s1.m4.p1 **Crystal structures of the S64C mutant monomer and dimer from *desulfovibrio vulgaris* in the semiquinone state.** [a]Gabriella Bombieri, [a]Roberto Artali, [b]Davide Cavazzini, [c]Gianfranco Gilardi, [a]Nicoletta Marchini, [b]Gian Luigi Rossi, *[a]Institute of Pharmaceutical Chemistry University of Milano,Italy, [b]Department of Biochemistry and Molecular Biology University of Parma.Italy [c]Department of Biological Sciences, ImperialCollege, London. Email: gabriella.bombieri@unimi.it*

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Flavodoxins are small flavoproteins of 14-23 kDa having as cofactor flavin mononucleotide (FMN), found in many microorganisms, where they act as electron carriers at low oxidation-reduction potentials. FMN can accept two electrons and exist in the oxidized, semiquinone and hydroquinone states. Its interactions with the apoprotein modify the two one-electron redox potentials with positive shifts of the midpoint potential at pH 7 of the oxidized/semiquinone couple and negative for the semiquinone/hydroquinone couple. As general trend for the structures of flavodoxins in different oxidation states[1], the reduction to the semiquinone state is accompanied by a movement of the peptide at a glicine residue allowing the formation of a hydrogen bond between the carbonyl oxygen of the glicine and the N(5)H of the reduced FMN. The monomer and dimer of S64C in the semireduced forms crystallize in the tetragonal system space group $P4_32_12$ a=b=50.49,c=137.03 Å (monomer) and $P4_12_12$ a=b=55.05,c=122.62 Å (dimer). The semireduced forms were obtained from the oxidized ones by reduction in aerobic conditions with sodium dithionite. Data sets were collected with syncrotrone radiation (ELECTRA beam line, Trieste, Italy). Structural changes were observed in both monomer and dimer forms with conformational changes at the 60-loop similar to those occurring in the S35C mutant[2] upon reduction to the semiquinone form.

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s1.m4.p2 **Kinetic crystallography on cholinesterases.** J.P. Colletier[1], A. Royant[2], A. Specht[3], F. Nachon[4], G. Zaccai[1], M. Goeldner[3], J.L. Sussman[5], I. Silman[6], D. Bourgeois[2] and M. Weik[1]. *[1]LBM & [2]LCCP, IBS, Grenoble (France) - [3]LCB, ULP, Strasbourg (France) - [4]UE, CRSSA, La Tronche (France) - [5]DSB & [6]DNB, WIS, Rehovot, (Israel). E-mail: colletie@ibs.fr*

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Acetylcholinesterase (AChE) hydrolyses the neurotransmitter acetylcholine very rapidly, as required by its biological function, which is the termination of impulse transmission at cholinergic synapses. The 3D structure of *Torpedo californica* AChE (TcAChE) revealed a buried active site, accessed by a deep and narrow gorge [1]. This structure raises questions concerning the traffic of substrates and products to and from the active site. Substantial 'breathing' motions of the protein are required for penetration of substrates in the active site gorge and for product exit. For AChE, the existence of a 'back-door' has been suggested [2], whose transient opening would allow for rapid choline clearance from the active site. Butyrylcholinesterase (BChE) is a structurally similar enzyme that, unlike AChE, can also hydrolyse butyrylcholine efficiently.

We employ a combination of cryo-cleavage of nitro-phenyl-ethyl-caged choline (photolabile precursors of choline) and temperature-controlled protein crystallography [3] with the ultimate goal of identifying and characterizing structural reaction intermediates in both ChEs.

Caged choline molecules, complexed with crystalline *Human* BChE (*Hu*BChE), were cleaved at 100 K by means of X-ray radiolysis. Subsequently, the temperature was raised transiently to either below (170 K) or above (180 K) the dynamical transition of crystalline *Hu*BChE, which has been determined by temperature-derivative microspectrofluorimetry to take place at 175 K [4]. At 170 K, neither the choline molecule, nor the released "cage" were cleared from the active site, indicating that protein and solvent dynamics are too reduced to allow traffic of products to occur. Above the dynamical transition, in contrast, occupancies of both the cage and the choline molecules were almost zero, thus structurally evidencing the importance of this transition. As anticipated, however, severe X-ray radiation damage to the protein was induced by cryo-radiolysis at 100 K and subsequent temperature increase.

Employing cryo-photolysis (at 100 K) by laser illumination instead of cryo-radiolysis, X-ray radiation damage could be minimized. After heating to 180 K (above the dynamical transition), and despite reduced cleavage efficiency relative to radiolysis, occupancies of photolysis products had decreased in both *Tc*AChE and *Hu*BChE. The exit of choline was concomitant with the development of local disorder, observed specifically for some residues whose counterparts in TcAChE are putatively involved in forming a backdoor.

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