

Structural biology and oxidative stress: X-ray crystallography of aggregates and proteins



Our laboratory has a long tradition in structural biology research, using mainly X-ray crystallography. Over the years, we have worked on the structure determination and analysis of a number of biological systems spanning in size from small peptides and oligonucleotides to large molecular aggregates, such as virus antibodies and receptor complexes or vaults. In many cases, we have established fruitful collaborations with groups working on the biological or biomedical aspects of these systems. In addition, the laboratory has focused on a number of biological issues. For example, in recent years, we have been deeply involved in structural and functional studies of proteins related to oxidative stress, using both theoretical (computational) and experimental approaches, in particular X-ray crystallography. We also consider it a priority to work on methodologically challenging problems of structural biology, both for their intrinsic scientific interest and the new avenues they often open but also as a way to maintain and increase the professional skills and specialisation of the laboratory.

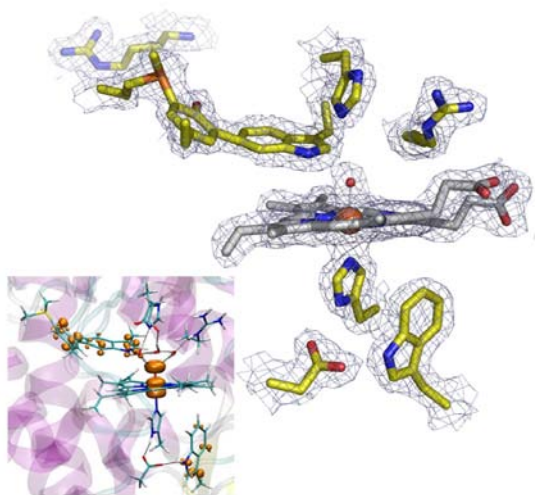


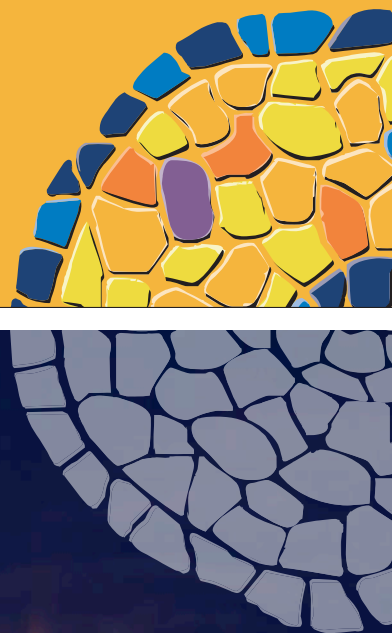
Figure 1. We have shown that the KatG-specific adduct formed by a Met, a Tyr and a Trp and located at the active site acts as a one-electron donor towards the high-valent iron-oxo porphyrin (as illustrated by the spin density distribution shown in the insert). This result is in line with recent experimental findings suggesting the participation of protein-based radicals in the activation of isoniazid, a front line drug in the treatment of tuberculosis, by KatG.

Structure determination of oxidative stress systems and other large molecular aggregates

We have continued our research into systems related to oxidative stress. In particular, we have done extensive work on the catalase-peroxidase system in an attempt to clarify the biochemical mechanisms that allow the function of these moonlight enzymes and also because of its crucial role in the activation of isoniazide, one of the main anti-tubercular treatments. This work has been done in close collaboration with Peter C Loewen at the University of Manitoba (Canada) and with Carme Rovira (an ICREA scientist at the Barcelona Science Park). Also in the field of oxidative stress, we have continued our scientific collaboration with Xavier Parés and Jaume Farrés (at the Autonomous University of Barcelona, UAB), concentrating efforts mainly on the human enzymes p53-inducible quinone oxidoreductase (PIG3) and aldo-keto reductase AKR1B10.

Our group has also been involved in the structure determination of a number of large molecular aggregates. In particular, in a collaboration project led by Nuria Verdaguer (Institute of Molecular Biology of Barcelona, IBMB-CSIC), we have determined the structures of the seven N-terminal domains of the major protein from the ribonucleoprotein vault particles at almost atomic resolution, and of the intact vault particles at 8 Å resolution. These results have been submitted for publication and might explain (among other things) the opening mechanisms of these large particles. In a continuing collabo-

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ration we have had for many years with Vicente Rubio (Instituto de Biomedicina de Valencia, IBV-CSIC), we have also contributed to the structure determination and analysis of a number of kinase complexes. The paper in *Current Opinion in Structural Biology* (by Llacer, Fita and Rubio, 2008) reviews some of the most relevant recent results on arginine and nitrogen storage that we (and others) have obtained in these systems.

