01 Macromolecular Structures

The activity of the department is focussed on the area of Structural Biology. The groups are involved in different aspects of the determination of the structure of macromolecules, their interactions and the molecular basis of their function.

One of the main strengths in this department is the presence of several groups with ample experience in advanced microscopy methods, ranging from cryo-electron microscopy and three-dimensional single particle reconstruction, tomography and correlative methods. This unique critical mass of expert microscopists host the Instruct Image Processing Centre, and maintain strong collaborations with ALBA and other European synchrotrons to develop X-ray imaging methods.

X-ray crystallography has grown in the department in the last few years to become a major activity, developing interfaces with the microscopy groups as well as with many other groups in the CNB and abroad. The analysis and manipulation of isolated macromolecules and complexes is also a main topic in the activity of the department, including a formal collaboration with the IMDEA Nanoscience.

The department also hosts the coordinating node for the Spanish Proteomic Network, running several projects based on advanced methods in mass spectrometry, with emphasis on high-throughput analyses of post-translational modifications. The incorporation of a Functional Bioinformatics Unit has reinforced the activity of the department in different “Omics” projects.

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SELECTED PUBLICATIONS

R. Navajas, A. Paradela, J. P. Albar, Immobilized metal affinity chromatography/reversed-phase enrichment of phosphopeptides and analysis by CID/ETD tandem mass spectrometry. Methods Mol Biol 681, 337-48 (2011).

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Functional proteomics

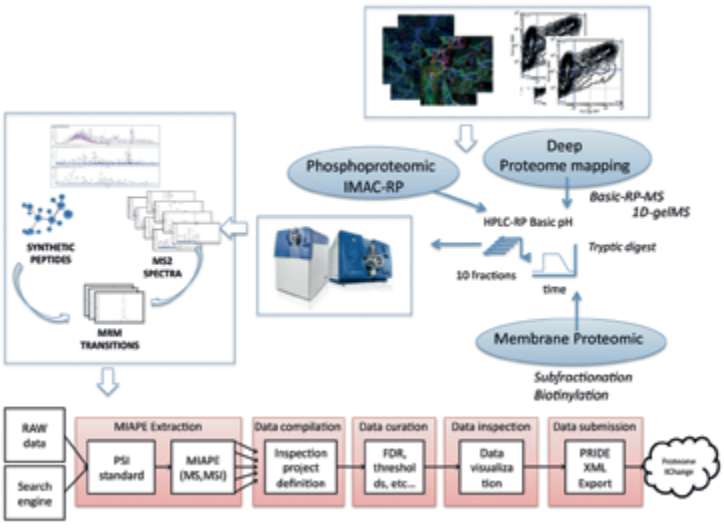
Functional proteomics aspires to draw a complete map of protein dynamics, interactions and posttranslational modifications that take place in the cell. Our goals within the CNB Functional Proteomics Group are to develop and apply state-of-the-art tools to monitor proteins involved in molecular interactions and pathways relevant to pathologies in a variety of tissues, cell types and organisms after various experimental treatments/conditions. We incorporate the latest methodologies to specific functional proteomic projects:

1. Human Proteome Project: This project was launched by the HUPO to systematically map the whole human proteome. The Chromosome-Centric HPP focusses on constructing a protein catalogue on a chromosome-to-chromosome basis. Our main goal is to design experimental approaches to detect and quantify both the "conspicuous" and the "hidden" proteome. This is being driven by the most advanced unbiased shotgun approaches and targeted profiling by selected reaction monitoring (S/MRM).

2. Signal transduction networks untangled by phosphoproteomic analyses: We are focussing on TCR signalling and the role of diacylglycerol production on the control of this response. A combination of phosphopeptide enrichment and SILAC labelling has been implemented for accurate phosphoprotein and phosphopeptide analysis and quantitation.

3. Interactomics: The CAM project "Interactomics of the Centrosome" aims to characterise interactions between centrosomal proteins and to identify macromolecular complex components by proteomics approaches based on affinity tags, stable isotopic labelling, mass spectrometry and peptide arrays.

4. Computational proteomics covers data analysis obtained from large-scale experiments and meta-annotation of proteins and protein complexes. This includes: a) probability-based methods for large-scale peptide and protein identification and quantitation from mass spectrometry data, b) strategies for data mining visualisation, and c) data analysis tools for integration, validation, inspection, deposition and reporting. See ProteoRed MIAPE WTK available at http://www.proteored.org/MIAPE). All within the EU ProteomeXchange project.



5. Quality control and experimental standardisation: Reproducibility and robustness of proteomics workflows are key issues that are being addressed through participation in multi-laboratory studies within the "ProteoRed-ISCIII" project led by our group.

6. Prolamin characterisation in foods using classical and mass spectrometry approaches is being carried out in the context of coeliac diseases.

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SELECTED PUBLICATIONS

Patwardhan A, Carazo JM, Carragher B, Henderson R, Heymann JB, Hill E, Jensen GJ, Lagerstedt I, Lawson CL, Ludtke SJ, Mastronarde D, Moore WJ, Roseman A, Rosenthal P, Sorzano CO, Sanz-García E, Scheres SH, Subramaniam S, Westbrook J, Winn M, Swedlow JR, Kleywegt GJ. Data management challenges in three-dimensional EM. Nat Struct Mol Biol. 2012 Dec, 19(12):1203-7.

