regulatory proteins essential for this process. SM proteins are thought to regulate fusion by interaction with the SNARE protein Syntaxin (Sx). Different binding modes for the SM-Sx interaction have been observed: binding to the closed conformation (neuronal system [1]) binding to the N-terminus of the Sx protein (yeast Golgi-ER system [2] and GLUT4 system [3],[4]) and to the SNARE ternary complex (yeast exocytotic system [5]). The role of SM proteins in vesicle fusion has been disputed partly due to the different binding modes. Binding to the closed mode inhibits the formation of the ternary complex, negatively regulating vesicle fusion. Whereas binding via the N-terminus of Sx facilitates binding to the ternary complex and positively regulates fusion [3]. Our research focus is on Munc18c and Sx4, the SM and Sx protein respectively, involved in GLUT4 vesicle transport to the plasma membrane in response to insulin signalling. We have used biophysical techniques such as ITC and small angle scattering (SAS) to further characterize the molecular details of the Munc18c-Sx4 interaction. The findings using these techniques, specifically the possible conformational changes of Sx4 on binding to Munc18c will be discussed.

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Keywords: protein-protein interactions, biophysical methods, SAS

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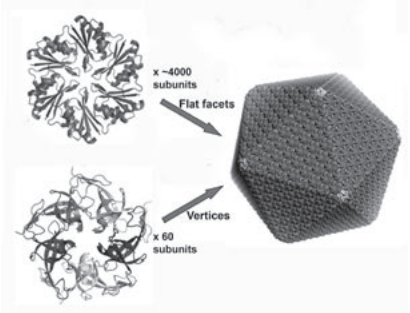
Atomic-level models of the bacterial carboxysome shell

Shiho Tanaka[1], Cheryl A Kerfeld[3], Michael R Sawaya[2], Todd O Yeates[4]

[1]University of California, Los Angeles, Dept. of Chemistry and Biochemistry, 607 Charles E. Young Dr., Los Angeles, CA, 90095, USA, [2]UCLA-DOE Institute for Genomics and Proteomics (611 Charles Young Dr., Los Angeles, CA, 90095, USA), [3]Department of Energy - Joint Genome Institute (2800 Mitchell Drive, Walnut Creek, CA, 94598, USA), [4]UCLA Dept. of Chemistry and Biochemistry (611 Charles Young Dr., Los Angeles, CA, 90095, USA), E-mail：shihot@mbi.ucla.edu

The carboxysome is a bacterial microcompartment, roughly 1000Å in diameter, that sequesters enzymes involved in carbon fixation. The shell is built from several thousand protein subunits and resembles a viral capsid. We have previously solved the crystal structures of hexameric carboxysome shell subunits, which suggest their roles in forming flat facets of the polyhedral shell (Kerfeld, *et al.*, *Science* 2005). There are three structures of homologous hexameric shell proteins solved from one class of carboxysome (beta-type) and one structure solved from another class of carboxysome (alpha-type) (Tsai, *et al.*, *PloS Biol* 2007). The comparison of these hexameric proteins shows interesting characteristics in sheet packing and residue interactions between adjacent hexamers. Furthermore, the recently determined structures of CcmL and CsoS4A subunits from the two classes of carboxysomes possess predominantly β-sheet structures and assemble as pentamers whose size and shape are compatible in forming icosahedral vertices of the protein shell. Combining these pentamers with the hexamers gives two plausible, preliminary atomic models for the carboxysome shell (Tanaka, *et al.*, *Science* 2008).



Keywords: bacterial organelles, carboxysome, CO_2 fixation

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Coordination structure of two Cl^--binding sites in oxygen-evolving photosystem II

Keisuke Kawakami[1], Yasufumi Umena[2], Nobuo Kamiya[2], Jian-Ren Shen[1]

[1]Okayama University, Division of Bioscience, Graduate School of Natural Science and Technology/Faculty of Science, Tushimanaka 3-1-1, Okayama, Okayama, 700-8530, Japan, [2]Osaka City University Sugimoto Sumiyoshi 3-3-138, Osaka, 558-8585, Japan., E-mail：dns19403@cc.okayama-u.ac.jp

