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.

[1] T. Nakamura, K. Torikai, K. Uegaki, J. Morita, K. Machida, A. Suzuki, Y. Kawata, *FEBS J*., **2011**, *278*, 598-609

Keywords: cambialistic, superoxide dismutase, Aeropyrum pernix

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"ON" and "OFF" States of Reversibly Phoswitchable Fluorescent Protein rsTagRFP

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Reversibly photoswitchable fluorescent proteins (rsFPs) are a new class of FPs changing their spectroscopic characteristics under illumination with the light of a specific wavelength. This unique property holds a great potential for rsFPs to be used as biomarkers to study protein migration and protein-protein interactions in living cells. rsTagRFP is the first red fluorescent protein with reversibly photoswitchable absorbance spectra. Irradiation of rsTagRFP with blue (440 nm) and yellow (567 nm) light switches it between the fluorescent (ON) and non-fluorescent (OFF) states, respectively. Transition of rsTagRFP from the OFF to the ON state is accompanied by 200-fold increase of fluorescence intensity at 585 nm, disappearance of absorbance band at 440 nm and appearance of absorbance band at 567 nm.

We have determined the structures of rsTagRFP in the fluorescent and the dark states at 2.2 and 1.8 Å resolution, respectively. The analysis of X-ray structures have shown that photoswitching of rsTagRFP is accompanied by *cis-trans* isomerization of the chromophore. *Cis*- and *trans*- chromophore isomers correspond to the ON and OFF states of rsTagRFP, respectively. Both *cis*- and *trans*- chromophore conformers are essentially coplanar. The nearest aminoacid environment of the *p*-hydrohyphenyl site of the chromophore is essentially hydrophobic providing no direct hydrogen bond between the chromophore and the protein scaffold. The lack of a distinct stabilization of either *cis*- or *trans*- conformation of the chromophore is a key factor responsible for the photoswitching phenomenon. Additionally, *cis-trans* isomerization of the chromophore is accompanied by a slight expansion/contraction motion of the β-barrel. This is especially noticeable for the loop comprised of residues 31-40 and 68-80, indicating that this area requires a certain freedom in order to provide the efficient chromophore isomerization.

Coplanarity of the chromophore in both ON and OFF states, its essentially hydrophobic environment, and the absence of direct hydrogen bonds with the protein scaffold are unique features of rsTagRFP that have not been previously observed in other rsFPs reported up today.

Keywords: photoswitchable fluorescent proteins, X-ray structure, cis-trans isomerization.

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Structural Basis of Different Substrate Preferences of Yeast Old Yellow Enzymes

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Old yellow enzyme (OYE) is an NAD(P)H-dependent reductase that can catalyze asymmetric reduction of C=C bond of enone substrates. Thus, OYE is applicable to the synthesis of chiral compounds. Although the molecular mechanism of the asymmetric reduction catalyzed by OYE has been proposed, the structural basis of different substrate preferences of different OYEs has not been fully understood. For example, the two OYEs from the two yeast strains, *Candida macedoniensis* (CYE) and *Torulopsis* sp. (TYE), show 46% sequence identity, but they show different substrate preferences to 3,5,5-trimethyl-2-cyclohexene-1,4-dione (ketoisophorone) and 4-hydroxy-2,6,6-trimethyl-2-cyclohexanone ((4*S*)-phorenol); CYE catalyzes the asymmetric reduction of ketoisophorone only, whereas TYE catalyzes the asymmetric reductions of both ketoisophorone and (4*S*)-phorenol. To reveal the structural basis of the different substrate preferences between CYE and TYE, we analyzed their crystal structures in the absence and presence of a substrate analogue, *p*-hydroxybenzaldehyde (*p*-HBA), and performed mutational analysis. As a result, we demonstrated that the two loop regions, Loops 5 and 6 located between β_5 and α_5 and between β_6 and α_6, respectively, function as the substrate filters which confer their different substrate preferences (Fig. 1). Detailed structural basis of their different substrate preferences will be presented and discussed.

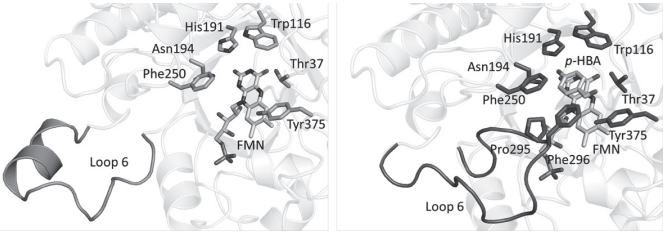

Fig. 1. Open (left) and closed (right) forms of CYE.
Mutation analysis indicates that Loop 6 plays significant roles in substrate preferences.

Keywords: old yellow enzyme, substrate preferences

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Stabilization of a full A-T hairpin oligonucleotide

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The human genome contains many non-coding DNA regions which are A-T rich. Since the function of those regions is still unclear it appears of interest to study A-T rich oligonucleotides at the atomic structural level. The formation of stable DNA hairpins could be involved in the condensation of mitotic chromosomes and the recognition of homologous chromosomes in meiosis. They could also be involved in the regulation of DNA function. For all these reasons, we have undertaken the study of a DNA hairpin which only contains A-T bases. With a representative non-coding sequence of 16 bases d(ATATATTTAATATAT) it has been predicted the formation of a DNA hairpin with a 6 base pairs stem and a tetra-loop (T_3A) [1]. We have simulated its formation by kinetic folding. Followed by crystallization