The concept of the cell as a collection of multi-subunit protein complexes are emerging as a cornerstone of modern biology. Transcription by RNA polymerase II (Pol II) is a prime example for this concept as it is regulated by large protein assemblies comprising many subunits, including Mediator. Structure determination of these multi-protein complexes is essential to understand gene regulation mechanism.

We have solved crystal structure of the Mediator Head module (7 subunits, 223 kDa) at 4.3 Å resolution [1]. Our Mediator Head structure reveals the striking complex assembly mechanism: the multi-helical bundle with five different Mediator subunits is formed as a single structure unit, thereby ensuring stable assembly of the Head subunits, as well as providing the binding sites for general transcription factors (GTFs) and Pol II. Such interactions could not have been determined from structures of individual subunits alone, or from analyzing pairwise small domain-domain interactions, but only by study of the multi-protein complex in its entirety.

[1] T. Imasaki, G. Calero, G. Cai, K.L. Tsai, K. Yamada, F. Cardelli, H. Erdjument-Bromage, P. Tempst, I. Berger, GL. Kornberg, FJ. Asturias, RD. Kornberg, Y. Takagi, *Nature* **2011**, *in press*.

Keywords: transcription, macromolecule, complex

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Substrate fingerprint and the structure of NADP+ dependent serine dehydrogenase from *Saccharomyces cerevisiae* complexed with NADP+

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NADP+-dependent serine dehydrogenase [EC 1.1.1.276] from *Saccharomyces cerevisiae* (YMR226C) was determined to a resolution of 2.36 Angstroms. The protein is the first structure solved of the NADP+ serine 3-dehydrogenase group with the conformation of all three substrate binding loops fully resolved.

This protein contains a 5 substrate-fingerprint of AG-YTG, which is one of the five most observed substrate-fingerprints in the TGYK-SCOR family comprising over 150 members from different species of bacteria and lower eukaryotes. The binding of the cofactor and a bond between the substrate fingerprint residues Y162 and R209 stabilizes the 3rd substrate binding loop forming the binding pocket. Although all residues in the predicted 5-substrate binding fingerprint may not directly contact the substrate, the structure revealed their importance to forming the secondary shell to the binding pocket and verifying the predicted residues in clustering and characterizing members in this subfamily.

Keywords: NADP+ dependent serine dehydrogenase, substrate fingerprint, short chain oxidoreductase

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Transcriptional activator DmpR - combining BioCrystallography and BioInformatics

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Our goal is to understand the molecular mechanisms by which bacteria sense and respond to environmental pollutants. The *Pseudomonas putida* DmpR protein is an aromatic compound sensor and a σ^{54}-dependent transcriptional regulator that belongs to the AAA^+ superfamily of ATPases. DmpR controls a multitude of physiological processes in response to environmental signals. Its ATPase activity is essential for the activation of the isomerisation incompetent σ^{54}-RNA polymerase. The evolutionary highly conserved catalytic C-domain of DmpR harbours the ATPase activity that defines the family and interacts with the transcriptional apparatus.

Using X-ray crystallography, small angle X-ray scattering, SAXS, and bioinformatics methods we elucidate the structure of the inactive dimer and the active hexameric form. In addition, by designing single point mutations and deletion mutations in the active site environment of the C-domain of DmpR we explore the contribution of certain amino acids to ATP binding and ATPase activity.

Keywords: AAA+ ATPase, DNA biding, signalling

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Crystal Structure of Archaeal Cambialistic Superoxide Dismutase

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Superoxide dismutases (SODs) play a protective role against oxidative stress by catalyzing disproportionation of the superoxide anion radical to hydrogen peroxide and dioxygen. The SOD-catalyzed reaction proceeds through a redox cycle of metal ions. SODs are grouped into four classes according to their metal cofactors: Cu,Zn-SOD, Fe-SOD, Mn-SOD, and Ni-SOD. Fe- and Mn-SODs are closely related in amino acid sequences. Several SODs, which are termed cambialistic SODs, are active in the presence of either Fe or Mn. A hyperthermophilic archaeon *Aeropyrum pernix* K1 produces a cambialistic SOD (ApeSOD), with more activity in the presence of Mn than Fe. Here we present the crystal structures of ApeSOD in the apo, Mn-bound, and Fe-bound forms determined at the resolutions of 1.56, 1.35, and 1.48 Å, respectively [1].

The overall structure consisted of a homotetramer both in crystal and solution. The tetrameric assembly of ApeSOD contained significantly more intersubunit ion pairs than SOD from a thermophilic bacterium *Thermus thermophilus* (TthSOD); 24 and 4 intersubunit ion pairs were found from ApeSOD and TthSOD, respectively. In accordance with this, ApeSOD was more stable than TthSOD under organic conditions, although these enzymes showed similar thermostability in aqueous conditions. Next we focused on differences in active site structures of ApeSOD depending on binding of metal cofactors. While Mn was in trigonal bipyramidal coordination with five ligands, the Fe-bound form contained additional water and the metal was in octahedral coordination with six ligands. The additional water occupied the position of superoxide binding in the Fe-bound form. Upon binding of Fe, the OH of Tyr residue in the outer sphere of the active site shifted 1.1 Å to the central metal cofactor, whereas the shift upon Mn binding was negligible. These features are discussed in relation with lower activity of ApeSOD in the presence of Fe.