CRYSTALLOGRAPHY OF BIOLOGICAL MACROMOLECULES

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Keywords: glycosylation, TPR repeat, protein-protein interactions

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Structural Studies of Quinolinate Synthase

Erika Soriano, Ethan Settembre, Tadhg P. Begley, Steven E. Ealick, *Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA*. E-mail: evs4@cornell.edu

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

<u>Larissa M. Podust,</u> Michael R. Waterman, *Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146, USA.* E-mail: larissa.m.podust@vanderbilt.edu

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

<u>Dohyun Han</u>¹, Jongchul Yoon^{1,2}, Yup Kang^{3,2}, Kyunggon Kim¹,

Jungeun Park¹, Youngsoo Kim^{1,4}, Division of ¹Molecular Genomic Medicine and ⁴Cancer Research Institute, Seoul National University. ³Insitute for Medical Sciences, Ajou University School of Medicine. E-mail: hdh03@snu.ac.kr

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

Ralf Flaig^a, Holger Greschik^b, Carole Peluso-Iltis^b, Dino Moras^b, ^aDiamond Light Source, UK. ^bIGBMC, Strasbourg, France. E-mail: ralf.flaig@diamond.ac.uk

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 typedependent 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

Yoshinori Hirano^a, Sosuke Yoshinaga^a, Ryu Takeya^b, Nobuo N. Suzuki^a, Masataka Horiuchi^a, Motoyuki Kohjima^b, Hideki Sumimoto^b, Fuyuhiko Inagaki^a, ^aDepartment of Structural Biology, Graduate School of Pharmaceutical Sciences, Hokkaido University, N-12, W-6, Kita-ku, Sapporo, Japan. ^bMedical Institute of Bioregulation, Kyushu Universuty, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Japan. E-mail: y-h@pharm.hokudai.ac.jp