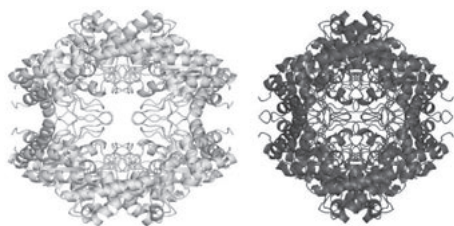


After the transition, the crystal totally recovers its crystalline state and diffraction power. The symmetry is reduced from space-group  $I222$  to its subgroup  $P2_12_12$  but the effects of this symmetry breaking on the structure are subtle.

The decrease of the unit-cell volume by more than 15 percents produces more intense and interesting structural rearrangements in the crystal (see figure).



Keywords: phase transitions, enzyme structure, polymorphism

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#### A comparison of hydrated protein models obtained by crystallography, SAXS and other techniques

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Crystallographic or NMR techniques provide information on the precise 3D structure of proteins and reveal the position of some water molecules preferentially bound to certain amino acids. Knowledge of the hydration sites is required for understanding behavior and interactions of hydrated proteins in context with flexibility, dynamics and functionality. A critical comparison of anhydrous and hydrated protein models obtained by crystallography with models from quite different experimental techniques and calculation approaches allows comparing the quality of the models under analysis. Both models and molecular parameters were analyzed: (i) Conventional and ab initio modeling approaches signify satisfactory agreement between crystal- and SAXS-based protein models, provided hydration contributions are taken into account. (ii) Recourse to crystallographic data also allows hydrodynamic modeling; in the case of multibead assemblages efficient bead reductions have to be adopted. (iii) The creation of hydrated models from cryo-electron microscopy data necessitates qualified assumptions regarding hydration. (iv) Combining surface calculation programs and our recent hydration algorithms allows the prediction of individual water molecules; a critical comparison of the water sites on the surface or buried in crevices and channels proves far-reaching identity of crystallographic data and predictions. The good agreement of the results found for hydrated models offers the possibility to complement different techniques and to predict details such as the localization of potential water sites (even in those cases where no crystallographic waters have been identified). Examples presented include proteins ranging from simple proteins to complex, multisubunit, liganded proteins.

Keywords: protein water analysis, small-angle X-ray scattering, modelling

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#### Flagellar and SOS structural genomics of *Xanthomonas campestris*

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Structural genomics is crucial for understanding the intricate interactions among proteins in a whole organism. We have studied the structural genomics of *Xanthomonas campestris* (Xcc), a gram-negative bacterium that is phytopathogenic to cruciferous plants and causes worldwide agricultural loss. Currently we are working on its flagellar and SOS structural genomics. In the flagellar system, we have solved the first crystal structure of a hook-capping protein FlgD. The core structure reveals a novel hybrid comprising a tudor-like domain interdigitated with a fibronectin type III domain. In the crystal, the monomers form an annular pentamer of dimers of pseudo five-fold symmetry. The resulting asymmetrical star-like decamer complex has a outer dimensions of approximately 110 Å × 90 Å × 65 Å, and a shortest diameter of approximately 20 Å in the center. The outer dimensions of the atomic Xcc hook-capping FlgD complex turn out to be very similar to those of the *Salmonella* filament cap complex observed by electron microscopy. SOS has been the most intensively studied system induced under DNA damage, and is characterized by the induction of more than 20 genes, which are under the control of LexA. In response to DNA damage, RecA is activated to induce the auto-cleavage of LexA, resulting in de-repression of genes in the SOS regulon. The recX gene is co-transcribed with recA and its product is suggested to regulate RecA function by directly interacting with RecA protein. We have solved the first RecX structure to a resolution of 1.6 Å. It comprise three tandem repeats R1, R2 and R3 of three-helix bundles. Model studies indicate RecX can fit into the helical groove of the RecA filament very well, similar to that reported for the cryoEM image of the RecA/RecX/ATP/ssDNA complex.

Keywords: RecA, RecX, three-helix bundle

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#### The structural basis of calcium transport by the calcium pump

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The sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase 1a (SERCA) actively pumps calcium across the SR membrane through formation and break-down of a phosphoenzyme intermediate. The pumping of Ca<sup>2+</sup> from the muscle cell cytoplasm induces muscle relaxation and the SERCA pump consumes about 25% of the ATP hydrolyzed during muscle activity. The protein has 10 membrane spanning helices, with a cytoplasmic head consisting of three domains (A-actuator, P-phosphorylation and N-nucleotide binding). The vectorial translocation of two Ca<sup>2+</sup> ions is secured by stringent movements of the cytoplasmic domains coupled to movements of

the transmembrane segments harboring the two  $\text{Ca}^{2+}$  transport sites. Previous work has identified the mechanism for phosphorylation of the enzyme coupled to occlusion of  $\text{Ca}^{2+}$  ions in the membrane, and also the dephosphorylation step coupled to counter-cation occlusion has been described. The missing link of the reaction cycle has been the E2P 'ground state' where the  $\text{Ca}^{2+}$  ions after translocation across the membrane are exchanged for protons via a 'long sought' luminal opening. Here we present functional studies and a new crystal structure of  $\text{Ca}^{2+}$ -ATPase in complex with beryllium fluoride representing the phosphorylated E2P form with full exposure of the luminal exit pathway. The exit path has developed as a trilobed opening between the transmembrane segments M1 through M6 which are found in all P-type pumps and it exposes the  $\text{Ca}^{2+}$  transport sites to the luminal space. The conformational change is actuated by a rotation of the A-domain bringing the dephosphorylation TGES motif in close apposition to phosphorylation site in recognition of phosphorylation. The release of  $\text{Ca}^{2+}$  ion is promoted by movement of the M4 helix, exposing the  $\text{Ca}^{2+}$  coordinating directly towards the lumen