Photosystem II (PSII) performs light-induced electron transfer and water-splitting reactions, leading to the formation of molecular oxygen which is essential for the survival of most of the life on the earth. The components of oxygen-evolving PSII from cyanobacteria include 17 membrane-spanning subunits, 3 hydrophilic, peripheral subunits, and over 70 cofactors such as chlorophylls, carotenoids, Mn, Ca, Fe, and plastoquinones, which give rise to a total molecular mass of 350 kDa for a monomer. The structure of PSII has been reported at 3.8-3.0 Å resolution by X-ray crystallographic analysis. The catalytic center for water splitting of PSII, namely, the oxygen-evolving complex, is located in the membrane surface and composed of 4 Mn atoms and 1 Ca atom coordinated by the protein matrix of PSII. Cl^- is one of the essential cofactors for oxygen evolution of PSII, and is closely associated with the Mn_4Ca-cluster. Its detailed location and function, however, have not been identified. In order to elucidate the coordination structure and function of Cl^- in PSII, we substituted Cl^- with Br^- or I^- in a PSII dimer from *Thermosynechococcus vulcanus*, and analyzed the crystal structure of Br^- or I^--substituted PSII. Our results showed that two Cl^- are bound to each PSII in positions surrounding each side of the Mn_4Ca-cluster at equal distances to the Mn atoms (7.0 Å) as well as to the Ca (10.0 Å). Among these two Cl^-, one is located in the entrance of a proton exit pathway, and another one is located close to the backbone of CP43-Glu354, the side chain of which is coordinated to the Mn_4Ca-cluster. These results well explain the various Cl^--effects on PSII oxygen evolution observed, and provides a basis for fully understanding the mechanism of oxygen evolution.

Keywords: photosynthesis, membrane proteins, anomalous diffraction

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Crystallization and structure determination of the phycobilisome complex

Liron David[1], Noam Adir[2]

[1]Technion, Israel Institute of technology, Chemistry, Shulich faculty of chemistry, Haifa, 32000, Israel, Haifa, Israel, 32000, Israel, [2]Technion, Israel Institute of technology, Shulich faculty of chemistry, Haifa, 32000, Israel, Haifa, Israel, 32000, Israel, E-mail：ldavid@tx.technion.ac.il

The photosynthetic process is initiated by the absorption of light energy by protein complexes called light harvesting antennas. In cyanobacteria and red algae the major antenna is called the phycobilisome (PBS). The PBS is an extremely large complex, with a molecular weight of 3-7MDa which is made up of pigmented proteins known as phycobiliproteins (PBPs) and unpigmented proteins known as linker proteins. Our goal is to obtain an atomic resolution structure of the entire PBS complex from the cyanobacterium Thermosynechococcus vulcanus using x-ray crystallography. Intact PBS was isolated in high phosphate buffer by sucrose gradient ultracentrifugation. Small blue crystals shaped like half moons were obtained in stabilization buffer in two to four weeks. Material obtained from the dissolving of extensively washed crystals was analyzed by fluorescence, SDS-PAGE and mass spectrometry (MS). The results of these experiments indicate that the crystals contain intact, functional PBS complex. Dynamic light scattering indicates a molecular weight of at least 2.8MDa. Preliminary diffraction experiments have indicated that the present crystals diffract poorly. A structure of phycocyanin (one of the PBP components) was determined as a half hexamer in the asymmetric unit. Within the phycocyanin disks, unstructured electron density could be identified in the position thought to be occupied by the linker. MS results for those crystals suggest that this is a phycocyanin rod structure contain the phycocyanin and three different linker proteins.

Keywords: light harvesting antenna, protein complex, energy transfer

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Crystal structure of the human GINS complex

Hye Seong Lim, Jung Min Choi, Yong Hyun Cho, Yunje Cho
Pohang University Of Science and Technology, Life Science, 204ho department of life science San 31 Hyojadong, Pohang, Kyungbuk, 790-784, Korea (S), E-mail：star8122@postech.ac.kr

The GINS complex mediates the assembly of the MCM2-7 (minichromosome maintenance) complex with proteins in a replisome progression complex. The eukaryotic GINS complex is composed of Sld5, Psf1, Psf2, and Psf3, which must be assembled for cell proliferation. We determined the crystal structure of the human GINS complex: GINS forms an elliptical shape with a small central channel. The structures of Sld5 and Psf2 resemble those of Psf1 and Psf3, respectively. In addition, the N-terminal and C-terminal domains of Sld5/Psf1 are permuted in Psf2/Psf3, which suggests that the four proteins have evolved from a common ancestor. Using a structure-based mutational analysis, we identified the functionally critical surface regions of the GINS complex.

