-disordered *Escherichia coli* GyrA-CTD, both adopting a non-planar β-pinwheel fold composed of 6 seemingly spirally arranged β-sheet blades. Interestingly, structural analysis revealed that the non-planar architecture mainly stems from the tilted packing seen between blades 1 and 2, with the packing geometry likely being defined by a conserved and unusual β-strand-bearing proline. Consequently, the GyrA-box-containing blade 1 is placed at an angled spatial position relative to the other DNA-binding blades, and an abrupt bend is introduced into the otherwise flat DNA-binding surface. Mutagenesis studies support that the proline-induced structural twist contributes directly to gyrase’s (-) supercoiling activity. To our knowledge, this is the first demonstration that a β-strand-bearing proline may impact protein function. Potential relevance of β-strand-bearing proline to disease phenylketonuria is also noted.

IL05

Potential Application of Coherent Electron Diffractive Technique in the Imaging of a Single Bio Molecule

Roman Dronyak[1,3], Jin-Sheng Tsai[1], Yen-Ju Chen[1], Ting-Kuo Lee[2], Fu-Rong Chen[3], and Keng S. Liang[1]

[1]*National Synchrotron Radiation Research Center, 101 Hsin-Ann Road, Hsinchu, Taiwan*

[2]*Institute of Physics, Academia Sinica, Nankang, Taipei, Taiwan*

[3]*Department of Engineering and System Science, National Tsing Hua University, Hsinchu, Taiwan*

The structure determination of proteins has become a major research issue in structural biology, aiming to build up a database of all proteins in nature. Crystallography is an effective tool for solving the structure of soluble proteins which can form large crystals. The alternative tools for solving the structure of single macromolecule are a cryo-electron microscopy (cryo-EM) and a newly emerged coherent diffractive imaging (CDI) technique. The CDI requires the employment of a coherent source of x-rays or electrons. In coherent diffraction experiments of a single molecular specimen, the measured diffuse diffraction pattern can be phased to obtain the atomic arrangement of the sample. In recent years, the CDI has been intensively investigated as coherent x-ray sources become available from low-emittance synchrotrons and free electron lasers [1]. Recently, we have performed the CDI of nanocrystals using coherent electron source from a field-emission TEM [2,3]. The results are quite promising, especially because the advantage of high scattering cross sections of electrons as compared to x-rays. In this report, we will discuss the potential of electron CDI on bio molecules.

Simulation and preliminary experiment of electron CDI on a single nano-sized molecule have been performed using Holliday Junction Binding Protein Ruva (1HJP) from E. coli bacteria [4] with the size of about 8.5 nm on a graphene substrate. The single layer of graphene is chosen as a substrate to reduce the background scattering. Simulated diffraction intensity shows Bragg peaks from periodic structure of graphene and the speckle pattern from the molecule due to the coherent nature of illumination. The real-space structure of the sample is recoverable with iterative algorithms. The results of two-dimensional reconstruction reveal the projected atomic arrangement of the sample. The resolution of recovered image is about 0.05 nm which is limited by the maximal scattering angle of recorded intensity. The three-dimensional structure of the sample, which can be obtained by rotating the specimen or acquiring diffraction intensities from similar molecules at different orientations, will be discussed.

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IL06

Viral proteins: how to form a pore in a lipid membrane

Wolfgang B. Fischer

Institute of Biophotonics, School of Biomedical Science and Engineering

National Yang Ming University, 155, Sec. 2, Li-Nong St., Taipei 112, TW

Correspondence e-mail address: wfischer@ym.edu.tw

Viruses encode special membrane proteins which form homo-oligomers in lipid membranes. As a consequence the membrane becomes permeable for ions and small substrates. With this technique the virus steers the host cell enhancing its own replication. Until recently viral channel proteins have been resistant to the 'visualization' of their oligomeric structure due to their small size. Even though some of the proteins are now been 'seen' due to experimental techniques, computational methods deliver essential support-tools to understand the mechanism of function of these proteins and also generate the platform for structure based drug development.

