MAP kinases respond to a wide variety of extracellular stimuli. These are first phosphorylated by MAP/ERK kinase kinase (MAP2K) and then the MAP kinases in turn phosphorylate specific nuclear transcription factors. These events are mediated by docking peptides, which are remote from the phosphorylation site in substrates or active site of the activators. Earlier work from our laboratory on the crystal structures of p38 with docking peptides from substrate MEF2A and activator MKK3B (Chang et al., 2002); and ERK2 with a similar peptide derived from a phosphatase (Zhou et al., 2006) showed conformational changes local to the binding site. In addition, large unexpected allosteric changes in the active site were seen in both p38 and ERK2. Recently Vogtherr et. al (Vogtherr et al., 2005) assigned 64% of the backbone and Cb shifts for p38. To see the relevance of the allostery observed in crystal structure, we conducted N15 NMR studies on p38 with docking peptides derived from MEF2A and MKK3b. Residues distal from the binding site showed moderate chemical shifts indicating docking and allostery are present in solution and is a mechanism by which specificity is achieved in these kinases.
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Keywords: allostery, MAP kinases, NMR

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Time-resolved X-ray crystallography captures transition-state-like intermediate in PYP photocycle

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Since the geometrical isomerization within the chromophore pocket of a protein is spatially restricted via media constraints such as hydrogen bonding network and confined space, the usual one-bond-flip mechanism observed in the gas and solution phases no longer holds and instead the volume-conversing mechanism has been hypothesized. Previous studies on photoactive yellow protein (PYP) predicted that a twisted form of chromophore by volume-conversing model in picoseconds regime, but its exact 3D structure has been elusive. Here we report the earliest intermediate structure (IT) of trans-cis isomerization in PYP and detailed atomic motions by picosecond X-ray crystallography. The IT intermediate is distorted such that the planarity of chromophore is broken while all three original hydrogen bonds are still intact, and resembles a theoretically predicted transition-state. Hydrogen bonds networking make this distorted structure stable as an intermediate rather than a transition state detectable with time-resolved crystallography. The carbonyl oxygen of IT is along the pathway connecting the ground state to the next intermediates, ICT and pR1, via the bicycle-pedal mechanism and hula-twist mechanism, respectively.

Keywords: time-resolved crystallography, time-resolved Laue diffraction, reaction mechanisms

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Slow ligand migration dynamics in carbonmonoxy myoglobin at cryogenic temperature

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Myoglobin (Mb) is a small globular heam protein in muscle, which reversibly binds ligands at the heam iron site deeply inside the protein matrix. This ligand dissociation can be triggered by photo irradiation [1]. The ligand binding reaction in Mb has been studied by a variety of techniques. Photolysis of carbonmonoxy myoglobin (MbCO) has been extensively studied by X-ray diffraction experiments (e.g. Laue diffraction experiments at room temperature [2,3] or monochromatic X-ray diffraction experiments at cryogenic temperature [4]). In spite of lots of known details regarding the gas ligand molecules trapped in internal cavities of Mb, there exists no direct evidence to show the migration pathways connecting these cavities. In order to explore the ligand migration pathways in myoglobin induced by ligand dissosiation, we have carried out cryogenic X-ray crystallographic investigations of carbonmonoxy myoglobin (native sperm whale MbCO) crystals illuminated by a laser. Slow ligand migration in Mb was observed at the cryogenic temperatures.
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Keywords: myoglobin, time-resolved X-ray diffraction, ligand binding

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Crystal structure of FlgD from Xanthomonas: Insights into the hook capping for flagellar assembly

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Structural genomics is crucial for understanding the intricate interactions among proteins in a whole organism. We have studied the structural genomics of Xanthomonas campestris (Xcc), a gram-negative bacterium that is phytopathogenic to cruciferous plants and causes worldwide agricultural loss. Xcc is the only bacterium known to lack a cAMP signaling system, and uses a cAMP-receptor protein like protein (CLP) system instead. Currently we are working on its flagellar structural genomics. The first crystal structure of a hook-capping protein FlgD of a microbial flagellum from the plant pathogen Xanthomonas campestris has been determined to a resolution of 2.5 Å; crystallography. The monomer comprises 221 amino acids with a MW of 22.7kD, but the disordered N-terminus