Keywords: GINS, MCM, replisome

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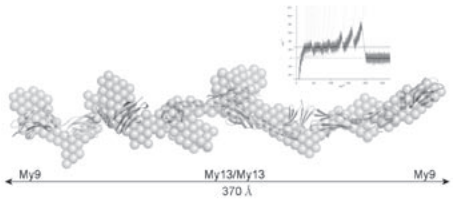
Myomesin forms a 370 Å long two-chained, antiparallel filament across the muscle M-band region

Nikos Pinotsis[1,2], Spyros D Chatziefthimiou[2], Felix Berkemeier[3], Dmitry S Svergun[2], Matthias Rief[3], Matthias Wilmanns[2]
[1]Institute of Cancer Research, Section of Structural Biology, 237 Fulham Road, London, London, SW3 6JB, UK, [2]EMBL-Hamburg c/o DESY, Hamburg, Germany., [3]Physik Department E22, Technische Universitaet Muenchen Germany., E-mail：Nikos.Pinotsis@icr.ac.uk

Sarcomeric filament proteins display extraordinary properties in terms of protein length and mechanical elasticity, requiring specific anchoring and assembly mechanisms. The M-band protein myomesin links the major sarcomeric filaments titin and myosin and is a unique filament model in terms of function and assembly. Our crystal structure of the domains My12 and My13 revealed a dimeric end-to-end filament of 143 Å. The two domains are connected by a six-turn alpha-helix, resembling a three body beads-on-the-string model with potentially elastic properties (Pinotsis et al, 2008 EMBO J.). We are currently presenting the entire C-terminal Ig domain array My9-My13 of myomesin. The crystal structures of the domains My9-My11 and My11-My13 combined with an overall low resolution model derived from small angle X-ray scattering data, reveal an unprecedented 370 Å long, two-chained filament composed of repetitive Ig-domain/alpha-helix motifs. AFM measurements confirm the elastic properties of the filament ascribed mainly to the helical linkers between the Ig-domains. Overall, our experiments provide a unique architectural insight for the tension-relaxation cycle of the muscle sarcomeres.



Keywords: muscle proteins, SAXS, AFM

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Electrostatic interaction explains D-staggered structure of collagen

Tatsuya Kawaguchi, Kouichi Masakiyo, Haizhe Jin, Kenji Okuyama
Osaka University, Macromolecular Science, Graduate School of Science, 1-1 Machikaneyama, Toyonaka, Osaka, 560-0043, Japan, E-mail：kguchi@chem.sci.osaka-u.ac.jp

Single collagen molecules with ca 300 nm length self-assemble to form collagen fibrils in the extracellular matrix, which in turn form larger fibers. The details of the self-assemble process were unclear for many years, although it was known from electron microscopic and small angle X-ray diffraction observations that the fibers show a 67 nm (1 D) repeat pattern. As now known, this is because adjacent molecules are staggered in 67 nm intervals along the fiber axis. Though there were several preceding analyses to explain D-staggered structure in terms of electrostatic and hydrophobic interactions, none of them explained satisfactorily on the basis of the molecular interaction. Based on the single crystal structures of collagen-model peptides together with the fiber diffraction data from native collagen, we confirmed that the average molecular structure of collagen is not the prevailing Rich and Crick 10/3-helical conformation but the 7/2-helical conformation (Okuyama, et al., Biopolymers, 2006). Using this conformation, we examined electrostatic interactions between two collagen molecules arranged in the parallel fashion by changing relative offset along the molecular axis systematically. The amino acid sequences of alpha chain of human Type I, II and III collagen were used to form a triple-helical conformation with 7/2-helical symmetry. Charged residues were supposed to have +1 or -1 charge on their C(beta) atoms for convenience. The local minimum of Coulomb energy for homotrimers of Type I, II and III collagens clearly showed that the electrostatic interaction is one of important driving forces to form D-staggered structure.

Keywords: collagen, D-staggered, electrostatic interaction