A potential rout of the assembly of viral channel forming proteins will be outlined using a combination of molecular dynamics simulations and docking approaches. The idea is to compare possible simplified biological pathways such as a concerted versus sequential assembly pathway. The proteins shown will be taken from HIV-1, Hepatitis C virus and SARS-CoV to model proteins with increasingly complex transmembrane topology. Computational data will be compared with experimental results.

IL07

Membrane Proteins and Drug Discovery

Che Ma

Genomics Research Center, Academia Sinica, Taipei, Taiwan

Correspondence e-mail address: cma@gate.sinica.edu.tw

Membrane proteins are important drug targets and they represent 20-30% of all open-reading frames encoded in genomes. The approach using structural studies in combination with chemical biology methods will be presented, including two following topics in infectious diseases. First, in order to overcome the problems of drug-resistant bacterial infection, a new enzyme target for antibiotic development, the membrane-bound bifunctional transglycosylase, has been chosen for structural and functional analysis. We have recently determined the X-ray crystal structure of this membrane-bound enzyme in complex with its inhibitor moenomycin, and studied its mechanism of peptidoglycan synthesis. In addition, a high-throughput screening method for finding new antibiotics has been developed using the purified full-length membrane protein. In parallel, structure-based drug design with our crystal structure is ongoing. Second, we have studied the effect of glycosylation on influenza virus major surface protein hemagglutinin (HA) with regards to its role in receptor binding and immune response, and developed a new strategy for molecular vaccine design.

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IL08

Fabrication of 3D Plasmonic Biodevices

Chi-Hsiang Lien,[1] Wen-Shuo Kuo,[1] Chun-Yu Lin,[1] Keng-Chi Cho,[2] Chia-Yuan Chang,[2] and Shean-Jen Chen[1,3],*

[1]*Department of Engineering Science, National Cheng Kung University, Tainan 701, Taiwan*

[2]*Institute of Electro-Optical Science and Engineering, National Cheng Kung University, Tainan 701, Taiwan*

[3]*Center for Micro/Nano Science and Technology, National Cheng Kung University, Tainan 701, Taiwan*

*Corresponding author: sheanjen@mail.ncku.edu.tw

In this study, nonlinear multiphoton photocrosslinking and photopolymerization of rose bengal and trimethylolpropane triacrylate monomers in solution have been used to direct the three-dimension (3D) assembly of microdevices; moreover, we also presents the first example of containing gold nanorods within the photopolymerization. The experimental results show that the two-photon excited photopolymerization containing the nanorods improves more efficiently of decreasing the power density of femtosecond laser, and also provides a great diversity of optical properties. The doped nanorods with two-photon luminescence act as label molecules for internal microdiagnosis of 3D polymer microdevices.

Keywords: Three-dimensional fabrication, photopolymerization, gold nanorods, microdevice.

IL09

Intravital imaging of dynamic liver metabolism and hepatocellular death in mice using multiphoton microscopy

Hsuan-Shu Lee,[1,3] Chen-Yuan Dong,[2]

[1]Institute of Biotechnology [2]Department of Physics, National Taiwan University, [3]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.

Recently we have successfully established a technique allowing multiphoton microscopic observation of liver through a window device installed on living mice. Taking advantage of this high-resolution and high-magnification microscopic system, we have measured dynamic changes of a fluorogenic bile solute carboxyfluorescein diacetate (CFDA) in hepatocytes and sinusoids of mouse livers in vivo.

Disruption of plasma membrane is the hallmark of cell necrosis. However, the sequential morphological changes of hepatocytes or other cells have so far only been recorded on cells in culture. Using this high-resolution multiphoton microscopic system, we recorded in living mice serial changes of hepatocyte necrosis in relevance to metabolism of a bile solute carboxyfluorescein. Initial change was increasing basal membrane permeability of the injured hepatocytes. Blistering and rupturing of adjacent bile canaliculi might occur and result in flooding of bile into these hepatocytes and followed by inflammatory cell invasion or extrusion of cytoplasm blebs into sinusoids. Our results demonstrated an unrecognized role of apical membrane rupture during hepatocyte necrosis.

