Title: NMR Spectroscopy in Solution and X-ray Diffraction in Crystals for Structural Biology and Structural Genomics

Presenter: **Kurt Wüthrich** (ETH Züthrich, Switzerland)

Biography

Kurt Wüthrich is currently Cecil H. and Ida M. Green Professor of Structural Biology at The Scripps Research Institute, La Jolla, CA, USA and Professor of Biophysics at the ETH Zürich, Zürich, Switzerland. His research interest includes molecular structural biology and structural genomics. His specialty is nuclear magnetic resonance (NMR) spectroscopy with biological macromolecules, where he contributed the NMR method of three-dimensional structure determination of proteins and nucleic acids in solution. The Wüthrich groups have solved more than 70 NMR structures of proteins and nucleic acids, including the immunosuppression system cyclophilin A-cyclosporin A, the homeodomain-operator DNA transcriptional regulatory system, and prion proteins from a variety of species.

Kurt Wüthrich was born in Switzerland on October 4, 1938, is married to Marianne Briner, and has two children, Bernhard Andrew and Karin Lynn. He studied chemistry, physics and mathematics at the University of Bern from 57-62, obtained the Eidgenössiches Turn-und Sportlehrerdiplom and a Ph.D. in inorganic chemistry with Prof. Silvio Fallab at the University of Basel in 64, was postdoctoral fellow in Basel (Prof. S. Fallab), at the University of California in Berkeley, CA, USA (Prof. R.E. Connick) and at Bell Telephone Laboratories in Murray Hill, NJ, USA (Dr. R.G. Shulman) before joining the ETH Zürich in 69 (Privatdozent 70, Assistant Professor 72, Associate Professor 76, Professor of Biophysics 80, Chairman of the Department of Biology 95-00). Since 2001 he shares his time between ETH Zürich and The Scripps Research Institute. Kurt Wüthrich's achievements have been recognized by the Prix Louis Jeantet de Médecine, the Kyoto Prize in Advanced Technology, the Nobel Prize in Chemistry, and by a number of other awards and honorary degrees

Abstract

Kurt Wüthrich, The Scripps Research Institute, La Jolla, CA 92037, USA, and Institute of Molecular Biology and Biophysics, ETH Zürich, CH-8093 Zürich, Switzerland

X-ray diffraction in single-crystals and NMR spectroscopy with protein solutions have generated nearly all of the more than 40'000 atomic-resolution structures in the Protein Data Bank (PDB). Many of these Data Bank deposits are associated with "classical" structural biology projects, but during the last few years the yield of new structures from structural genomics projects has greatly increased. Discussions on structural genomics often tend to focus on "high-throughput" methods. Crystallographic techniques have already been established that can efficiently solve a wide variety of protein structures. In solution NMR, innovative ideas are still being implemented to improve the efficiency of structure determination, and a brief survey of the current state of these approaches in my research groups will be presented. However, the main focus of this presentation will be on the different philosophies and strategies of structural biology and structural genomics in their efforts to unravel the protein universe.

Title: Structural Basis for the Human Bifunctional Enzyme PAICS in Purine Biosynthesis

Presenter: **Ru-Chang Bi** (Institute of Biophysics, Chinese Academy of Sciences, China)

Biography

Ru-Chang Bi, working at Center for Computational and Systems Biology, Institute of Biophysics, Chinese Academy of Sciences, graduated as master from the Leningrad University in the former USSR (Russia) in 1965. Then he worked in Institute of Biophysics, Chinese Academy of Sciences, and became an important member of research group to determine the first protein crystal structure in China, the three-dimensional structure of pig insulin. 1980-82, as visiting scholar, he continued studies on protein crystallography in University of York, England. 1986-92, he was in charge of Department of Protein Crystallography, Institute of Biophysics, and was a member of expert group of protein engineering for the national high-tech development program. 1990-2003, as person responsible for relevant project, he opened up a direction of space biotechnology in China, and performed successfully space experiments of protein crystallization aboard the Chinese recoverable spacecrafts. Entering into 21th century, his research group was involved in structural and functional studies of several proteins such as human epidermal growth factor, the bacterioferritin from *Azotobacter vinelandii* and human bifunctional enzyme PAICS in purine biosynthesis. 1995-2006, he was a member of international advisory committee for the International Conference of Crystallization of Biological Macromolecules held generally every two years.

Abstract

R.C. Bi, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang Dist., Beijing 100101, China, Email: rcbi@ibp.ac.cn

PAICS is an important bifunctional enzyme in de novo purine biosynthesis in vertebrate with both 5-aminoimidazole ribonucleotide carboxylase (AIRc) and 4-(N-succinylcarboxamide)-5-aminoimidazole ribonucleotide synthetase (SAICARs) activities. It becomes an attractive target for rational anticancer drug design, since rapidly dividing cancer cells rely heavily on the purine de novo pathway for synthesis of adenine and guanine, whereas normal cells favor the salvage pathway. The crystal structure of human PAICS, the first in the entire PAICS family, at 2.8 Å resolution has been determined in our group (Li et al. J. Mol. Biol. (2007) 366, 1603–1614). It revealed that eight PAICS monomers, each composed of distinct AIRc and SAICARs domains, assemble a compact homo-octamer with an octameric-carboxylase core and four symmetric periphery dimers formed by synthetase domains. Based on structural comparisons and functional complementation analyses, the active sites of SAICARs and AIRc were identified, including a putative substrate CO_2-binding site. Furthermore, four symmetry-related, separate tunnel systems in the PAICS octamer were found that connect the active sites of AIRc and SAICARs. In accord with the fact that PAICS exists as octamer in solution, this study has illustrated the octameric nature of the bifunctional enzyme. Each carboxylase active site is formed by structural elements from three AIRc domains, demonstrating that the octamer structure is essential for the carboxylation activity. Furthermore, the existence of the tunnel system implies a mechanism of intermediate channeling and suggests that the quaternary structure arrangement is crucial for effectively executing the sequential reactions.

Title: Heterodimeric Restriction Endonucleases

Presenter: **Geoffrey G. Wilson** (New England Biolabs, USA)

Biography

Geoffrey Wilson grew up in the U.K. graduated from the University of Sussex with a B.Sc. in Biology and a Ph.D. in Molecular Biology. Following post-doctoral work at Edinburgh University and Yale, he joined New England Biolabs in 1980 to establish a research and development program to clone, overexpress, and characterize restriction and modification enzymes. The primary goal was to simplify the purification of these enzymes to provide molecular biologists with better tools for DNA analysis and manipulation. The secondary goal was to learn enough about them to permit the fabrication of 'custom' enzymes with properties of choice. In collaboration with many others, the primary goal has been largely met: several hundred restriction and modification enzymes have been cloned and characterized, and many hundreds more are known from sequenced microbial genomes. In the process, much has been learned about their organizations, structures, modes of action, and variety. Changing their properties and fabricating custom enzymes has proved more difficult, however, and achieving this goal is still some way into the future. Current projects in Geoffrey's lab include the identification of restriction enzymes that use two different catalytic sites to cleave DNA, and their conversion to enzymes that nick DNA rather than cleave it; the analysis of multi-subunit, combination restriction-and-modification enzymes; and the conversion of modification enzymes into modular restriction-like enzymes by catalytic-domain grafts. The challenges here are daunting, just as the prospects are exciting, but a wealth of new insights, and opportunities undoubtedly lie ahead.

Abstract

Shuang-Yong Xu, Zhenyu Zhu, Daniel F. Heiter, Keith D. Lunnen, Penhua Zhang, Siu-hong Chan, James C. Samuelson, Jianping Xiao and Geoffrey G. Wilson, New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938, USA, E-mail: wilson@neb.com

Restriction endonucleases bind to specific sequences of base-pairs in duplex DNA and cleave the DNA wherever these sequences occur. Thousands of such enzymes are known, many unique with respect to the sequence bound or to the positions of cleavage. From a restriction enzyme's point of view, DNA is a double substrate whose cleavage requires two catalytic events, one to hydrolyze each strand. Most restriction enzymes accomplish this using two catalytic sites, hydrolyzing the two strands simultaneously, in parallel reactions that proceed independently. Frequently such enzymes are homodimers comprising two identical subunits that interact in opposite orientations and bind to DNA in concert. Because the binding sites and catalytic sites of the subunits of homodimers are identical, these enzymes characteristically recognize sequences that are symmetric and cleave them internally in a symmetric manner.

We have uncovered four restriction enzymes of a quite different kind—BssIMI (GGGTC -3/0), BstNBI (GAGTC 4/4), BsrDI (GCAATG 2/0), and BtsI (GCAGTG 2/0)—that appear to act as heterodimers rather than homodimers. These enzymes comprise two different subunits, one large (average 450 aa) and one small (average 170 aa), each with a different catalytic site. On their own, we find, the large subunits of all four enzymes display efficient and specific DNA-nicking activity: they bind to the recognition sequence and hydrolyze one strand of the duplex, but not the other. The small subunits on their own display no endonucleolytic activity, but in the presence of the large subunits they hydrolyze the other strand to produce complete DNA cleavage. We refer to the large subunits of these unusual restriction enzymes as 'hemi-dimeric' (= half a dimer) nicking endonucleases.

The heterodimeric enzymes are considerably more difficult to purify than are regular homodimeric restriction enzymes. To varying degrees, cleavage activity vanishes during column chromatography and little of the activity applied to columns can be recovered by elution. We think this instability is

due to dissociation of the subunits on the column and their subsequent chromatographic separation, and we find that it can be readily reversed. Mixing the small and large subunits together after purifying them individually reconstitutes the enzymes and restores their double-strand cleavage ability. Two further restriction enzymes, BsrI (ACTGG 1/-1) and BsmI (GAATGC 1/-1), cleave DNA as monomers but appear distantly related to BsrDI and BtsI in amino acid sequence and organization. These enzymes incorporate two different catalytic sites into the same protein chain and might have arisen during evolution by fusion of the two subunits that in the other enzymes remain separate.

