

NIST Biomolecular Crystallization Database (BMCD): Adenylyl Cyclase Structure and Mechanism Measurements

NIST scientists are studying the bacterial enzyme adenylyl cyclase as a model system for the development of NIST's biomolecular crystallization database (BMCD) of proteins. Crystallization is a key step to protein structure analysis. The BMCD plays a central role in making this process rational and scientific, rather than simply a matter of random screening over thousands of chemical conditions, which can be very expensive in the case of difficult-to-purify human proteins. The predictive value of the BMCD is increased greatly by comparisons between structures and crystal growth effects. The current work on the AC enzyme includes a systematic examination of its crystal growth conditions and the interactions between structure and crystal growth; these data are then used to develop the capacity of the BMCD to utilize such information.

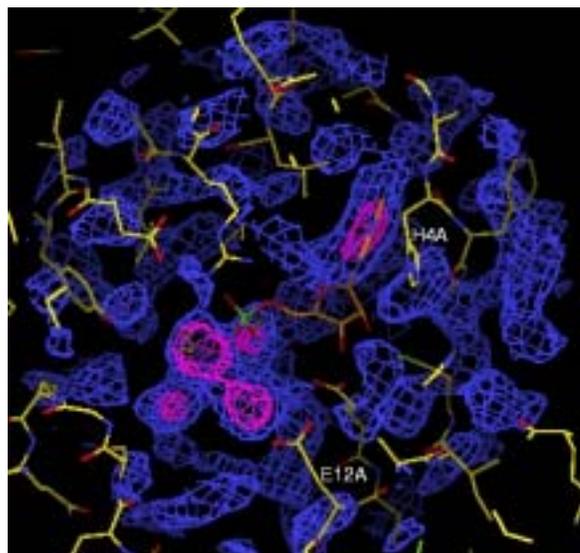
**T. D. Gallagher, N. Smith, S.-K. Kim, and
P. Reddy (Div. 831)**

The enzyme adenylyl cyclase (AC) performs key signaling functions in humans and also in pathogenic bacteria such as those that cause anthrax and tuberculosis. The plague-causing pathogen *Yersinia pestis* contains an AC belonging to a newly characterized class, class IV. Of the six distinct classes of AC enzymes that have been found (distinct protein families that perform the same enzymatic reaction), three have been structurally characterized. The form present in both anthrax and whooping cough bacteria (bacillus anthracis and bordetella perussis), which belongs to class II, the human form, belonging to class III, and the newly characterized form in *Yersinia pestis*, belonging to class IV, all show distinct protein folds. All are active scientific research foci; the class IV enzymes are of special interest because of the pathogenicity of *Y. pestis* and other organisms that contain them, and because of their small size (smallest known AC subunits; in *Y. pestis* the chain length is 179 amino acids) and thermostability (this AC class is also found in hyperstable archaeal organisms).

The BCMD is used by researchers worldwide for development of strategies to crystallize proteins and thus determine the structure of new molecules of interest.

We have expressed, crystallized and structurally characterized the class IV AC from *Y. pestis* at 1.9 Å resolution (PDB:2FJT and J. Mol. Biol. 362, 114-122, 2006). The general region of the active site was identified

based on the structure and on conserved residues in multiple alignments. Recently we have obtained additional diffraction data at high resolution from crystals containing the substrate analog dideoxy-ATP, enabling modeling of the substrate complex and detailed analysis of the catalytic mechanism. **Figure 1** shows a preliminary view of the electron density map for this substrate analog in the active site.



The figure shows the active site region, with initial electron density for the substrate analog ddATP. The cluster of pink balls reports the locations of the three phosphate moieties in ddATP and a manganese ion that stimulates enzyme activity, while the additional pink blob on the upper right represents the adenine ring.

Impact: Even before complete refinement of this active-site complex structure, it reveals several features of the mechanism. For example, the residue E12A is observed as the key anchor for the manganese ion, and the adenine moiety binds near the N-terminus (near histidine H4A in Figure 1). This work will lead directly to further publications of widespread interest, especially regarding protein structure analysis, pathogen biology, enzyme mechanism, and crystallogenesis.

Future Plans: After completing the refinement at 1.8 Å (the resolution of our data), the structural analysis will be combined with our earlier measurements of activity under various conditions and in several key mutants, enabling a thorough treatment of the catalytic mechanism in this novel and important enzyme.