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PL

Reminiscing 40 years of structural biology: Bioactive small molecules, DNA and enzymes

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The study of myoglobin and hemoglobin by x-ray diffraction by Kendrew, Perutz and colleagues in the early 1950s was a truly remarkable feat, considerably way ahead of its time. At that time, the structure determination of small molecules, with fewer than one hundred non-hydrogen atoms, was already a challenge, not to mention protein molecules which have thousands of atoms. My interest in x-ray crystallography was provoked by the simplicity of the diffraction theory of Bragg equation. I learnt the theory and practice of x-ray crystallography through the examples of minerals. Interestingly, my university chemistry background allowed me to enter the structural world of bioactive small molecules such as antibiotics, natural products, bioinorganic compounds during my Ph.D. study. Fortunate to the field of structural biology, many requisite technologies, such as molecular biology (cloning), high speed computing, theory developments (phase determination, refinement, modeling, graphics) and rapid data collection (including synchrotron and cryo protection), began to emerge. I went to MIT in 1974 to learn the competitive field of nucleic acids, particularly transfer RNA structure. Later I wandered into DNA structures, and discovered the new structure of the left-handed Z-DNA, not necessary by design. Moreover my early interest in small molecule antibiotics attracted me back to study antibiotic-DNA complexes. Through the combination of NMR and x-ray crystallography several interesting antibiotic-DNA complexes were analyzed.

When I returned to Taiwan in 2000, it was clear that in order to help the biotechnology industry, important protein drug targets should be the focus. Moreover, necessary infrastructures, particularly high throughput strategy using efficient protein expression and crystallization systems, synchrotron data collection, and powerful structure analysis softwares, should be integrated and established in Taiwan. Through those efforts, during the past ten years, a number of important protein structures, covering enzymes for various diseases such as infectious diseases, cancers, inflammation, etc. have been solved.

In this lecture I will glide through my 40 years of odyssey of structural biology with examples from different periods of research career. Hopefully this may provide some inspiration to our young scientists.

PL01

NMR Studies of Retrovirus Assembly

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In cells infected by HIV-1, newly synthesized retroviral Gag polyproteins are directed to specific cellular membranes where they assemble and bud to form immature virions. Membrane binding is mediated by Gag's matrix (MA) domain, which contains an N-terminal myristyl group that can adopt sequestered and exposed conformations. Membane specificity is regulated by phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P_2), a cellular factor abundant in the inner leaflet of the plasma membrane (PM). We now have evidence (1) that phosphoinositides, including soluble analogs of PI(4,5)P_2 with truncated lipids, bind HIV-1 MA and trigger myristate exposure. The phosphoinositol moiety and one of the fatty acid tails binds to a cleft on the surface of the protein. The other fatty acid chain of PI(4,5)P_2 and the exposed myristyl group of MA bracket a conserved basic surface patch implicated in membrane binding. Thus, PI(4,5)P_2 can act as both a trigger of the myristyl switch and as a membrane anchor, suggesting a structure-based mechanism for the specific targeting HIV-1 Gag to PI(4,5)P_2-enriched membranes. The Gag protein is responsible for specifically recruiting two copies of the viral genome into assembling virions. We now have evidence that diploid genome selection is mediated by an RNA structural switch mechanism, in which dimerization-dependent changes in RNA base pairing expose conserved UCUG sequences that are targeted by Gag (2). Recent studies of the structures responsible for genome selection will be presented.

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PL02

Structural insights into mRNA decapping

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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 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.

PL03

Structure of P-glycoprotein

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P-glycoprotein (Pgp) detoxifies cells by exporting hundreds of chemically unrelated toxins but has been implicated in multidrug resistance in the treatment of cancers. Substrate promiscuity is a hallmark of Pgp activity, thus a structural description of polyspecific drug-binding is important for the rational design of anticancer drugs and MDR inhibitors. The x-ray structure of apo-Pgp at 3.8 Å reveals an internal cavity of ~6,000 $Å^3$ with a 30 Å separation of the two nucleotide binding domains (NBD). Two additional Pgp structures with cyclic peptide inhibitors demonstrate distinct drug binding sites in the internal cavity capable of stereo-selectivity that is based on hydrophobic and aromatic interactions. Apo- and drug-bound Pgp structures have portals open to the cytoplasm and the inner leaflet of the lipid bilayer for drug entry. The inward-facing conformation represents an initial stage of the transport cycle that is competent for drug binding. We will present our latest findings on P-glycoprotein and present strategy on obtaining other conformations, extending the diffraction resolution, and new co-crystal structures with inhibitors/drugs.

