Example

Structure of Cytochrome c Oxidase

Fusao Takusagawa

The inner mitochondrial membrane contains various proteins including redox proteins involved in electron transport and oxidative phosphorylation. In the electron-transport process, the free energy of electron transfer from NADH and $FADH_2$ to O_2, *via* protein-bound redox center is coupled to ATP synthesis. Cytochrome c oxidase (Complex IV) catalyzes the one-electron oxidations of four consecutive reduced cytochrome c molecules and the concomitant four-electron reduction of one O_2 molecule:

$$4\text{Cytochrome } c^{2+} + 4H^+ + O_2 \rightarrow 4\text{cytochrome } c^{3+} + 2H_2O.$$

The crystal structure of bovine heart cytochrome c oxidase reveals 13 subunits, five phosphatidyl ethanolamines, three phosphatidyl glycerols, two cholates, two hemes A, three coppers, one magnesium, and one zinc. A hydrogen-bonded system, including a propionate of a heme a, part of peptide backbone, and an imidazole ligand of Cu_A, could provide an electron transfer pathway between Cu_A and heme a. Two possible proton pathways for pumping, each spanning from the matrix to the cytosolic surfaces, were identified, including hydrogen bonds, internal cavities likely to contain water molecules, and structures that could form hydrogen bonds with small possible conformational change of amino acid chains. Possible channels for chemical protons to produce H_2O, for removing the produced water, and for O_2, respectively, were identified.

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September 13, 2016

Crystal Structure of the Calcium Pump of Sarcoplasmic Reticulum at 2.6 Å Resolution

Ian Bell

Calcium ATPase is a member of the P-type ATPases and some are responsible for transporting calcium against a concentration gradient in the skeletal muscle sarcoplasmic reticulum, also known as SERCA1a. These pumps are partially responsible for muscle movement by providing the sarcomeres with calcium, which they use indirectly to contract.

The structure of the ATPase was determined by first growing crystals and using electron microscopy. The full structure was discovered by using standard multiple isomorphous replacement methods. It was confirmed that the protein did indeed span a lipid bilayer and by looking at the distribution of water molecules the position of the membrane boundary was deduced.

The cytoplasmic region consists of three separated domains. Domain A is believed to act as an anchor for domain N, which is responsible for binding nucleotides. Domain P is responsible for phosphorylation.

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Chikashi Toyoshima, Masayoshi Nakasako, Hiromi Nomura, & Haruo Ogawa. “Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 A Ê resolution”. Nature. Vol 405. June 8, 2000. Web. September 3, 2016.

September 13, 2016

The Structure and Mechanism of Molybdenum Nitrogenases

Max Combest

Nitrogen fixation -the catalytic reduction of atmospheric N_2 gas into NH_3- is one of the most important chemical processes that occurs within the earth's biosphere. It turns otherwise unreactive nitrogen gas into a form that is available to be metabolized into proteins and other nitrogen containing biomolecules. Only a select few varieties of organisms such as Rhizobium and Cyanobacteria possess the Nitrogenase enzymes required to fix atmospheric nitrogen and as such, these organisms play crucial roles in the ecosystems they inhabit.

The Nitrogen Fixation reaction follows the scheme:

$$N_2 + 8\ e^- + 16\ ATP + 8\ H^+ \rightarrow 2\ NH_3 + 16\ ADP + 16\ P_i + H_2$$

This reaction is catalyzed by Nitrogenase, a multimeric metalloenzyme. Molybdenum dependent nitrogenases use an Iron-Molybdenum-Sulfur cofactor called the FeMo-cofactor in the active site that facilitates the N_2 reduction. The holoenzyme is composed of a central heterotetrameric (a_2b_2) component called the MoFe protein that contains two FeMo-cofactors, and two homodimeric (a_2) Fe-protein components that associate one to each end of the MoFe protein. The Fe proteins are detachable, and each contain two bound MgATP molecules. The detached Fe protein is the oxidized form, when it is reduced by an electron transfer protein, it binds to the MoFe protein, this binding triggers the hydrolysis of the two MgATPs and facilitates the transfer of an electron from the Fe protein to the MoFe protein and then to the bound N_2 in the active site. Eight such reduction events make up the reaction that fully changes N_2 into two ammonia molecules.

