

m11.p01**Structure of a group III truncated hemoglobin from *Camphylobacter jejuni***Martino Bolognesi^a, Marco Nardini^a, Alessandra Pesce^b, Michel Guertin^c^aDept. of Biomol. Sciences & Biotechn. CNR-INFN, U. of Milano Italy.^bDept. of Physics, U. of Genoa, CNR-INFN, Italy. ^cDept. of Biochem. and Microbiol., U. Laval, Canada. E-mail: martino.bolognesi@unimi.it**Keywords: heme proteins, protein homology, evolution**

Truncated hemoglobins (trHbs) build a protein family loosely related to hemoglobin, showing smaller size and extensive residue substitutions relative to conventional Hbs (< 20% sequence identity). TrHbs, widespread in unicellular organisms and in some plants, bind oxygen, and display diversified functions, including NO-detoxification. The trHb family is divided in three groups, based on amino acid sequences and evolutionary considerations. We have shown in the past that trHbs from group I and II adopt a 2-on-2 alpha-helical sandwich fold, hosting the heme [1]. Moreover, a protein matrix tunnel connecting the heme to the protein surface has been identified [2]. Here we present the first crystal structure of a group III trHb (from *Camphylobacter jejuni*, a micro-aerobic prokaryote parasite; data at 2.5 Å resolution, R-gen 21.9%, R-free 26.6%). We show that the trHb fold is modified mostly at the N-terminus, at the C-CD helical region, and in the matrix tunnel, that is closed and filled by core residues in this protein. We present these results at the light of evolution of the trHb and globin folds, and of the two protein families.

[1] Milani, M., Pesce, A., et al. *J. Inorg. Biochem.*, 2005, 99, 97.[2] Milani, M., Pesce, A., Ouellet, Y., Ascenzi, P., Guertin, M., Bolognesi, M., 2001, *EMBO J.* 20, 3902.**m11.p02****Structural and functional characterization of two new class D β-lactamases: OXA-46 and OXA-48**Y. Calderone¹, F. Giuliani¹, M. Benvenuti¹, J.D. Docquier^{1,2}, P. Nordmann³, G.M. Rossolini¹, S. Mangani¹¹Univ. of Siena, Italy; ²Univ. of Liège, Belgium; ³Univ. of Paris-Sud, France**Keywords: β-lactamases, antibiotic resistance, OXA-lactamases**

The class D β-lactamases represent a heterogeneous family of more than 80 variants exhibiting important functional differences. Some of them have the notable ability to hydrolyze carbapenems, compounds which usually behave as inactivators of these enzymes.

Although some structures of class D enzymes have been determined (e.g. OXA-1, OXA-10 and OXA-13), no structural information on class D carbapenemases is currently available. OXA-46 is a narrow-spectrum enzyme identified in *Pseudomonas aeruginosa* whereas OXA-48, found in a *Klebsiella pneumoniae* clinical isolate from Turkey, is one of the most active class D carbapenemases. Both OXA-46 and OXA-48 were produced in *E. coli* and purified by chromatography.

Diffraction data were collected using the BW7A X-ray beamline at DESY (Hamburg, Germany) and the two structures solved at 2.4 Å resolution (OXA-46) and 2.0 Å resolution (OXA-48) by molecular replacement using the OXA-13 structure as template.

The quaternary structure of OXA-46 and OXA-48 is dimeric both in solution and in the crystal. In contrast with other class D enzymes, both dimers occur without the intervention of metal binding at the dimer interface.

Comparison of the crystal structures of OXA-46 and OXA-48 with that of other class D β-lactamases reveals original features which provide clues to understand the different enzymatic profile and specificity towards β-lactam substrates.