s8a.m1.p39 **The Structure of Serotonin N-Acetyltransferase: Illustrating Catalysis by the GCN5-Related N-Acetyltransferase (GNAT) Enzyme Superfamily.** F. Dyda[1], M.A.A. Namboodiri[2], D.C. Klein[2], A.B. Hickman[1], [1]*LMB, NIDDK,*[2]*LDN, NICHD, NIH, Bethesda MD 20892 USA.*
Keywords: N-acetyltransferase, seratonin, acetyl coenzyme A.

Serotonin N-acetyltransferase (also known as arylalkylamine N-acetyltransferase, or AANAT) is the penultimate enzyme in the convertion of serotonin to the neurohormone, melatonin. As part of a program to understand the circadian regulation of AANAT activity, we have recently determined the crystal structures of AANAT in both the substrate-free form and as a complex with a bisubstrate analog.

The structures illuminate several aspects of catalysis. For example, substrate binding is accompanied by a dramatic conformational change involving approximately forty residues. This re-ordering provides a structural basis for the known kinetics of the reaction which requires ordered substrate binding: the conformational change is driven by the binding of acetyl coenzyme A. Only then is the binding site for serotonin completed.

Another feature of catalysis which can be rationalized on the basis of the structures of AANAT is the issue of a general base for the reaction. It had been assumed that a crucial role of the enzyme is to provide a mechanism to deprotonate the primary amine of the substrate (pKa ~10), yet site-directed mutagenesis of residues surrounding the active site failed to identify a single amino acid which was required for deprotonation. Rather, the 1.8 Å crystal structure of the bisubstrate analog complex reveals a clearly defined network of hydrogen-bonded water molecules leading from the site of acetyl transfer, located deep within the protein, to the protein surface. We propose that this "proton wire" serves as the means to remove a proton from the primary amine substrate prior to acetyl transfer, and that a structurally conserved general base is not required for catalysis. This view of catalysis is supported by the crystal structure of the inactive ternary complex between *Tetrahymena* GCN5, CoA, and a histone H3 peptide. In contrast, it has recently been reported that another member of the superfamily, the putative tumor suppressor Fus-2, proceeds through a ping-pong mechanism involving transfer of the acetyl group to an active site nucleophile. On the basis of known structures of members of the GNAT superfamily, such a mechanism appears unlikely.

s8a.m1.p40 **The Structure of 5-Aminolaevulinate Dehydratase (ALAD)**. P. T. Erskine, P. M. Shoolingin-Jordan, S. P. Wood and J. B. Cooper. *School of Biological Sciences, University of Southampton, Southampton, SO16 7PX, UK*, and M. J. Warren, *Biological Sciences, Queen Mary and Westfield College, London, E1 4NS, UK*.
Keywords: haem biosynthesis, 5-aminolaevulinate dehydratase.

ALAD is a key early enzyme of the porphyrin biosynthetic pathway. It catalyses the condensation of two 5-aminolaevulinic acid (ALA) molecules to form the pyrrole porphobilinogen (PBG). The yeast ALAD structure[1], solved by MAD, was used to solve the *E. coli* enzyme at 2.0 Å by molecular replacement. Both ALADs form large homo-octameric structures with 422 symmetry in which each subunit adopts the TIM-barrel fold with a 20-30 residue N-terminal arm forming extensive inter-subunit interactions. The octamer has all eight active sites exposed on the surface. At the base of each active site are two lysine residues, one of which forms a Schiff base link to the substrate ALA. The catalytic zinc ion is held by three cysteines and a solvent molecule. The *E. coli* enzyme possesses another well defined zinc binding site in which the metal ion is coordinated by a Glu and five solvent molecules buried at a subunit interface. This metal binding site must account for the activating effects of magnesium ions. The structures of the several bound inhibitors reveal that a large loop over the active site undergoes a substantial ordering on binding the inhibitor.

[1] Erskine, P., *et al*, "X-ray structure of 5-aminolaevulinate dehydratase, a hybrid aldolase.", Nat. Str. Biol. (1997) 4: 1025-1031.