Title: The Centre for Protein Science and Crystallography and Research Activities

Presenter: **Pang-Chui Shaw** (Chinese University of Hong Kong, Hong Kong)

Biography

Pang-Chui Shaw was trained as a molecular biotechnologist and obtained his Ph.D. degree at the Imperial College of Science, Medicine and Technology, University of London, UK. He is now Professor in the Department of Biochemistry of the Chinese University of Hong Kong, Director of the Centre for Protein Science and Crystallography and Deputy Director of the Institute of Chinese Medicine. He also serves as the chairperson of the Hong Kong Bioethics Association, council member of the Chinese Genetics Society and member of the editorial boards of several scientific journals. His research interest includes structure-function studies of proteins with medical significance, in particular on ribosome-inactivating proteins and proteins of influenza viruses. He also works on the authentication and quality control of medicinal herbs and their activities on neural cell growth and Alzheimer's disease. He has published 120 refereed papers, two books and four patents.

Abstract

Pang-Chui Shaw and Wing-Ping Fong, Department of Biochemistry and Centre for Protein Science and Crystallography, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China, Email: pcshaw@cuhk.edu.hk

The Centre for Protein Science and Crystallography was established in 2005 by the Department of Biochemistry of CUHK. Researchers employ multi-disciplinary techniques including protein engineering, biophysical characterization, and X-ray crystallography to study the structure-function relationship of proteins. The Centre has facilities for protein crystallization and houses the first state-of-the-art X-ray crystallographic setup in Hong Kong for high-resolution protein structure determination. It is also equipped for the study of the biophysical properties of proteins and protein-protein interaction. The aim is to provide a platform to promote collaboration on structure-function research of proteins within the local community and with China. Projects on proteins of biotechnological significance, proteins related to diseases and viral infections are being carried out and selected projects will be introduced in the talk.

Title: Role of FTIR Spectroscopy in Structural Proteomics

Presenter: **Parvez Haris** (De Montfort University, UK)

Biography

After completing his PhD under the guidance of Professor Dennis Chapman FRS, at the Royal Free Hospital School of Medicine (University of London) in 1989, Parvez Haris spent seven more years as a post-doctoral research fellow in the same laboratory. Subsequently, he was appointed as an academic at De Montfort University, Leicester, UK where he continues his research on protein structure-function analysis. He is one of the pioneers in the development of FTIR spectroscopy for protein structural analysis and has demonstrated how this technique can be used to monitor subtle changes in protein conformation through a combination of secondary structure analysis and hydrogen-deuterium exchange kinetics in a wide range of environments and systems. He also introduced the concept of studying protein-protein interaction using FTIR spectroscopy via ^{13}C and ^{15}N isotopic labelling of one of the interacting proteins. He is currently developing FTIR spectroscopy as a tool for proteomics research including suitable methods for rapid secondary structure prediction, fold classification. He is also developing an infrared protein spectra database for proteomics research. He has published over 120 scientific articles in diverse journals including *Nature Biotechnology*. He has also edited two books, including one on *Biomembrane Structures*. He is an editor of the *Biochemical Journal*, the Editor-in-Chief of *Spectroscopy – Biomedical Applications*, and the Editorial Advisory Panel member of *Molecular Membrane Biology*.

Abstract

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With the completion of the sequencing of the human genome, the major challenge confronting structural biochemists is rapid characterisation of protein structure which in turn necessitates the development of biophysical techniques that can aid this process. For over two decades I have been developing Fourier transform infrared (FTIR) spectroscopy for understanding protein structure and dynamics and this work continues into the post-genomic era. Whilst this technique cannot provide the complete 3D structure of a protein at atomic resolution, it has the advantage of being able to probe subtle conformational changes and transient molecular interactions in diverse environments that are not readily amenable for X-ray crystallographic or NMR analysis. FTIR spectroscopy has been used in my laboratory to analyse protein structure in diverse physical environments including aqueous solution, organic solvents, detergents, phospholipids membranes as well as in thin films, solid powders and in the crystalline state. Protein amide bands have been used for monitoring protein secondary structure and hydrogen-deuterium exchange kinetics. A database of protein infrared spectra, recorded in aqueous media, has been produced and artificial intelligence methods are being used to rapidly predict secondary structure content, lengths of different secondary structures as well as fold classification. One of the major advantages of FTIR spectroscopy is its suitability for probing membrane proteins, which constitutes a third of most genomes, as it not restricted by the size or membrane-bound nature of these proteins. In addition to membrane proteins, work in my laboratory has also focused on other systems that are difficult to study using most physical techniques, including protein aggregates, such as amyloids, and the development of isotope-edited FTIR spectroscopy for protein-protein interaction analysis. In this presentation, the pros and cons of FTIR spectroscopy as a tool for protein structure determination, and its potential role in structural proteomics, will be discussed.

Title: Solving Enzyme Structure by Metabolic Pathways

Presenter: **Xiao-Dong Su** (Peking University, China)

Biography

Education and Employment:1980-1985, B.Sc. in solid state physics. Department of Physics, Peking University, China; 1985-1987,Graduate study (M. Sc.) at the Dept. of Biophysics, Beijing Medical College, Peking University, China; 1988-1994, Ph.D. candidate at the Department of Cell and Molecular Biology, Karolinska Institute, Sweden; 1995-1998, Research associate with Howard Hughes Medical Institute (HHMI) at Division of Biology, California Institute of Technology,USA; 1998-2001,Assistant professor at Department of Molecular Biophysics, Chemistry Center, Lund University, Sweden;2001-2002, Associate Professor at Department of Molecular Biophysics, Chemistry Center, Lund University, Sweden; 2002-present, Professor at Department of Biochemistry and Molecular Biology, College of Life Sciences, Peking University.

Current Positions: Secretary-general of Chinese crystallography Society (CCrS); Associate Director, National Lab. of Protein Engineering & Plant Genetic Engineering; Associate Director, the Center for Protein Science, Peking University.

Research Interest: The lab is interested in structural and functional studies of genes related to human diseases or with important functions. We have built up technological platforms for high-throughput (HTP) methods for structural genomics (structural biology) studies, including target selection; HTP and automated gene cloning; protein expression; protein purification; crystallization and crystal structure determinations. Using the Single-wavelength Anomalous Diffraction methods on the copper rotating anode (in-house S-SAD), we can now make an *ab initio* crystal structure determination in weeks. And we have both automatic protein purification system (ÄKTA Xplorer with 3D) and Beckman-Coulter Robot System for large scale structural genomics study, furthermore, we have home-built robotic system for protein crystallization screening and imaging. In addition to *E. coli* expression systems, eukaryotic systems such as baculovirus system have been tried as well to express proteins that are hard to do in prokaryotic expression systems. Main research topics include: Solving 3-D structures of proteins related to signal transduction pathways in cell apoptosis, tumor genesis and metastasis of various human diseases including SARS; Functional studies of the above proteins; Develop and use of different host systems to express and purify glyco- and membrane proteins; Structure-based drug design.

Abstract

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It has been a dream-like goal for biochemists to have all enzyme structures solved and catalytic mechanisms worked out, the results will not only satisfy academic curiosity, but also serve to the numerous practical fields related, such as structural-based drug design for one. In the post genomic era, rapid development of novel and efficient techniques, particularly the emerging and development of the so-called structural genomics has made the goal of solving all enzyme structures reachable. Towards achieving this goal we have been trying to solve enzyme structures by their metabolic pathways on a large-scale, high-throughput structural genomics platform at Peking University. I will present in this meeting over a dozen enzyme structures in the pathways of amino acid and carbohydrate metabolism pathways we have worked over the last a few years, particularly on enzymes involved in a universal histidine degradation pathway, and enzymes in the biosynthesis pathway of an essential amino acid, tryptophan.

Title: Metal Transport and Storage Proteins: Implication for Metal Homeostasis

Presenter: **Hongzhe Sun** (University of Hong Kong, Hong Kong)

Biography

Hongzhe Sun received his Ph.D from the University of London in 1996. After two years as a GlaxoWellcome Research Fellow at the University of Edinburgh, he joined the Department of Chemistry at the University of Hong Kong in 1998 as an Assistant Professor and later Associate Professor.

Dr. Sun's research interest lies in the chemistry of metals in biology and medicine. He has characterized several important metallo-transport and storage proteins in microorganisms which provide a basis for mechanism-based drug design. Dr. Sun recently has focused on structural biology of metallo-proteins by NMR spectroscopy. He has been invited to deliver lectures in various international conferences including the prestigious Gordon Conferences and International Conference on Biological Inorganic Chemistry. He has published over 70 papers in international journals. Dr. Sun received the "*Outstanding Young Researcher Award*" of the University of Hong Kong (2005) and "*Outstanding Young Scholar Award*" from NSFC (2005), and currently serves as an editorial (advisory) board member in several journals.

Abstract

Hongzhe Sun, Hongyan Li, Yibo Zeng, Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, E-mail: hsun@hku.hk

More than one third of the known proteins require metal ions as cofactors to perform their functions. However, metal ions are also toxic at the elevated concentration and therefore uptake, trafficking and storage must be strictly regulated [1]. Various diseases have been found to directly or indirectly relate to metal ions. The divalent metal ion transporter (DMT1) is a membrane protein and mutation of glycine at TM4 leads to malfunction of the protein. The structure of the wild-type and G185R mutant of TM4 of DMT1 shows that the single mutation results the protein change from a trimer to a hexamer [2]. Using a metalloproteimic approach, we have identified several Bi-binding proteins in *Helicobacter pylori* including a histidine-rich protein HspA [3]. The histidine-rich proteins, Hpn is a small protein found in *H. pylori* with 28 His residues out of 60 amino acids. It may be an analog of metallothionein in terms of metal-binding and functions. Hpn is present as a multimer with 20-mer being the predominant species in solution and binds to around 4.8(±0.2) Ni^{2+} per monomer moderately (K_d of 7 μM) [4]. Although *in vitro,* it binds to Cu^{2+} stronger than Ni^{2+} and Bi^{3+}, the *in vivo* protection by the protein is in the order of Ni^{2+}> Bi^{3+}> Cu^{2+} [5]. This indicates that the primary role of Hpn is nickel storage and homeostasis.

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Title: Protein Crystallography Beamlines at SPring-8

Presenter: **Masaki Yamamoto** (RIKEN SPring-8 Center, Japan)

Biography

Position held: Head of Division
Division of Synchrotron Radiation Instrumentation

Research Interest:
1) Research and development of instrumentations for synchrotron radiation beamlines.
2) Research and development of new technologies for structural biology research.

