Saturday, March 21, Session VII

L25

FLUORESCENT PROTEINS AS MOLECULAR ANTENNAS: STRUCTURE AND DIRECTIONALITY

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The most important fluorescent molecules in biology are fluorescent proteins [1]. Despite their widespread use, it is largely unappreciated that they do not behave as point sources of light, but as antennas. The rate at which fluorescent proteins absorb light depends on their orientation with respect to the incoming light wave [2], and the emission of light by fluorescent proteins is directional. Directionality of optical properties of molecules is described by a vector, the transition dipole moment (TDM). Here we present the results of our optical measurements of FP crystals that, when combined with knowledge of the crystal structures, allowed us to determine the molecular orientations of absorption TDMs in several representative fluorescent proteins. Knowing the TDM orientations will allow quantitative interpretations of fluorescence resonance energy transfer experiments, determinations of molecular orientations by polarization-resolved microscopy, rational development of molecular probes, and other novel applications of these exceedingly useful molecules.

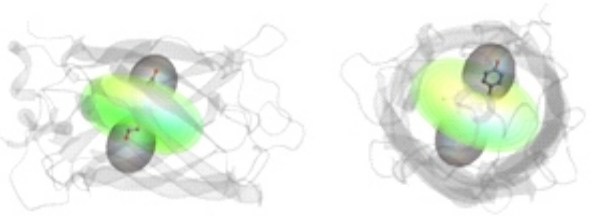

Figure 1. Structure of the molecule of the enhanced green fluorescent protein (eGFP), with the directionality of its light absorption and emission indicated.

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L26

STRUCTURAL BASIS FOR ENGINEERED THERMOSTABILITY IN HALOALKANE DEHALOGENASES

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Enhancing protein robustness is crucial for fundamental research as well as for technological applications. Our previous computational protein design effort yielded an 11-point mutant haloalkane dehalogenase DhaA115 with outstanding thermostability (T_m = 73.5 °C, T_m = 24 °C). Precise understanding of molecular basis for this thermostability remained sparse. Here we report 1.55 Å and 1.6 Å resolution structures of DhaA115 obtained by X-ray crystallography. We show that the placement of bulky aromatic amino acids on the protein surface triggered novel long-distance backbone changes, establishing a new double-lock system that: (i) closed access gates, (ii) reduced volumes of both main and slot access tunnels, and (iii) made the active site occluded. Despite of these extensive structural changes, experimental tracking of entry pathways by high-pressure krypton derivatization of DhaA115 crystals revealed transport of small ligands through enzyme's tunnels. Experimental observations are in full agreement with the results from computer simulations. Our findings unravel a novel structural basis of enzyme thermostabilisation, which will pave the way for designing highly thermostable biocatalysts and therapeutics.

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STRUCTURE-FUNCTION ASPECTS OF THE T1SS-MEDIATED SECRETION OF LARGE RTX PROTEINS

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Repeats-in-Toxins (RTX) protein family is a heterogeneous group of proteins that are secreted by Gram-negative bacteria. The polypeptides vary in size including molecular weights from about 10 kDa to larger than 1000 kDa and exhibit a wide range of biological and biochemical activities. While many of classical RTX proteins function as toxins/cytolysins involved in bacterial pathogenesis, the others comprise secreted proteases, lipases, bacteriocins, or play a role in plant nodulation, motility, adhesion and biofilm formation. Here, we examine the structure-function aspects of the RTX domain of the *Bordetella pertussis* adenylate cyclase toxin (CyaA) and its role in the T1SS-mediated secretion of the toxin polypeptide. We show that unfolded RTX repeats keep the stability of CyaA polypeptide in the Ca^{2+}-depleted bacterial cytosol and demonstrate that secretion levels of CyaA are proportional to the number of RTX motifs retained in the different CyaA constructs. Furthermore, we describe the crystal structure of the RTX block IV-V of CyaA ($CyaA_{1372-1681}$) containing a continuous assembly of two parallel â-roll motifs not previously seen in RTX lipases or other RTX proteins. Our data provide structural insight into the architecture of the RTX motifs of large RTX proteins and support the push-ratchet mechanism for the T1SS-mediated secretion of very large RTX proteins [1].

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L28

CRYSTAL STRUCTURE OF HEME-BASED OXYGEN SENSOR *AF*GCHK IN COMPLEX WITH IMIDAZOLE

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*Af*GcHK [1] is a globin coupled histidine kinase from a soil bacterium *Anaeromyxobacter* sp. Fw109-5. It is an intracellular enzyme which serves as an oxygen sensor. It has two domains: the N-terminal domain contains heme with an oxygen binding site and the C-terminal domain exhibits autophosphorylation activity induced by the oxygen binding. Both domains are connected by a flexible linker, which makes crystallization of the full-length *Af*GcHK difficult. The enzyme is active in dimeric form.