Sorzano CO, de la Rosa Trevín JM, Otón J, Vega JJ, Cuenca J, Zaldívar-Peraza A, Gómez-Blanco J, Vargas J, Quintana A, Marabini R, Carazo JM. Semiautomatic, high-throughput, high-resolution protocol for three-dimensional reconstruction of single particles in electron microscopy. Methods Mol Biol. 2013; 950:171-93.

Oton J, Sorzano CO, Pereiro E, Cuenca-Alba J, Navarro R, Carazo JM, Marabini R. Image formation in cellular X-ray microscopy. J Struct Biol. 2012 Apr;178(1):29-37.

Melero R, Rajagopalan S, Lázaro M, Joerger AC, Brandt T, Veprintsev DB, Lasso G, Gil D, Scheres SH, Carazo JM, Fersht AR, Valle M. Electron microscopy studies on the quaternary structure of p53 reveal different binding modes for p53 tetramers in complex with DNA. Proc Natl Acad Sci USA. 2011 Jan 11;108(2):557-62..

Jiménez-Lozano N, Segura J, Macías JR, Vega J, Carazo JM. Integrating human and murine anatomical gene expression data for improved comparisons. Bioinformatics. 28(3):397-402. 2012 doi: 10.1093/bioinformatics/btr639.

Three-dimensional electron and X-ray microscopies: image processing challenges

During this period, we initiated a strong refocusing of our activities, centering on our role as image processing infrastructure providers for Instruct, the Structural Biology project of the European Strategic Forum for Research Infrastructures. We have thus been particularly active in the area of algorithmic inventions and technological development, aiming to provide not only image processing capabilities, but to link them to the exponentially growing fields of genomics and proteomics. Indeed, Xmipp, the software suite we developed, is being used increasingly in the field of three-dimensional electron microscopy, with hundreds of individualised downloads per year from all over the world; it is the software of choice for a large percentage of all 3D maps being deposited in structural databases.

In 2012, the soft X-ray microscope of the Spanish synchrotron ALBA began operation, the third instrument of its kind in the world. We have studied the image processing issues associated to this instrument in depth, developing the first formulation of its image formation model under incoherent illumination, and have begun to develop tailored 3D reconstruction approaches suited to this new imaging modality.

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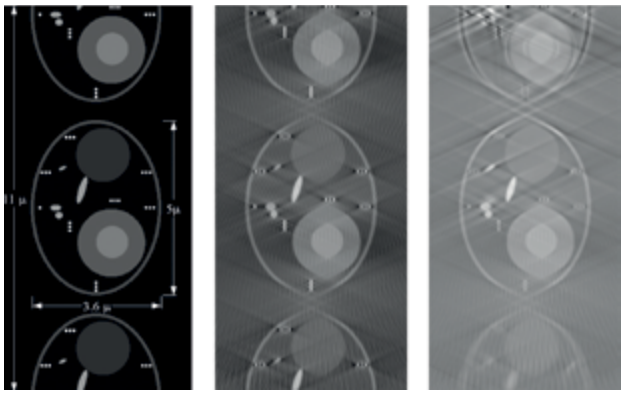
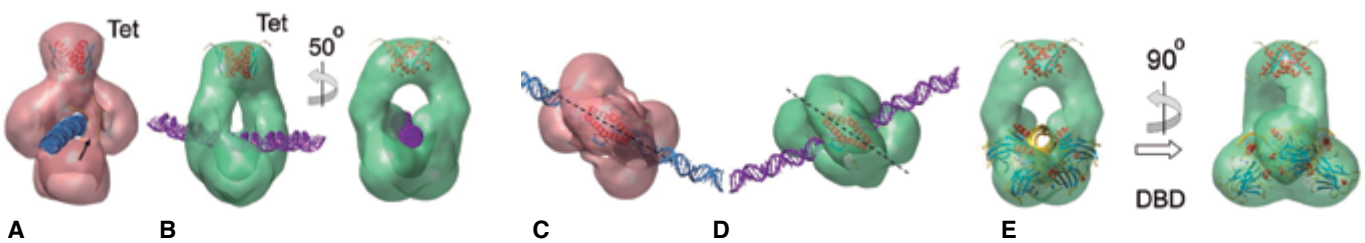


1 *Simulations of different 3D reconstruction from soft X-ray microscopy images under several conditions (left, oiginal phantom, center and right, 3D reconstructions with standard EM algorithms)*

2 *Different DNA-binding modes of p53 tetramers solved by 3D electron microscopy.*

2



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SELECTED PUBLICATIONS

Reguera J, Santiago C, Mudgal G, Ordoño D, Enjuanes L, Casasnovas JM. Structural bases of coronavirus binding to host aminopeptidase N and its inhibition by neutralizing antibodies. PLoS Pathog. 2012;8(8):e1002859

Manangeeswaran M, Jacques J, Tami C, Konduru K, Amharref N, Perrella O, Casasnovas JM, Umetsu DT, Dekruyff RH, Freeman GJ, Perrella A, Kaplan GG. Binding of Hepatitis A Virus to its Cellular Receptor 1 Inhibits T-Regulatory Cell Functions in Humans. Gastroenterology. 2012 Jun;142(7):1516-25

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Reguera J, Ordoño D, Santiago C, Enjuanes L, Casasnovas JM. Antigenic modules in the N-terminal S1 region of the Transmissible Gastroenteritis Virus spike protein. J Gen Virol. 2011 May;92(Pt 5):1117-26

Cell-cell and virus-cell interactions

A large variety of glycosylated molecules engaged in cell-cell and virus-cell interactions populate cell and viral membranes. Cell surface glycoproteins connect the cell with its environment; they participate in cell-cell contacts and in virus entry processes.

