psychrophilic (cold-adapted) subtilisin which shares high sequence identity (60%) with Sph, as well as the same calcium binding mode. We suggest that the five calcium ions found in these two subtilisins act as counter ions for the acidic residues found on their surface. Since Sph is a mesophilic subtilisin and S41 is a psychrophilic subtilisin and their structure is identical, we conclude that Sph and S41 most probably have the same origin and are evolutionally related.

**MS07 P04**

**New insights for the catalytic and inhibition mechanisms of periplasmic Nitrate reductase (Nap) from *Desulfovibrio desulfuricans* from structural and spectroscopic análisis** Shabir Najmudin[a], Pablo J. González[a], Catarina Coelho[a], José Trincão[a], Isabel Moura[a], José J. G. Moura[a], Carlos D. Brondino[b]* and Maria J. Romão[a] [a]*REQUIMTE/CQFB, Departamento de Química, FCT-UNL, 2829-516 Monte de Caparica, Portugal.* [b]*Departamento de Física, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, 3000 Santa Fe, Argentina.*
E-mail: shabir@dq.fct.unl.pt

**Keywords: periplasmic nitrate reductase, Mo-sulfido ligand, novel catalytic mechanism**

The periplasmic nitrate reductase from *Desulfovibrio desulfuricans* ATCC 27774 was isolated from cells grown in anaerobiosis and in the presence of nitrate. This enzyme is isolated as a soluble monomeric protein of 80 kDa having a Mo atom in the active site, coordinated to a *bis*-MGD moiety [1]. Previous EPR studies in both catalytic and inhibiting conditions showed that this Mo ion has a high flexibility of coordination when reacted with reducing agents, substrates or inhibitors. In the present work, samples both in as-purified conditions and reacted with substrates and inhibitors were crystallized and the corresponding structures were solved from 1.99 to 2.45 Å resolution. These results together with a single-wavelength anomalous dispersion experiment at above the iron edge (λ= 1.77 Å) of the as-purified (native) enzyme strongly suggests the presence of a sulfur atom instead of oxygen at the sixth position of coordination of the Mo ion. Analysis of the crystallographic data of the reacted samples of Nap indicates that neither azide nor cyanide binds to the Mo atom, and that perchlorate blocks the funnel-like cavity, hindering the substrate entrance and product release. In addition, EPR studies indicate that the turnover Mo(V) signal is not produced by a Mo-substrate. Based on these results, new hypotheses for the catalytic mechanism are proposed.

[1] Dias J.M., Than M. E., Humm A., Huber R., Bourenkov G. P., Bartunik H. D., Bursakov S., Moura J. J.G., Moura I.,Romão M. J. *Structure*,1999, **7**, 65-79..

**MS07 P05**

**PURY: The database of geometric restraints of hetero compounds** Miha Andrejašič[a], Dušan Turk[a] , [a]*Jožef Stefan Institute, Ljubljana, Slovenija.*
E-mail: miha.andrejasic@ijs.si http://pury.ijs.si/

**Keywords: macromolecular structure refinement, structure determination using X-ray diffraction; topology**

The paper of Engh and Huber (1991) [1] with description of accurate geometrical parameters of amino acid residues has set a new standard in macromolecular crystallography. A similar step forward in the area of nucleic acids was made by Parkinson et al. (1996) [2]. With the increasing number of macromolecular structures also complexes with "hetero" ligands and their variety is growing. Obtaining correct geometry of the hetero ligands is often crucial for understanding of biological relevance of a structure. The correctness of conclusions may however be hampered by the error contained in parameters describing geometry of a compound.
Therefore we have developed a "PURY" database, which contains lists of atom classes, bonds connecting them as well as angle and chirality, planarity and conformation parameters. The three last in the forms of improper and dihedral angles. Each entry in the list has associated target value and a force constant derived from the standard deviation of the list entry. The database is compiled from close to 162540 entries present in the latest release of small molecule crystal structures deposited in Cambridge Crystal Structure Database [3]. PURY database contains about 1946 atom classes, and lists of 28882 bonds, 223059 bonding angles and 235764 dihedral and 62437 improper angle terms.
Direct comparison with Engh Huber parameter set revealed that PURY parameters essentially correspond to EH target values in spite the fact that they have not been generated on a an expert selected list of entries with a much higher number of repetitions. Coordinative bonds with metal ions are included too. The database and the server allow generating parameters also for hydrogen atoms, although these parameters have much larger standard deviations due lower precision of their positions and lack of proper atom class assignment, which does not yet include hydrogen bond analysis.
The database can be used through web server "http://pury.ijs.si/", where from a deposited coordinates in a PDB format, topology and parameter files in forms for refinement programs MAIN, CNS and RefMac are generated. SHELEX output is in progress too. The server will in near future provide topology and geometry parameter files for all currently deposited hetero compounds in Protein Structure Database.
Basics of the server use as well as analysis of accuracy, reliability of the derived terms will be presented and demonstrated.

[1] Engh, R.A. and Huber, R. (1991): Accurate bond and angle parameters for X-ray protein structure refinement. Acta. Cryst. A47, 392-400.
[2] G. Parkinson, J. Vojtechovsky, L. Clowney, A.T. Brunger, H.M. Berman: New Parameters for the Refinement of Nucleic Acid Containing Structures, Acta Cryst. D, 52, 57-64 (1996).
[3] Allen, F.H., Bellard, S., Brice, M.D., Cartwright, B.A., Doubleday, A., Higgs, H., Hummelink, T., Hummelink-Peters, B.G., Kennard, O., Motherwell, W.D.S., et al (1979): The Cambridge Crystallographic Data Centre: computer-based search, retrival, analysis and display of information. Acta Cryst. **B35**, 2331-2339.