**Structure, dynamics and interactions of flexible proteins and ligands**

*E pur si muove* was the dramatic claim of Galileo Galilei after he was forced to retract his ideas of a universe not centred on a static Earth. Dynamic macromolecular structures and interactions are now recognised as key elements of life and the basis of function. Efficient regulation relies on narrow equilibrium conditions that can be shifted in response to stimuli. To maintain responsiveness, the energy changes must be small, *i.e.*, involving weak and dynamic interactions. Intrinsically unfolded proteins (IUPs) exist in a dynamically exchanging ensemble of multiple conformations, which are easily perturbed by interactions with specific targets. Eukaryote leverage of IUPs results in 30% of their proteins having unfolded regions, in contrast to 4% in eubacteria and 2% in archaea. In this regard, 80% of the proteins associated with human cancer have unfolded regions. A recent report in *Science* demonstrates that IUPs are themselves tightly regulated and describes their central physiological role. Our group addresses the challenge of flexible systems by combining powerful techniques, such as NMR, Small Angle X-ray Scattering (SAXS), and fluorescence spectroscopy with new interpretation paradigms, taking into account the emerging properties of ensembles and networks. Biomedical applications of our research are pursued in the fields of cardiovascular diseases, bacterial pathogenicity, antibiotic resistance, and network pharmacology.

*Figure 1.* (a) Pure curves for the major (red) and minor (green) species in the oligomerisation equilibrium of bovine low molecular weight phosphatase. (b) Low resolution structures (cyan spheres) superimposed to the X-ray structures of the monomer (red) and dimer (green). The two sets of images are orthogonal views.
Nucleoid-associated proteins and horizontal gene transfer

Our group maintains a stable collaboration with Antonio Juarez (University of Barcelona and Institute for Bioengineering of Catalonia) for the structural study of nucleoid-associated proteins of the H-NS and Hha families, and their interaction partners.

H-NS is a general regulator of gene expression in response to environmental challenges, such as changes in osmolarity or temperature. It has also been described as a genome guardian because of its role in providing a stealth effect by facilitating the acquisition of horizontally transferred genes. Horizontal gene transfer is the process of intercellular exchange of DNA fragments, thereby providing the bacteria with new capacities. Transfer can take place between individuals of the same species or between distinct bacterial species. One of the most studied cases of this transfer is the spread of antibiotic resistance to cells that have not previously been exposed to the drug. Mobile genetic elements may occur in autonomously replicating plasmids or be integrated in the bacterial chromosome. Many of the genes involved in pathogenicity are associated with horizontally transferred genes.

H-NS is an abundant protein encoded in the bacterial chromosome of many gram-negative bacteria. Related proteins are also encoded in plasmids associated with bacteria belonging to the enteric group. One of the current challenges is to elucidate the differential regulation of two large groups of genes that are under the control of H-NS but that are repressed under different conditions. On the one hand, there are the ‘house-keeping’ genes, which ensure the metabolic response to temperature and osmolarity, for example, and on the other hand, the horizontally transferred genes - often associated with pathogenic phenotypes - that are de-repressed during host colonisation. While changes in temperature and osmolarity are among the environmental cues used by bacteria to signal host colonisation, the differentiation between house-keeping and pathogenicity-related genes remains poorly understood. Two recent advances in this regard have been provided by observations made by Antonio Juarez’s group: the role of the helper protein Hha and the differential regulation by plasmidic forms of H-NS. In both cases, the gene-pool regulated
Our group is studying several paralogues of Hha, including YmoA, the paralogue of Hha in Yersinia, and Ydgt. YmoA and Ydgt show interactions with other proteins that do not belong to the H-NS family. These intriguing results may indicate moonlighting behaviour of the Hha family of proteins or point to a higher level integration of the H-NS-based regulatory system. Jesús García leads this line of research. Other ongoing collaborations are with Marc Baldus’ group in Utrecht, which has already succeeded in the observation and partial assignment of solid-state NMR spectra of full length H-NS. At present, we are comparing the apo- and DNA-bound forms. We are also collaborating with Juan Recio (Barcelona Supercomputing Center) to include Paramagnetic Relaxation Enhancement (PRE) information, as well as the effect of point mutations and NMR chemical shifts to derive a docking model of the Hha-H-NS complex. Additional collaborations are maintained with Modesto Orozco (IRB Barcelona) for the prediction of differential DNA features of HTG and house-keeping genes, and with Félix Ritort, in the use of optical tweezers to study DNA-H-NS complexes.

