

## 03-Crystallography of Biological Macromolecules

## 03.01 – Nucleic Acid Protein Interactions

**MS-03.01.01** HIGH RESOLUTION ANALYSIS OF THE TRP REPRESSOR/OPERATOR INTERACTION: THE SPECIAL ROLE OF WATER MOLECULES by R.G.Zhang\*, C.K.Kundrot, Z.Otwinowski and P.B.Sigler, The Dept. of Molecular Biophysics and Biochemistry and The Howard Hughes Medical Inst., Yale Univ., New Haven, CT 06536 USA.

The trp repressor/operator complex crystallized in space group P2<sub>1</sub>, with unit cell dimensions a=43.65Å, b=72.43Å, c=107.39Å and  $\beta$ =94.96. Originally the structure was solved at 2.4Å (Otwinowski et al., Nature 335,321–329,1988). We have extended the resolution to 1.9Å by usubg data collected on imaging phosphor plates at the Photon Factory synchrotron facility in Japan.

A clear picture of the hydration pattern of the trp repressor/operator complex, made possible by high resolution refinement of the crystal structure, shows that fixed water sites mediate most of the interactions between the repressor and the bases that specify the operator's identity. Thus water molecules, firmly bound in a stereospecific but reversible manner, represent a noncovalent and dynamic extension to the functional surfaces that participate in macromolecular recognition.

**MS-03.01.02** tRNA<sup>Asp</sup> ASPARTYL-tRNA SYNTHETASE RECOGNITION by J.Cavarelli\*, B.Rees, M.Ruff, J.C.Thierry, D.Moras, UPR de Biologie Structurale, 15 rue rene Descartes, 67084 Strasbourg Cedex, France

Our present understanding of the molecular mechanisms responsible for the recognition of tRNAs by their cognate amino-acyl-tRNA synthetases (aaRS) is essentially based on three sources of information: (i) the characterization of tRNA identity determinants using *in vivo* and *in vitro* approaches, (ii) the classification of synthetases from primary sequence analysis: aaRS can be partitioned into two classes according to the spatial structure of their ATP binding domain, (iii) the structure results of crystallographic investigations and solution studies. The crystal structures of three aaRS and two complexes, one of each class, are known to atomic resolution.

AspRS, like six other members of class II, is an  $\alpha$ 2 dimer. Yeast tRNA<sup>Asp</sup> exhibits five identity determinants: the three anticodon bases, the discriminator base G73 and the base pair G10-U25. The refined crystal structure of AspRS complexed with tRNA<sup>Asp</sup> at 2.9Å resolution reveals three regions of contact, each involving a domain of AspRS and at least one identity determinant of tRNA<sup>Asp</sup>. Each monomer binds one tRNA; the anticodon region of the tRNA is recognized by the N-terminal module (residues 90 to 201) and the C-terminal module (residues 242 to 557) anchors the acceptor stem. The synthetase approaches the tRNA on the variable loop side, thus interacting with the major groove side of the helical amino acid acceptor stem.

The mode of binding of the acceptor stem of tRNA<sup>Asp</sup> by AspRS can be generalized to class II aaRS, whereas the deciphering of the anticodon, which involves a large conformational change of the loop and the formation of a bulge, is more specific to the aspartic system.

**MS-03.01.03** HIGH RESOLUTION STRUCTURE OF A GCN4 bZIP-ATF/CREB SITE DNA COMPLEX. by P.Konig, W.Keller, and T.Richmond\*, Institut für Molekularbiologie und Biophysik, ETH-Honggerberg, CH-8093 Zurich, Switzerland

The X-ray structure of the DNA binding domain of the yeast transcriptional activator protein GCN4 bound to a DNA fragment containing the sequence of the perfectly symmetrical ATF/CREB site has been solved to 3.0Å resolution. The basic domain and coiled-coil leucine zipper of this protein are homologous to over 30 other eukaryotic transcription regulatory factors. Specific recognition of the DNA sequence is achieved by insertion of a single  $\alpha$ -helix from each subunit of the dimer into the DNA major groove on opposite sides of the double helix. The structure reveals how this protein can tightly bind to the symmetrical ATF/CREB site in addition to the pseudo-symmetrical AP-1 site lacking one central base pair. Crystals of this complex that diffract to approximately 2Å resolution have been obtained, and refinement of the structure at high resolution is in progress.

**MS-03.01.04** STEREOCHEMICAL PRINCIPLES OF DNA TARGET SELECTION BY THE STEROID/NUCLEAR RECEPTOR by Paul B.Sigler\*, Yale University, Department of Molecular Biophysics and Biochemistry, and The Howard Hughes Medical Institute, New Haven, Ct., 06510, USA

Transcriptional regulatory proteins interact with one another, and with the basal transcription apparatus, to produce a near continuum of responses. These processes require specific high affinity interactions between proteins that occur only after one or more of the proteins bind to the DNA regulatory element. The steroid/nuclear receptor family is a well studied system that exhibits such DNA-dependent specific protein-protein interactions including those responsible for DNA target selection.

The DNA targets of the steroid/nuclear receptor family are distinguished by: (1) the base sequence of the 6-basepair 'half-sites'; and, (2) the orientation of the half-sites as well as the number of basepairs between them. We have studied the crystal structure of the glucocorticoid receptor's DNA-binding domain (GR-DBD) in complexes with a variety of DNA targets. We have also studied DNA complexes of mutational variants of the GR-DBD designed to recognize alternative half-site sequences and alternative half-site arrangements. These studies reveal: (1) the stereochemistry of half-site recognition, and (2) structural features influenced by DNA-binding that select for targets with appropriate orientation and spacing of half-sites.