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September 20, 2016
Structure and Mechanism of the Lactose Permease of *Escherichia coli*

Tisha Hayes

Membrane transport proteins are an important class of integral membrane proteins. Membrane transport proteins are involved in the movement of ions across the membrane, and are classified into two subsets. One of these subsets is the major facilitator superfamily (MFS) of transporters which includes lactose permease of *E.coli*. Lactose permease has a crystal structure at 3.5 angstroms in *E. coli*. Lactose permease transports ions across the phospholipid bilayer by transducing free energy that is stored in the proton gradient. The lactose permease of E.coli is a highly studied molecule that is composed of N and C-terminal domains each with 6 transmembrane helices. Therefore the crystal structure of lactose permease in *E.coli* has a total of 12 transmembrane helices that together form a large hydrophilic cavity open to the cytoplasm showing the inward movement of the transported ions against a concentration gradient. Lactose permease requires the binding of a lactose homolog to induce structural changes necessary in moving specific ions across the membrane. Once the lactose homolog binds to the protein the sugar binding site within the water-filled cavity is accessible; as well as specific amino acid residues that work in substrate recognition and proton translocation revealed. The lactose permease membrane transport protein has a specific lactose/proton symport process, and within this article a possible mechanism for that process is proposed based on data and structure.

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Lipid-Linked Oligosaccharides in Membranes Sample Conformations That Facilitate Binding to Oligosaccharyltransferase

Suwoo Kim

Lipid-linked oligosaccharides (LLOs) studied in this publication are the substrates that bind to oligosaccharyltransferase (OST), which are the enzyme responsible for catalyzing the transfer of the oligosaccharides onto the nascent proteins during N-glycosylation.

The study focuses on exploring LLOs' preferred location, orientation, structure, and dynamics in three different types of membrane bilayer by modeling and simulating eukaryotic and bacterial LLOs. The LLOs compose of an isoprenoid moiety and an oligosaccharides that are bonded by pyrophosphate.

The results of the simulation present no strong correlation between the hydrophobic thickness and the orientation, structure, and dynamics of the LLO. In addition, the isoprenoid moiety displays high flexibility when located inside the hydrophobic core, which can suggest that it acts as a tentacle in searching for the oligosaccharltransferase. The properties of the simulation such as the oligosaccharide conformation and dynamics are in match with those in solution; however, LLO sugar orients parallel to the bilayer surface due to favorable interactions between the sugar and the bilayer head groups. Such orientation aids in binding of LLOs to OST.

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September 27, 2016

Crystal Structure of Inhibitor-Bound Human 5-Lipooxygenase-Activating Protein

Eric Lightfoot

The role of biological molecules called Leukotrienes have been explored as one the important causes of inflammation in the immune system response. The precursor molecule to Leukotrienes is known to be Arachidonic acid, and a particular enzyme involved in the oxidation of this precursor and the proinflammatory cellular response is called 5-Lipooxygenase (5-LOX). The integral membrane protein called FLAP is essential to the facilitation of binding Arachidonic acid, and sequentially transferring it to 5-LOX. This paper takes a look at the crystal structure of FLAP with two different Leukotriene biosynthesis inhibitors bound in the complex at 4.0 and 4.2 Angstrom resolutions. The crystal structures revealed the inhibitors to be bound to FLAP in the membrane embedded alpha helices, and are thereby preventing the binding pocket access to binding with Arachidonic acid. The importance of exploring the crystal structure of FLAP in regard to Leukotriene biosynthesis can help develop new lead compounds for the treatment of respiratory and cardiovascular diseases.

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Andrew D. Ferguson,1 * Brian M. McKeever,1,5* Shihua Xu,1 Douglas Wisniewski,2 Douglas K. Miller,3,6 Ting-Ting Yamin,3 Robert H. Spencer,4,7 Lin Chu,1 Feroze Ujjainwalla,1 Barry R. Cunningham,2 Jilly F. Evans,3,8 Joseph W. Becker. “Crystal Structure of Inhibitor-Bound Human 5-Lipoxygenase-Activating Protein”. Science AAS,317, 510 (July 27th,2007).