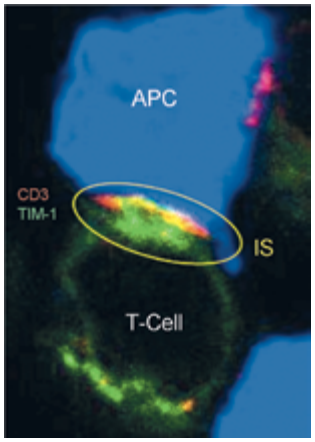
We study cell surface molecules engaged in immune system regulation and virus entry into host cells. We analyse receptor-ligand interactions related to immune processes such as cell adhesion and phagocytosis, as well as to virus binding to cells. We are also characterising virus neutralisation by humoral immune responses and its correlation with virus cell entry. Our research has provided key insights into immune receptor function and has identified viral epitopes essential for virus infection, some of which are targeted by neutralising antibodies. The group carries out multidisciplinary research using structural (X-ray crystallography), biochemical and cell biology approaches. Below we highlight some recent results related to immune processes and viral infections.

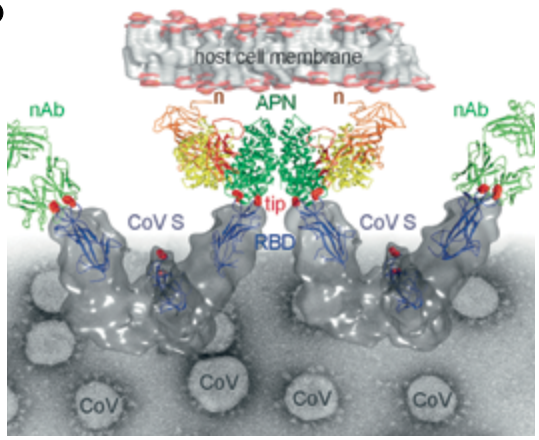
TIM proteins: a family of PtdSer receptors that regulate immunity

The transmembrane, immunoglobulin and mucin domain (TIM) gene family has a critical role in regulating immune responses, including transplant tolerance, autoimmunity, allergy and asthma, and the response to viral infections. We demonstrated that the TIM proteins are pattern recognition receptors, specialised in detecting the phosphatidylserine (PtdSer) cell death signal. The TIM protein bears a motif, the MILIBS, which determines its specificity for phospholipids such as PtdSer. We recently determined that TIM-1 traffics to the immune synapse during antigen presentation (Figure 1), where it may function as a costimulatory molecule.

Virus-receptor interactions and virus neutralisation by antibodies

Our group has been analysing virus-receptor interactions in measles virus and coronavirus, and has determined crystal structures of virus-receptor complexes. These structures define the way in which measles virus and certain coronaviruses bind to cell surface proteins, and identify the major receptor recognition determinants in those viruses. Moreover, our analysis of how antibodies prevent and neutralise virus infections showed that potent measles- and coronavirus-neutralising antibodies target virus residues engaged in binding to cell surface receptors. This indicates that prevention of virus entry into host cells is a major mechanism used by the immune system for virus neutralisation. Figure 2 illustrates how an antibody (nAb) prevents coronavirus (CoV) binding to the aminopeptidase N (APN) receptor.





1 *TIM-1 traffic toward the immunological synapse (IS), formed by a T cell and an antigen presenting cell (APC).*

2 *Structural view of coronavirus (CoV) binding to its host cell aminopeptidase N (APN) receptor and its inhibition by neutralising antibodies.*

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SELECTED PUBLICATIONS

Ionel A, Velázquez-Muriel JA, Luque D, Cuervo A, Castón JR, Valpuesta JM, Martín-Benito J, Carrascosa JL. Molecular Rearrangements Involved in the Capsid Shell Maturation of Bacteriophage T7. J Biol Chem. 2011 Jan 7;286(1):234-42

Molero LH, López-Montero I, Márquez I, Moreno S, Vélez M, Carrascosa JL, Monroy F. Efficient Orthogonal Integration of the Bacteriophage Ø29 DNA-Portal Connector Protein in Engineered Lipid Bilayers. ACS Synth. Biol. 2012; 1: 414-24

Chichón FJ, Rodríguez MJ, Pereiro E, Chiappi M, Perdiguero B, Guttmann P, Werner S, Rehbein S, Schneider G, Esteban M, Carrascosa JL. Cryo X-ray nano-tomography of vaccinia virus infected cells. J Struct Biol. 2012 Feb;177(2):202-11

Morin JA, Cao FJ, Lázaro JM, Arias-Gonzalez JR, Valpuesta JM, Carrascosa JL, Salas M, Ibarra B. Active DNA unwinding dynamics during processive DNA replication. Proc Natl Acad Sci USA. 2012 May 22;109(21):8115-20

Fumagalli L, Esteban-Ferrer D, Cuervo A, Carrascosa JL, Gomila G. Label-free identification of single dielectric nanoparticles and viruses with ultraweak polarization forces. Nat Mater. 2012 Sep;11(9):808-16

Structure of macromolecular assemblies

The activity of the group has focussed on the study of the molecular bases of virus assembly and maturation. We used 3D-cryo-EM and single particle reconstruction approaches to describe, at subnanometer resolution, the reorganisation involved in maturation of the shell of the icosahedral dsDNA caudovirales group. Using the phage T7 as a model, we are presently using a combination of biochemical and microscopy approaches to study structural changes in viral components involved in DNA packaging, virus-cell interaction and DNA ejection.

