List of Abstracts

Felicia Chen-Wu Memorial Lecture

PL Sunney I. Chan
Structure/Function Studies of Membrane Proteins: Challenges and Opportunities

PLENARY LECTURES

PL01 Michael M.C. Lai
Hepatitis C Virus and Cells: Entry, Replication and Translation

PL02 Kuniaki Nagayama
Phase Contrast Cryo-electron Tomography Reveals Close-to–Life Ultrastructures in Three-Dimension

PL03 Pan-Hsien Kuo, Lyudmila G. Doudeva, Yi-Ting Wang, Che-Kun James Shen and Hanna S. Yuan
Structural Insights into TDP-43 — a New Player in Neurodegenerative Diseases

PL04 Stephen L. Mayo
Computational Protein Design: Enzymes that Work!

PL05 Zang-Hee Cho
Some Results of Ultra High Field MRI- 7.0 T-Human Brain MRI

PL06 Tai-huang Huang
Probing Molecular Interactions by NMR and X-ray Crystallography

PL07 Tzyh-Chang Hwang
Molecular Biophysics of the CFTR Chloride Channel

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Structure/Function Studies of Membrane Proteins: Challenges and Opportunities

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Structure/function studies of membrane proteins are challenging. First, the isolation and purification of a protein from the plasma membrane in which it resides is not straightforward. A membrane protein is typically present in low relative abundance. Second, the methods used to fractionate one of these proteins from their native environment for purification and reconstitution into a lipid bilayer or a detergent micelle for biochemical/biophysical studies often lead to depletion of essential cofactors that might be part of the system, disruption of the three-dimensional protein fold, and chemical and structural heterogeneity, with concomitant loss of specific activity. While one could scale up production of the protein by cloning and heterologous expression of the gene in a suitable host, the expressed protein often ends up as inclusion bodies in the cytoplasm of the host cell, and attempts to transfer the protein to a membrane-like environment are fraught with difficulties. The problems encountered are accentuated with a protein containing metallic cofactors, or a protein complex comprised of multiple subunits. Perhaps, a better strategy would be to introduce the heterologous gene, and genes that are required for the biosynthesis of membrane lipids, into a single operon that is placed under the control of a strong inducible promoter. This grouping of multiple, related genes into a common operon, is apparently the way that methanotrophic bacteria use to overproduce the particulate membrane monooxygenase (pMMO) for the facile conversion of methane to methanol. Upon strong induction of the promoter, these bacteria produce high levels of the plasma membranes that are enriched in the pMMO. In this lecture, I will describe how we have exploited this approach to obtain high quality pMMO in high yields for biochemical and biophysical studies. This highly complex enzyme is notoriously difficult to purify because of its instability outside the lipid bilayer and the tendency to lose its essential metal cofactors. For this reason, pMMO has resisted both initial identification and subsequent isolation and purification for biochemical and biophysical characterization. I will summarize the structure and function of this interesting and important enzyme (1), and highlight the progress that we have made toward developing a catalyst for the facile conversion of methane to methanol in the laboratory (2) based on the novel chemistry that has emerged from our understanding of the enzyme.

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PL01

Hepatitis C Virus and Cells: Entry, Replication and Translation

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Hepatitis C virus (HCV) contains a single-stranded RNA genome of 9.5 kilobases. It consists of a single open reading frame encoding a polyprotein of approximately 3100 amino acids, which is processed post-translationally by viral and cellular proteases into 10 structural and nonstructural proteins. HCV infects primarily hepatocytes, using CD81 and several other molecules (including tight-junction molecules, Claudin and Occludin) as receptors. However, we have isolated an HCV strain (SB strain), which is capable of infecting lymphocytes, particularly B lymphocytes. This lymphotropism is determined by the nature of viral envelope proteins. A new cellular receptor has been identified that mediates the entry of HCV into lymphoid cells. HCV RNA replication is associated with a cytoplasmic multi-layered vesicle structure, which is composed of detergent-resistant membrane. Surprisingly, translation of viral proteins also takes place in the same membrane complex as RNA replication. We termed this structure “replicasome”. This membrane structure is probably derived from the endoplasmic reticulum. This “virus factory” contains replicating viral RNA and nonstructural proteins and is the site of viral replication. The virus factory is transported from the site of virus replication to the site of virus assembly on lipid droplets by cytoskeleton and microtubules. These new concepts will be discussed.