IL10

Prediction of Interfaces for Molecules on Protein Surfaces Based on Protein Sequences and 3D-Structures

Kei Yura

Center for Informational Biology, Ochanomizu University, Tokyo, Japan

Correspondence e-mail address: yura.kei@ocha.ac.jp

Genome sequencing and structural genomics projects have provided a huge number of protein sequences and protein three-dimensional (3D) structures, both of which are the fundamental data to understand mechanisms of protein functions. Out of many types of biological functions, we are interested in the protein functions realized by interaction with a small molecule, which can be a drug candidate. Computational structural biology plays substantial roles in this field.

Many methods to model a three-dimensional structure of a protein in complex with a ligand have been developed and extensively used. These methods generally employ a docking technique based on the first principle or an empirical potential. These methods often suffer from the issues of many false positive, difficulty in taking dynamics of the protein and the ligand into account, and computational time[1]. To overcome these issues, we started to collect many types of empirical rules in protein-ligand complex structures.

Het-PDB Navi. is a freely accessible database we launched about nine years ago[2]. The database rearranges the protein 3D data in Protein Data Bank focusing on types of ligand. Using this database, we built an empirical interaction potential for each ligand and found that the potential had a reasonable capability to predict ligand-binding sites on a protein surface[3]. To perform a docking simulation, conformational change of both proteins and ligands may need to be considered. We found unexpected differences between the structures of the bound ligand and of the free ligand. The finding may help improve our understanding of the dynamics in the protein-ligand docking process.

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IL11

Accommodating protein dynamics for prediction of enzyme substrates and inhibitors

Jung-Hsin Lin [1,2,3]

[1] *Division of Mechanics, Research Center for Applied Sciences, Academia Sinica, Taiwan*

[2] *Institute of Biomedical Sciences, Academia Sinica, Taiwan*

[3] School of Pharmacy, National Taiwan University, Taipei, Taiwan

Correspondence e-mail address: jhlin@gate.sinica.edu.tw, jlin@ntu.edu.tw

Molecular docking has been applied for the prediction of inhibitors and substrates with considerable success. In particular, it has been shown that docking the high energy intermediates of candidate molecular structures can predict the substrates of an enzyme and infer functions of uncharacterized proteins (J. Am. Chem. Soc. 2006, 128: 15882-15891, Nature 2007, 448: 775-779). However, due to the apparent limitation of docking to a static protein structure, proper incorporation of protein flexibility has received increasing attention to include the effects of induce-fit and the conformation selection of ligands. In the past we have proposed the relaxed complex scheme (J. Am. Chem. Soc. 2002, 124: 5632-5633, Biopolymers 2003, 68: 47-62) to incorporate the protein flexibility by rapid docking to the conformation ensemble generated by molecular dynamics simulations, which has been demonstrated as a computational analogue of the “SAR-by-NMR” technique.

In this talk, the advances of computational methodologies in the scope of the relaxed complex scheme will be reviewed and discussed. Two major approaches can be adopted to enable more efficient use of the relaxed complex scheme: one is to implement a more efficient global search algorithm, and the other is to apply the clustering method to reduce the number of protein conformations for docking. Recently we have proposed a novel global optimization algorithm that could work very efficiently for most protein-ligand interaction problems (Nucleic Acids Research, 2005, 33, W233-W238). Several clustering schemes have been compared and their relative performance been benchmarked. Besides, we have also curated a large dataset for binding information of protein-ligand interactions, and with this large dataset, we are now able to design an improved scoring scheme within the QSAR framework. Finally, we have also proposed an iterative docking scheme to automatically predict multiple binding sites or contiguous conformations of substrates along the pathways or channels inside the proteins (Nucleic Acids Research, 2009, 37, W559-W564).