Abstract

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In the third generation synchrotron radiation, structural biology research is one of the major subjects in the past decade. At SPring-8, 8 beamlines are operating for protein crystallography. We have been constructing and developing the beamlines with the view of two objects. One object is improvements of the applicable crystals size and the data quality, and the other is the high throughput data collection. BL41XU is an undulator beamline with the high brilliance of 4.8×10^{14} photons/sec/mm^2. The new K/B mirror system was installed for micro crystals (~ 25 μm) using a micro beam. The beam size at sample position is controllable from 25 × 25 to 70 × 100 μm^2 using two quadrant slits. SAD measurements of Se-methionine samples with the crystal size from 15 to 50 μm were performed using multiple positions on a crystal, and we solved the initial phase at the resolution from 2.7 to 3.9 Å. A new micro focus beamline, aimed at the target beam size of 1 × 1 μm^2, is planning. BL26B1&B2 have been constructed for Structural Genomics research. The beamline operation is automated cooperating with the sample changer robot named SPACE, the operation software BSS and beamline management database D-Cha. Combination of BSS, SPACE and D-cha also enables Mail-in data collection, and the Web interface of D-Cha allows users to deposit measurement condition or to observe recorded images from distant place. We will present the present status and the future plane of protein crystallography beamlines at SPring-8.

Title: Structural Genomics Studies of *Xanthomonas campestris* at NCHU, Taiwan

Presenter: **Shan-Ho Chou** (National Chung-Hsing University, Taiwan)

Biography

Shan-Ho Chou got his Ph. D. in 1984 from the Department of Chemistry, U. of Washington, Seattle, USA. Since then, he has been working there as a research assistant professor and research associated professor in the Department of Biochemistry, Howard Hughes Medical Institute, U. of Washington, before returning to Taiwan in 1996 to serve as a professor at the Institute of Biochemistry, National Chung-Hsing University. He has been working on the unusual nucleic acid structures using NMR approach, and has published extensively in this field and written several review articles in the *JMB* (267, 1055-1067, **1997**), *Nucleic Acids Research* (31, 2461-2474, **2003**), and *Trends in Biochemical Science* (30, 231-234, **2005**). He has been awarded several prestigious awards in Taiwan, including twice the National Science Council Outstanding Award. Currently he is a chair professor of the National Chung-Hsing University on the field of microbial structural genomics. He has been working on the structural genomics of a plant pathogen *Xanthomonas campestris*, which infects most economically important crop plants and causes their black rot diseases, by using X-ray crystallography and NMR approaches, since 2005.

Abstract

Chin, KH[1], Lee, YC[1], Tu, ZL[1], Yang, CY[1], Li, TN[1], Liao, SJ[1], Su, YC[1], Kuo, WT[1], Liao, CJ[1], Chen, JN[1], Teng, YC[1], Tsai, DY[1], Lyu, PJ[2], Tsai, P[3], Chou, CC[4], Wang, AHJ[4], Chou, SH[1], [1] Institute of Biochemistry, National Chung-Hsing University, Taichung, 40227, Taiwan, [2] Dept. of Life Science, National Tsing-Hwa University, Shin-Chu, Taiwan, [3]Dept. of Life Science, National Yang-Ming University, Pei-Tou, Taiwan, [4] Institute of Biological Chemistry, Academia Sinica, Nankang, Taipei, Taiwan, Email: shchou@nchu.edu.tw.

The flood of sequence information available from the various genome projects coupled with the recent advances in molecular and structural biology has led to the concept of structural genomics on a genome-wide scale. Determining three-dimensional structures of proteins is crucial for understanding their biological functions. In this respect, structural genomics is expected to pave way for understanding the intricate interactions among proteins in a whole organism, and is emerging as a powerful approach of functional annotation. We have initiated a coordinated program to study the structural genomics of *Xanthomonas campestris* pv. campestris, a gram-negative bacterium that is phytopathogenic to cruciferous plants and causes worldwide agricultural loss. Although pathogenic, it, however, also produces exopolysaccharide (xanthan gum) that is of great industrial importance. Thus, due to the academic importance, immense economic impact, and simplicity of its genetic constitution (lacking introns) of this organism, we have endeavored to identify and characterize the structures and functions of proteins encoded in Xcc using X-ray crystallography and high resolution NMR techniques.

Until now, approximately 500 target genes of this bacterium have been successfully constructed in expression vectors. Among them, about 50% gene products are soluble, and 200 proteins have been purified. By screening crystallization condition using a robot system, we obtained about 100 crystals, of which ~30% were found to be suitable for structure determination. So far, more than ten structures have been solved using this structural genomics approach, many of which provide clues to biochemical functions that can be experimentally confirmed. Several novel fold or subfamilies of well-known folds have been identified. A pathogenic structural genomics of Xcc regarding the Clp (cAMP-receptor like protein) related proteins, and a flagellar structural genomics regarding the flagellar structural proteins, are also being actively pursued.

Title: **Studying Structure and Function of Proteins by Microfluidic Techniques**

Presenter: **Bo Zheng** (Chinese University of Hong Kong, Hong Kong)

Biography

Bo Zheng received his B.S. in chemistry in 1997 from Peking University, he then went to the Chemistry Department of Duke University for graduate study. He obtained his Ph.D. degree in 2002 with research in materials chemistry. He spent two and half years in Professor Ismagilov's group in the Chemistry Department of the University of Chicago as a postdoc research associate, working on the development of microfluidic methods for protein crystallization. In August 2005, he joined the Chemistry Department of the Chinese University of Hong Kong as an assistant professor. Currently his research focuses on bioanalysis and study of soft materials by using microchannels and microreactors.

Abstract

Bo Zheng, Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China, Email: bozheng@cuhk.edu.hk

This talk will present two microfluidic techniques for studying the structure and function of proteins in nanoliter volumes. First, a method of generating nanoliter droplets containing the mixture of protein and precipitant or substrate in microfluidic channels will be described. The chemical composition and the concentration can be controlled in individual droplets for screening or reaction. In the second method, nanoliter volume of reagents is dispensed into microwells through microchannels. Multiplex screening or reaction can be performed by binding two such microwells together. Several known and unknown water-soluble proteins have been crystallized using these methods, and the crystals of the proteins have been harvested for X-ray diffraction studies.

Title: HIV Reverse Transcriptase: High Resolution Crystal Engineering, Inhibitor Strategic Flexibility, and Fragment Screening

Presenter: **Eddy Arnold (**CABM & Rutgers University, USA**)**

Biography

Eddy Arnold received his Ph.D. in Organic Chemistry from Cornell University in 1982 with Prof. Jon Clardy and pursued postdoctoral studies with Prof. Michael Rossmann at Purdue University. In 1987 Arnold established his research group at the Center for Biotechnology and Medicine (CABM) and Rutgers University, where he is Professor of Chemistry and Chemical Biology. Currently Dr. Arnold and his group are working to develop and apply structure-based drug and vaccine designs for the treatment and prevention of serious human diseases, especially HIV/AIDS. Arnold and coworkers, including Dr. Stephen Hughes (NIH), study the structure and function of reverse transcriptase (RT), an essential component of the AIDS virus and target of many of the most widely used anti-AIDS drugs. Another major effort in the laboratory, co-directed by Dr. Gail Ferstandig Arnold, consists of engineering a human common cold virus, rhinovirus, to display appropriate segments from more dangerous pathogens for the purpose of developing vaccines against AIDS and other infectious diseases.

Abstract

Eddy Arnold, Professor of Chemistry and Chemical Biology, CABM & Rutgers University, 679 Hoes Lane, Piscataway, NJ 08854, arnold@cabm.rutgers.edu

TMC278/rilpivirine is highly effective in treating HIV-1 infections at relatively low doses (~25-75 mg/day) in clinical trials. TMC278 is a potent inhibitor of HIV-1 strains resistant to approved NNRTI drugs (Janssen et al., J. Med. Chem. 2005, **48**:1901-1909). Strategic flexibility (Das et al., J. Med. Chem. 2004, **47:**2550-2560) helps TMC278 and other DAPY analogs overcome the effects of drug-resistance mutations. Based on promising Phase II trial results, TMC278/rilpivirine is being entered into Phase III clinical trials.

In structural studies, the inhibitor flexibility interferes with the formation of well-diffracting crystals of the RT/TMC278 complex. Systematic protein engineering was performed to improve diffraction from crystals of the HIV-1 RT/TMC278 complex. Crystals of the engineered RT/TMC278 complexes diffracted X-rays to 1.8 Å resolution. Crystal structures of wild-type, and L100I+K103N and K103N+Y181C double mutant RTs in complexes with TMC278 were determined at 1.8, 2.1, and 2.9 Å resolution, respectively. The strategically positioned cyanovinyl group of TMC278 is important in retaining potency of the drug against drug-resistant mutants. Loss of stabilizing aromatic ring interactions by Y181C mutation is largely compensated by new interactions developed between the cyanovinyl group of TMC278 and Y183. The structure of the I100L+K103N mutant RT/TMC278 complex demonstrated that TMC278 undergoes significant conformational (wiggling) and positional (jiggling) changes when binding to mutant RT.

High-resolution structures of RT are critical for reliable description of inhibitor-protein interactions, better understanding of resistance mutations effects, and systematic structure-based drug design. The structures demonstrate the important role of strategic flexibility of TMC278 in evading effects of drug-resistance mutations. Incorporation of strategic flexibility into ligands appears to be an important consideration in designing drugs against rapidly evolving targets.

We are now pursuing systematic screening of drug-like fragments to HIV-1 RT using multiple crystal forms with different conformations and bound ligands. This information should be useful in developing novel drugs for the treatment of AIDS.

Title: Structure Bases on the Druggability of Nuclear Hormone Receptors

Presenter: **Jinsong Liu** (Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, China)

Biography

Jinsong Liu received his B.S. degree from Peking University in 1992 before he went on his graduate study in US. He then received his Ph.D. degree from Rutgers University early 2000. During his Ph.D. study, Jinsong worked on three diverse projects and determined three crystal structures, including a triple helical collagen-like peptide, a ternary complex of protein/DNA, and a RNA-binding protein. After a short period of postdoctoral research, he joined Tularik Inc, a biotech company in Bay Area. In the summer of 2004, Tularik was merged into Amgen and became Amgen San Francisco. During his stay in Tularik and Amgen for almost six years, Jinsong solved numerous protein structures, involved in many structure based drug design programs. Among the structures he solved there are several key protein kinases, nuclear hormone receptors, and other enzymes. After serving in Amgen San Francisco as senior scientist for more than one year, Jinsong decided to make the next career move. Dr. Liu returned to a newly founded institute in southern China, Guangzhou Institute of Biomedicine and Health (GIBH) at the end of 2005. Bringing his experiences from both academic and industrial settings, Jinsong has been setting up a new structural biology laboratory in Guangzhou with the ultimate goal of structure based drug design.

