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Keywords: glycosylation, TPR repeat, protein-protein interactions

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Structural Studies of Quinolinate Synthase
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Nicotinamide adenine dinucleotide (NAD) is an essential cofactor in several metabolic pathways and has recently been shown to play a role in several signaling pathways [1]. Consequently, there is great interest in the biosynthesis of NAD. Quinolinate is the universal precursor in the de novo biosynthesis of NAD and can be synthesized from either tryptophan in the case of eukaryotes or aspartate in most prokaryotes [2].

The aspartate pathway begins with L-aspartate oxidase which converts aspartate to iminoaspartate. Quinolinate synthase (QS) catalyzes the condensation of iminoaspartate and dihydroxyacetone phosphate to form quinolinic acid [3]. This enzyme has been difficult to characterize due to either instability or inactivity when it is overexpressed and purified.

QS is the last enzyme in this pathway to be structurally characterized. We have determined the crystal structure of QS at 2.8 Å resolution. The crystal structure and sequence alignments provide insights into the details of the active site and the enzyme's evolution.

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Keywords: quinolinate synthase, NAD biosynthesis, quinolinate

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A Second FMN-binding Site in Yeast CPR Suggests a Novel Mechanism of Electron Transfer by Diflavin Reductases
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NADPH-cytochrome P450 reductase (CPR) transfers two reducing equivalents from NADPH via FAD and FMN to the large super family of microsomal cytochrome P450 monooxygenases (CYPs) in one-electron transfer steps. Mechanism of electron transfer by diflavin reductases remains elusive and controversial. We determined the crystal structure of CPR from *Saccharomyces cerevisiae*, which is functionally active toward its physiological substrate cytochrome P450 and discovered a second FMN-binding site at the interface of the connecting and FMN-binding domains. We propose that during catalytic turnover a single FMN molecule shuttles twice between two protein sites that accommodate two different semiquinone forms, neutral (blue) and anionic (red). Oscillating between two sites FMN presumably swings along the interface between the reductase domains circumscribing about half a circle of the 10 Å radius around invariant D187 as the center of rotation, so that the FMN N5-reference atom relocates approximately 20 Å, while the ribityl moiety remains within interaction distances from the carboxyl of D187 and T71. Yeast CPR loses the ability to support the catalytic function of CYP51 upon substitution of D187 or T71 with alanine. We believe that the proposed mechanism will move forward our understanding of electron transfer by diflavin reductases (including nitric oxide synthase (NOS)) since these electron transporters are highly homologous genetically, structurally, and functionally to CPR.
Keywords: diflavin reductase, electron transfer, FMN-binding

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Identification and Purification of a Soluble Region of BubR1
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The mitotic checkpoint complex (MCC) ensures the fidelity of chromosomal segregation, by delaying the onset of anaphase until all sister chromatids have been properly attached to the mitotic spindle. In essence, this MCC-induced delay is achieved via the inhibition of the anaphase-promoting complex (APC). Among the MCC components, BubR1 plays two major roles in the functions of the mitotic checkpoint. First, BubR1 is able to inhibit APC activity, either by itself or as a component of the MCC. Second, BubR1 activates mitotic checkpoint signaling cascades.

To determine the structure of BubR1, we obtained a soluble BubR1 constructs using a three-step expression strategy. First, we obtained two constructs from BLAST sequence homology searches, both of which were expressed abundantly in the inclusion bodies. Second, we adjusted the lengths of the two constructs by secondary structure prediction, thereby generating partially soluble constructs. Third, we optimized the solubility of the two constructs by modification at the C-terminus. Finally, we obtained a highly soluble BubR1 protein via the *E. coli* expression system.

This report may provide insight into the design of highly soluble constructs of insoluble multi-domain proteins.
Keywords: protein secondary structure analysis, cell-cycle proteins, solubility

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Structural Basis for the Cell-specific Activity of NGFI-B/Nurr1 Ligand-binding Domains
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NGFI-B is a ligand-independent orphan nuclear receptor of the NR4A subfamily that displays important functional differences with its homolog Nurr1. In particular, the NGFI-B ligand-binding domain (LBD) exhibits only modest activity in cell lines in which the Nurr1 LBD strongly activates transcription. To gain insight into the structural basis for the distinct activation potentials, we determined the crystal structure of the NGFI-B LBD at 2.4 Å resolution. Superimposition with the Nurr1 LBD revealed a significant shift of the position of helix 12, potentially caused by conservative amino acid exchanges in helix 3 or helix 12. Replacement of the helix 11-12 region of Nurr1 by that of NGFI-B dramatically reduces the transcriptional activity of the Nurr1 LBD. Mutation of M414 in helix 3 to leucine, or of L591 in helix 12 to isoleucine (the corresponding residues found in NGFI-B) significantly affects Nurr1 transactivation. Swapping the helix 11-12 region of Nurr1 into NGFI-B results in a modest increase of activity. These observations reveal a high sensitivity of LBD activity to changes that influence helix 12 positioning. Mutation of hydrophobic surface residues in the helix 11-12 region (outside the canonical co-activator surface constituted by helices 3, 4 and 12) severely affects Nurr1 transactivation. Together, our data suggest that a novel co-regulator surface that includes helix 11 and a specifically positioned helix 12 determine the cell type-dependent activities of the NGFI-B and the Nurr1 LBD.
Keywords: ligand-binding domain, transcription, NGFI-B