PL02

Phase Contrast Cryo-electron Tomography Reveals Close-to–Life Ultrastructures in Three-Dimension

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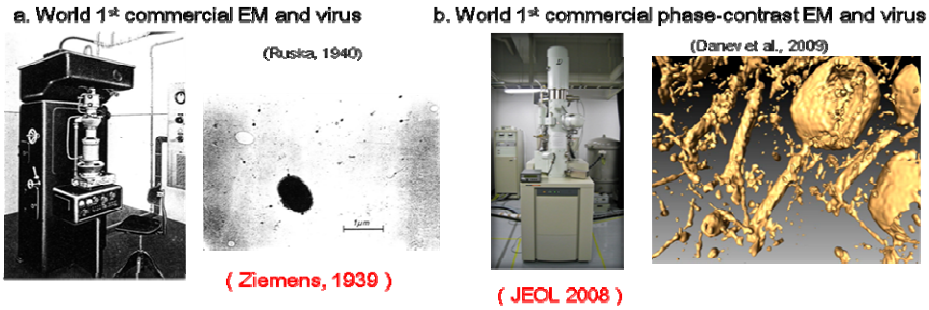
Generally speaking, quantitative analysis is not a major task in the biological microscopy where staining is profoundly employed for the enhancement of imaging contrast. Staining processes, however, as selective chemical reactions easily conceal what kinds of materials actually are in specimens. In this respect, the imaging contrast faithful to the material quantity involved is the phase contrast employed to date for the visualization of unstained specimens. Phase contrast electron microscopes (EM) recently developed using phase plates can quantify the mass density of objects through phase contrast. This novel methodology, once combined with cryo- and tomographic techniques, is able to reveal close-to-life 3D structures at a high resolution owing to its *in vivo* nature in the specimen preparation. In this report, phase contrast 3D tomograms of various biological samples such protein nano-machines, viruses, organelles, neural cells and brain tissues are to be shown.

Fig. 2D visualization of viruses in 1940s and 3D visualization of viruses in 2000s



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PL03

Structural insights into TDP-43 — a new player in neurodegenerative diseases

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Proteins constitute and carry out all kinds of intra-cellular and extra-cellular events and therefore mutations, deletions, misfolding and aggregation of protein molecules, leading to gain or loss of protein functions, are related to numerous genetic and sporadic diseases. TDP-43 is an example of a pathogenic protein: its normal function in binding to UG-rich RNA is related to cystic fibrosis, and inclusion of its C-terminal fragments in neuronal cells is directly linked to neurodegenerative diseases, including frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). To address the role of TDP-43 in RNA binding and pathogenic aggregation, we characterized its biochemical and biophysical properties and determined the crystal structure of one of the truncated TDP-43 mutants in complex with DNA. We show that TDP-43 is a dimeric protein with two RRM domains, both involved in RNA binding. The crystal structure reveals the basis of TDP-43's UG preference in RNA binding. It also reveals that the C-terminal RRM domain in TDP-43 has an atypical RRM fold with an additional β-strand involved in making protein-protein interactions. This self association of RRM2 domains produces highly thermal-stable RRM2 assemblies that are likely related to the pathogenic inclusion identified in neuronal cells. *In vivo* cell transformation assays and *in vitro* EM studies of various TDP-43 deletion mutants support a model of RRM2 assembling into fiber-like aggregates. These studies thus characterize the recognition between TDP-43 and nucleic acids and the mode of RRM2 self association, and provide molecular models for understanding the role of TDP-43 in the neurodegenerative diseases related to TDP-43 proteinopathy.

PL04

Computational Protein Design: Enzymes that Work!

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Computational protein design has "evolved" from an early focus on the development and application of methods aimed at designing sequences that are stable on target folds to the development and application of methods aimed at designing functional proteins. This talk will briefly cover some of the early history in the field, but will focus primarily on recent results related to the development and application of computational enzyme design methodology. Results, including wet-laboratory characterization, for model enzymatic systems will be highlighted.

PL05

Some Results of Ultra High Field MRI- 7.0 T-Human Brain MRI

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Recent progress in Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) for molecular imaging and its applications to Brain Research, Medicine and Biology will be reviewed and discussed. Among the interesting topical areas the ultra high field MRI (7.0 T), dedicated for the brain imaging will be highlighted. Increased field strength in MRI in the last few years has accumulated a large amount of experiences in, both imaging as well as biological effect of Ultra high magnetic field. With high field MRI, such as the 7.0T brain imager, one can now able to visualize submillimeter cortical laminae in-vivo hitherto unable to do with existing MRI systems. With molecular imaging, now, it is possible to visualize quantitatively molecular mechanisms. Another front of interests of ultrahigh field MRI is the functional brain imaging with markedly improved sensitivity suggests the possibility of imaging or finding true neuronal activation sites hitherto unable to observe and will further facilitate our understanding of BRAIN, the central focus of modern science.

PL06

Probing Molecular Interactions by NMR and X-ray crystallography

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Molecules exert their function by interacting with other molecules. Understand how molecules interact is essential for unraveling their functions and for designing drugs. However, molecular interactions are complex multidimensional processes and require temporal as well as spatial information for complete characterization. In this lecture I will use the following examples to illustrate how NMR and X-ray crystallography can be combined effectively to probe molecular interactions.

(1)The Role of Intrinsic Disorder and Coupled Allosteric Effect in Viral Nucleocapsid Packaging (1): The nucleocapsid protein (N) of the severe acute respiratory syndrome coronavirus (SARS-CoV) packages the viral genomic RNA and is crucial for viability. We showed that the N protein contains two structural domains—the N-terminal domain (NTD) and the C-terminal dimerization domain (CTD)—flanked by long stretches of disordered regions accounting for almost half of the entire sequence. We have also determined the structure of CTD by both NMR and crystallography and have showed that all disordered regions are capable of binding to RNA in a way that can be explained by the “coupled-allostery” model. These features may be conserved across different groups of *Coronaviridae* and underscore the important roles of multisite nucleic acid binding and intrinsic disorder in N protein function and RNP packaging.

