PS04.07.12 CRYSTALLOGRAPHIC STUDY OF THE TETRAMERIZATION DOMAIN OF A *SHAKER*-TYPE POTASSIUM CHANNEL A. Kreusch, #P.J. Pfaffinger, *C. F. Stevens, S. Choe. Structural Biology and *Molecular Neurobiology Laboratories, The Salk Institute, La Jolla, CA 92093, #Baylor College of Medicine, Houston, TX 77030

Functional diversity of ion channels arises not only from multiple genes and gene splicing, but also from diverse formation of heterotetramers. Potassium channels are grouped into four subfamilies: *Shaker, Shab, Shaw*, and *Shal*. Members of the same family share high sequence homology and can coassemble, whereas members from different subfamilies do not. The N-terminal domains of *Shaker*-type K+-channels has been shown to be crucial for subunit assembly into functional channels and are able to tetramerize in solution when isolated.

Studies by Shen *et al.* (1995) identified the core region responsible for the tetramerization of the *Aplysia* potassium channel protein. We purified and crystallized this tetramerization domain derived from *Aplysia*. Crystals have been obtained by hanging drop method and belong to the space group I4 with a=b=51.034, c=65.605 Å, with one molecule per asymmetric unit. These crystals diffract to beyond 2.0 Å resolution at room temperature. A complete native data set to 2.0 Å has been collected at SSRL with an R_{merge} of 6.3% and an overall completeness of 99%. The search for heavy atom derivatives and structure determination are in progress. Three-dimensional structure can reveal the structural basis for subfamily-specific recognition motif underlying the formation of diverse channel properties.

Shen, N. V., & Pfaffinger, P. J. (1995) *Neuron* 14, 625-633.

PS04.07.13 CRYSTAL STRUCTURE OF THYMIDYLATE KINASE FROM *SACCHAROMYCES CEREVISIAE* WITH SUBSTRATE BOUND. Arnon Lavie, Ingrid Vetter, Manfred Konrad, Roger Goody, Joachim Reinstein, Ilme Schlichting, Max-Planck Institut für Molekulare Physiologie, Rheinlanddamm 201, Dortmund 44139, Germany

The three-dimensional X-ray structure of Thymidylate Kinase (TmpK) from *Saccharomyces cerevisiae* with TDP bound has been solved at 2.5Å resolution. TmpK plays a crucial role in cell proliferation catalyzing the phosphorylation of TMP to TDP, which is then further phosphorylated by Nucleoside Diphosphate Kinase to TTP. It is this activated form of thymidine which is the substrate for DNA polymerase, thus making TmpK an essential enzyme for DNA synthesis. In addition, nucleoside and nucleotide monophosphate kinases are important for pro-drug activation of nucleoside antivirals.

The overall fold of TmpK is similar to that of Adenylate Kinase even though there is very low sequence homology between AK and TmpK. TmpK, which is a dimer in solution, crystallizes in space-group $P2_1$ with a dimer in the asymmetric unit. The dimer interface is composed of an hydrophobic core made up by 6 leucines, 4 trptophanes, and 2 isoleucines, with additional binding energy contributed by 2 pairs of argenine-glutamic acid salt bridges. The structure of the P-loop is similar to that of Adenylate Kinase. The substrate is seen bound in the monophosphate binding pocket with the base moiety sandwiched between Phe69 and Try102, and the phosphate making ionic interactions with Lys37 and Arg94.

In addition to the TDP bound TmpK crystals, substrate-enzyme complex crystals with TMP and with the bi-substrate inhibitor TP5A have been obtained, and native data sets collected. The refinement of these structures is in progress, and together should provide a clue to the different conformations of TmpK upon substrate binding, in analogy to the different states found for AK.

PS04.07.14 CRYSTAL STRUCTURE OF SAICAR SYNTHASE FROM *SACCHAROMYCES CEREVISIAE*. V.M. Levdikov+, V.V. Barynin+, A.I. Grebenko+, W.R. Melik-Adamyan+, V.S. Lamzin* and K.S. Wilson*, +Institute of Crystallography, Russian Academy of Sciences, Leninsky pr. 59, Moscow 117333, Russia; *European Molecular Biology Laboratory (EMBL), c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany

The three-dimensional crystal structure of SAICAR synthase of *Saccharomyces cerevisiae* has been solved by multiple isomorphous replacement and refined at 1.9 Å resolution to an R=0.154. SAICAR synthase is a monomeric enzyme comprising a single polypeptide chain of 306 amino acids arranged into three domains. The cores of the first two domains comprise antiparallel β-sheets and the third is composed of two long α-helices. There is a long deep cleft in the middle of the molecule which is made up of residues from all three domains. The positions of two sulphate ions bound in the cleft and comparison of SAICAR synthase structure with known structures of other nucleotide binding proteins indicate the most probable binding sites of the phosphate moieties of ATP and phosphoribosylcarboxyaminoimidazole.

Sequence alignment of SAICAR synthases from different organisms reveals 24 fully conserved amino acid residues, 14 of which are charged. Almost all of them are located at the surface of the interdomain cleft. Some of them are presumably implicated in substrata binding.

Comparison of the SAICAR synthase with other nucleotide binding proteins shows that this protein does not belong to the protein families with classical di- and mononucleotide-binding fold. However SAICAR synthase structure have some resamblance with glutathione synthetase, D-alanine:D-alanine ligase and cyclic AMP-dependent protein kinase. The probable ATP phosphate anchor in the structure of SAICAR synthase made up of a β-loop-β motif typical for the actin and heat-shock cognate protein.

Studies of the enzyme-substrata complexes is in progress.

PS04.07.15 STRUCTURE OF THE BACTERIOCHLOROPHYLL *a* PROTEIN FROM *CHLOROBIUM TEPIDUM*. Y. F. Li, W. Zhou, R. E. Blankenship, J. P. Allen, Department of Chemistry and Biochemistry, Center for The Study of Early Events in Photosynthesis, Arizona State University, Tempe, AZ 85287 USA

The bacteriochlorophyll (BChl) *a* protein accepts energy from the chlorosome antenna complex and then transfers the excitation energy to the reaction center in green photosynthetic bacteria. Studies on the antenna system from *Chlorobium tepidum* have shown the unusual property that energy transfer efficiency could be modulated by the redox potential. The nearly 100% efficiency of energy transfer in reducing conditions is reduced to 10% or less under oxidizing conditions due to unknown changes of the BChl *a* protein (Blankenship *et al.*, 1993, *Photochem. Photobiol.* 57, 103-107).

The BChl *a* protein from *C. tepidum* has been crystallized using the sitting drop method of vapor diffusion. These crystals belong to the cubic space group $P4_132$ with cell dimensions of a = b = c = 169.5 Å. A native data set has been collected to a resolution of 2.0 Å. An initial solution has been determined by using the molecular replacement with X-PLOR. The search model was the structure of the BChl *a* protein from *Prosthecochloris aestuarii* (Tronrud, D. E. & Matthews, B. W., 1993, *The Photosynthetic Reaction Center,* Norris, J. & Deisenhofer, J., eds., pp. 13-21, Academic Press, NY). The model was rebuilt and refined to a current R factor of 28.0%. The structure is similar to that of *P. aestuarii* with three identical subunits related by an approximate 3-fold axis of symmetry. In each subunit the polypeptide backbone forms large β-sheets and encloses a central core of seven BChl *a* molecules. Model building and refinement is in progress. To identify structural changes of the BChl *a* protein upon reduction data sets have been collected to a resolution of 2.0 Å for crystals in the presence of dithionite. The structure of the BChl *a* protein under reducing and oxidizing conditions will be compared.