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

is cleaved for up to 75 residues during crystallization automatically. The final core structure reveals a novel hybrid comprising a tudor-like (Sm-like) domain interdigitated with a Immunoglbolubin-like (Ig) or fibronectin type (Fn) III domain. In the crystal, the monomers form an annular pentamer of dimers of pseudo five-fold symmetry, due to different dimer-dimer interactions employed. The resulting asymmetrical star-like complex has a outer dimensions of approximately 110 Å × 90 Å × 65 Å, and a shortest diameter of approximately 20 Å; in the center. The outer dimensions of the atomic Xanthomonas hook-capping FlgD complex turn out to be very similar to those of the Salmonella filament cap complex observed by electron microscopy. This atomic hook cap structure may help understand hook protein-cap protein interactions, hook protein insertion, and hook length control, the three features that are crucial for understanding the bacterial flagellar biogenesis.

Keywords: FlgD, hook capping, flagellar biogenesis

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Structure of DFA0005 complexed with α -ketoglutarate: A novel member of the ICL/PEPM superfamily

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The pale-pink alkali-tolerant Deinococcus ficus (DF strain CC-FR2-10T) is a novel bacterium isolated from the rhizosphere of the sacred tree Ficus religiosa L. It is found to possess high degrading power for food and organic waste at pH 10 and is being applied to industrial usage. It contains one chromosome of 2801970 bp, and three mega-plasmids of 642681, 395118, 311736 bp, respectively. The total genome sequence has been recently completed (Lin et al, manuscript in preparation), and a structural genomics project is followed to study the structures of proteins encoded in this genome and to study the alkali adaptation mechanism exhibited by this alkali-tolerant bacterium compared with their non-alkaline counterparts. The crystal structure of the DFA0005 protein complexed with α -ketoglutarate (AKG) from an alkali-tolerant bacterium Deinococcus ficus has been determined to a resolution of 1.62 Å. The monomer forms an incomplete α7/ β8 barrel, with a protruding α8 helix that interacts extensively with another subunit to form a stable dimer of two complete α8/ β8 barrels. The dimer is further stabilized by four glycerol molecules situated at the interface. One unique AKG ligand binding pocket per subunit is detected. Fold match using the DALI and SSE servers identifies DFA0005 as belonging to the isocitrate lyase / phosphoenolpyruvate mutase (ICL/PEPM) superfamily. However, further detailed structural and sequence comparison with other members in this superfamily and with other families containing AKG ligand indicate that DFA0005 protein exhibits considerable distinguishing features of its own and can be considered a novel member in this ICL/PEPM superfamily.

Keywords: Deinococcus ficus, ICL/PEPM superfamily, alpha-ketoglutarate ligand

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RecX adopts a tandem repeats of three-helix bundle:Insights into RecX inhibition of RecA activities

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The bacterial RecA protein plays a key role in recombinational DNA repair in bacteria. it is a DNA-dependent ATPase that promotes DNA strand exchange reactions, and induces SOS response. However, the activities of RecA protein must be regulated in the cell to avoid aberrant DNA recombination. Currently, many additional proteins are known to regulate RecA function such as RecF, RecO, and RecR and DinI proteins. RecX protein is an inhibitor of RecA function both in vivo and in vitro. However, RecX protein is among the least understood of these RecA modulator proteins. But it has been found that RecX protein inhibits RecA protein ATPase, coprotease, and recombinase activity. Direct interaction between RecA and RecX has also been well demonstrated. Reconstituted RecA/RecX nucleoprotein filament from EM and RecA crystal structure show that RecX protein bind to the helical groove of RecA filament, spanning the monomer-monomer interface from the C-terminal domain of one RecA monomer to the core domain of the second. However, detailed RecX crystal structure is still unavailable until to date. We have solved the first RecX crystal structure from Xanthomonas campestris to a resolution of 1.6 Å; crystallography. The tertiary structure of RecX indicates it adopts a novel three tandem repeats of three helix bundle. Enzyme assays indicate that RecX can efficiently inhibit the ATPase, coprotease, and recombinase activity of the RecA protein. A docking study of RecA dimer and RecX monomer reveals the tight interactions between these two proteins. Model study shows that RecX proteins can fit well into the major helical groove, with its C-terminus fitted into the notch between two adjacent RecA monomers in the RecA nucleofilament, as revealed by the cryoEM study.

Keywords: RecA, RecX, recombination

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Structure of Xcc UMPK/GTP complex reveals a novel GTP-binding site and allosteric mechansim

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Uridylate kinase (UMPK) is a key enzyme in pyrimidine biosynthesis. Due to its importance in nucleic acid biosynthesis, UMPK is ubiquitous in every living organism, including bacteria, archaea, and eukarya. However, UMPKs of bacterial origin are very different from the UMPKs of eukaryotic origin. In general, the eukaryotic enzymes have a broader substrate preference, exhibiting