Report BM-30, Proposal 30.01.766.

Initial and essential data collections were done at BM30-A (crystal screening, initial diffraction data of the native and heavy atom soaked crystals). High-resolution data sets were obtained on ID23-1.

Abstract of the submitted paper:

Photosynthesis is intrinsically linked with the production of reactive oxygen species (ROS) that are deleterious to the elemental bricks of life. In chloroplasts, a central mechanism of photoprotection is constituted by the xanthophyll cycle in which conversion of the carotenoid Violaxanthin (V) into Zeaxanthin (Z) contributes to both light energy dissipation as heat and ROS scavenging. This conversion is triggered by an acidification of the thylakoid's lumen due to a high photosynthetic activity that switches on the activity of Violaxanthin de-epoxidase (VDE), the enzyme responsible for Zeaxanthin formation. We report here the crystal structure of the central lipocalin domain of VDE (VDE_{cd}) in a closed and open state. At neutral pH, VDE_{cd} is monomeric with the lipocalin barrel closed and the active site partially occluded by loop L1. Upon acidification, not only the top of the barrel is opened, but also its side, allowing a functional dimerisation with two active sites connected by a channel that could readily accommodate and de-epoxidate the two ends of the violaxanthin at once. This rare example of pH-dependant oligomeric switch, confirmed in solution by mutagenesis on the full-length, also provides an elegant example of enzyme adaptation to its centrosymmetric substrate.

Data collection statistics:

		pH5 – Gd (BM30-A)		pH7 (ID23-1)
Space Group		I4122		P1
Wavelength, Å		1.71116	0.98	0.97625
Resolution range, Å		40-2.5		50-2.0
Cell parameters:	a	122.1Å		34.6Å
	b	122.1Å		52.5Å
	c	158.2Å		54.6Å
	α, β, γ	90°, 90°, 90°		82.5°, 76.0°, 74.5°
No. of measured reflections		161,878		46,388
No. of unique reflections		39,942		23,478
R_{sym} [a,b]		0.43 (0.18)		0.069 (0.383)
I/σI [b]		9.9 (3.2)		6.4 (1.7)
Completeness, % [b]		97.4 (98.2)		96.9 (96.1)
Sites (n)		3	3	
Refinement				
Resolution range, Å				
R_{cryst} / R_{free}				
No. of non hydrogen atoms:				
Total				
Protein				
Glycerol				
Pyruvate				
Ion				
Water				
Average B-factors, Å² :				
Main-chain			26.9	31.8
Side-chain			31.2	36.0
Ion			27.1	-
Water			36.9	44.0
RMSD bonds, Å				
RMSD angles, °				
PDB code				

[a] $R_{sym}=\Sigma_h\Sigma_i|I_i(\mathbf{h}) - \langle I(\mathbf{h})\rangle / \Sigma_h\Sigma_i I_i(\mathbf{h})$

[b] Number in parenthesis refer to the last 0.1Å shell