Deciphering membrane proteins through X-ray crystallography

For the last few years, the laboratory has focused consider-

able efforts on membrane proteins, one of the main challenges in protein crystallography. Some very promising results have now been obtained thanks to a close collaboration headed by Manuel Palacín (Molecular Medicine Programme, IRB Barcelona) on the structure-function relationship in heteromeric amino acid transporters (HATs). The structural studies of HATs has allowed us to define their oligomeric state, membrane topology and the key residues involved in amino acid transport. The new structural and mechanistic insights into PKC α -C2 domain association (Guerrero-Valero *et al*, *PNAS*, in press) and the prokaryotic secreted lipoygenases (Carpena *et al*, in preparation) contribute to our knowledge of membrane-related proteins.

Publications

Brucet M, Querol-Audí J, Bertlik K, Lloberas J, Fita I and Celada A. Structural and biochemical studies of TREX1 inhibition by metals. Identification of a new active histidine conserved in DEDDh exonucleases. *Protein Sci*, 17, 2059-69 (2008)

Llácer JL, Fita I and Rubio V. Nitrogen storage and arginine. *Curr Opin Struct Biol*, 18, 673-81 (2008)

Querol-Audí J, Konecsni T, Pous J, Carugo O, Fita I, Verdaguer N and Blaas D. Minor group human rhinovirus-receptor interactions: geometry of multimodular attachment and basis of recognition. *FEBS Lett*, Epub ahead of print (2008)

Richardson JS, Carpena X, Switala J, Perez-Luque R, Donald LJ, Loewen PC and Oresnik IJ. RhaU of *Rhizobium leguminosarum* is a rhamnose mutarotase. *J Bacteriol*, 190(8), 2903-10 (2008)

Rovira C, Rodríguez-Fortea A, Alfonso-Prieto M, Vidossich P, Carpena X, Fita I and Loewen PC. Electronic state of the molecular oxygen released in the catalase reaction. *J Phys Chem*, 112, 12843-48 (2008)

Ruiz FX, Gallego O, Ardèvol A, Moro A, Domínguez M, Álvarez R, de Lera AR, Rovira C, Fita I, Parés X and Farrés J. Ring-oxidised retinoid specificity and retinoic acid signalling of aldo-keto reductase AKR1B10. *Chem Biol Interact*, Epub ahead of print (2008)

Large molecular aggregates

Nuria Verdaguer, Institute of Molecular Biology of Barcelona (Barcelona, Spain)

Oxidative stress-related systems

Peter C Loewen, University of Manitoba (Winnipeg, Canada)

Pathogenicity in Mycoplasmas

Enric Querol and Jaume Piñol, Autonomous University of Barcelona (Barcelona, Spain)

Structural and biochemical studies of TREX1 inhibition

Antonio Celada, IRB Barcelona (Barcelona, Spain)

Structural characterisation of enzymatic systems involved in cellular detoxification and regulation

Xavier Parés and Jaume Ferrés, Autonomous University of Barcelona (Barcelona, Spain)

Structure determination and analysis of kinase complexes

Vicente Rubio, Institute of Biomedicine of Valencia (Valencia, Spain)

Structure-function relationship in heteromeric amino acid transporters (HATs)

Manuel Palacin and Modesto Orozco, IRB Barcelona (Barcelona, Spain)

Research networks and grants

Estructura y función de hemo-enzimas

Spanish Ministry of Science and Innovation, BFU2005-08686-C02-01 (2005-2008)

Principal investigator: Ignasi Fita

Unravelling the molecular mechanism of nitrosative stress resistance in tuberculosis

European Commission, HEALTH-F3-2008-223335 (2008-2011)

Principal investigator: Ignasi Fita

Collaborations

Catalytic mechanism and regulation of glycogen synthase

Joan Guinovart, IRB Barcelona (Barcelona, Spain), Miquel Pons, IRB Barcelona (Barcelona, Spain), Joan Carles Ferrer, University of Barcelona (Barcelona, Spain)