Weak protein-protein oligomers. NMR and SAXS studies

Our group has a long-standing interest in mammalian low molecular weight tyrosine protein phosphatases (lmwPTPs) and their oligomerisation through the active site. We have hypothesised that inactive oligomers are supramolecular pro-enzymes that are maintained in an inactive form until competition with a phosphorylated substrate triggers the release of the active monomer. This hypothetical mechanism would allow signalling pulses to be transmitted, as in the absence of substrate the phosphatase would return to its inactive oligomeric form. The high dissociation constant of the dimer in vitro causes two types of problems: a technical one derived from the low concentration of the oligomeric forms in vitro, even at high protein concentrations, and a conceptual one, namely how to demonstrate the biological relevance of such a weak interaction mechanism.

During 2008, work in the group carried out under the direct supervision of Pau Bernadó has provided answers to both problems. Concerning the technical issue, we have developed a method to deconvolute SAXS curves recorded at a range of protein concentrations in order to extract the SAXS profiles of the ‘pure’ species (Blobel et al., J Am Chem Soc, in press, 2008). Low resolution structures of both species can be derived from the pure curves, in perfect agreement with the independently determined X-ray structures, thereby confirming that it is possible to extract structural information from a minor species in an equilibrium mixture (Figure 1).

With regard to the physiological relevance of the weak oligomerisation, we have demonstrated that a low molecular weight phosphatase from Bacillus subtilis, initially studied by Chang-wen Jin (Beijing) by means of NMR, shows a completely analogous behaviour to the eukaryotic form, in spite of non-trivial sequence differences and distinct substrate selectivity (Blobel et al., in preparation). To the best of our knowledge, this is the first demonstrated example of the conservation of a weak protein-protein interaction and it reinforces our previous hypothesis of a physiological role for this interaction.

by H-NS can be divided in two groups, one containing mostly horizontally transferred genes (Baños et al, submitted, PLoS Genet, 2008).

Structural studies performed by our group are shedding light on both mechanisms. The active role of Hha is demonstrated by the lack of activity of a Hha mutant that retains the three-dimensional (3D) structure and the capacity to bind H-NS of the wild-type (Cordeiro et al, 2008). Structural differences have been observed by NMR in the C-terminal domain of plasmidic and chromosomal H-NS (Fernández de Alba et al, in preparation). These results are reinforced by complementary work with the C-terminal domain of Ler, which shows high sequence homology with the DNA-binding domain of the two forms of H-NS. After a careful evaluation of a variety of DNA sequences, the 3D structure of DNA-bound Ler is currently being determined by solution NMR by Tiago Cordeiro in collaboration with the Griesinger group in Göttingen (Germany).
Our group also addresses another protein, STAT5a, involved in signal transduction pathways. This protein has been studied by SAXS in order to differentiate between distinct proposed models for the dimer present in equilibrium with the monomer. Like the low molecular weight phosphatase, STAT5a shows a large dissociation constant. (Bernadó et al, in press, 2009).

Intrinsically unfolded proteins: the unique domain of c-Src

The unfolded unique domain of human tyrosine kinase c-Src has become a focal point in our group.