Two crystal structures of the globin domain of the enzyme, comparing its oxidized and reduced state [2], were presented in this conference two years ago. This year, we would like to present crystal structure of the *Af*GcHK globin domain in a monomeric form, in complex with imidazole [3].

The crystal grew in the Morpheus C4 condition containing 50 mM imidazole as a part of a buffer system. The diffraction data were collected at the Diamond Light Source and they were processed to resolution 1.8 Ĺ. The structure has been deposited in the Protein Data Bank under the code 6OTD.

The *Af*GcHK globin domain has monomeric form in this crystal structure. Imidazole is bound to the heme oxygen binding site and, moreover, there is a second bound imidazole molecule. It is also near the heme and occupies position of Tyr15 of the partner chain in dimer. This observation led us to study the importance of the Tyr15 residue for the globin domain dimerization and enzyme activity.

We discovered that mutation of Tyr15 is a simple way to disrupt the dimerization interface of the *Af*GcHK globin domain. It was found that the dimerization of the globin domain is necessary for internal signal transduction in the full-length *Af*GcHK and for its autophosphorylation activity (Fig. 1).

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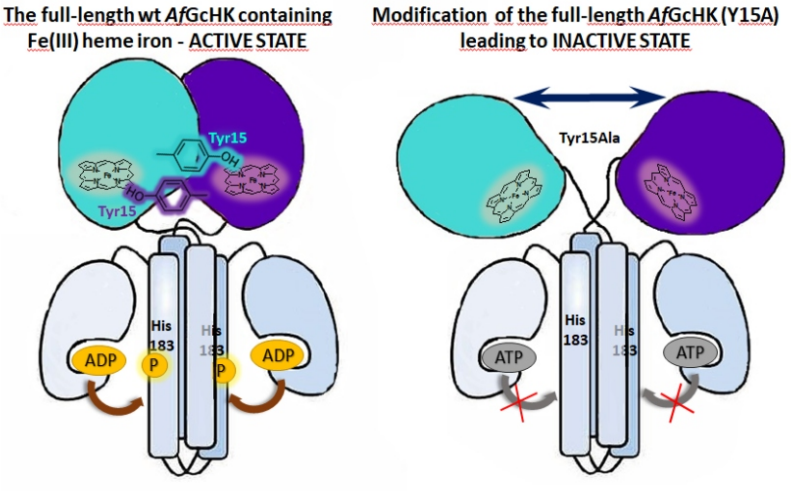

Figure 1. Scheme of two-domain dimeric histidine kinase *Af*GcHK (left). When globin domain dimerization is disrupted, the enzyme loses its activity (right). This figure was originally published in the Journal of Biological Chemistry [3]. © the American Society for Biochemistry and Molecular Biology

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O-METHYLATED SACCHARIDES AS A NEGLECTED TARGET FOR *PHOTORHABDUS* SPP. LECTINS

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Lectins are carbohydrate-interacting proteins involved in numerous processes in living organisms, including cell development, cell-cell communication, recognition of a pathogen by a host and vice versa. Frequently, single organism produces several different lectins and, especially in complex environments, single lectin might be involved in several processes. *Photorhabdus* is a genus of gram-negative bioluminescent bacteria living in a symbiosis with *Heterorhabditis* nematodes forming a highly entomopathogenic complex that is used in agriculture as a nature-based insecticide. Some members of *Photorhabdus* genus act as human pathogens as well. It is not surprising, that *Photorhabdus* bacteria encode several lectins in their genome [1-3].

There are several well-known specificities for lectins. Commonly studied are lectins recognizing L-fucose or sialic acid, saccharides that are found on mammalian tissues. However, in bacteria, fungi and invertebrates, many other potentially targeted saccharides exist. One such group of rarely studied saccharides are *O*-methylated glycans that are modified by methyl group on one or more of their hydroxyls [4]. They were recently described as pathogen-associated molecular patterns, i.e. molecules that serve for pathogen recognition. They were found in bacteria, fungi, plants, worms and molluscs but not in arthropods or mammals.

We analysed several homologous lectins from *P. asymbiotica* and *P. laumondii* revealing their ability to recognize *O*-methylated sugars. We examined the binding using glycan array, ITC and SPR techniques and investigated their effect on lectin-based stimulation of insect immune system. Since each of studied lectins harbours several binding sites of non-equal specificity, we also solved X-ray structure with a few *O*-methylated glycans. The combination of approaches allows us to investigate these interactions in further details. It may shed a light onto the complex interaction between *Photorhabdus* and its symbionts/hosts and possibly find applications in biotechnologies, clinical research and drug development.

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