IL12

Design of integrins-specific disintegrins

Woei-Jer Chuang, Chiu-Yueh Chen, Jia-Hau Shiu, Yi-Chun Chen, Yao-Tsung Chang, Chun-Hau Huang, and Yung-Sheng Chang

Department of Biochemistry and Molecular Biology, National Cheng Kung University College of Medicine

Correspondence e-mail address: wjcnmr@mail.ncku.edu.tw

Integrins are αβ heterodimers that are expressed on virtual all cells with adhesive capacity. They are involved in fundamental cellular processes such as attachment, migration, proliferation, differentiation, and survival. Disintegrins are a family of RGD-containing proteins found in snake venoms that contain 47 to 84 amino acids with 4-7 disulfide bonds. Rhodostomin (Rho) is obtained from *Calloselasma rhodostoma* venom and belongs to the family of disintegrins. Our study showed that Rho expressed in *P. pastoris* possesses the same function and structure as native protein. In order to design integrin-specific disintegrins, we expressed >300 Rho mutants in *P. pastoris* and used platelet aggregation and cell adhesion assays to identify the mutant proteins that can selectively inhibit integrins αIIbβ3, αvβ3, and α5β1. We found that the mutant proteins containing the AKGDWN and ARLDDL motifs can selectively inhibit integrins αIIbβ3 and αvβ3, respectively. We also determined 3D structures and backbone dynamics of integrin-specific disintegrins using NMR spectroscopy. According to the results of animal disease models, we found that integrin αvβ3-specific disintegrins can be used for the treatment of integrin αvβ3-related diseases, such as osteoporosis, age-related macular degeneration, and metastatic tumors. This drug candidate is now in preclinical trial for the treatment of metastatic bone tumor.

IL13

Folding Stability and Native Conformation of Amyloid-β Monomer are Important Determinants of the Nucleation Kinetics and Fibril Formation

Chun-Lun Ni[1], Hoi-Ping Shi[1], Hui-Ming Yu[1], Yun-Chorng Chang[2], and Yun-Ru Chen*[1]

Contribution from the Genomics Research Center, Academia Sinica, 128, Academia Road, Sec. 2, Nankang Dist., Taipei 115, Taiwan[†]

[‡], Tainan 701, Taiwan[‡].

[1]Genomics Research Center, Academia Sinica, Taipei, Taiwan

[2]Institute of Electro-Optical Science and Engineering, National Cheng Kung University, Taipei, Taiwan

Correspondence e-mail address: yrchen@gate.sinica.edu.tw

Amyloid formation is initiated with protein misfolding followed by self-association to form oligomers and fibrils. The discovery of toxic pre-fibrillar oligomers in many amyloidosis provokes the importance of understanding the folding mechanism prior to aggregation. Here, we investigated the folding properties of the natively unfolded Amyloid-β peptide (Aβ) and the familial mutants (A21G, E22Q, E22G, E22K, and D23N) in Alzheimer's disease (AD). In combination of native electrophoresis, analytical ultracentrifugation, fluorescence emission, and far-UV circular dichroism, we found all Aβ40 variants are predominantly monomeric containing similar residual secondary structures, but distinct hydrophobic-exposed protein surfaces. Guanidine hydrochloride (GdnHCl) denaturation showed that Aβ variants adopt an apparent two-state folding mechanism with different folding stabilities in which wild type is less stable than A21G but more stable than D23N and E22 mutants. By correlating the folding stability with their nucleation phase in fibrillization, we found the more stable the Aβ variant the slower the nucleation except D23N. Besides, unfolding native Aβ leads to reduction of mature fibrils, but increase of amorphous aggregates. The wild type (WT) Aβ and A21G show good correlation between native conformation and the amount of fibrils, whereas the folding equilibrium of E22 mutants and D23N may be shifted toward fibrillization pathway. Our results demonstrated that the folding stability of Aβ is an important determinant of the nucleation kinetics and native Aβ conformation implicates mature fibril formation.