Keywords:  $\text{Ca}^{2+}$ -ATPase, luminal opening, proton/ion exchange

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### Recognition and catalysis of ribosomal protein L11 by the protein trimethyltransferase PrmA

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Bacterial ribosomal protein L11 is post-translationally trimethylated at multiple residues by a dedicated methyltransferase, PrmA. Here, we describe multiple apoenzyme structures and enzyme-substrate complexes of PrmA from the extreme thermophile *T. thermophilus*. We have determined two apo-PrmA structures at 1.59 and 2.3 Å and two structures with bound cofactor AdoMet at 1.75 and 2.9 Å resolution. A PrmA-L11 enzyme-substrate complex structure at 2.4 Å resolution illustrates the highly specific interaction of the PrmA N-terminal domain with its substrate and places the L11  $\epsilon$ -amino group of Lys39 in the active site in a pre-catalytic state. Two other complex structures at 2.56 and 1.75 Å resolution show the di- and trimethylated  $\alpha$ -amino group of Met1 and AdoHcy in the intermediate and post-catalytic states. In addition, we have determined the structures of two active site mutants H104A and T106Y complexed with L11 at 2.1 and 2.35 Å resolution. A structure of the truncated PrmA substrate recognition domain complexed with the L11 N-terminal domain at 1.37 Å suggests that a single recognition mode is sufficient to place all substrate side chains in the active site. All structures of PrmA exhibit distinct relative positions of the substrate recognition and catalytic domains, revealing how the remarkable plasticity of PrmA can position the L11 substrate for multiple, consecutive side-chain methylation reactions. The presence of a unique flexible loop in the cofactor-binding site suggests how exchange of AdoMet with the reaction product AdoHcy can occur without necessitating the dissociation of PrmA from L11. Finally, the mode of interaction of PrmA with L11 explains its preference for L11 as substrate before its assembly into the 50S ribosomal subunit.

Keywords: post-translational modification, methylation, multi-specificity

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### The structural analysis of BUB1 and BUBR1 reveals their role in the mitotic checkpoint

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Premature separation of sister chromatids results in the loss or gain of chromosomes in daughter cells. This condition is a prevalent form of genetic instability observed in various classes of human cancer. BUB1 and BUBR1 are central components of the mitotic checkpoint control, the regulatory mechanism of the cell cycle that ensures the even distribution of chromosomes during the transition from metaphase to anaphase and prevents dividing cells from such disastrous scenario. BUB1 and BUBR1 are multidomain proteins consisting of a conserved N-terminal region that is essential for efficient mitotic checkpoint function, an intermediate disorder region that contains the binding site of the upstream regulator BUB3, and a catalytic C-terminal domain of serine/threonine kinase activity. We present the high-resolution crystal structures of the N-terminal region of BUB1 and BUBR1. The two domains are organised as a divergent tandem arrangement of alpha-helices and show conserved residues that are important in determining the relative orientation between neighbour alpha-helices and, consequently, of the overall shape of the protein. The projection of the degree of sequence conservation onto the surface of BUB1 and BUBR1 reveals characteristic features, including an alternating pattern of loop conservation throughout most of the sequence. We mapped onto the structure the various mutations that have been identified and associated with aneuploidy and cancer progression and provide a structure-based explanation of how these mutants lead to the impairment of mitotic checkpoint activity. The crystal structures contribute to the understanding of how genetic alterations of this domain impair the functions of these proteins, resulting in a defective mitotic checkpoint response.

Keywords: BUB1/BUBR1, mitotic checkpoint, cancer

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### Structure of a Swi2/Snf2 protein (RapA) and mechanism of RNAP recycling during transcription

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Members of the Swi2/Snf2 family mediate mobilization of various nucleic acid-protein complexes to facilitate gene expression. Bacterial RapA is an RNA polymerase (RNAP)-associated Swi2/Snf2 enzyme with ATPase activity. It stimulates RNAP recycling during transcription. Here, we report the first structure of RapA, which is also the first full-length structure for the entire Swi2/Snf2 family. Our structure and in vitro competition experiment establish that RapA and  $\sigma 70$  share a common binding site on RNAP and that  $\sigma 70$  exhibits higher affinity for the core RNAP (CORE). The structure also indicates that RapA may facilitate the release of sequestered RNAP from DNA template, which is demonstrated by our kinetic template-switching assay, showing the recycling of RNAP in vitro