	Experiment title: The Active Conformation of Glutamate Synthase and its Binding to Ferredoxin	**Experiment number**: LS2183
Beamline: ID14-EH1 ID14-EH2 ID14-EH4	**Date of experiment**: from: 28 February 2002 to 01 March 2002 from: 12 June 2002 to 14 June 2002 from: 15 July 2002 to 16 July 2002 from: 17 July 2002 to 18 July 2002	**Date of report**: 15/7/03
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Report:

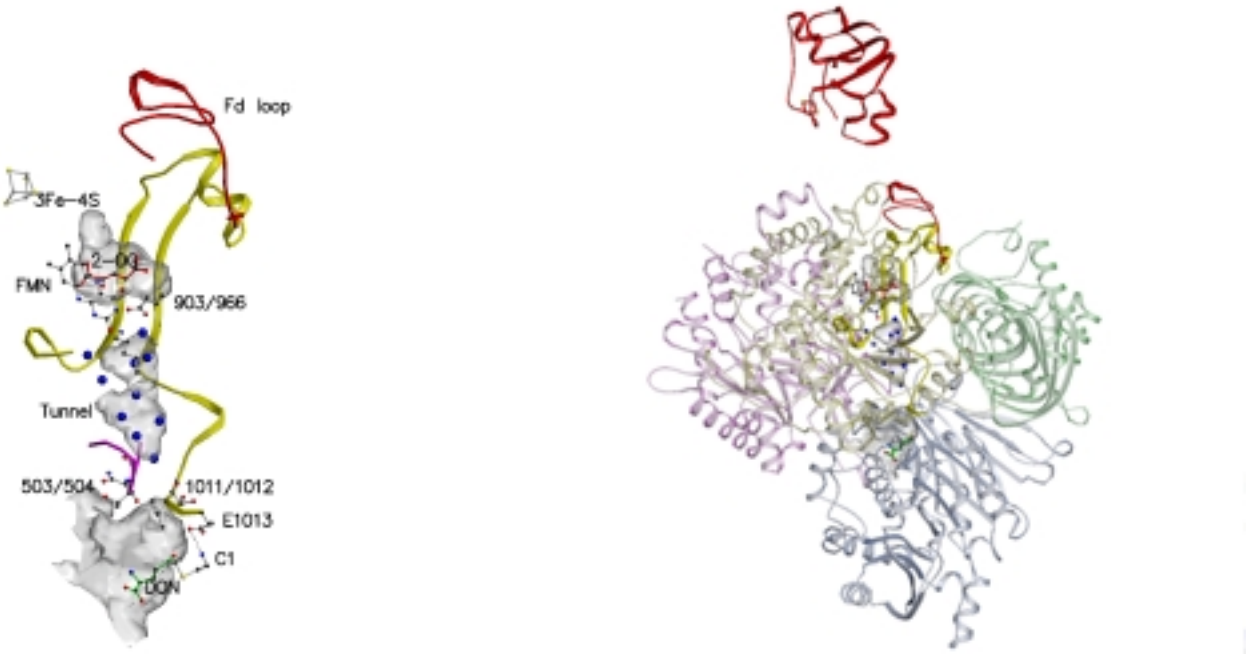
The results obtained thanks to the experiments carried out at the ESRF have been published in 2003:

van den Heuvel, R.H.H., Svergun,D.I., Petoukhov, M.V. Coda, A., Curti, B., Ravasio, S., Vanoni, M.A., Mattevi, A. (2003) The Active Conformation of Glutamate Synthase and its Binding to Ferredoxin. *J. Mol Biol*, 330, 113-128

As a summary of the work carried out at the ESRF, we include here the abstract of the published article.

Abstract

Glutamate synthases (GltS) are crucial enzymes in ammonia assimilation in plants and bacteria, where they catalyze the formation of two molecules of L-glutamate from L-glutamine and 2-oxoglutarate. The plant-type ferredoxin-dependent GltS and the functionally homologous α subunit of the bacterial NADPH-dependent GltS are complex four-domain monomeric enzymes of 140-165 kDa belonging to the NH_2-terminal nucleophile family of amidotransferases. The enzymes function through the channeling of ammonia from the N-terminal amidotransferase domain to the FMN-binding domain. Here, we report the X-ray structure of the *Synechocystis* ferredoxin-dependent GltS with the substrate 2-oxoglutarate and the covalent inhibitor 5-oxo-L-norleucine bound in their physically distinct active sites solved using a new crystal form. The covalent Cys1-5-oxo-L-norleucine adduct mimics the glutamyl-thioester intermediate formed during L-glutamine hydrolysis. Moreover, we determined a high resolution structure of the GltS:2-oxoglutarate complex. These structures represent the enzyme in the active conformation. By comparing these structures with that of GltS α subunit and of related enzymes we propose a mechanism for enzyme self-regulation and ammonia channeling between the two active sites. X-ray small-angle scattering experiments were performed on solutions containing GltS and its physiological electron donor ferredoxin (Fd). Using the structure of GltS and the newly determined crystal structure of *Synechocystis* Fd, the scattering experiments clearly showed that GltS forms an equimolar (1:1) complex with Fd. A fundamental consequence of this result is that two Fd molecules bind consecutively to Fd-GltS to yield the reduced FMN cofactor during catalysis.



A **B**

Figure 1. *A.* Ammonia channeling between glutaminase and synthase sites. From bottom to top, the diagram shows the Cys1-5-oxo-L-norleucine covalent adduct (red) and the open cavity in the glutaminase site, residues Thr503-Asn504 and Ser1011-Ile1012, the closed tunnel lined by residues from the central and FMN-binding domain with the ordered water molecules, residues Glu903 and Lys966 and the closed 2-oxoglutarate (green) binding cavity in the synthase site. Surfaces are shown for the open cavity in glutaminase site, the closed tunnel and the closed cavity in the synthase site *B.* The positions of the cavities in Fd-GltS and the structure of ferredoxin. The position of ferredoxin relative to Fd-GltS is not based on experimental evidence. The coloring is identical to that of Figure 1*A* and Fd is depicted in red.