Conformational Dynamics of Specific Aβ Oligomers Govern Their Ability to Replicate and Induce Neuronal Apoptosis

Martinez, Erica

Even though oligomers of amyloid-β have been noted to be the main toxic compounds responsible for early synaptic dysfunction and neuronal cell death in Alzheimer's disease, the molecular mechanism underlying the problem remain unknown. Understanding traits of oligomers is key to unraveling the molecular mechanism involved in Alzheimer's pathogenesis. It was previously established that a 12-24mer neurotoxic oligomer of Aβ42 (LFAO's) shows a method of replication in which they replicate when in contact with monomers. This replication method somewhat resembles that of prions. The experiment aims to observe the effects of concentration on the structure LFAO's and how this plays a role in the triggering of neuronal apoptosis. The experiments demonstrated that LFAO's transition between 11mers and disperse 12-24mers with a 0.1μM dissociation constant, all of this based on the concentration gradient. The two species vary in their tertiary and quaternary structures except for their secondary structures. The changes in structure seem to be what determines their capacity to replicate and cause apoptosis in SH-SY5Y human neuroblastoma cells. It was also established that the 12mers are more neurotoxic and susceptible to 12-24mers. This means that the LFAO replication process dominates at low physiological concentrations. The results of this experiment can be used as a base for further research on the Aβ oligomer's prion like reproduction in Alzheimer's disease pathology.

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October 4, 2016

Crystal Structure of Bacterial Multidrug Efflux Transporter AcrB

Taylor Moldenhauer

Multidrug efflux pumps are used by many bacteria, including *Escherichia coli*, to pump antibacterial substances and other potentially compounds out of the cell. If these pumps are expressed enough or, more than likely overexpressed, in the cell resistance can be caused. Sequencing of *E. coli's* genome has shown the presence of genes associated with a RND type transporter now known as AcrB that allows this bacterium to be resistant against many dyes, detergents, and lipophilic antibiotics. The discovery of the crystal structure has helped others in determining just how the pump functions to export drugs and detergents through the interaction of AcrB with TolC. As well as how this pump very well may function to export compounds from the periplasmic space.

The crystal structure of the AcrB-TolC system shows a membrane fusion protein, AcrA, an outer membrane channel called TolC, and three promoters that form a homotrimer and function as a multidrug exporter, AcrB. With a resolution of 3.5 Å, the AcrB structure shows that the funnel-like top functions to insert into the TolC channel as well as three alpha-helices that connect the funnel to a central cavity. The cavity is what leads to the periplasm and may have the ability to pump out beta-lactam antibiotics that do not cross the cytoplasmic membrane and therefore must reside in the periplasmic space.

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Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside

Hailey Myers

A molecular assembly in the inner mitochondrial membrane carries out the synthesis of ATP. The ATP-ADP carrier was discovered through its catalysis of the reverse reaction, the hydrolysis of ATP. ATP is exported into the cytoplasm while ADP is imported into the cellular matrix.

The carrier has been studied for over four decades and consists of 297 residues and the transmembrane domain consists of six transmembrane alpha-helices. With respect to the sequence, the carrier was predicted to consist of six transmembrane helices. To provide an insight into the nucleotide transport mechanism, an understanding of the structure of the carrier at high resolution is necessary.Research now report that this structure, determined by X-ray crystallography, has a resolution of 2.2 A˚.

The ATP–ADP carrier is very specifically inhibited by very few compounds. One of these inhibitory compounds which includes carboxyatractyloside (CATR), binds to the ATP–ADP translocase from the cytoplasmic side, locking it in a cytoplasmic side open conformation. The negatively charged groups of the inhibitors bind strongly to the positively charged residues deep within the binding pocket. The high affinity makes each inhibitor a deadly poison by obstructing cellular respiration/energy transfer to the rest of the cell.

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October 18, 2016

Crystal Structure of the Sodium-Potassium Pump

Mallory Paxson

The sodium-potassium pump is a vital membrane-bound ion pump in all animal cells. The pump, belonging to the P-type ATPases, uses energy from ATP hydrolysis to create electrochemical gradients for sodium and potassium, which is required for: electrical excitability, cellular uptake of ions, nutrients and neurotransmitters, and regulation of cell volume and intracellular pH.

The sodium, potassium-ATPase consists of two subuints. The alpha subunit contains the sites for binding of sodium, potassium and ATP. The beta subunit is required for routing of the alpha subunit to the plasma membrane. A third subunit, gamma, is often associated with the pump and regulates pumping activity. It can be seen that rubidium and potassium bind similar sites and are the first two ions visualized in a P-type ATPase structure. The beta subunit completely covers two of the alpha subunit's extracellular loops, which may play a role in the occlusion of potassium. It was found that the sodium-potassium-ATPase structurally resembles the calcium-ATPase. Also, the differences in side chains and water molecules contribute to cation selectivity.