Abstract

Jinsong Liu, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou Science Park, Guangzhou, China, E-mail: liu_jinsong@gibh.ac.cn

Structural biology has been a great tool to assess the druggability of a potential drug target. Finding the binding pocket is the first step for the assessment of druggability, and crystal structure will provide the most detailed picture of the pocket. Nuclear hormone receptor superfamily which comprises 48 members is one of the major drug target classes. Classical ligand binding site for several nuclear hormone receptors have been very well defined. Structural studies of those proteins have elucidated the detail of the interaction between the small molecular drug and receptors. An excellent example is Peroxisome Proliferator-Activated Receptor (PPAR) which has more than 40 structures of protein/ligand complex deposited in PDB and has several drugs on the market targeting this receptor. Since the publication of structure of ligand binding domain (LBD) of Nurr-1, a nuclear hormone receptor with virtually no binding cavity, similar findings have been observed on other nuclear hormone receptors. Drug discovery program on Nurr-1 was terminated in Tularik due to the lack of binding pocket. Recently, a unique binding pocket was determined for LBD of Estrogen Related Receptor-α, which previously was shown to have no binding pocket similar to Nurr-1. This intriguing relationship of the druggability of a protein and structural characterization of binding sites will be discussed.

Title: Structural Analysis of Fluorophore-Labeled Beta-Lactamase as a Biosensor for Beta-Lactam Antibiotics

Presenter: **Yanxiang Zhao** (Hong Kong Polytechnics University)

Biography

Yanxiang Zhao is currently an assistant professor in the Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University. The research program in Dr. Zhao's lab focuses on structural studies of protein complexes involved in signal transduction and cell cycle regulation, employing a combination of x-ray crystallography, molecular biology, protein biochemistry and cell biology approaches. Dr. Zhao obtained her Ph.D. from the Rockefeller University in New York City in 2002. She received her postdoc training in Weill Medical College of Cornell University during 2002-2006. She is the author/co-author of 8 papers, including papers in leading journals as *Cell* and *Nature Struct. Mol. Biol*.

Abstract

Wai-Ting Wong, Pui-Kin So, Yun-Chung Leung, Kwok-Yin Wong, Yanxiang Zhao, Department of Applied Biology and Chemical Technology, Central Laboratory of the Institute of Molecular Technology for Drug Discovery and Synthesis, Hong Kong Polytechnic University, Hung Hom, Hong Kong, P.R. China, Email: bcyxzhao@inet.polyu.edu.hk

Beta-lactamase is an enzyme produced by bacteria that have acquired resistance to beta-lactam antibiotics (such as the popular penicillin and ampicillin). It binds to and hydrolyzes the four-member beta-lactam ring in these antibiotics, rendering them ineffective in their antibacterial function. A large number of beta-lactamases have been identified from various strains of bacteria, most of them displaying broad substrate spectrum to hydrolyze multiple antibiotics, as well as the ability for rapid evolution to recognize and deactivate new antibiotics invented.

Identification of novel compound that can serve as beta-lactamase inhibitor holds the promise to restore the therapeutic efficacy of the currently used antibiotics. To screen for such compounds, a sensitive and fast assay that can detect the binding of candidate compounds to their targeted beta-lactamases is needed. Previous work in Prof. KY Wong's lab constructed a biosensor to detect such a binding by using a fluorophore-labeled beta-lactamase from *Bacillus cereus* 569/H (PenPC). Binding of antibiotics to this biosensor causes a change of fluorescence signal emitted, which is detectable for compound as little as 50 nM.

Here we report our recent structural studies on this biosensor, using both computational modeling and experimental x-ray crystallography, to understand the mechanism of fluorescence change. Such information will aid our future work in rational design of new biosensors with improved substrate spectrum and sensitivity.

Title: Structural Basis for Therapeutic Intervention against the SARS Coronavirus

Presenter: **Zihe Rao** (Nankai University and Tsinghua University, China)

Biography

Zihe Rao, current President of Nankai University, is a renowned molecular biophysicist and structural biologist in China. A graduate of the University of Science and Technology of China (USTC), he received his Master's degree from the Graduate School of the Chinese Academy of Sciences and his doctoral degree from the University of Melbourne, Australia. Following a long period of research in the University of Oxford, he returned to China as a Professor of Structural Biology in Tsinghua University. From March 2003 to April 2007, he served as Director-general of the Institute of Biophysics, Chinese Academy of Sciences, and Director of the National Laboratory of Biomacromolecules. He was awarded the "Chen Jiageng Science Prize" for his work on the structure of mitochondrial respiratory membrane protein Complex II, and the "Trieste Science Prize" for his SARS research. Zihe Rao was elected as a Member of the Chinese Academy of Sciences in 2003 and a Member of the Third World Academy of Sciences in 2004.

Abstract

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The 2003 outbreak of severe acute respiratory syndrome (SARS), caused by a previously unknown coronavirus called SARS-CoV, had profound social and economic impacts worldwide. Since then, structure-function studies of SARS-CoV proteins have provided a wealth of information that increases our understanding of the underlying mechanisms of SARS.

No effective therapy is currently available, and considerable efforts have been made by our group towards preventing SARS-CoV infection. We have determined 14 protein and complex structures from SARS-CoV and other coronaviruses to date, with a view towards anti-viral drug discovery targeting coronavirus proteins. These include: the first structure of the SARS-CoV M^{pro} and its complex with an inhibitor (Yang et al., 2003); broad-spectrum inhibitor design targeting coronavirus M^{pro} (Yang et al., 2005); the SARS-CoV and MHV spike (S) protein fusion cores (Xu et al., 2004a; Xu et al., 2004b); the SARS-CoV nsp7-nsp8 super-complex (Zhai et al., 2005); SARS-CoV nsp10 (Su et al., 2006), a novel zinc-finger protein; and MHV nsp15, an endoribonuclease (Xu et al., 2006). The structure of another replicase protein was recently determined by our group.

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Title: Structure and Function of a Calcium Signalling Enzyme - CD38

Presenter: **Hon-Cheung Lee** (University of Hong Kong)

Biography

Lee, Hon Cheung received his Ph.D. degree in Biophysics in 1978 from the University of California in Berkeley and postdoctoral training in Stanford University. He joined the University of Minnesota in Minneapolis in 1981 and was promoted to Professor of Physiology in 1990. In 2006, he came back to Hong Kong, his home town, and has taken up the appointment of Chair Professor of Physiology at the University of Hong Kong.

His research area is cellular physiology, with particular emphasis on the mechanisms of calcium signalling. He is credited with the discovery of two calcium messenger molecules, cADPR and NAADP, for mobilizing intracellular calcium stores. His current research focuses on the structure and functions of the calcium signalling pathway mediated by these two novel messengers.

He was awarded the Distinguished McKnight University Professorship by the University of Minnesota in 1996 and was inducted into the Academy of Excellence for Health Research in 2004, the highest recognition in faculty research at that institution. He was bestowed an honorary degree in Medicine and Surgery in 1997 by the University of Genoa, Italy. He is currently an editorial board member of the Journal of Biological Chemistry.

Abstract

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CD38 is a multi-functional enzyme catalyzing the synthesis and hydrolysis of two calcium messengers, cyclic ADP-ribose (cADPR), a cyclic nucleotide derived from NAD, and nicotinic acid adenine dinucleotide phosphate (NAADP), a linear metabolite of NADP. Evidence accumulated in the past decade indicates that cADPR targets the ryanodine receptor in the endoplasmic reticulum, while separate acidic calcium stores are mobilized by NAADP. The cADPR/NAADP-signalling pathway is now known to be present and functional in a wide variety of cell types, spanning three biological kingdoms from protist, plant to animal, including human. The cellular functions it regulates are equally diverse and, most recently, the pathway has been shown to be involved in regulating neuronal oxyntocin secretion and social behaviour of mice. Despite the differences in structures and functions, both cADPR and NAADP are not only synthesized by CD38, but also degraded by it. This presentation will summarize the current findings of this novel calcium signalling pathway, focusing particularly on the recent crystallography studies of the structure and function of CD38.

Title: Synaptic Signalling Complex Organization by Scaffolding Proteins

Presenter: **Mingjie Zhang** (Hong Kong University of Science and Technology)

Biography

Mingjie Zhang is a Professor in the Department of Biochemistry at the Hong University of Science & Technology. His research interest is in the molecular basis of protein complexes regulating neuronal signal transduction and controlling cell polarity. Dr. Zhang has published extensively in the leading journals including *Science*, *Nature Structural Biology/Nature Structural & Molecular Biology, EMBO J, etc.* Dr. Zhang has been very successful in attracting basic research grants both from local (e.g., Research Grant Council of Hong Kong) and international (e.g., Human Frontier Science Program) grant agencies. In 2003, Dr. Zhang was awarded as a Croucher Senior Fellow and an Outstanding Young Scientist by the National Science Foundation of China for his excellent research achievement. In 2006, Dr. Zhang won the State Natural Science Award, which is the most prestigious award in the areas of science and technology in China.

Abstract

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Transduction of neuronal signals from receptors at the plasma membrane to their targets in cytoplasm and nucleus relies on specific protein-protein interactions. A common strategy used by cells is to organize proteins in the same signaling cascade or several related signaling pathways into large molecular weight, multi-protein complexes. Such large signaling complex organization often relies on multi-domain scaffolding proteins. These scaffold proteins can further oligomerize to form supramolecular structures. Many PDZ domain proteins are multi-domain scaffold proteins, and have been shown to play important roles in assembling various signaling complexes. In addition to binding to their respective targets, multiple PDZ domains in these scaffold proteins are not just a simple attachment of "beads on a string", but often represent functional supramodules spatially organized for coordinated binding to specific targets. We also demonstrate that L27 domains can further organize PDZ domain scaffold proteins into very large protein complexes. I will discuss the structural basis of neuronal signaling complex organization mediated by several PDZ domain scaffold proteins in my talk.

