

Ignasi Fita



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 structural 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 skills and specialisation of the laboratory.

Structure determination of oxidative stress systems and other large molecular aggregates

Our group works on the structural 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 (Querol-Audí, Casañas *et al*, 2009). With a mass of 13 Megadaltons and overall dimensions of 400*400*700 Å, the vault complex is the largest ribonucleoprotein particle found in eukaryotes. In mammals, vaults contain three proteins: the 100-kDa major vault protein (MVP), the 193-kDa vault poly(ADP-ribosyl)ating polymerase and the 240-kDa telomerase-associated protein. In addition, at least one small and untranslated RNA is found as a constitutive component. Approximately 75% of the vault particle mass is due to MVP. When rat MVP is expressed in insect cells it has the capacity to produce vault-like particles similar to endogenous vaults. Despite their diverse origin, vaults are uniform in size and morphology, presenting a barrel-like structure with an invaginated waist and two protruding caps, as observed by electron microscopy. The finding that the murine MVP is orthologous to the earlier described human lung resistance-related protein, known to be overexpressed in multiple chemotherapy resistance models, prompted the association of vaults with intrinsic drug resistance. Vaults have also been implicated in the regulation of several cellular processes, including transport mechanisms, signal transduction and immune responses. A large and increasing number of proteins have been found to interact with vaults, in particular, it is now well established that the tumour-suppressor

phosphatase PTEN binds to N-terminal repeats R3-R4 of MVP in a Ca²⁺ dependent manner.

In continuing and highly productive collaboration with Vicente Rubio (Instituto de Biomedicina de Valencia, IBV-CSIC) for more than ten years, we have participated in the structural determination and analysis of a number of kinase and large kinase complexes. Two new papers have derived from this research, one is now in press (Ramon-Maiques *et al*) and the other under revision (Gil-Ortiz *et al*).

We have also continued our research into systems related to oxidative stress, including mammalian peroxidases (Carpena *et al*, 2009) and plant peroxidases (Vidossich *et al*, in press). 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 Carme Rovira (ICREA scientist at the Barcelona Science Park).

Also in the field of oxidative stress, we have continued our collaboration with Xavier Parés and Jaume Ferrés (Autonomous University of Barcelona-UAB) on the human enzymes P53-inducible quinone oxidoreductase (Porte *et al*, 2009) and aldo-keto reductase AKR1B10 (Xavier-Ruiz *et al*, 2009). The critical tumour-suppressor P53 regulates the expression of P53-induced genes (PIGs), which trigger apoptosis. PIG3 is the only known member of the medium-chain dehydrogenase/reductase super-



family induced by P53 and it is used as a pro-apoptotic marker as the participation of PIG3 in the apoptotic pathway is extensively documented. In the study by Porte *et al*, we found that *in vitro* activity and *in vivo* overexpression of PIG3 leads to the accumulation of reactive oxygen species (ROS). Accordingly, an enzymatically inactive PIG3 variant (Ser151Val) did not produce ROS in cells. This observation supports the notion that PIG3 action is exerted through oxidative stress produced by its enzymatic activity, thus providing essential information for the potential control of apoptosis. Aldo-keto reductases (AKRs) are monomeric NAD(P)H-dependent enzymes. These molecules exert detoxifying activity and therefore contribute to phase I drug metabolism. AKR1B10 (aldose reductase-like or human small intestine reductase) is the most active AKR with all-trans-retinaldehyde, a crucial molecule in the retinoic acid synthesis pathway. AKR1B10 is induced in several types of cancer and is proposed to be involved in the onset of carcinogenesis, thus stressing its potential use as a diagnostic marker for smoking-related lung cancer and as a potential therapeutic target.

Deciphering membrane proteins through X-ray crystallography

For the last few years, our laboratory has devoted considerable effort to membrane proteins, a major challenge in protein crystallography. Some very promising results have now been obtained thanks to close collaboration with Manuel Palacín (Molecular Medicine Programme, IRB Barcelona), who leads research on the structure-function relationship of amino acid transporters.

