s8a.m1.p35 **Temperature-controlled crystallographic experiments on acetylcholinesterase**. M. Weik[1], P. Gros[1], J. Kroon[1], T. Ursby[2], D. Bourgeois[2], R. Ravelli[3], S. McSweeney[3], L. Peng[4], A. Specht[4], M. Goeldner[4], G. Kryger[5], I. Silman[6], J. Sussman[5], *[1]Dept. of Crystal and Structural Chemistry, Bijvoet Center for Biomolecular Research, 3584 CH Utrecht, The Netherlands ; [2]Institut de Biologie Structurale, LCCP, 38027 Grenoble Cedex 1, France ; [3]EMBL Grenoble outstation, B.P. 165, 38042 Grenoble Cedex 9, France ; [4]Lab. de Chimie Bio-organique, Univ. Louis Pasteur Strasbourg, B.P. 24, 67401 Illkirch, France and Depts. of [5]Structural Biology and [6]Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel.*

Keywords: protein dynamics, enzyme function, caged compounds.

Despite its high turnover number, the active site of *Torpedo californica* acetylcholinesterase (*Tc*AChE) is buried at the bottom of a deep and narrow gorge [1]. This raises cogent questions concerning traffic of substrate and products to and from the active site. We are employing cryo X-ray crystallography, in conjunction with caged compounds, to attack this issue. Proteins in flash-cooled crystals gain internal flexibility as the temperature is raised above a dynamical transition temperature. Our approach is to photolyze *Tc*AChE-caged compound complexes at 100K, and to increase the temperature stepwise, collecting structural data at each temperature, to seek a temperature at which putative reaction intermediates accumulate at specific sites.

Caged heavy-atom cholinergic ligands were synthesised [2], and we show that they bind to the *Tc*AChE active site within the crystal. Microspectrophotometry shows that they are photocleaved at cryotemperatures, and X-ray crystallography reveals that, upon raising the temperature, the cleaved products exit the active site.

Control experiments, performed to validate our methodology, showed that intense synchrotron radiation causes specific chemical and structural damage to proteins [3], which is enhanced at higher temperatures.

Photolysis of caged compounds at cryotemperatures, together with temperature controlled X-ray crystallography, thus provide a valuable tool for studying the dynamics of the catalytic activity of AChE.

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s8a.m1.p36 **Crystal Structure of Nitrite Reductase from *Rhodobacter sphaeroides.*** H. Guo[1], K. Olesen[2], Y. Xue[3], L. Sjölin[1]. *[1]Göteborg University, Center for Structural Biology, Dept of Inorganic Chemistry, SE-412 96 Göteborg, Sweden [2] Göteborg University, Dept of Biochemistry and Biophysics, SE-405 30 Göteborg, Sweden [3] Structrual Chemistry Laboratory, AstraZeneca R&D Mölndal, Sweden.*

Keywords: enzyme catalysis, protein engineering.

Nitrogen is introduced into the biosphere by biological and chemical fixation of nitrogen gas, and removed from there again by the process of denitrification. Denitrification is one of the main steps of the global nitrogen cycle that is sustained by prokaryotic organisms. Denitrifying bacteria use two entirely different enzymes in the denitrification step from nitrite (NO_2^-) to nitric oxide (NO), one based on haem cd1 prosthetic groups and the other on type I – type II Cu centres. Nitrite Reductase (Nir) is part of a denitrification pathway and it is a key enzyme in denitrification, since it catalyses the first committed step of denitrification that leads to the gaseous products NO, N_2O or N_2.

High resolution of three-dimensional crystallographic structure of the copper-containing Nir from *rhodobacter sphaeroides* 2.4.3 in *Escherichia coli* (1) has been determined to 1.46 Å resolution by molecular replacement using the Nir from *Achromobacter cycloclastes* as search model (2).

Our copper-containing Nir is a trimeric enzyme that contains copper in two distinct binding sites per monomer and a single high molecular mass peak of 140 ± 15kDa analysis by gel filtration. One of these is essentially a standard type I copper centre(Cu-I; two His, one Cys, and one Met ligands) and is believed to be the redox center that accepts electrons from donor proteins. The other copper center (Cu-II; three His and one solvent ligands) is approximately 12.5 Å distant and can be assigned as the catalytic site, not least because nitrite has been suggested to bind at this site. This finding is based on a number of crystal structure determinations of the enzyme from several sources.

Of particular interest for us concerning the mechanism of action are mutants in the first copper site. We are now preparing the novel M182T mutant since this methionine residue seemingly plays a central role for a suggested gating mechanism.

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