Title: Structural and Functional Insight into mRNA Decapping

Presenter: **Haiwei Song** (Institute of Molecular and Cell Biology, Singapore)

Biography

Haiwei Song obtained his PhD (1998) in Leeds University, UK. He was a Postdoctoral Research Associate in Oxford University and Institute of Cancer Research in London before he joined the former Institute of Molecular Agrobiology, Singapore as a senior scientist and principal investigator in 2001. He joined Institute of Molecular and Cell Biology, Singapore as a principal investigator in 2002. He is currently an Associate Professor.

Abstract

Haiwei Song[1], Meipei She[1], Carolyn J. Decker[2] , Dmitri I. Svergun[3] , and Roy Parker[2], [1]Institute of Molecular and Cell Biology, 61 Biopolis Drive, Proteos, Singapore 138673, [2]Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, University of Arizona, Tucson, AZ 85721, USA, [3]Hamburg Outstation, European Molecular Biology Laboratory, Hamburg, Germany, Email:haiwei@imcb.a-star.edu.sg

mRNA degradation plays an important role in post-transcriptional regulation of gene expression. Decapping is a key step in both general and nonsense-mediated 5'-3' mRNA decay pathways, and plays an important role in the AU-rich element (ARE)-mediated decay pathway and has been implicated in miRNA-mediated RNA decay. Removal of the cap structure is catalyzed by the Dcp1/Dcp2 complex, consisting of at least two subunits Dcp1p and Dcp2p which act as regulatory and catalytic subunits respectively. We have solved the crystal structures of Dcp1p, Dcp2p, and most recently the Dcp1p-Dcp2p complex. The crystal structure of Dcp1p from *S. cerevisiae* shows that Dcp1p is a small protein containing an EVH1 domain, which is generally a protein-protein interaction module. The crystal structure of Dcp2p from *S. pombe* reveals that a conserved N-terminal region of approximately 250 residues forms a bi-lobed structure with a N-terminal α-helical domain, which interacts with Dcp1p, preceding a Nudix domain, which contains the active site with a cluster of conserved glutamates characteristic of this family of pyrophosphatases. The crystal structure of a *S. pombe* Dcp1p-Dcp2p complex combined with small-angle X-ray scattering analysis (SAXS) reveals that Dcp2p exists in open and closed conformations, with the closed complex being, or closely resembling the catalytically more active form, suggesting that a conformational change between these open and closed complexes might control decapping. These structures combined with mutagenesis provide insights into how Dcp1p recognizes Dcp2p and stimulates the activity of Dcp2p.

Title: Chaperonin Action studied by Single Particle Cryo-Electron Microscopy

Presenter: **Helen Saibil** (Birkbeck College, University of London, UK)

Biography

Helen Saibil was educated in biophysics at McGill University, Montreal and then at Kings College London, where she did her PhD on the structure of retinal photoreceptor membranes. She continued to work on photoreceptors at Kings College and at the Centre d'Etudes Nucleaires, Grenoble, and then at Oxford University. After moving to her current position at Birkbeck College, she moved into the field of molecular chaperones and protein misfolding into amyloid fibrils. Her main interest is in analyzing conformational changes in macromolecular machines, using cryo-electron microscopy and image processing. Her cryo-EM analysis of the structure and movements of the *E. coli* chaperonin has elucidated the allosteric mechanism of this protein folding machine. Another major interest is in studies of the conformational changes in membrane pore formation by bacterial toxins. She is a member of EMBO and was elected as a Fellow of the Royal Society in 2006.

Abstract

DK Clare[1], N Elad[1*], NA Ranson[2], G Farr[3], AL Horwich[3], HR Saibil[1], [1] Department of Crystallography and Institute of Structural Molecular Biology, Birkbeck College, Malet St, London WC1E 7HX, UK, [2] Astbury Centre for Structural Molecular Biology and Institute of Molecular & Cellular Biology, University of Leeds, Leeds, LS2 9JT, UK, [3] Department of Genetics &Howard Hughes Medical Institute, Yale School of Medicine, New Haven, Connecticut, CT 06510, USA, *Present address: Ben Gurion University, Beersheva, Israel, Email: h.saibil@mail.cryst.bbk.ac.uk

The molecular chaperone GroEL and its co-chaperone GroES provide essential assistance for the correct folding of a subset of cellular proteins. The barrel-shaped chaperonin complexes use binding and hydrolysis of ATP to drive an allosteric cycle that alternately binds and releases non-native substrate proteins, transiently encapsulating them in an enclosed chamber for folding. Substrates are captured on hydrophobic binding sites and may be mechanically unfolded by the large domain movements of the chaperonin subunits, before being allowed to refold inside the folding chamber. We have used cryo-electron microscopy (EM) to analyse the conformational changes that take place during the chaperonin ATPase cycle and to examine complexes of chaperonins with bound, non-native substrate proteins. Cryo EM and single particle analysis provide a versatile approach to macromolecular structure determination. Isolated complexes with a distribution of orientations in vitrified solution are individually detected and aligned by image processing. Using statistical analysis, it is possible to sort out mixtures of complexes in different structural states and determine their three-dimensional (3D) density maps. Using these methods, different functional states of macromolecular machines can be resolved. By docking atomic structures of chaperonin subunit domains into cryo EM maps at 7-10 Å resolution, we can characterize pathways of allosteric transmission and determine the distribution of density for non-native substrates in the chaperonin cavity.

Title: Contribution of Charge-Charge Interactions in Thermostability of Proteins

Presenter: **Kam-Bo Wong** (Chinese University of Hong Kong)

Biography

After obtaining his B.Sc. and M.Phil. degrees in Biochemistry in the Chinese University of Hong Kong, Kam-Bo Wong moved to pursuit a Ph. D. degree in the University of Cambridge under the supervision of Prof. Alan Fersht, where he worked on the structure determination of proteins and characterization of residual structures in denatured proteins using NMR. After post-doctoral training in University of Washington and University of Cambridge, he moved back to Hong Kong in 1999 and he is now an Associate Professor in the Department of Biochemistry, CUHK. His research interest is on structure/function studies of proteins, which use various multi-disciplinary techniques like structure determination by NMR and X-ray crystallography, protein engineering, biophysical characterization, and computational methodology to study how proteins function on the atomic and molecular levels. One main area of his research focuses on how thermophilic proteins remain stable and active at elevated temperatures.

Abstract

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It has been long assumed that protein is stabilized mainly by the hydrophobic interaction among core residues, while the surface charge-charge interactions contribute little to protein stability. Here we use a ribosomal protein L30e from *Thermococcus celer* as a model to investigate the electrostatic contributions to protein thermostability. Structure of *T. celer* L30e determined by both NMR spectroscopy and X-ray crystallography reveals that the thermophilic homologue has more charge-charge interactions. Site-directed mutagenesis studies demonstrated that charge-charge interactions contribute to the thermostability of *T. celer* L30e. Comparison of thermodynamics parameters of the thermophilic L30e with those of a mesophilic homologue suggests that *T. celer* L30e achieves thermostability by having a much smaller value of heat capacity change of unfolding (ΔC_p), which differences cannot be explained by established theory that ΔC_p is correlated with change of solvent-accessible surface area. Our work has established for the first time that the reduction in the ΔC_p values commonly found in thermophilic proteins is a result of increased favorable charge-charge interactions.

This work was supported by a grant from the Research Grants Council of the Hong Kong SAR, China, CUHK4254/02M.

Title: Crystal Structures of Mini-Inteins Reveals Conserved Catalytic Modules and Dynamic Features of Protein Splicing

Presenter: **Ming-Qun Xu** (New England Biolabs, USA)

Biography

Ming-Qun Xu is currently a senior scientist at New England Biolabs, Inc. (USA). He obtained his B.S. degree in 1982 at University of Science and Technology of China and his Ph.D. in 1989 at State University of New York at Albany. During his postdoctoral research with David Shub at SUNY at Albany he discovered the first eubacterial intron (Xu et al. Science, 1990). In 1992 he demonstrated the first *in vitro* protein self-splicing experiment (Xu et al., Cell 1993) after joining NEB. His lab has been conducting structural and mechanistic studies of self-splicing inteins that led to the invention of intein-based affinity purification (IMPACT), intein-mediated protein ligation (IPL) and trans-splicing technologies for protein semisynthesis, site-specific protein labeling, and protein backbone cyclization.

Abstract

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Inteins are naturally occurring proteins that precisely break and form peptide bonds in a process termed protein splicing. We have determined the crystal structures of two mini-inteins derived from cyanobacteria *Synechocystis* sp. strain PCC6803. The X-ray structure of the 154-residue mini-intein derived from the *Synechocystis* sp. *dna*B gene reveals two catalytic modules that appear to be separately responsible for cleavage of the N- and C-terminal scissile bonds. The imidazole ring of His143 is implicated in activating the side chain Nδ atom of the last intein residue, Asn154. The penultimate intein residue, His153, along with Asp136 and a water molecule appear to constitute an oxyanion binding site that contacts the carbonyl oxygen of Asn154 to stabilize the transition state. This catalytic module is conserved in the C-terminal subdomains of inteins from diverse organisms. We have also conducted structural studies of a naturally occurring split intein, without a penultimate histidine residue, found in the *Synechocystis* sp. *dnaE* gene (Ssp DnaE intein, 159 residues). Comparison of the crystal structure of the excised intein and a second structure from an unspliced intein precursor reveals an orientation change of the aromatic ring of Tyr-1 (the last N-extein residue) following scission at the N-terminal splice junction. This switching event alleviates steric hindrance to the alignment of Arg73 and the C-terminal scissile bond and thus may account for the sequential reaction property of protein self-splicing.

Title: Redox Regulation of SUMO Proteases

Presenter: **Shannon W.N. Au** (Chinese University of Hong Kong)

Biography

Shannon Au received her PhD degree on the structure of human glucose-6-phosphate dehydrogenase at The University of Hong Kong. After completing her postdoctoral training in The University of Hong Kong, University of Oxford and Institute of Cancer Research in London, she joined The Chinese University of Hong Kong in 2003 as an Assistant Professor in the Department of Biochemistry. Her research interest focuses on molecular basis of sumoylation/desumoylation and flagellar assembly pathway of *H. pylori*. She also serves as the honorary secretary of the Hong Kong Society of Biochemistry and Molecular Biology.

