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*

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*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.

[1] Linder *et al.* (2002), *JBC* **277**, 15271-6
[2] Sunahara *et al.* (1997), *Science* **278**, 1943-7
[3] Tesmer *et al.* (1999), *Science* **285**, 756-60