IL14

Relaxation and aggregation of biopolymers, and q-statistics

Chin-Kun Hu[1,2]

[1]*Institute of Physics, Academia Sinica, Taipei 11529, Taiwan*

[2]*Center for Nonlinear and Complex Systems and Department of Physics, Chung-Yung Christian University, Chungli 32023, Taiwan ,*

Correspondence e-mail address: huck@phys.sinica.edu.tw

Many important biological macromolecules, such as starch, DNA, RNA, protein, etc are biopolymers. Relaxation and aggregation of biopolymers are closely related to biological functions of biological systems, e.g. many neurodegenerative diseases in humans or animals, such as Alzheimer's disease, Huntington's disease, Parkinson's disease, frontotemporal lobar degeneration, etc. are related to the aggregation of proteins. Here we propose simple models for relaxation and aggregation of biopolymers. We have used molecular dynamics to simulate various systems of polymer chains and Lennard-Jones molecules; the neighboring monomers along a polymer chain are connected by rigid bonds [1] or spring of strength ks [2]. We find that the velocity distributions of monomers in a wide range of simulation time can be well described by Tsallis q-statistics [3] with q larger than or equal to 1 and a single scaling function. The value of q is related to the conformation constraining potential, the interactions with background fluid, the destruction of chain homogeneity or the value of ks; when q becomes 1, the velocity distribution of monomers becomes Maxwell-Boltzmann distribution. We have computed some correlation functions along the polymer chains to understand the geometrical origin of q-statistics with q>1. We also find that the polymer chains tend to aggregate as neighboring monomers of a polymer chain have small or zero bending-angle and torsion-angle dependent potentials [4-6]. Our results are useful for understanding mechanism of protein aggregation.

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IL15

Symmetries and the trajectory of Listeria monocytogenes

Hsuan-Yi Chen[12] , Fu-Lai Wen[1], and Kwan-Tai Leung[2]

[1]*Department of Physics, National Central University, Jhongli, Taiwan*

[2]*Institute of Physics, Academia Sinica, Taipei, Taiwan*

Correspondence e-mail address: hschen@phy.ncu.edu.tw

In vivo, Listeria monocytogenes hijack the actin polymerization machinery of the host cell by their surface protein ActA. In vitro, ActA coated plastic beads are also propelled by similar comet-like tails made by actin gel. This provides a much simplified model system for the study of the dynamics and organization of actin cytoskeleton.

An interesting experimental observation is that, in a homogeneous environment, the trajectories of both Listeria and ActA-coated beads, instead of being straight, are often curved. Circular, figure-eight, helical, and more complicated trajectories are examples of these non-straight trajectories. In the absence of a commonly accepted molecular model for the physical mechanism of actin-based motility, the symmetry principles often emphasized in the physics of non-living world is a powerful concept for building a phenomenological theory for the motion of Listeria monocytogenes.

In this talk, I will show that the transition of an ActA-coated bead from rest to moving state is associated with a broken (front-tail) symmetry in the actin density on the bead surface. The transition from moving straight to a circular motion is associated with a broken (left-right) symmetry in the actin density and force per filament on the bead surface. These simple observations set physical constraints on the possible molecular level mechanisms of actin network organization dynamics for the emergent phenomena (bead trajectories) observed in the experiments.

IL16

Uncovering small RNA-mediated responses to stress conditions by deep sequencing-based expression analysis

Li-Ching Hsieh[1], Shu-I Lin[2], Chun-Chieh Shih[3], Wen-Hsiung Li[4,5] and Tzyy-Jen Chiou[2,6]

[1]*Genomics Research Center, Academia Sinica, Taipei, Taiwan*

[2]*Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan*

[3]*Institute of Information Science, Academia Sinica, Taipei, Taiwan*

[4]*Biodiversity Research Center, Academia Sinica, Taipei, Taiwan*

[5]*Department of Ecology and Evolution, University of Chicago, Chicago, USA*

[6]*Department of Life Sciences, National Chung-Hsing University, Taichung, Taiwan*