Abstract

Zheng Xu, Levina Suk Mi Lam, Ho Yin Chan, Kwok Ho Lam, So Fun Chau, Lok Hei Lam, Tzi Bun Ng and Shannon Wing Ngor Au, Centre for Protein Science and Crystallography, Department of Biochemistry and Molecular Biotechnology Program, Faculty of Science, The Chinese University of Hong Kong, Hong Kong, China, Email: Shannon-au@cuhk.edu.hk

Sumoylation is an indispensable post-translational modification modulating the functions of a broad spectrum of proteins. SUMO proteases catalyzing SUMO maturation and deconjugation are crucial in maintaining a proper balance of sumoylation-desumoylation. To understand the regulatory basis of SUMO deconjugation, we investigate the effect of oxidative stress on various SUMO proteases. We have showed that H_2O_2 triggers formation of inter-molecular disulfide linkage of human SUMO protease SENP1, via the active-site Cys 603 and a unique residue Cys 613. Such reversible modification confers higher enzyme activity recovery, is also observed in yeast Ulp1, but not in human SENP2, suggesting its protective role against irreversible sulfhydryl oxidation. The modifications are further elucidated by the crystal structures of Ulp1 with catalytic cysteine oxidized to sulfenic, sulfinic and sulfonic acids. Our findings suggest that, in addition to SUMO conjugating enzymes, SUMO proteases may act as redox sensors and effectors modulating the desumoylation pathway and specific cellular response to oxidative stress.

Title: From β-catenin to PP2A: Structural and Mechanistic Studies of the Wnt Signaling Pathway

Presenter: **Wenqing Xu** (University of Washington, USA)

Biography

University of Science and Technology of China	B.S.	1985	Molecular Biophysics
Institute of Biophysics, Chinese Acad. of Science	M.S.	1989	Biochem & Crystallogr.
Massachusetts Institute of Technology,	Ph.D.	1995	Biology
Harvard Medical School	Postdoc	1999	Protein Crystallography

Positions and Employment

1985-1989	Student Researcher, Institute of Biophysics, Chinese Academy of Sciences
1989-1991	Research Assistant, Dept. of Biophysics, Johns Hopkins University School of Medicine
1991-1995	Research Assistant, Department of Biology, Massachusetts Institute of Technology
1995-1999	Postdoctoral Fellow, Children's Hospital, Harvard Medical School
1999-2004	Assistant Professor, Department of Biological Structure, University of Washington
2004-present	Associate Professor, Department of Biological Structure, University of Washington
2000	Consultant, Structural Genomix, CA
1999-2001	Consultant, AstraZeneca Pharmaceuticals, UK
2007-	Consultant, Novartis Institute of BioMedical Research, Cambridge, MA
2005-	President, Seattle Chinese Biomedical Assocaition

Abstract

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A main focus of my laboratory has been the structural and mechanistic analysis of the canonical Wnt pathway. We focused on two aspects in this pathway: (1) How does β-catenin recognize its partners, and is it possible to design compounds that specifically disrupt the β-catenin-Tcf complexes but not other β-catenin complexes? Such compounds may be useful for cancer treatment. Our crystal structures of the β-catenin/Tcf, β-catenin/ICAT, β-catenin/BCL9 complexes and related biochemical studies provided the essential structural basis and the proof of principle for designing Wnt pathway inhibitors. (2) How does APC, which accounts for >80% of colon cancers, play a critical role in β-catenin turnover? Our crystallographic and biochemical studies on the β-catenin/APC and β-catenin/Axin complexes suggested that APC is both the "recruiter" and the "rejuvenator" of the β-catenin destruction complex, and that the phosphorylation of the APC 20aa repeats could be a critical switch for APC function.

More recently, we have focused on the structural and functional analysis of protein phosphatase 2A (PP2A), a critical tumor suppressor, in the Wnt signaling pathway. PP2A is composed of the scaffolding A subunit, regulatory B subunit and the catalytic C subunit. Our crystal structure of a PP2A holoenzyme reveals that the 15 HEAT repeats of the scaffolding A subunit form a horseshoe-shaped fold, holding the catalytic C and regulatory B' subunits together on the same side. The regulatory B' subunit forms pseudo-HEAT repeats and interacts with the C subunit near the active site, thereby defining substrate specificity. The catalytic C subunit interacts directly with Axin and regulates the phosphorylation states of proteins in the β-catenin destruction complex. Together, our structural and biochemical results establish a crucial foundation for understanding PP2A assembly, substrate recruitment, and regulation in the Wnt signaling pathway.

Title: SWIRM Domain of LSD1 Interacts with N-terminal Residues of H3

Presenter: **Guang Zhu** (Hong Kong University of Science and Technology)

Biography

Guang Zhu attended Northwest University (BS), P.R. of China and obtained a PhD from University of Maryland, College Park, USA. Currently is an Associate Professor in the Department of Biochemistry, HKUST.

Guang Zhu is a biomolecular NMR spectroscopist and his current research interest focuses on applying biomolecular NMR spectroscopy and biochemical approaches to the structure-function studies of nucleic acids and proteins involved in epigenetic control. Working with histone methyl-transferase SET8, his group has determined the substrate and product specificities of this enzyme and demonstrated that the N-terminal of SET8 can binds to histone 4 N-terminal tail causing Hela cell cycle arrest at late G1 phase. His current research also involves other histone methyl-transferases and protein domains that recognize the modified histone tails, and investigates their possible roles in cancers.

He is also interested in the development of bio-NMR methods to facilitate the structure-function studies of biomolecules, such as proteins and tRNAs. In the past, has developed many applicable NMR methods, such as linear prediction methods, and multiple-quantum and TROSY based NMR experimental techniques.

Abstract

Jing Wang, Changdong Liu, Yinliang Yin, Xing Wu and Guang Zhu, Department of Biochemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China, Email: gzhu@ust.hk

The evolutionarily conserved SWIRM (Swi3p, Rsc8p and Moira) domain is a module found in many chromatin modification or remodeling proteins, such as the Ada2 and LSD1 subunits of chromatin modification complexes and the Swi3 and Rsc8 subunits of the SWI/SNF-family of the chromatin remodeling complexes. Both *in vivo* Co-Ip and *in vitro* pull down assay demonstrated that the SWIRM domain of LSD1 bound to H3-acK9. The luciferase activity assay indicated that SWIRM domain was required for LSD1 related DNA repression activity. NMR titration and studies revealed that both H3NT and H3-ack9 bind with the SWIRM domain of LSD1 (Kd≈40mM). Here we also present the high-resolution NMR structure of the SWIRM domain from LSD1 and the NMR structural model of the SWIRM and the histone H3-AcK9 N-terminal tail complex in which interacting residues were identified. Together, these data suggest that the SWIRM domain is a nucleosome targeting module that functions through binding with Histone H3 N-terminal tails and plays an important role in the LSD1 related transcriptional activity

This work is supported by the Research Grants Council of Hong Kong (HKUST6436/06M).

Title: Structural Basis of Dscam isoform Specificity

Presenter: **Jiahuai Wang** (Dana-Farber Cancer Institute, USA)

Biography

Jia-huai Wang received his BS from the University of Science and Technology of China in 1963. He joined the Beijing Biophysics Institute and was the member of Beijing Insulin Group, He was appointed as an assistant professor in Beijing Biophysics Institute in 1979. He then became associate professor there in 1986 and full professor in 1988. He went to United States in 1979 as a visiting scholar first in University of Wisconsin working on structure of tRNA, and then moved to Harvard University in 1981, joining a group, cracking the "code" of how protein recognizes DNA. He was back in Beijing late in 1982. During the period of 1982 and 1988, he also worked on structure of trichosanthin, and was the first one to identify this protein as a ribosome inactivating protein. He was awarded as an outstanding scientist by the State Council of China in 1987, and appointed as member of the National 863 Committee of Biotechnology in 1986. He went to Harvard again in 1988 as a visiting scientist. There he determined the structure of CD4, the HIV primary receptor. In 1996 he took a position at Dana-Farber Cancer Institute, working on structural immunology, solving a series of structures of immune receptors, including class I and II T cell receptors in complex with MHC molecules, their co-receptor complexes and many cell adhesion molecules. He has been an associate professor at Harvard Medical School since 2001. His major interest is on cell surface receptors and structural virology.

Abstract

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The *Dscam* gene gives rise to thousands of diverse cell surface receptors thought to provide homophilic and heterophilic recognition specificity for neuronal wiring and immune responses. Mutually exclusive splicing allows for the generation of sequence variability in three immunoglobulin (Ig) ecto-domains (D2, D3, D7). X-ray structures of the N-terminal four Ig domains (D1-D4) of two distinct Dscam isoforms have been determined. The structures reveal a horseshoe configuration, with variable residues of D2 and D3 constituting two independent surface-epitopes on either side of the receptor. Both isoforms engage in homo-dimerization coupling variable domains D2 with D2 and D3 with D3. These interactions involve symmetric, antiparallel-pairing of identical peptide segments from epitope I that are unique to each isoform. Structure-guided mutagenesis and swapping of peptide segments confirm that epitope I but not II confer homophilic binding specificity of full-length Dscam receptors. Phylogenetic analysis shows strong selection of matching peptide sequences only for epitope I. We propose that peptide-complementarity of variable residues in epitope I of Dscam is essential for homophilic binding specificity.

Title: Chelerythrine and Sanguinarine Bind at Novel Sites on BclXL and Mcl-1 that are Not the Classic "BH3 Binding Cleft"

Presenter: **Yu-Keung Mok** (National University of Singapore, Singapore)

Biography

Henry Y.K. Mok obtained his M.Phil. degree from the Chinese University of Hong Kong and Ph.D. degree from the University of Cambridge. He is now assistant professor of the Department of Biological Sciences, National University of Singapore. His general interest is on the elucidation of protein structure-function relationship using Nuclear Magnetic Resonance (NMR), particularly on proteins related to human diseases. His current projects include:

1. Determination of the binding sites of small molecule inhibitor on anti-apoptotic proteins such as Bcl_{XL} and Mcl-1.
2. Structure-based IgE epitope mapping of allergens from dust mite and cockroaches for the preparation of hypoallergen for immunotherapy.
3. NMR structure determination of chaperones and their substrates in the Type III secretion system of pathogenic bacteria.
4. Structure determination of NPM and its interaction with caspase-6, p53 and Bax in acute myeloid leukaemia.