The new structural and mechanistic insights into the PKC α -C2 domain association (Guerrero-Valero *et al*, 2009) and on the prokaryotic secreted lipoxygenases (Carpena *et al*, in preparation) have also contributed to our experience on X-ray crystal studies of membrane and membrane-related proteins.

Research Group Members

Group Leader:

Ignasi Fita

Postdoctoral Fellows:

Xavi Carpena, Antonio Rodriguez

PhD Students:

David Aparicio, Bárbara Machado, Luca Martinelli

Lab Technician:

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Visiting Student:

Maria Adell (Spain)



Scientific output

Publications

Carpena X, Vidossich P, Schroettner K, Calisto BM, Banerjee S, Stampfer J, Soudi M, Furtmüller PG, Rovira C, Fita I and Obinger C. Essential role of proximal histidine-asparagine interaction in mammalian peroxidases. *J Biol Chem*, **284**(38), 25929-37 (2009)

Guerrero-Valero M, Ferrer-Orta C, Querol-Audí J, Marin-Vicente C, Fita I, Gómez-Fernández JC, Verdaguer N and Corbalán-García S. Structural and mechanistic insights into the association of PKC α -C2 domain to PtdIns(4,5)P₂. *Proc Natl Acad Sci USA*, **106**(16), 6603-07 (2009)

Porté S, Valencia E, Yakovtseva EA, Borràs E, Shafqat N, Debreczeny JE, Pike AC, Oppermann U, Farrés J, Fita I and Parés X. Three-dimensional structure and enzymatic function of proapoptotic human p53-inducible quinone oxidoreductase PIG3. *J Biol Chem*, **284**(25), 17194-205 (2009)

Querol-Audí J, Casañas A, Usón I, Luque D, Castón JR, Fita I and Verdaguer N. The mechanism of vault opening from the high resolution structure of the N-terminal repeats of MVP. *EMBO J*, **28**(21), 3450-57 (2009)

Querol-Audí J, Konecni 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*, **583**(1), 235-40 (2009)

Ruiz FX, Gallego O, Ardèvol A, Moro A, Domínguez M, Álvarez S, Álvarez R, de Lera AR, Rovira C, Fita I, Parés X and Farrés J. Aldo-keto reductases from the AKR1B subfamily: retinoid specificity and control of cellular retinoic acid levels. *Chem Biol Interact*, **178**(1-3), 171-77 (2009)

Research networks and grants

Ajuts a grups de recerca reconeguts
Agency for Administration of University and Research Grants (AGAUR), 2009-SGR-1309 (2009-2012)
Principal investigator: Ignasi Fita

Análisis estructural de las proteínas peroxisomales: Enzimas metabólicos y peroxinas
Spanish Ministry of Science and Innovation, BFU2009-09268 (2009-2012)
Principal investigator: Ignasi Fita

Biología estructural del peroxisoma
Spanish Ministry of Science and Innovation, BFU2008-01539 (2009)
Principal investigator: Ignasi Fita

Unraveling the molecular mechanism of nitrosative stress resistance in tuberculosis (NOSTRESS)
European Commission, HEALTH-F3-2008-223335 (2008-2011)
Principal investigator: Ignasi Fita

Collaborations

Catalytic mechanism and regulation of glycogen synthase
Joan Carles Ferrer, University of Barcelona (Barcelona, Spain); Joan Guinovart, IRB Barcelona (Barcelona, Spain); Miquel Pons, IRB Barcelona (Barcelona, Spain)

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); Carme Rovira, ICREA-PCB (Barcelona, Spain)

Pathogenicity in mycoplasmas
Enric Querol and Jaume Piñol, Autonomous University of 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 Palacín and Modesto Orozco, IRB Barcelona (Barcelona, Spain)