In contrast to the well-folded multidomain core, which is well conserved among diverse family members, the unique domain of the proteins belonging to the Src family differ completely. Although the unique domain is essential for substrate specificity, very few structural studies have been performed as a result of the technical and interpretative difficulties associated with IUPs. The complete NMR assignment of the domain, achieved by Yolanda Pérez using carbon-detected NMR experiments, has allowed us to study the conformational ensembles of the unique domain using PRE of spin-labelled derivatives and Residual Dipolar Couplings (RDC). We have also studied the forms phosphorylated in Ser17, Thr37 and Ser75 (Figure 2).

We have recently started a new study funded by the ‘Marató de TV3’ Foundation call for research projects on cardiovascular diseases. In this project we examine the unique domains of several members of the Src family of kinases and their possible cardioprotective action. The underlying hypothesis is based on the observation of a cardioprotective interaction between the ND2 mitochondrial protein and the unique domain of c-Src.

New tools for leveraging the power of small molecules in structural biology

The LINGO concept for efficient molecular similarity calculations has been combined with a new clustering method published in Science in 2007 by Frey and Dueck. We have developed a new method by which we have been able to derive the intrinsic structure of the NIH PubChem database, which contains 19 million compounds. The method identifies around 50 eigenmolecules, molecules that show very little similarity between them but that, collectively, show similarity to most (99.9%) of the molecules in the database. Although no information on biological function was used for the classification, the clusters derived succeed in grouping the ligands of most of the 40 targets in the Directory of Useful Decoys (DUD) database (Figure 3; Cincilla et al, in preparation).

Physicist Cristina Gabellieri has joined the group this year to explore the use of Dynamic Nuclear Polarisation to increase the sensitivity of NMR, especially for small molecules. High nuclear polarisation is transferred from that of unpaired electrons at temperatures close to 1K (~272°C) and the polarised nuclei are rapidly (less than two seconds) transported to a conventional NMR instrument and measured at room temperature. Signal enhancements higher than 2000 can now be easily achieved for slowly relaxing nuclei.

**Publications**


Research networks and grants

**Acceso externo a la ICTS de Barcelona**
**Principal investigator:** Miquel Pons

**Acción de mejora de la infraestructura científico-técnica singular de RMN de Barcelona**
Spanish Ministry of Science and Innovation, ICTS2006-05 (2007-2008)
**Principal investigator:** Miquel Pons

**Dominis únics de quinases de la família Src implicats en malalties cardiovasculars**
‘La MTV3’ Foundation, 81510 (2008-2011)
**Principal investigator:** Miquel Pons

**EMAR-Multidisciplinary frontiers of magnetic resonance support**
**Principal investigator:** Miquel Pons

Collaborations

**Bacterial nucleoid-associated proteins**
Antonio Juárez, University of Barcelona and Institute for Bioengineering of Catalonia (Barcelona, Spain)

**Computational studies in drug design**
Michael Thormann, Origenis (Munich, Germany)

**Characterisation of unfolded states**
Javier Sancho, University of Zaragoza and Institute for Biocomputation and Physics of Complex Systems-BIFI (Zaragoza, Spain)

**NMR-based protein-protein docking**
Juan Recio, Barcelona Supercomputing Center (Barcelona, Spain)

**NMR, Hoffmeister effects and uroporphyrinogen**
Oscar Millet, CIC bioGUNE (Bilbao, Spain)

**Ribosomal proteins**
Mikael Akke, Lund University (Lund, Sweden)

**SAXS**
Dimitry Svergun, European Molecular Biology Laboratory (Hamburg, Germany)

**Solid state NMR studies of H-NS**
Marc Baldus, University of Utrecht (Utrecht, The Netherlands)

**Target characterisation in drug design**
Andrew Marsh, University of Warwick (Coventry, UK)

**Unfolded proteins and residual dipolar couplings**
Martin Blackledge, Institut de Biologie Structurale (Grenoble, France)

Honours

Steering committee member, ISMAR
Board of Trustees member, EUROMAR