Abstract

Yong-Hong Zhang[1], Anirban Bhunia[1], Krishnamoorthy Janarthanan[1], Kah Fei Wan[2], Mei Chin Lee[2], Shing-Leng Chan[2], Victor C.-K. Yu[2] and Yu-Keung Mok[1], [1]Department of Biological Sciences, National University of Singapore, Singapore 117543, and [2]Institute of Molecular and Cell Biology, 61 Biopolis Drive (Proteos), Singapore 138673, Email: dbsmokh@nus.edu.sg

Proteins of the Bcl-2 family are central regulators of apoptosis. High levels of expression of pro-survival members such as Bcl_{XL} were frequently found in human cancers. The pro-survival members of the Bcl-2 family mediate their effects through heterodimerization with the BH3 region of the pro-apoptotic members using a hydrophobic groove termed "BH3 binding cleft". Chemical mimetics of the BH3 region such as BH3I-1 that target the "BH3 binding cleft" indeed exhibit pro-apoptotic activities. Chelerythrine (CHE) and sanguinarine (SAN) are natural benzophenanthridine alkaloids that are structurally homologous to each other. CHE was previously identified as an inhibitor of Bcl_{XL} function from a high throughput screen, but its mode of interaction with Bcl_{XL} has not been determined.

By determining the effect of site-directed mutagenesis on ligand binding based on saturation transfer difference (STD) NMR experiments, chemical shift perturbation and BH3 peptide displacement, we have verified locations of these docked ligands. Surprisingly, CHE and SAN bind separately at the "BH groove" and BH1 region of Bcl_{XL} respectively, different from the "BH3 binding cleft" where other known inhibitors of Bcl_{XL} target. Mcl-1 (myeloid cell leukaemia-1) is another anti-apoptotic member of the Bcl-2 family that functions distinctly from Bcl_{XL}. Drugs that effectively and selectively inhibit Bcl_{XL}, e.g. ABT-737, did not bind Mcl-1 and cannot effectively induce apoptosis in cancer cells that over-express both Bcl_{XL} and Mcl-1. The binding sites of CHE, SAN and BH3I-1 on Mcl-1 are determined using various NMR approaches and their possibilities to act as "Pan-Bcl2" inhibitors will be discussed.

Title: Resonance Assignments and Solution Structure of a 28 kDa Active Mutant of Maize Ribosome-Inactivating Protein (MOD) Studied by NMR Spectroscopy

Presenter: **Kong-Hong Sze** (University of Hong Kong)

Biography

Kong Hung Sze received his BSc in Chemistry from the Chinese University of Hong Kong and his PhD in Chemical Physics from the University of British Columbia. He moved to the University of Oxford as a Postdoctoral fellow and subsequently joined the Biological NMR Center in the University of Leicester as a research associate.

In 1997, Kong Hung Sze moved back to Hong Kong and joined the Hong Kong University of Science and Technology to assist the setting up of the first Biological NMR Center in Hong Kong. He is currently a faculty member at the University of Hong Kong. His major research interest includes: structure-function studies of biomolecules, biomolecular NMR spectroscopy, and the development of computational and experimental methods for NMR studies.

Abstract

Yang Yinhua[1], Mak Amanda Nga-Sze[2], Shaw Pang-Chui[2], Sze Kong Hung[1], [1]Chemistry Department and Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China, [2]Department of Biochemistry and Centre for Protein Science and Crystallography, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR, P.R. China, Email: khsze@hku.hk

N-glycosidase ribosome-inactivating proteins (RIP) cleave the N-glycosidic bond of A4324 in the 28S ribosomal RNA. Removal of this base abolishes the binding of eukaryotic elongation factor EF1 and EF2 to the ribosome and inhibits protein synthesis. RIPs are therefore highly cytotoxic, and they can be used to design immunotoxins, anti-viral and anti-tumor agents. Maize ribosome-inactivating protein belongs to a unique class of RIP with an internal 25-amino acid inactivation fragment. It is synthesized and stored in the kernel as a 34 kDa inactive precursor (Pro-RIP). During germination, the 25-amino acid fragment is removed to generate a two-chain biologically active RIP (MOD). We have cloned MOD with an engineered short loop linking the two chains and expressed the protein in *E. coli*. Overall, 92.6% of ^{13}CO, NH, ^{15}N, C_α and $C_\alpha H$ backbone resonances were assigned. For side chain assignment, 91.4% of ^{1}H, ^{13}C and ^{15}N chemical shifts were assigned. The 3D solution structure of MOD and its comparison with the structures of other RIPs will be discussed in the present study. Figure 1 displays the full ^{1}H, ^{15}N-HSQC spectrum of MOD recorded at 310 K showing very good ^{1}H-^{15}N cross peaks dispersion.

This work was supported by grants from the Hong Kong University Research Grant (KH Sze), the Research Grants Council of Hong Kong for KH Sze (HKU 7350/04M, HKU 7533/06M) and PC Shaw (CUHK4606/06).

Title: Crystal Structures of [NiFe] Hydrogenase Maturation Proteins, HypC, HypD, and HypE

Presenter: **Kunio Miki** (Kyoto University, Japan)

Biography

Degrees:
B.Eng. (1975), Osaka University
M.Eng. (1977), Osaka University
Dr.Eng. (1981), Osaka University

Appointments:

1978-1990	Research Associate, Osaka University, Faculty of Engineering
1982-1983	Visiting Scientist, Max-Planck-Institut für Biochemie, Germany (Lab. Robert Huber)
1991-1994	Associate Professor, Tokyo Institute of Technology, Research Laboratory of Resources Utilization
1994-1995	Professor, Kyoto University, Faculty of Science, Department of Chemistry
1995-present	Professor, Kyoto University, Graduate School of Science, Department of Chemistry
1999-2002	Director, Kyoto University, Graduate School of Science, Research Center for Instrumental Analysis
1999	Visiting Professor, Tohoku University, Center for Interdisciplinary Research
1999-present	Laboratory Director (1999-2007: Chief Scientist / 2007-: Team Leader) RIKEN SPring-8 Center at Harima Institute
1999-present	Visiting Professor, Department of Chemistry, Zhejiang University, Hangzhou, China

Abstract

Kunio Miki, Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan and RIKEN SPring-8 Center at Harima Institute, Koto 1-1-1, Sayo, Hyogo 679-5148, Japan, Email: miki@kuchem.kyoto-u.ac.jp

Hyp proteins (HypABCDFE) are required for the maturation of [NiFe] hydrogenases. The whole pathway of the maturation by Hyp proteins has been recently elucidated, but each step in the maturation is not fully understood. We have determined the crystal structures of HypC, HypD, and HypE from *Thermococcus kodakaraensis* KOD1 at 1.8Å, 2.07Å, and 1.55Å resolution, respectively (S. Watanabe, R. Matsumi, T. Arai, H. Atomi, T. Imanaka, and K. Miki, ***Mol. Cell***, 27, 29-40, 2007). HypC, HypD, and HypE catalyze the insertion and cyanation of the active site iron center of [NiFe] hydrogenases. Structural features of these proteins revealed functional roles of their conserved motifs. The structure of HypD reveals its probable iron-binding and active sites for cyanation. An extended conformation of each conserved motif of HypC and HypE allows the essential cysteine residues of both proteins to interact with the active site of HypD. The C-terminal tail of HypE is shown to exist in an ATP-dependent dynamic equilibrium between outward and inward conformations. The 4Fe-4S cluster environment of HypD is shown to be quite similar to that of ferredoxin: thioredoxin reductase (FTR), indicating the existence of a redox cascade similar to the FTR system. On the basis of these structures, we propose a cyanation reaction mechanism via unique thiol redox signaling in the HypCDE complex.

Title: Enzymatic Acylation of *N*-benzoyl-L-arginine Ethyl Ester in Binary Aquo-Organic Solvent Mixtures

Presenter: **Yuen-Kit Cheng** (Hong Kong Baptist University)

Biography

Prior joining at Hong Kong Baptist University (HKBU) at 1999, Yuen-Kit Cheng finished a postdoctoral research with Dr. P. J. Rossky at University of Texas at Austin on *Hydrophobic Hydration* of biomolecules via computer simulation. Dr. Cheng obtained his B.Sc. (Chemistry) in late 80s at the Chinese University of Hong Kong and subsequently pursued a Ph.D. under the supervision of Dr. B. M. Pettitt focusing on the molecular modeling of triple DNA helices.

Dr. Cheng turned his interest to three-dimensional quantitative structure-property relationship (3D-QSPR) on the bio-interfacial properties of small biomolecules (nucleobases) by performing experiments and modeling on HPLC when he first started his career at HKBU, then to a study in the enantioseparating mechanism of polysaccharide-based chiral stationary phase using Monte-Carlo simulation. Intermittently he revived his former interest in extending the issue in protein hydration to nonaqueous enzymology using serine proteases as the model protein systems. Recently, he has participated in a joint effort --- molecular biology, ligand-receptor through TIRFM and computer simulation --- in unraveling the therapeutic secret of ginsenosides via the cellular signaling pathway(s) of steroid hormone receptors. Using QM/MM hybrid computational technique, he also tried to dissect the cAMP versus cGMP specificity in phosphodiesterases (PDEs).

For the last half year, he has been amused by the fascinating electroluminescence of small amorphous organic materials used in OLEDs and attempted some small-scale calculations in understanding the charge-carrier transport property.

Abstract

Kai-Tai Yeung and Yuen-Kit Cheng, Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China, Email: ykcheng@hkbu.edu.hk

The energetics of the rate-limiting acylation step of *N*-benzoyl-L-arginine ethyl ester catalyzed by trypsin in pure acetonitrile (ACN), 95:5% ACN:water (ACN95), 50:50% ACN:water (ACN50) and pure water (WAT) are investigated by Molecular Dynamics (MD) simulation and hybrid Quantum Mechanics/Molecular Mechanics (QM/MM) calculations. MD analysis revealed that there is water stripping phenomena in the two binary aquo-organic solvent systems (ACN95 and ACN50) with respect to the aqueous system (WAT). There are on average significantly more solvent molecules (both water and acetonitrile) in the active site of the ACN95 than the ACN50 system. The calculated potential energy barriers for the rate-limiting step at the QM/MM ONIOM(B3LYP/6-311+G(d,p):AMBER) level are 13.2, 19.0, 21.2 and 26.2 kcal/mol in the WAT, ACN95, ACN50 and ACN systems respectively, which corroborate with the order of the Michaelis constants observed experimentally (Simon et al., *Biochem. Biophys. Res. Comm*. **2001**, *208*, 1367). The major reason contributed to the difference in activities is ascribed to the electrostatic stabilization of transition state by hydration within the binding pocket and the active site. The lower activation energy predicted in the ACN95 than that in the ACN50 counterpart is likely caused by the increased electrostriction of water molecules within the active site due to an average lower dielectric constant of the solvent mixture.