PL04

In vivo cell tracking

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Cell trafficking is a complex biological process that is central to immunity, cancer metastasis, and stem cell therapy. Questions such as how Immune cells traffic to the site of inflammation, how cancer cells seed distant organs, and how stem cells home to their proper niches, can only be addressed in the complex setting of the live, intact host organisms. In order to capture the dynamic unfolding of these cellular processes in real time and in three dimensions, *in* vivo imaging techniques must be developed with single cell sensitivity and resolution both spatially and temporally. While whole-body imaging modalities such as MRI and bioluminescence imaging are powerful ways to localize whole cell populations on the macroscale, optical microscopy is uniquely capable of tracking of individual cells *in vivo*, with the added advantage that multiple cell types can be tracked simultaneously by color-coding using appropriate fluorescent probes and reporters. Here I will describe a unique multichannel video rate confocal and multiphoton laser scanning microscope that is deigned specifically for cell tracking in live animals, and an *in vivo* flow cytometer that is developed for continuous, real-time monitoring of circulating cells without the need to draw blood samples. Using examples from transplantation biology, I will discuss how advanced optical technology can be applied to help uncover fundamental biological processes that have previously only been postulated but can now be directly visualized.

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(2) Lo Celso C, Fleming HE., Wu JW, Zhao CX, Miake-Lye S, Fujisaki J, Côté D, Rowe DW, Lin CP, Scadden DT. Live-animal tracking of individual hematopoietic stem cells in their niche. Nature 457:92-6 (2009).

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PL05

Design of Protein Interfaces and Switches

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Strategies will be discussed for designing protein-protein interfaces and creating genetically encoded photoactivatable proteins. In both cases, we make extensive use of the sequence and backbone optimization protocols in the molecular modeling program Rosetta. To design novel protein interactions we have developed a computational protocol for creating directed libraries that are enriched in high affinity binders for target proteins. Using this approach we have identified low nanomolar binders between proteins that do not naturally interact. To build photoactivable proteins we are making use of the naturally occurring LOV2 domain from the plant protein phototropin. When activated with blue light, the C-terminal helix (Jalpha helix) of the LOV2 domain unfolds. We are using this unfolding event to control the activity of proteins or peptides that are either embedded in the Jalpha helix or placed adjacent to it. Studies by collaborators indicate that these switches are functional in living cells and animals.

PL06

Cryo-EM of Viruses and Cells

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We use cryo-EM to determine structures of several ds-DNA viruses (epsilon15, P-SSP7, P22 phages and herpes simplex virion) with and without any symmetry imposition (Chang et al., 2006; Jiang et al., 2008; Jiang et al., 2006; Zhou et al., 2000). The resulting structures of the phages yield backbone traces of the shell proteins and spatial organization of the portal vertex protein complex. We also use cryo-EM to determine structures of bacterial cells being infected by viruses. Our combined structural observations suggest a mechanism whereby, upon binding to the host cell, the tail fibers induce a cascade of structural alterations of the portal vertex protein complex that triggers DNA release.

This research has been supported by NIH grant (P41RR00250) and the Welch Foundation (Q1242).

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PL07

The Development of Hybrid Core-Shell Nanostructures for Theranostics

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Nanomedicines are complex material of nanoscale size that can be used for in vitro diagnosis, whole body imaging and effective disease treatment. In this decade, several kinds of novel imaging modalities and hybrid nanoparticles have been developed to achieve the purpose of theranostics. However, how to apply new materials and the new techniques to study or confirm the important biological phenomenon is still an important issue. In the classical nanotechnology, lipid-based drug delivery systems have been studied extensively to increase therapeutic index of chemotherapy. We have developed several lipid nanocarriers with sizes less than 150 nm for anticancer drug delivery and imaging. To facilitate receptor targeting and improve intracellular transport of hybrid nanoparticles, Tat peptide, organelle-specific ligands and adaptor proteins were conjugated first for layer-by-layer assembly of targeted nanoparticles. Recently, these targeted nanocarriers were further enclosed monocrystalline iron oxide nanoparticles or fluorescent nanoparticles in the core for molecular imaging simultaneously. The lipid-coating iron oxide nanoparticles or fluorescent nanoparticles not only preserved their optical and MRI properties, but also displayed a strong third harmonic generation (THG) signals. By using biocompatible lipids as the outer shell, cells can be labeled by the hybrid nanoparticles efficiently without significant cytotoxicity. The labeled cells were detectable by multimodal imaging systems such as fluorescent imaging, MRI and THG. Thus, these multifunctional nanoparticles may have the potential for future tracking effectiveness of drug delivery and cell therapy in vivo.

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