Viral maturation of complex viruses has been studied at the subcellular level using mainly the vaccinia virus as a model system. Analysis by electron tomography of infected cells has shown the existence of extensive membrane reorganisation during vaccinia maturation. To overcome the intrinsic limitation of electron microscopy in imaging samples thicker than 0.5 microns, we developed novel methods for soft X-ray microscopy of frozen whole cell samples. Successful X-ray imaging of 5- to 10-micron samples in the frozen state allowed us to produce cryo-X-ray tomographic 3D reconstructions of cells. The resolution obtained so far for the tomograms has been sufficient to detect different virus types within the cytoplasm of unfixed, uncontrasted cells. We are currently developing methods for correlative combination of light, electron and X-ray tomography to improve quantitative 3D microscopy.

The structural data obtained by 3D-cryo-microscopy from the experimental systems under study in our group are combined with analysis of the nano-mechanical properties of individual viral particles, using atomic force microscopy and spectroscopy. We are also exploring the application of optical tweezers to test small forces involved in specific viral functions. We are using isolated viral components to study their properties as nanomachines and to build with them synthetic tools. We are studying the deformation behaviour and material properties in individual viral particles for systematic studies to correlate molecular structure, nanoscopic behaviour, and macroscopic properties of viral containers.

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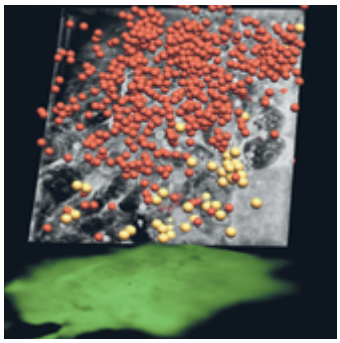

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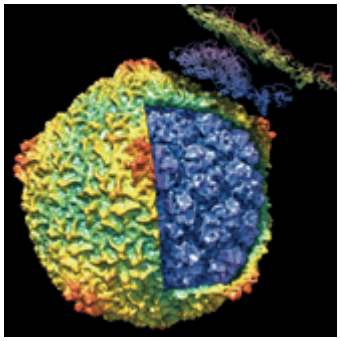

1 *Composite image showing correlative merging of a light microscopy image of a cell (fluorescent green), a plane of an X-ray cryo-tomogram of the same cell, and the segmented virus types (yellow: immature vaccinia virus; red: mature virus) from an X-ray cryo-tomogram of the cell.*

2 *View of the mature capsid of bacteriophage T7 obtained by three-dimensional reconstruction from electron cryo-microscopy at 1 nm resolution.*

3 *Reconstruction of the capsid of bacteriophage T7 by electron cryo-microscopy at 1 nm resolution. The outer shell corresponds to the mature capsid. The inner blue shell is from the immature prohead. The ribbon models are two related structures of the monomers from each shell type, showing the domain reorganisation involved in virus maturation.*

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Ionel A, Velázquez-Muriel JA, Luque D, Cuervo A, Castón JR, Valpuesta JM, Martín-Benito J, Carrascosa JL. Molecular rearrangements involved in the capsid shell maturation of bacteriophage T7. J Biol Chem. 2011 Jan 7;286(1):234-42

Garriga D, Pickl-Herk A, Luque D, Wruss J, Castón JR, Blaas D, Verdaguer N. Insights into minor group rhinovirus uncoating: the X-ray structure of the HRV2 empty capsid. PLoS Pathog. 2012 Jan;8(1):e1002473

Luque D, González JM, Gómez-Blanco J, Marabini R, Chichón J, Mena I, Angulo I, Carrascosa JL, Verdaguer N, Trus BL, Bárcena J, Castón JR. Epitope insertion at the N-terminal molecular switch of the rabbit hemorrhagic disease virus T = 3 capsid protein leads to larger T = 4 capsids. J Virol. 2012 Jun;86(12):6470-80

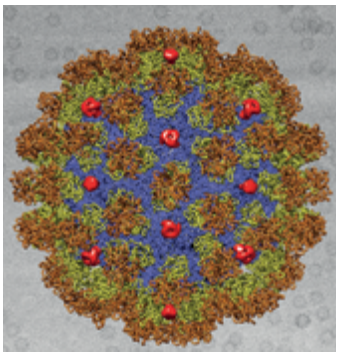
Irigoyen N, Castón JR, Rodríguez JF. Host proteolytic activity is necessary for infectious bursal disease virus capsid protein assembly. J Biol Chem. 2012 Jul 13;287(29):24473-82

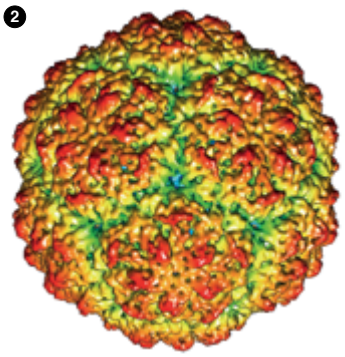
Gómez-Blanco J, Luque D, González JM, Carrascosa JL, Alfonso C, Trus B, Havens WM, Ghabrial SA, Castón JR. *Cryphonectria nitschkei* virus 1 structure shows that the capsid protein of chrysoviruses is a duplicated helix-rich fold conserved in fungal double-stranded RNA viruses. J Virol. 2012 Aug;86(15):8314-8