Correspondence e-mail address: lchsieh@gate.sinica.edu.tw

We developed an analysis pipeline for small RNA deep sequencing data obtained from samples under normal or stress conditions, and filtered out the small RNAs showing different expression patterns among the samples using two approaches, a cluster-based approach for finding novel differentially expressed small RNAs and a gene-model-based approach for analyzing small RNAs from known gene models, especially for miRNA genes and ta-siRNA genes. This pipeline was then applied to four small RNA libraries from root and shoot tissues of Arabidopsis (*Arabidopsis thaliana*) under phosphate (Pi)-sufficient or -deficient conditions. From the results of analysis, one new Pi starvation-induced miRNA family (miR2111) was identified in dicotyledons and a newly evolved Pi-responsive small RNA derived from the long terminal repeat of a retrotransposon was also identified. Importantly, we observed an increased abundance of TAS4-derived trans-acting siRNAs (ta-siRNAs) in Pi-deficient shoots and uncovered a negative feedback regulatory mechanism for the control of a set of transcription factors that regulate the biosynthesis of anthocyanin. This study not only demonstrates how the analysis pipeline works but also exhibits that the results of the analysis pipeline are very informative and are helpful in uncovering small RNA-mediated responses to stress conditions.

IL17

Error-tracking clustering gives quantitative statistics to DNA segmentation analysis

Chih-Hao Chen[1,2], Hsing-Chung Lee[1,3], Qingdong Ling[1,4], Hsiao-Rong Chen[1], Yi-An Ko[1], Tsong-Shan Tsou[4,5], Sun-Chong Wang[1], Li-Ching Wu[1] and H.C. Lee[1,2,4,6]

[1]*Graduate Institute of Systems Biology and Bioinformatics, National Central University, Chungli, Taiwan 32001*

[2]*National Center for Theoretical Science, Shinchu, Taiwan 30043*

[3]*Department of Surgery, Cathay General Hospital, Taipei, Taiwan*

[4]*Cathay Medical Research Institute, Cathay General Hospital, Taipei, Taiwan*

[5]*Graduate Institute of Statistics, National Central University, Chungli, Taiwan 32001*

[6]*Department of Physics, National Central University, Chungli, Taiwan 32001*

Correspondence e-mail address: hclee12345@gmail.com

Inferences acquired by applying clustering analysis of microarrays cannot be reliably assessed before data-originated errors are quantified, an exacting task that is often not performed. Here, we present a novel and fast clustering technique, pair-wise Gaussian merging (PGM), suited for this purpose. Designed for systems with normally distributed error, PGM treats each observation as a Gaussian distribution function, with error as width, uses a simple but exact mathematical relation to track error at every step of clustering, and gives results from which quantitative statistics are easily extracted. PGM is built on a framework of agglomerative hierarchical clustering, uses *t*-value as distance and requires no linkage criteria. We demonstrate the merits of PGM by applying it to a segmentation algorithm for DNA copy number analysis (SAD) which, by comparing it's performance to existing algorithms, we verify that it: provides quantitative statistics for predictions; is simpler in formulation; is less thirsty for memory; offers higher accuracy; and for today's typical array size, is faster by orders of magnitude than its nearest competitor. With only two user-adjusted and easily comprehended parameters, SAD is highly user friendly. SAD's running time scales linearly with data size and is therefore ideally suited to the challenge of ever-growing array resolution. On a typical modern notebook, SAD completes high-quality copy number analysis for a 250 thousand-marker array in ~1 second and a 1.8 million-marker array in ~8 seconds.