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Structure of a Cell Polarity Regulator, an aPKC and Par6 PB1 Domain Complex
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A complex of atypical PKC and Par6 is a common regulator for cell-polarity related processes, which is an essential clue to evolutionary conserved cell-polarity regulation. Here, we determined the crystal structure of the aPKC and Par6 PB1 domain complex to a resolution of 1.5 Å. Both PB1 adopt a ubiquitin fold. aPKC PB1 presents an OPCA motif, 28 amino acid residues with acidic and hydrophobic residues, which interacts with the conserved lysine residue of Par6 PB1 in a front-and-back manner. Structural comparison of the aPKC and Par6 PB1 complex with the $p40^{phox}$ and $p67^{phox}$ PB1 complex, subunits of neutrophil NADPH oxidase, reveals that the specific interaction is achieved by tilting the interface so that the insertion or extension in the sequence is engaged in the specificity determinant. The PB1 domain develops the interaction surface on the ubiquitin fold to increase the versatility of molecular interaction.

Keywords: protein complex structure, domain structure, cell polarity

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The Allergenic Non-specific Lipid Transfer Protein from Peach: Structural Studies

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A large number of proteins can bind small molecules with a poor solubility in water. The function of many of these proteins is to transport small molecules which would not be soluble in the biological environment. The ligands often present a strong lipophilic characters, as, for example, retinoids and fatty acids. In addition to the well known calycins, this group of protein includes the so-called non-specific lipid transfer proteins (*ns*-LTPs). They are plant proteins of about 9 kDa molecular mass, and they bind fatty acids, steroids and other types of lipids. However, their function has not been precisely defined as yet [1].

Our study is focused on the determination of the three dimensional structure of peach ns-LTP. Recently, several biological and medical studies have stimulated interest in the *ns*-LTP from peach. This protein, like others *ns*-LTPs, binds fatty acids, but it has been also identified as an important food allergen [2].

Despite its strong sequence similarity with others ns-LTPs whose structure is already known [3], its crystal structure determination was not straightforward. It required the measurement of synchrotron anomalous data with enhanced sulphur f´´ by using a synchrotron X-ray wavelength of 2Å from SRS MPW beamline 10. In addition a stronger <I/sig(I)> was then achieved using the APS undulator at SBC CAT. The molecular model is a single compact domain, made up by four *α-helices*, which form a hydrophobic pocket where the lipid is bound. The 3-D structure is strongly stabilized by four disulphide bridges. Possible immunogenic epitopes associated with the allergenic reaction will be proposed by this study.

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Keywords: ns-LTP, non specific lipid transfer protein, food allergen

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LRRs: A platform to build a Protein Recognition Motif

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The leucine-rich repeat domains (LRRs) of cell-surface receptors often constitute the binding regions for small protein ligands. Examples are found in many types of medically important receptors, e.g. Insulin and growth-factor receptors, G-coupled protein receptors and Toll-like receptors. Thus they are important therapeutic targets.

The architecture of LRRs generally consists of a β-helix or solenoid with a prominent β-sheet down one face forming the ligand-binding surface. Side chains on this face are tightly packed to form a sterically well-defined surface with the chemical composition dictated by sequence. The opposite face shows considerable structural variability and here, the space occupied by the main chain appears to dictate the curvature of the ligand-binding face. Thus LRRs have a simple but elegant design, where the main chain provides a regular framework of variable size and shape and chemical nature of the site is under genetic control.

Now that a considerable number of these structures have been determined, with or without their ligands, a detailed analysis has revealed the factors which control the overall architecture for *ab initio* design of a protein-binding surface. Naturally-occurring augmentations of this standard architecture provide additional ways of creating a protein-docking site.

Keywords: receptor-ligand interactions, molecular recognition, protein engineering

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Cracking of the Targeting Signal Embedded in Mitochondrial Presequences

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Most mitochondrial proteins are synthesized in the cytosol as precursor proteins with a cleavable N-terminal presequences and are imported into mitochondria. Protein import into mitochondria is mediated by protein assemblies in the mitochondrial membranes. A subunit, Tom20, functions as a general protein import receptor by recognizing presequences of preproteins. Although no consensus sequence is found, Tom20 recognizes a wide variety of presequences.

To understand the structural basis of the presequence recognition, we determined the NMR and crystal structures of Tom20 in a complex with a presequence peptide. Note that the presequence was fixed to Tom20 via a designed intermolecular disulfide bond to obtain crystals. The bound presequence forms an amphiphilic a-helix. NMR titration experiments indicated the presence of a unique presequence binding site in Tom20, and defined a common five-residue pattern in different presequences. To refine this pattern, we introduced a new peptide library approach using the formation of an intermolecular disulfide bond. We propose that a presequence is regarded as a collective entity of short amino acid sequences that are recognized by several proteins including Tom20. The organization (position, order, and overlapping) of these binding segments is unique for each presequence. This view explains why no consensus sequences are found by simple sequence comparisons.

Keywords: protein transport, molecular recognition, crystallographic and NMR solution structures

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Crystal Structure of Decameric Peroxiredoxin (AhpC) from *Amphibacillus xylanus*

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Peroxiredoxins (Prxs), also referred to as AhpCs, are a ubiquitous family of antioxidant enzymes. Bacterial AhpC is recognized as the primary scavenger of endogenously generated hydrogen peroxides. AhpC purified from *Amphibacillus xylanus* shows extremely high scavenging activity for both hydroperoxide and alkyl hydroperoxide in