s1.m7.p7 **Crystal structures of conserved hypothetical protein YLBA from three different bacteria.** A.A. Fedorov, E.V. Fedorov, S.C. Almo, *Albert Einstein College of Medicine, Bronx, NY 10461, USA. E-mail: fedorov@aecom.yu.edu*

Keywords: Structural genomics; NYSGXRC; Crystal structures

The structures of three functionally uncharacterized YLBA homologs from Escherichia Coli, Enterococcus faecalis and Deinococcus radiodurans are described. The first structure was solved by SeMet MAD and refined to R(cryst) =0.232, R(free)=0.267 at 2.6Å resolution. The second structure was solved by molecular replacement using first structure as the search model (~55% identity) and refined to R(cryst)=0.208, R(free)=0.220 at 2.0Å resolution. The third structure (~28% identity with first two) was solved by Hg SAD and refined to R(cryst)=0.229, R(free)=0.278 at 2.4Å resolution. All data were collected at NSLS beam line X9A. All three structures have a common fold and differ only in the placement of outer loops segments. The molecule is composed of two similar domains positioned face to face around a pseudo two-fold axis. Each domain contains two antiparallel beta-sheets forming a beta-sandwich. Details of the structures and functional predictions will be presented.

s1.m7.p8 **PEG200 improved crystallisation of a mycobacterial adenylyl cyclase regulatory domain.** Felix Findeisen[a], Irmgard Sinning[a], Joachim Schultz[b], Juergen Linder[b], Ivo Tews[a], [a]*Biochemiezentrum der Universität Heidelberg, INF 328, 69120 Heidelberg, Germany,* [b]*Pharmaceutical Biochemistry, Institute of Pharmacy, University of Tübingen, Morgenstelle 8, 72076 Tübingen, Germany. E-mail: felix.findeisen@bzh.uni-heidelberg.de*

Keywords: Adenylyl cyclase; PEG200; SAD

Mycobacterium tuberculosis is a pathogen causing a million deaths every year. As a human pathogen it encounters many different environmental challenges in its life cycle and each requires a measured and regulated response. Accordingly, it has 15 adenylyl cyclases (compared with 10 in *Homo sapiens*), many of them with predicted attached regulatory domains. We have focussed on Rv1264, which has an N-terminal auto-inhibitory domain and a C-terminal catalytic domain [1]. Each of the two domains contributes about 200 residues to the enzyme. We determined the structure of the intrinsic, auto-inhibitory regulatory domain.

Adenylyl cyclases (AC) are dimers with the active site at the dimer interface and residues from both monomers contributing. For Rv1264 we observe a very tight dimer of the regulatory domains and hypothesise that this dimerisation can result in a reversible misalignment of catalytic domains, thus directly regulating cyclase activity like a protein switch. We present mutagenesis studies to back this hypothesis.

Structural studies of AC catalytic domains of various organisms have elucidated a good deal about the mechanism of the cyclization reaction [2,3] and AC activation by G-proteins and forskolin has been determined in structural terms. However, essentially nothing is known concerning the structure or a potential function of the N-terminally attached huge membrane anchors of mammalian ACs. Here, we present the first structure of an AC regulatory domain present in *Mycobacterium tuberculosis.*

For structure determination with SAD we used a fixed wavelength of 0.934A at ID14.2 at ESRF on selenomethionine substituted protein corresponding to a wavelength 620eV high remote from the absorption edge of selenium. We used the program Shake'n'Bake to find selenium positions. Pivotal in improving crystals from initially 2.7A diffraction to 1.7A, was a change of precipitant from PEG4000 to PEG200, resulting in a refined structure with an R factor of 17.0% and a free R factor of 21.8%. A PEG200 molecule can be modelled into the structure at the interface between crystallographic dimers. Furthermore we find density compatible with a PEG200 buried in the middle of the protein.

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[2] Sunahara *et al.* (1997), *Science* **278**, 1943-7
[3] Tesmer *et al.* (1999), *Science* **285**, 756-60