MS9-01 **Packaging of the membrane-containing thermophilic virus STIV2.** Sarah J. Butcher,[a] Lotta J. Happonen, [ab] Esko Oksanen,[c] Lassi Liljeroos,[ab] Adrian Goldman,[a] Tommi Kajander [a] [a]*Institute of Biotechnology, University of Helsinki, Finland,* [b]*Department of Biosciences, University of Helsinki, Finland,* [c]*European Spallation Source ESS AB, Sweden*
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Icosahedral viruses package their genome into preformed procapsids with the help of a genome translocating NTPase. Such NTPases have been characterized in detail from both RNA and tailed DNA viruses. We have studied the structure of the non-tailed, membrane-containing, DNA, hyperthermo-acidophilic, archaeal virus Sulfolobus turreted icosahedral virus 2 by three-dimensional electron microscopy [1] and now present the crystal structure and activity of its NTPase, B204. The protein binds both single stranded and double stranded nucleic acids, and has an optimum activity at pH 4.5, 80°C. The overall fold of B204 places it in the Ftsk-HerA superfamily and sheds light on the evolution of viruses from the PRD1-lineage of viruses.

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MS9-02 **Fatty acid synthase: insights in the substrate shuttling mechanism by cryo-EM.** Janet Vonck,[a] Preeti Gipson,[a] Luciano Ciccarelli,[a] Deryck J. Mills,[a] Sean Connell,[b] Remco Wouts,[a] Martin Grininger,[b] Werner Kühlbrandt[a] [a]*Max Planck Institute of Biophysics, Frankfurt, Germany,* [b]*Goethe University, Frankfurt, Germany*
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Fatty acid synthase (FAS) in yeast and fungi is a 2.6 MDa barrel-shaped multienzyme complex of composition $\alpha_6\beta_6$ with D3 symmetry, which carries out cyclic synthesis of fatty acids from actetyl- and malonyl-CoA. The six α-subunits form an equatorial wheel, which divides the barrel into two separate domes, each consisting of three β-subunits. Each dome contains eight catalytic sites. The α-subunit contributes the phosphopantetheinyl transferase (PPT), acyl carrier protein (ACP), ketoacyl synthase (KS), ketoacyl reductase (KR), and part of the malonyl-palmitoyl transferase (MPT) domain. The β-subunit contributes the acetyl-transferase (AT), enoyl reductase (ER), dehydratase (DH), and the major part of the MPT domain. The ACP is tethered by two flexible linkers and shuttles the growing acyl chain between catalytic sites. By electron cryo-microscopy of single particles we obtained a 3D map of yeast FAS at 5.9 Å resolution [1]. Compared to the crystal structures of fungal FAS [2-4], the EM map reveals major differences and new features that indicate a considerably different arrangement of the complex in solution compared to the crystal structures. There is also a high degree of variance inside the barrel. Distinct density regions next to each of the catalytic domains fitted the ACP domain. In each case, the distance from the ACP substrate binding site to the active site of the catalytic domains was ~18 Å as expected. The multiple, partially occupied positions of the ACP within the reaction chamber provide direct structural insight into the substrate-shuttling mechanism of fatty acid synthesis in this large cellular machine.

Although most prokaryotes synthesize fatty acids via a set of individual enzymes, mycobacteria contain a multienzyme complex homologous to fungal FAS, composed of six identical chains assembled into a 2-MDa complex. A first cryo-EM reconstruction of mycobacterial FAS at ~20 Å resolution revealed a barrel-shaped structure with indications of high structural variability in the domes. In order to investigate the flexibility we sorted the dataset by Principal Component Analysis using SPARX [5] and determined three main conformations. In two of the EM maps, one of the six chains of the multi-enzyme complex is not in contact with the adjacent chain, suggesting an accordion-like movement of the barrel. Further refinement and fitting of the structure based on yeast FAS is in progress.

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