IL18

Size-Dependent Endocytosis of Gold Nanoparticles and Its Applications

Pei-Kuen Wei[1,2]

[1.] *Research Center for Applied Sciences, Academia Sinica, 128, section 2, Academic Road, Nankang, Taipei 11529, Taiwan*

[2.] *Institute of Biophotonics Engineering, National Yang Ming University, Taipei, 112, Taiwan*

We studied the endocytosis of gold nanoparticles (GNPs) with different sizes (13 nm, 45 nm, 60 nm, 80 nm and 110 nm) in various cells (Fibroblasts, CL1-0 and HeLa cells) by using a dark-field optical section microscope (DFSOM). The GNP has a very large scattering cross-section at the resonant wavelength of surface plasmon which is acted as the imaging signals. With this technique, we identify the endocytosis of GNPs is size-dependent. The 40 nm GNP has the highest ratio for the uptake of cells. On the contrary, GNPs with particle size larger than 80nm were most on the cellular membrane. We used the 40nm GNPs to deliver DNAs in to cells. The real-time images of the drug-carrying process by the 40 nm GNPs are demonstrated. In addition, The 80 nm GNPs were surface modified with poly (L-lysine) which attached to the membranes by way of electrostatic attractive force. Three-dimensional (3D) morphological image was obtained by tracking the peak scattering intensities of the GNPs at different focal planes. An algorithm for the reconstruction of 3D cell morphology was presented. With the measured GNPs images and calculations, we show morphologic changes of lung cancer cells under the interaction of cytochalasin D drug at different times.

IL19

Electrotactic signaling pathways in *Dictyostelium* cell

Masayuki J. Sato[1,2] and Masahiro Ueda[1,2,*]

[1]*Laboratories for Nanobiology, Graduate School of Frontier Biosciences, Osaka University*

[2]*Core Research for Evolutional Science and Technology, Japan Science and Technology Agency*

[*]Correspondence e-mail address: ueda@phys1.med.osaka-u.ac.jp

Switching between attractive and repulsive migration during cell movement in response to extracellular guidance cues has been found in various cell types and is an important cellular function for various cellular and developmental processes. One notable guidance cue is an electric signal. Many type of cells exhibit directed cell migration in response to a direct current electric field (dcEF). However, the underlying molecular mechanism remains unknown. Here we show that the preferential direction of migration during electrotaxis in *Dictyostelium* cells can be reversed by genetically modulating both guanylyl cyclase (GCase) and cyclic guanosine monophosphate (cGMP)-binding protein C (GbpC) in combination with the inhibition of phosphatidylinositol-3-OH kinase (PI3K). We found that the PI3K-dependent pathway is involved in cathode-directed migration under a dcEF, as too are the catalytic domains of soluble GCase (sGC) and GbpC via cGMP. On the other hand, the N-terminal domain of sGC was shown to mediate anode-directed signaling in conjunction with both the inhibition of PI3K and cGMP production. These observations identify the genes required for directional switching during electrotaxis and suggest that a parallel processing of electric signals, in which multiple-signaling pathways act to bias cell movement toward the cathode or anode, is used to determine the direction of migration.

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IL20

Active Waves and 3D Nanoparticle Motion on Cell Membranes Characterized by Wide-field Optical Profilometry

Chau-Hwang Lee[1,2]

[1]*Research Center for Applied Sciences, Academia Sinica, Taipei, Taiwan*

[2]*Institute of Biophotonics, National Yang-Ming University, Taipei, Taiwan*

Correspondence e-mail address: clee@gate.sinica.edu.tw

We employ a far-field optical technique, non-interferometric wide-field optical profilometry (NIWOP), on the observations and analyses of cell membrane activities and nanoparticle motion. NIWOP provides 20 nm depth sensitivity on cell membranes, and its image-acquisition rate can be up to 12 frames/sec with the setup using a liquid-crystal spatial light modulator. Hence it can be employed as a non-labeling and non-contact method for the studies of cell membrane dynamics (1,2). We characterize the retrograde propagation of active membrane waves on fibroblasts in culture dishes. We also verify that the membrane waves are driven by the interactions between the protrusion of actin filaments and the activities of myosin II (3). NIWOP is also able to track the 3D positions of sub-100 nm gold particles. The 3D positioning accuracy is about 20 nm in bright-field images. We analyze the internalization of transferrin–gold nanoparticles on the membrane (4) and the intracellular transportation of liposomes coated by fibroblast growth factor-1. In these observations the cellular phototoxic effects are minimized because no laser illumination is employed.

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