(2)Structure and antibody interaction of dust mite allergen from *Blomia trapicalis (2)*: Blo t 5 is the major allergen from *Blomia tropicalis* mites and shows strong IgE reactivity with up to 90% of asthmatic and rhinitis patients’ sera. The NMR solution structure of Blo t 5 comprises three long α helices, forming a coiled-coil, triple-helical bundle with a left-handed twist. TROSY-NMR experiments were used to study Blo t 5 interaction with the Fab’ of a specific monoclonal antibody, mAb 4A7. The results presented are critical for the design of a hypoallergenic Blo t 5 for efficacious immunotherapy of allergic diseases.

(3)SUMO-Daxx interaction: Small ubiquitin-like modifier (SUMO) modification has emerged as an important posttranslational control of protein functions. Daxx, a transcriptional corepressor, was reported to repress the transcriptional potential of several transcription factors and target to PML oncogenicdomains (PODs) via SUMO-dependent interactions. A SUMO-interacting motif (SIM) within Daxx was shown to be crucial for targeting Daxx to PODs and for transrepression of several sumoylated transcription factors, including glucocorticoid receptor (GR). We have applied NMR techniques to probe Daxx-SUMO interaction. The results of which will be reported.

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PL07

Molecular Biophysics of the CFTR Chloride Channel

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CFTR (Cystic Fibrosis Transmembrane conductance Regulator), a member of the ABC Transporter Family, is a protein kinas A (PKA)-activated chloride channel, the defect of which is responsible for cystic fibrosis, the most common fatal genetic disease in Caucasians. Opening and closing (gating) of the PKA-phosphorylated CFTR are controlled by ATP binding/hydrolysis at its nucleotide binding domains (NBDs). It is believed that opening of CFTR by ATP is coupled to the formation of NBD dimer in a head-to-tail configuration with two ATP molecules sandwiched at the dimer interface (Gadsby, et al., 2006). Each ATP binding pocket (ABP1 and ABP2), composed of the Walker A and B motifs of one NBD and the signature sequence of the partner NBD, is playing different roles in modulating CFTR gating (Zhou et al., 2006; Chen and Hwang 2008). Our recent data suggest that the interactions between the gamma-phosphate of ATP and the signature sequence in ABP2 are critical in controlling channel opening. Consistent with this hypothesis, pyrophosphate, which retains the beta-gamma phosphate structure of ATP, can effectively gate CFTR despite with a lower affinity than ATP (Tsai et al., 2009). Furthermore, G551D, the third most common disease-associated mutation located at the signature sequence of NBD1, completely loses its responsiveness to ATP (Bompadre et al., 2007). However, we fortuitously found that cadmium (Cd^{2+}), a soft metal ion with distinctive affinity for cysteine, can open G551D-CFTR at micromolar concentrations in the absence of ATP. A smaller but similar effect was seen with zinc (Zn^{2+}). This effect of Cd^{2+} or Zn^{2+} is not seen with wild-type CFTR. Pretreatment of the G551D channel with thiol-specific reagents abolished the effect of Cd^{2+}, suggesting the involvement of endogenous cysteine(s) in mediating this effect of Cd^{2+} on G551D-CFTR. Supporting the idea that a multi-valent coordination is responsible for the effect of Cd^{2+}, G551C-CFTR channels, which remain responsive to ATP, can be better gated by Cd^{2+} with a higher apparent affinity for Cd^{2+} than G551D. The mutants G551C, L548C, and S549C, all in the signature sequence of CFTR's NBD1, show robust response to Cd^{2+}. On the other hand, negligible effects of Cd^{2+} were seen with T547C, Q552C, and R553C, indicating that a specific region of the canonical LSGGQ sequence is involved in transmitting the signal of Cd^{2+} binding to the gate (Wang et al., 2009). In searching for the endogenous cysteine(s) responsible for the effect of Cd^{2+}, we first converted all cysteines in NBD2 to serines under the G551D background. Surprisingly, Cd^{2+} remains an effective ligand for this construct, suggesting that NBD dimerization is not involved in Cd^{2+}–dependent gating of G551D-CFTR. After an exhausted search for the

responsible cysteine, we identify cysteine 832, a cysteine residue in the regulatory domain, as the most likely candidate for coordinating cysteine. This result not only places C832 close to the signature sequence of NBD1, but also provides the first solid piece of evidence that opening of the CFTR channel does not require NBD dimerization (Hwang and Sheppard, 2009). Since the same region involved in Cd^{2+}-mediated gate opening interacts with the gamma-phosphate of ATP in numerous crystal structures of NBD dimers, we propose that the signature sequence serves as a switch that transduces the signal of ligand (ATP or Cd^{2+}) binding to the channel gate. Since many of the disease-associated mutations cause gating dysfunction by reducing the opening rate of CFTR, our results suggest that the signature sequence may serve as a drug target for developing CFTR potentiators.

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