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Crystal Structure of Mitochondrial Respiratory Membrane Protein Complex II

Hayley Tuggle

Mitochondrial respiratory system consists of five membrane protein complexes that produce energy in the cells. This paper looks at the crystal structure Complex II or succinate: ubiquinone oxidoreductase (SQR) is an integral membrane protein complex in the Kreb's Cycle. Complex II is composed of 2 hydrophilic proteins, flavoprotein (Fp) and iron sulfur protein (Ip), and two transmembrane proteins (CypL and CybS) and prosthetic groups required for electron transfer from succinate to ubiquinone. Complex II is a key membrane complex in the Kreb's cycle that catalyzes the oxidation of succinate to fumarate in the mitochondrial matrix as succinate dehydrogenase. This complex provides one part of the respiration electron transfer chain. Electrons are transferred from succinate to ubiquinone through the buried prosthetic groups flavin-adenine dinucleotide (FAD). The clusters and heme form an integral part of the complex.

The first crystal structure of porcine has been determined to be at 2.4 Angstrom as well as its complex structure with two inhibitors, 3-nitro-propionate (NPA) and 2-thenoyltrifluoroacetone (TTFA) at 3.5 Angstrom resolution. Providing a model for understanding Complex II at an atomic level. Two proposed ubiquinone binding sites were discussed. One proximal (Qp) ubiquinone binding pocket is found to be conserved from prokaryotes to eukaryotes. Another pocket by the C terminus of the transmembrane proteins is thought to be a ubiquinone binding site (Qd). The crystal structure provides framework for investigating these binding pockets. Residues in the four subunits of Complex II, are highlighted for their implications in mitochondrial diseases.

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October 25, 2016

Efficient CO_2-Reducing Activity of NAD-Dependent Formate Dehydrogenase from *Thiobacillus* sp. KNK65MA for Formate Production from CO_2 Gas

Brandon Tomás

Global climate change has become a major concern in recent years, instigating a movement towards producing methods to limit carbon dioxide into the atmosphere. Another approach aims to remove carbon dioxide via different means. One such methods utilizes biochemistry in the form of Formate Dehydrogenase (FDH), which is an enzyme that performs the following reversible reaction:

$$CO_2 + NADH \leftrightarrow COOH^- + NAD^+$$

This has the promise of both sequestering carbon dioxide, as well as synthesizing formate, which has the potential to be used in a carbon-neutral fuel cell. This paper looks at multiple FDH from different species, analyzing the forward and backwards reaction kinetics. Additionally, the environments were tested to determine which conditions favored and affected the reaction equilibrium. Focusing on two enzymes, from *Thiobacillus* and *Candida boidinii*, the FDH's were compared structurally to determine what structural deviations led to the higher reaction rate observed in the *Thiobacillus* FDH. Sequence and structural comparisons were made to support the reasoning for a significant deviation in the activities.

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Structure and mechanism of Glycerol-3-phosphate transporter from Escherichia coli

Morningstar Wagnehu

The glycerol-3-phosphate transporter is a member of the largest secondary transporter protein family called the Major facilitator superfamily. This transporter is a 12 transmembrane protein made of alpha helices, H1-12 and has connecting loops L1-2 and L11-12. The function of GlpT is to transport G3P into the cytoplasm and inorganic phosphate into the periplasm, therefore it acts as an organic phosphate/ inorganic phosphate antiporter. This process is driven by the Pi gradient and allows the protein to mediate Pi/ Pi exchanges.

GlpT has a pseudo two-fold symmetry on the amino and carboxyl ends. The substrate binding site, comprised of two arginine's, is closed to the periplasmic space. This single binding site operates by an alternating- access movement. The substrate binds in the detergent solution, reconstructs into proteoliposomes and then mediates G3P to Pi exchange. Not to be confused with E.coli's other transporter, hexose-6-phosphatetransporter.

Reference

Huang, Yafei. *Structure and Mechanism of the Glycerol-3-Phosphate Transporter from Escherichia Coli*. Rep. American Association for the Advancement of Science, 19 Feb. 2008. Web. 14 Oct. 2016. <http://crystal.res.ku.edu/taksnotes/Biol_750/memb_papers/p9.pdf>.