Title: The Structure and Function of AaHIV, a Metalloproteinase from the Venom of *Agkistrodon acutus*

Presenter: **Maikun Teng** (University of Science and Technology of China, China)

Biography

Maikun Teng graduated from University of Science and Technology of China, In 1992, he was recruited as associate professor in University of Science and Technology of China in structural biology and promoted to professor in 1998. He is now also the Vice Dean of School of Life Sciences, and the Chairman of Biotoxin Toxicology branch, Chinese Society of Toxicology. His major research interest includes: Structural biology of toxin proteins especially on snake venom toxins; Structure and function of initial transcription factor complexes such as acetyltransferase, RNA polymerase II complex related proteins; Structure and function of some immunological related protein and complexes of NK cell.

Abstract

Zhiqiang Zhu, Yingxiang Gao, Jianye Zang, Qun Liu, Gifeng Xu, Yang Yu, Liwen Niu and Maikun Teng, Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science & Technology of China, Hefei, Anhui 230026, China, Email: mkteng@ustc.edu.cn

AaHIV is a P-III type snake venom metalloproteinase from the venom of *Agkistrodon acutus,* which contains metalloproteinase domain, disintegrin-like domain and cysteine-rich domain. It can be auto-degraded to a new protein named acucetin which includes the carboxyl terminal two domains. Excluding haemorrhagic and caseinolytic activities related with the metalloproteinase domain, AaHIV is able to completely inhibit the platelets aggregation induced by Horm collagen, and partially affect the platelets aggregation induced by ADP. At the same time we have detected Acucetin have the same effects on platelet. These functions maybe resulted from the two proteins binding some receptor on platelets. Some experiments about the interaction between the two proteins and some cells such as alpha2beta1-CHO Cells and alphaIIbbeta3-CHO Cells have been carried out, which showed that AaHIV could bind to the two type cells with different affinity and Acucetin could have some effects on alphaIIbbeta3-CHO Cells only. The crystal structures of AaHIV and Acucetin have been solved. From the structure of AaHIV we could find a glycosylated site and two other calcium ions embed in disintegrin-like domain except the two ions in metalloproteinase domain. And cysteine rich domain is made of three special secondary structure units consisting of two beta sheets and one alpha helix. Comparing the structure of the disintegrin-like domain of Acucetin with the structures of some disintegrin peptides from various snake venoms, we could suggest that these type proteins maybe bind to receptors on platelets in a different way. Of course the structure of AaHIV may imply the reason why it can auto-degrade and the way how it do! The structure combined with function researches of other homologous SVMPs provides insight into the recognition of the interaction of the blood system.

Title: Structure and Catalytic Mechanism of Human ADP-Ribose Pyrophosphatase

Speaker: **Manwu Zha**

Biography

Man-Wu Zha is an assistant professor in the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. He received his B.S. degree from NanKai University and his Ph.D. in the Shanghai Institutes for Biological Sciences. He has been working on the structural & functional studies of human ADP-ribose Pyrophosphatase and several other proteins that are of biological significance and/or related to human diseases.

Abstract

Manwu Zha[1], Qing Guo[1], Chen Zhong[1], Yingjie Peng[1] and Jianping Ding[1], [1]State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, China, Email: mwzha@sibs.ac.cn

Nudix hydrolases, a superfamily of Mg^{2+}-requiring enzymes catalyze the hydrolysis of nucleoside diphosphates linked to other moieties, X, and contain the sequence motif or Nudix box, $GX_5EX_7REUXEEXGU$. Human NUDT5 (hNUDT5) is an ADP-ribose pyrophosphatase (ADPRase) belonging to the Nudix hydrolase superfamily and catalyzes the hydrolysis of potentially toxic substrates. It plays important roles in controlling the intracellular level of ADP-ribose (ADPR) to prevent non-enzymatic ADP-ribosylation by hydrolyzing ADPR to AMP and ribose 5'-phosphate and in sanitizing the DNA and RNA precursor pool through hydrolysis of 8-oxo-7, 8-dihydroguanine-containing nucleotides. We report here the crystal structures of hNUDT5 in apo form and in complexes with ADPR, AMP and analog AMPCPR with bound Mg^{2+}. hNUDT5 forms a homodimer with substantial domain swapping and assumes a structure more similar to *Escherichia coli* ADPRases than human ADPRase NUDT9. The adenine moiety of the substrates has extensive interactions with the enzyme and particularly forms strong π-π stacking interactions with Trp28 of one subunit and Trp46 of the other, providing the molecular basis for its high selectivity for ADP-sugars over other sugar nucleotides. To elucidate the substrate recognition and the catalytic mechanism, we have prepared mutants of hNUDT5 and performed kinetic analysis of the mutants. Structural and biochemical data both suggest that hNUDT5 may utilize a hydrolysis mechanism analogous to that of *E. coli* ADPRase.

Title: Structural Insights into the Inhibition of the SARS-CoV 3C-Like Peptidase by Phthalhydrazide-charged Peptidic Compounds

Speaker: **Jiang Yin**

Biography

Jiang Yin obtained his B.Sc. in Biochemistry from Nanjing University in 1992. After graduation, he worked for several years before joining the laboratory of Dr. Eckard Wimmer as a Ph.D. student at the State University of New York at Stony Brook. In 2002, he received his Ph.D. in Molecular and Cell Biochemistry. In 2004, Dr. Yin joined Dr. Michael James' group at University of Alberta, where he furthered my pursuit of viral protein structures. His current research focuses on the catalytic mechanism of 3C and 3C-like viral proteinases and their inhibitor design. He is also actively involved in several TB structural genomics projects.

Abstract

Jiang Yin[1], Chunying Niu[1], Maia M. Cherney[1], Jianmin Zhang[2], Carly Huitema[3], Lindsay D. Eltis[3], John C. Vederas[2] and Michael N. G. James[1,4], [1]Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, AB, Canada T6G 2H7. [2]Department of Chemistry, University of Alberta, Edmonton, AB, Canada T6G 2G2. [3]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada V6T 1Z3. [4]Alberta Synchrotron Institute, University of Alberta, Edmonton, AB, T6G 2E1.

The 3C-like main peptidase $3CL^{pro}$ is a viral polyprotein processing enzyme essential for the viability of the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV). While it is generalized that $3CL^{pro}$ and the structurally related $3C^{pro}$ viral peptidases cleave their substrates via a mechanism similar to that underlying the peptide hydrolysis by chymotrypsin-like serine proteinases (CLSPs), some of the hypothesized key intermediates have not been structurally characterized. In this report, we present three crystal structures of SARS $3CL^{pro}$ in complex with each of two members of a new class of peptide-based phthalhydrazide inhibitors. Both inhibitors form an unusual thiiranium ring with the nucleophilic sulfur atom of Cys145, trapping the enzyme's catalytic residues in configurations similar to the intermediate states proposed to exist during the hydrolysis of native substrates. Most significantly, our crystallographic data are consistent with a scenario in which a water molecule, possibly via indirect coordination from the carbonyl oxygen of Thr26, has initiated nucleophilic attack on the enzyme-bound inhibitor. Our data suggest that this structure resembles that of the proposed tetrahedral intermediate during the deacylation step of normal peptidyl cleavage.

Title: Structural Basis for Protein-Protein Interactions Associated with Neurodegenerative Diseases

Presenter: **Hong-Yu Hu** (Shanghai Institutes for Biology Sciences, Chinese Academy of Sciences, China)

Biography

Hong-Yu Hu obtained his Ph.D degree from Shanghai Institute of Biochemistry, Chinese Academy of Sciences. He is now Principal Investigator and professor of Biochemistry and Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The research group focuses on the mechanism of protein misfolding and amyloidogenesis (aggregation or fibrillization), and the interactions among the related proteins. On the basis of structural information combined with biochemical characterization, they are trying to understand the pathogenesis of these neurodegenerative diseases. The research areas are:
1. Mechanism of structural transformation, amyloidogenic aggregation and molecular recognition of Parkinson disease-related alpha-synuclein, and inhibition of alpha-synuclein fibrillization;
2. Structural basis for the protein interactions and biological functions associated with neurodegenerative diseases;
3. NMR structures and domain mediated interactions of proteins involved in ubiquitin-proteasome pathway.

Abstract

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Many proteins, such as α-synuclein (α-Syn), huntingtin (Htt) and ataxins, have been identified associated with neurodegenerative diseases. Aberrant interactions of these novel proteins with other cellular proteins may play important roles in the disease pathogenesis. By use of NMR and other biochemical techniques, we have studied interactions of Htt proline-rich region (PRR) with SH3GL3-SH3 domain and HYPA-WW1-2 domain pair, α-Syn with synphilin-1 (Sph1), and ataxin-3 (AT3) UIM domain with ubiquitin (Ub). Htt PRR binds with SH3 through its entire chain of three portions, and the binding sites on SH3 domain include the specificity pocket and its opposite concave surface besides the canonical PxxP-binding site. Htt PRR can also specifically bind to WW1-2 through its N-terminal portion preferential to WW1 domain and C-terminal to WW2. The N-terminal 12 amino-acid stretch of α-Syn specifically interacts with the central portion of Sph1. The positively charged surface of the dodecapeptide derived from the N-terminus of α-Syn is likely to provide a structural basis for binding with Sph1. The tandem UIMs of AT3 binds Ub with a cooperative manner between these two UIMs, suggesting that the cooperative interaction may have significant effect on the aggregation of polyglutamine tract adjacent to the tandem UIMs.

These studies provide structural insights into the specific interactions between amyloidogenic proteins and their functional binding partners, which may have implications for understanding of the related disease pathology.