

**P.18.01.1***Acta Cryst.* (2005). A61, C455**Electron Microscopic Single Particle Analysis of the Clamp Loading Complex from *Pyrococcus furiosus***

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Ring-shaped sliding clamps and clamp loader ATPases are essential factors for rapid and accurate DNA replication. The clamp ring is once opened and resealed at the primer-template junctions by ATP-fueled clamp loader function. Processivity of DNA polymerase is conferred by attachment to the clamp loaded onto DNA. In eukarya and archaea, the hetero-pentameric replication factor C (RFC) and the proliferating cell nuclear antigen (PCNA) trimer play crucial roles as the clamp loader and the sliding clamp, respectively [1]. Here we report an EM structure of an archaeal RFC-PCNA-DNA complex at 12 Å resolution. This complex exhibits excellent fitting of each atomic structure of RFC, PCNA, and a primed DNA with the convincing positions of 3' and 5' termini into the map. The PCNA ring is opened by extensive interactions with RFC, with the distorted structural view of a washer-like conformation. The RFC-PCNA contact mode is distinct from that in the yeast RFC-PCNA crystal structure [2]. Thus, the complex appears to represent a scene, where the PCNA ring is kept open before ATP hydrolysis by RFC.

[1] Waga S., Stillman B., *Annu. Rev. Biochem.*, 1998, **67**, 721. [2] Bowman G.D., O'Donnell M., Kuriyan J., *Nature*, 2004, **429**, 724-730.

**Keywords:** DNA replication, ATPase, electron microscopy

**P.18.01.2***Acta Cryst.* (2005). A61, C455**Correlation between Function and Oligomeric State of Human RECQ1 Helicase Revealed by Biochemical and Cryo-EM Analysis**

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DNA helicases are DNA unwinding enzymes that play an important role in cellular events such as replication, recombination, repair [1]. Human RecQ helicases are involved in the maintenance of chromosome stability, although their exact function is under investigation. Five members of the RecQ family are present in humans: RECQ1, BLM, WRN, RECQ4 and RECQ5. Mutations in BLM, WRN, and RECQ4 cause syndromes characterized by genomic instability, and cancer predisposition [2]. Our work focus on RECQ1 the first helicase of the family to be discovered in human cells, but also one of the less characterized in terms of its functional properties.

RECQ1 is a 3' to 5' helicase capable of unwinding short dsDNA substrates but also to promote strand annealing of complementary ssDNA molecules. These processes are modulated by nucleotide binding which alters RECQ1 protein conformation. We demonstrated that nucleotide binding inhibits the strand annealing activity of RECQ1 probably by inducing a change in the oligomeric state of the protein. The 3D structure of RECQ1 has been determined from a heterogeneous image population using cryo-electron microscopy. Our results indicated that RECQ1 forms hexameric rings in the presence of ATP $\gamma$ S and Mg<sup>2+</sup>, while it remains as a dimer in the presence of Mg<sup>2+</sup>. Moreover, EM data collected in the presence of DNA showed that its addition narrows the central channel of the hexameric ring. These results provide the first information on the structure of the RECQ1 helicase in the presence and absence of DNA opening a wide range of hypothesis concerning the role of RECQ1 in DNA metabolism.

[1] Hickson I.D., *Nat Rev Cancer*, 2003, **3**, 169. [2] Opresko P.L., Cheng W.H., Bohr V.A., *J Biol Chem.*, 2004, **279**, 18099.

**Keywords:** DNA repair and recombination enzymes, cryo-EM, oligomeric state

**P.18.02.1***Acta Cryst.* (2005). A61, C455**Electron Cryomicroscopy of Epsilon 15 Phage**

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We have used a JEM3000SFF liquid helium electron microscope to record images of Epsilon 15 phage which infects *Salmonella anatum*. Applying the icosahedral reconstruction, the fold of the capsid protein is derived from the subnanometer resolution density map and found to be homologous to the capsid proteins of HK97 and P22 phage. Using a novel reconstruction algorithm without imposing any symmetry, we are able to determine the complete structure of this phage, including the non-icosahedral components such as the viral genome, the portal complex and the phage tail. The density map is validated by the visualization of the icosahedrally arranged capsid proteins simultaneously with the non-icosahedral components. The viral genome is packed concentrically with a spacing ~25 Å as predicted by the x-ray solution scattering of P22 phage. The portal complex which is the entry point of the viral DNA into the procapsid has 12-fold symmetry spanning across one of the icosahedral 5-fold vertices. The phage fiber has a 6-fold symmetry extending from the portal complex outside the capsid particle. This structure shows for the first time the native structure of an entire virion without any bias in the reconstruction procedure.

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**Keywords:** 3D structure, cryoEM, phage particle

**P.18.03.1***Acta Cryst.* (2005). A61, C455**Using Transmission Electron Microscopy (TEM) to Complement Powder Diffraction Data**

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In recent years, a number of different approaches to structure solution from powder diffraction data have been developed, and they have been applied successfully to increasingly complex problems. Nonetheless, the structures of some polycrystalline materials remain intractable. In attempt to address such problems, we are currently investigating how electron microscopy techniques can be used to complement those of powder diffraction.

Not only can selected area electron diffraction (SAED) be used to facilitate the indexing of complex powder diffraction patterns, but the electron diffraction intensities themselves can sometimes be used to obtain a more correct partitioning of the intensities of reflections that overlap in the powder diffraction pattern. However, the effects of dynamical scattering can distort the intensities to the extent that this is no longer a reliable approach. In this case, a reconstructed potential distribution map from a high-resolution electron microscopy (HREM) image can be used to obtain the phases of the stronger reflections in that projection, and these, in turn, can be used to generate a structure envelope that defines where atoms are likely to be located within the unit cell. This envelope can then be used in conjunction with any direct-space structure determination algorithm. A test of this approach with the complex zeolite ZSM-5 showed that FOCUS (a zeolite-specific structure determination program) runs approximately five times faster with an envelope (generated from a single HREM image) than without it. Thus, even more complex structures become accessible.

**Keywords:** electron crystallography, powder diffraction, zeolite