Viral molecular machines

Our studies address the structure-function-assembly relationships of viral macromolecular complexes, also known as viral nanomachines, that control many fundamental processes in the virus life cycle. Our model systems are the viral capsid and other viral macromolecular complexes, such as helical tubular structures and ribonucleoprotein complexes. We study several viral systems with different levels of complexity: double-stranded (ds)RNA viruses such as infectious bursal disease virus (IBDV), *Penicillium chrysogenum* virus (PcV) and human picobirnavirus (HPBV), and single-stranded RNA viruses such as human rhinovirus 2 (HRV2) and rabbit haemorrhagic disease virus (RHDV). The structure of regular viral capsids, in which capsid proteins make extensive use of symmetry, is a paradigm of the economy of genomic resources. Capsids should not be considered inert closed structures, but as dynamic structures that define different functional states and participate in numerous processes, including virus morphogenesis, selection of the viral genome, recognition of the host receptor, and release of the genome to be transcribed and replicated. Some capsids even participate in genome replication. Structural analysis of viruses at the highest achievable resolution is therefore essential to understand their properties. We are carrying out nanoscopic studies of these biomachines by single-molecule manipulation techniques such as atomic force microscopy (AFM) to correlate structural features of capsomer interactions with their mechanical properties.

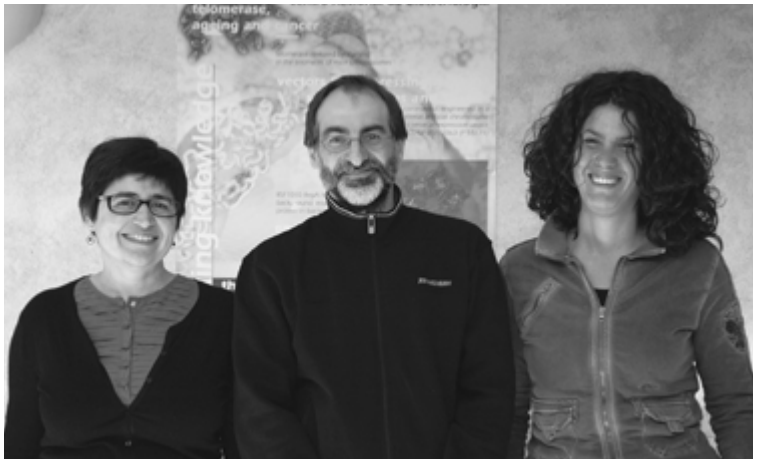
To determine the three-dimensional structure of such complex assemblies, we use hybrid methods that combine cryo-electron microscopy and image processing techniques with high-resolution X-ray structures. Our studies also intend to establish the basis of the conformational flexibility necessary to switch among almost identical conformational states (transient complexes), and their functional implications, which can provide clues for new vaccine design and/or immunisation strategies. We also focus on the structural basis of dsRNA virus replication. All dsRNA viruses, from the mammalian reoviruses to the bacteriophage phi6 and including fungal viruses, share a specialised capsid involved in transcription and replication of the dsRNA genome. Quasiatomic model of the RHDV virion. The RHDV capsid is based on a T=3 lattice containing 90 VP1 dimers. The cryo-EM map allowed modelling of the VP1 backbone structure from X-ray structures of other caliciviruses. Each VP1 monomer has three domains: an internal N-terminal arm, a shell composed of an eight-stranded b-sandwich (purple), and a flexible protruding domain subdivided into two subdomains, P1 (yellow) and P2 (orange).





❶ *Quasiatomic model of RHDV virion. The RHDV capsid is based on a T=3 lattice containing 90 VP1 dimers. The cryo-EM map allowed modeling of the VP1 backbone structure from X-ray structures of other caliciviruses. Each VP1 monomer has three domains, an internal N-terminal arm, a shell composed of an eight-stranded b-sandwich (purple), and a flexible protruding domain subdivided into two subdomains, P1 (yellow) and P2 (orange).*

❷ *Structure of Penicillium chrysogenum virus (PcV), a fungal double-stranded RNA virus. Radially color-coded outer surfaces of three-dimensional cryo-EM reconstruction of the PcV at 8 Å r esolution, which has a T=1 capsid formed by 60 copies of a single polypeptide. Structural subunits have two similar helical domains indicative of gene duplication.*



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SELECTED PUBLICATIONS

Agulleiro JI, Fernandez JJ. Fast tomographic reconstruction on multicore computers. Bioinformatics. 2011 Feb 15;27(4):582-3

Martinez-Sanchez A, Garcia I, Fernandez JJ. A differential structure approach to membrane segmentation in electron tomography. J Struct Biol. 2011 Sep;175(3):372-83

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Fernandez JJ. Computational methods for electron tomography. Micron. 2012 Oct;43(10):1010-30

Li S, Fernandez JJ, Marshall WF, Agard DA. Three-dimensional structure of basal body triplet revealed by electron cryo-tomography. EMBO J. 2011 Dec 13;31(3):552-62

Computational methods for 3D electron microscopy

Knowledge of the structure of biological specimens is essential to understanding their functions at all scales. Electron microscopy (EM) combined with image processing allows study of the three-dimensional (3D) structure of biological specimens over a wide range of sizes, from cell structures to single macromolecules, providing information at different levels of resolution. Depending on the specimen under study and the structural information sought, different 3D EM approaches are used. Single particle EM makes it possible to visualise macromolecular assemblies at subnanometer or even near-atomic resolution. Electron tomography is a unique tool for deciphering the molecular architecture of the cell. In all cases, the computational methods of image processing play a major role. Computational advances have contributed significantly to the current relevance of 3D EM in structural biology.

