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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 has 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 aminomacid environment of the p-hydroxyphenyl 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 of the protein scaffold is 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 macdoniensiis (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 (ketiosopherone) and 4-hydroxy-2,6,6-trimethyl-2-cyclohexanone ((4S)-phorenol); CYE catalyzes the asymmetric reduction of ketoisopherone only, whereas TYE catalyzes the asymmetric reductions of both ketiosopherone and (4S)-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 β1, and α1, and between β1, and α2, 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.

**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(ATATATTATAATATAT) it has been predicted the formation of a DNA hairpin with a 6 base pairs stem and a tetra-loop (TAT) [1]. We have simulated its formation by kinetic folding. Followed by crystallization

**Keywords:** cambialistic, superoxide dismutase, Aeropyrum pernix.