## KN01

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**Structural studies of macromolecular assemblies playing roles in transportation**

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Transportation of molecules is controlled in cells and tissues. Transport vesicles containing large amount of substances bud off from one compartment and fuse with another. The transport of molecules into and out of the nucleus is performed through the nuclear pore. Importins and exportins selectively transfer molecules into and out of the nuclear compartment, respectively. Membrane transport is another important transportation system. There are two types of membrane transport proteins: carrier proteins and channel proteins. All the channels allow molecules or ions to cross the membrane passively, and some carrier proteins pump certain substances across the membrane. We are working on structural studies of biological macromolecular assemblies playing in transport systems to understand transportation mechanism at an atomic level. We have determined the structures of exportin-5:RanGTP:pre-miRNA, vault and connexin-26 gap junction channel. Exportin-5: RanGTP transfers pre-miRNA through the nuclear pore. Vault may carry substances to certain regions of plasma membrane. Connexin-26 gap junction channel pierces two adjacent plasma membranes to exchange molecules between cells.

The structure of exportin-5:RanGTP:pre-miRNA complex shows that Exp-5:RanGTP recognizes the 2-nucleotide 3′overhang structure and the double-stranded stem of the pre-miRNA. Exp-5:RanGTP shields the pre-miRNA stem from degradation in a baseball mitt–like structure where it is held by broadly distributed weak interactions, whereas a tunnel-like structure of Exp-5 interacts strongly with the 2-nucleotide 3′overhang through hydrogen bonds and ionic interactions. RNA recognition by Exp-5:RanGTP does not depend on RNA sequence, implying that Exp-5:RanGTP can recognize a variety of pre-miRNAs. [1]

Vaults are among the largest cytoplasmic ribonucleo-protein particles and are found in numerous eukaryotic species. The cellular function of vault remains unclear. The x-ray structure of rat liver vault shows that the cage structure consists of a dimer of half-vaults, with each half-vault comprising 39 identical major vault protein (MVP) chains. The shoulder domain of MVP is structurally similar to a core domain of stomatin, a lipid-raft component in erythrocytes and epithelial cells.[2]

Gap junctions consist of arrays of intercellular channels between adjacent cells that permit the exchange of ions and small molecules. The structure of the human connexin-26 gap junction channel was determined. The pore is narrowed at the funnel, which is formed by the six amino-terminal helices lining the wall of the channel, which thus determines the molecular size restriction at the channel entrance. The structure of the Cx26 gap junction channel also has implications for the gating of the channel by the transjunctional voltage.[3]

[1] C. Okada, E. Yamashita, S. J. Lee, S. Shibata, J. Katahira, A. Nakagawa, Y. Yoneda and T. Tsukihara *Science* **2009,** *326*, 1275-1279. [2] H. Tanaka, K. Kato, E. Yamashita, T. Sumizawa, Y. Zhou, M. Yao, Kenji Iwasaki, M. Yoshimura and T. Tsukihara *Science* **2009,** *323*, 384-388. [3] S. Maeda, S. Nakagawa, M. Suga, E. Yamashita, A. Oshima, Y. Fujiyoshi and Tomitake Tsukihara *Nature* **2009,** *458*, 597-602

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**Time-resolved nanocrystallography with X-ray lasers**

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Our large collaborative effort [1] has recently demonstrated serial snapshot femtosecond diffraction (SFX) from membrane protein nanocrystals using the world's first hard X-ray laser, the LCLS at SLAC near Stanford, Ca [1]. Diffraction patterns from a single virus were also obtained and phased [2]. By recording patterns at 100 Hz from a stream of fully-hydrated submicron nanocrystals, it has since been found that near-atomic resolution data are obtained prior to vaporization of each nanocrystal, with little evidence of radiation damage for pulses consisting of $10^{13}$ photons in less than 40 fs. We outrun damage, since the pulse terminates before damage due to the photoelectron cascade commences. The dose is about 100 times the Henderson safe dose. This approach makes possible analysis of invisible nanocrystals from difficult-to-crystallize material, and, by using short pulses and room-temperatures (instead of freezing) to reduce damage, suggests many experiments in high-resolution snapshot time-resolved chemistry. Pump-probe experiments on Photosystem I - ferredoxin using our liquid jet particle injector [3] were attempted in June 2010. (Nanocrystals in a windowless liquid stream in vacuum are laser-excited before having their X-ray snapshot taken, in order eventually to provide a stroboscopic molecular movie). The destructive readout method and narrow bandwidth of the LCLS precludes the Laue method for time-resolved SFX; I will discuss an alternative coherent convergent-beam method, and new types of low-speed injectors with more viscous host media (e.g. lipid cubic phase).

Shape-transform effects on nanocrystal Bragg spots due to the full coherence of the LCLS, and crystal size variation, require new methods of data analysis [4], since each snapshot pattern shows only partial reflections. It may be shown that a suitable sum around Bragg spots over nanocrystal size and orientation converges to the wanted structure factors. These new intensity oscillations between Bragg reflections also offers a new iterative solution to the phase problem, which does not require atomic-resolution data, or risk biasing the PDB by returning to it structures based on it.

Z. Kam pointed out (see [6] for references) that SAX patterns recorded from particles frozen in space or time gain two-dimensional fluctuations, providing more information than conventional one-dimensional SAXS patterns. A sum of the angular correlation functions of these patterns converges to the correlation function for one particle, which may be inverted using new iterative phasing methods. We have demonstrated this experimentally for two-dimensional data [6]. Application of this approach to XFEL data will be discussed, since, unlike current single-particle XFEL experiments, the Kam method ensures 100% "hit rate". However, we find that the signal-to-noise ratio is independent of the number of particles per shot (but increases as the square root of the number of shots) [7]. Since temperature falls at about $10^{6}$ degrees per second along the liquid jet, this method is well suited to snap-shot chemistry experiments involving protein reactions with a substrate.

[1] Chapman, H., Fromme, P. et al (84 authors). Nature 470, 73 (2011). [2] Siebert, M., Hadju, J., et al (90 authors). Nature 470, 78 (2011). [3] Weierstall, U., Doak, B et al. Rev Sci Instr. http://arxiv.org/abs/1105.2104. [4] Kirian R. White, T. et al Optics Express 18, 5713 (2010). [5] Spence, J. et al Optics Express 19, 2866 (2011). [6] Saldin, D., Kirian, R. et al. Phys Rev Letts. 106, 115501 (2011). [7] Kirian, R. et al Phys Rev E Submitted. (2011).

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