Our research interests focus mainly on structural analysis of specimens of biological relevance, using 3D EM in general and especially electron tomography. We are exploring the structural alterations in the subcellular architecture in normal and pathological conditions in neurodegenerative diseases, particularly Huntington's disease. This project is led by Dr. MR Fernández Fernández, who has substantial expertise in different aspects of the molecular and cell biology of Huntington's disease neurodegeneration. We are also interested in structural elucidation of the microtubule-organising centre (MTOC), an important and complex cell organelle in eukaryotes. We are conducting projects to understand the spindle pole body and centriole/basal body in collaboration with Dr. Sam Li (UCSF, CA, USA). We also collaborate with other national and international groups in experimental structural studies. Another important focus of our research is the development of new image processing methods and tools for the advancement of electron tomography. In the last few years, we have worked on implementation of sophisticated tomographic reconstruction methods that are robust in our experimental conditions. We have also developed new methods to address an important challenge in electron tomography, that is, the automated segmentation of tomograms for 3D visualisation of subcellular landscapes.

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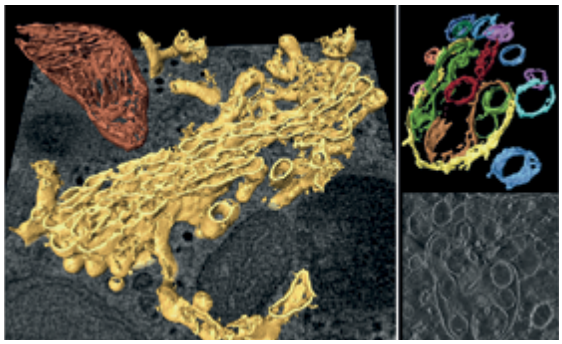
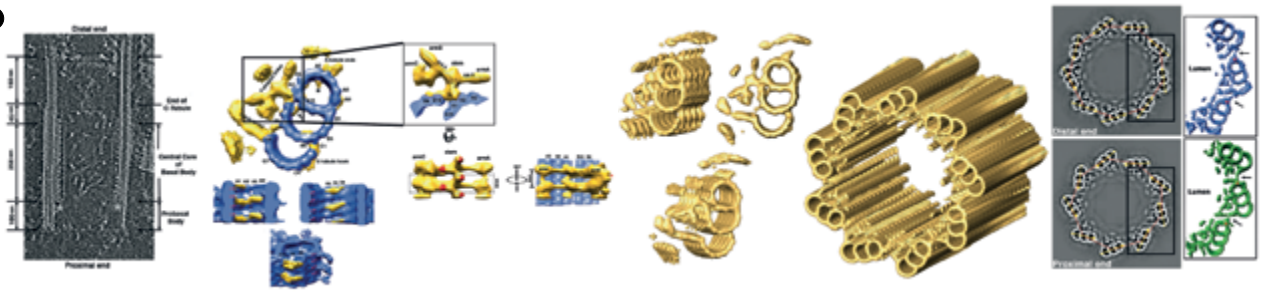


Three-dimensional visualisation of subcellular architecture with electron tomography and image processing techniques. Golgi complex and mitochondrion from a human cytotoxic T lymphocyte (left). Multivesicular body from a wild type mouse striatal sample (right).

2



Elucidation of the structure at close-to-molecular resolution of the basal body triplet and 3D model for the whole basal body.

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SELECTED PUBLICATIONS

Fuentes-Perez ME, Gwynn EJ, Dillingham MS, Moreno-Herrero F. Using DNA as a fiducial marker to study SMC complex interactions with the Atomic Force Microscope. Biophys J. 2012 Feb 22;102(4):839-48

Yeeles JT, van Aelst K, Dillingham MS, Moreno-Herrero F. Recombination hotspots and single-stranded DNA binding proteins couple DNA translocation to DNA unwinding by the AddAB helicase-nuclease. Mol Cell. 2011 Jun 24;42(6):806-16

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Molecular biophysics of DNA repair nanomachines

The molecular biophysics group aims to develop single-molecule techniques to study the mechanisms of protein machines involved in DNA repair processes. We also study the mechanical properties of nucleic acids and their interaction with proteins using these single-molecule approaches.

Over the last two years, we completed the construction of a magnetic tweezers (MT) machine that can manipulate single DNA molecules and measure force and torque applied by molecular motors. The group also has two custom-adapted atomic force microscopes (AFM) and a home-built optical tweezers setup (OT). We have used AFM and MT to image and monitor the dynamics of binding and processing of DNA breaks by the AddAB helicase-nuclease and to study the role of SSB in these reactions. We found that recombination hotspot sequences activate DNA unwinding by the translocating AddAB helicase-nuclease. This is the first example of stimulation of a DNA helicase by interaction with a specific sequence during translocation. This phenomenon will ensure the formation of ssDNA downstream of recombination hotspots as is required for homologous recombination. We have also investigated the structure and oligomerisation state of SMC (structural maintenance of chromosome) complex from *Bacillus subtilis*. Using a novel AFM method developed by the group, we have determined how the binding of ScpA and ScpB affect the overall structure of the SMC complex. Finally, we have further developed our knowledge in the mechanical properties of DNA and how these are affected by sequence and condensation.

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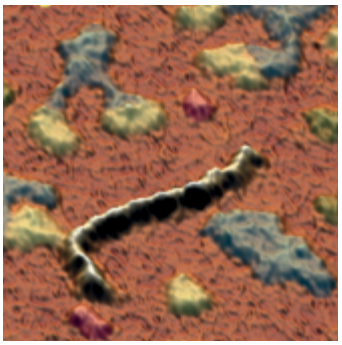

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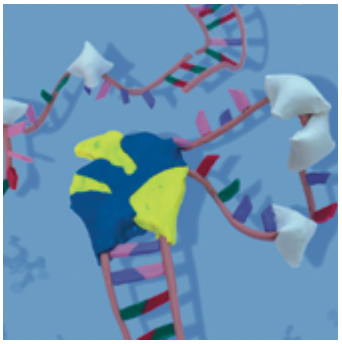

1 *SMC complex interactions studied with the AFM using DNA as a fiducial marker to quantify volumes of proteins with high precision. AFM results were used to color code the different protein components of the SMC complex: monomers of ScpA (red); monomers and dimers of ScpB (green); ScpA-ScpB complexes (yellow); and SMC proteins (blue). The fiducial DNA molecule used in the study appears in white at the bottom part of the picture. Size of the image is 500 nm x 500 nm.*

2 *Three-dimensional model illustrating that DNA translocation and unwinding are coupled through interactions of the AddAB helicase-nuclease with recombination hotspots (Chi). In the drawing, the AddAB complex pictured (yellow and blue) has recognised a Chi sequence, provoking the formation of a single-stranded DNA loop and thereby promoting stable DNA unwinding. Single-stranded DNA binding proteins, which also assist DNA unwinding, are shown in white.*

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SELECTED PUBLICATIONS

Zuklys S, Mayer CE, Zhanybekova S, Stefanski HE, Nusspaumer G, Gill J, Barthlott T, Chappaz S, Nitta T, Dooley J, Nogales-Cadenas R, Takahama Y, Finke D, Liston A, Blazar BR, Pascual-Montano A, Holländer GA. MicroRNAs control the maintenance of thymic epithelia and their competence for T lineage commitment and thymocyte selection. J Immunol. 2012 Oct 15;189(8):3894-904

Tabas-Madrid D, Nogales-Cadenas R, Pascual-Montano A. GeneCodis3: a non-redundant and modular enrichment analysis tool for functional genomics. Nucleic Acids Res. 2012 Jul;40(Web Server issue):W478-83

Muniategui A, Nogales-Cadenas R, Vázquez M, Aranguren XL, Agirre X, Luttun A, Prosper F, Pascual-Montano A, Rubio A. Quantification of miRNA-mRNA interactions. PLoS One. 2012;7(2):e30766

Fontanillo C, Nogales-Cadenas R, Pascual-Montano A, De las Rivas J. Functional analysis beyond enrichment: non-redundant reciprocal linkage of genes and biological terms. PLoS One. 2011;6(9):e24289

Pascual-Montano A. Gene classification, gene expression data processing and exploratory data analysis. Wiley Interdisciplinary Reviews Data Mining and Knowledge Discovery. Apr 04 2011. DOI: 10.1002/widm.29

Functional bioinformatics

To understand the biology underlying experimental settings, our group studies the development of new methodologies and analysis techniques to solve very oriented and specific biological questions. We concentrate our efforts in the functional bioinformatics area, which focusses its activities in the functional characterisation of genes and proteins in different experimental conditions. Our group focussed on the development of new methodologies for the analysis and interpretation of biological data. In particular, we have been working in two major areas: transcriptomics and functional analysis.

In the case of gene expression analysis, we have developed several techniques to determine the potential interactions between micro RNAs and their target transcripts. These interactions are predicted by sequence complementarity and quantified using expression information from both mRNAs and miRNAs in the same samples. We have also developed several very novel techniques to obtain the functional characterisation of list of genes or proteins. The novelty of our proposal lies in the fact that we combine several sources of information and determine which combination of functional annotations is significantly enriched in the list of genes or proteins. This has opened a new field of research know as modular functional enrichment. Methodologies to eliminate the redundancy of annotations as well as the existing bias in the annotations databases have been also developed.

In these years, we have continued our policy of developing high-quality bioinformatics software and making it available to the scientific community. The picture summarises the developments of our group in Functional Bioinformatics. More details can be found at http://bioinfo.cnb.csic.es

1 *General overview of the bioinformatics applications developed by the Functional Bioinformatics Group*

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Monserrat Lara Rojas

SELECTED PUBLICATIONS

López-Montero N, Risco C. Self-protection and survival of arbovirus-infected mosquito cells. Cell Microbiol. 2011 Feb;13(2):300-15

Risco C, Sanmartín-Conesa E, Tzeng WP, Frey TK, Seybold V, de Groot RJ. Specific, sensitive, high-resolution detection of protein molecules in eukaryotic cells using metal-tagging transmission electron microscopy. Structure. 2012 May 9;20(5):759-66

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PATENTS

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PCT 012/070864: Method for protein detection in cells with a clonable tag and sprectoscopic imaging.

Cell structure lab

Viruses manipulate cell organisation by recruiting materials to build factories, where they replicate their genomes, assemble new infectious particles, and conceal themselves from the antiviral defence sentinels of the cell. Our laboratory studies the biogenesis of virus factories to understand how viruses manipulate cell structure and create new organelles. The group works with important human pathogens such as Bunyaviruses, Togaviruses and Reoviruses; we are also interested in mechanisms of cellular immunity. With longstanding experience in structural biology, the lab is involved in developing new probes for correlative light and electron microscopy (CLEM) and electron tomography.

In the last two years, our group studied the structural transformations of mammalian cells during the last phase of the bunyavirus life cycle, and characterised two unreported structures involved in virus egress and propagation (Sanz & Risco, unpublished). Like many Arboviruses, Bunyaviruses are serious pathogens for mammals but cause little damage to their arthropod vectors. We studied the life cycle of a bunyavirus in mosquito cells (López-Montero & Risco, 2011) and are currently moving from cell culture systems to the arthropod hosts. These studies are necessary to understand key factors for virus spread in the arthropod vectors.

In collaboration with Dr. Raoul J. de Groot (University of Utrecht, The Netherlands), we described an approach, termed metal-tagging transmission electron microscopy (METTEM), that allows detection of intracellular proteins in mammalian cells with high specificity, exceptional sensitivity, and at molecular scale resolution. Based on the metal-binding protein metallothionein as a clonable tag, METTEM was combined with elemental gold imaging for simultaneous visualisation of ultrastructural details and protein molecule location. The applicability and strength of METTEM was demonstrated by a study of Rubella virus replicase and capsid proteins, which identified virus-induced cell structures not seen before (Risco *et al.*, 2012). With the help of METTEM, we recently characterised the biogenesis of the replication organelles of a Tombusvirus, in a study developed in collaboration with Dr. Peter D. Nagy (University of Kentucky, KY USA)(Barajas, Fernández de Castro, Risco & Nagy, unpublished).

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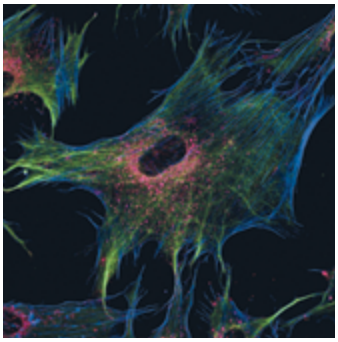

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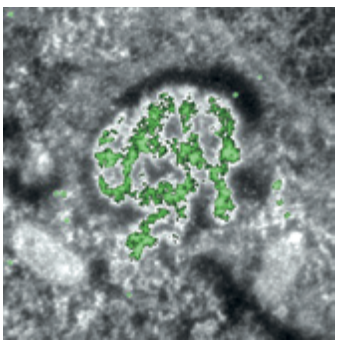

1 *Changes in the cytoskeleton of bunyavirus-infected cells*

2 *Localisation of proteins in cells with metal-tagging TEM and electron spectroscopic imaging*

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SELECTED PUBLICATIONS

Graziano V, Luo G, Blainey PC, Pérez-Berná AJ, McGrath WJ, Flint SJ, San Martín C, Xie XS, Mangel WF. Regulation of a Viral Proteinase by a Peptide and DNA in One-dimensional Space: II. Adenovirus proteinase is activated in an unusual one-dimensional biochemical reaction. J Biol Chem. 2012 Oct 7;288(3):2068-80

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Ortega-Esteban A, Horcas I, Hernando-Pérez M, Ares P, Pérez-Berná AJ, San Martín C, Carrascosa JL, de Pablo PJ, Gómez-Herrero J. Minimizing tip-sample forces in jumping mode atomic force microscopy in liquid. Ultramicroscopy. 2012 Mar;114:56-61

González-Aparicio M, Mauleón I, Alzuguren P, Bunuales M, González-Aseguinolaza G, San Martín C, Prieto J, Hernández-Alcoceba R. Self-inactivating helper virus for the production of high-capacity adenoviral vectors. Gene Ther. 2011 Nov;18(11):1025-33

Structural and physical determinants of adenovirus assembly

We are interested in the structural and physical principles that govern assembly and stabilisation of complex viruses. As a model system we use adenovirus, a challenging specimen of interest both in basic virology and nanobiomedicine. We approach the problem from an interdisciplinary point of view, combining biophysics, computational, structural and molecular biology techniques.

Adenoviruses are pathogens of particular clinical relevance in the immunocompromised population. They are also widely used as vectors for gene therapy, vaccination and oncolysis. The adenovirus genome, a dsDNA molecule, is bound to large amounts of positively charged proteins that help condense it to form the core, which is confined inside an icosahedral capsid composed of multiple copies of seven different viral proteins. The final stage of adenovirus morphogenesis consists of proteolytic processing of several capsid and core proteins. The immature virus, containing all precursor proteins, is not infectious due to an uncoating defect. To determine why the presence of precursor proteins impairs uncoating, we analysed *in vitro* disruption of mature and immature adenovirus capsids subjected to different types of stress: thermal, chemical, or mechanical (in collaboration with Dr. Pedro J. de Pablo, UAM, Madrid, Spain). The results indicated that precursor viral proteins act as scaffolds during assembly, explained how maturation primes the virus for stepwise uncoating in the cell, and revealed the structural changes the virion undergoes in conditions similar to those encountered during entry. In collaboration with Prof. Walter F. Mangel (Brookhaven Natl. Laboratory, NY, USA), we also helped to define the role of the viral genome as a cofactor of the adenovirus protease during maturation, in a newly described one-dimensional chemistry process. Our current research lines focus on the less understood aspects of adenovirus assembly, such as how the viral genome is packaged into the capsid, key elements that modulate virion stability and mechanical properties, how adenovirus evolution relates to that of its hosts, and finally, the organisation of the non-icosahedral virion components. Accurate knowledge of adenovirus structure and biology is fundamental both to the discovery of anti-adenovirus drugs and to the design of new, efficient adenoviral therapeutic tools.

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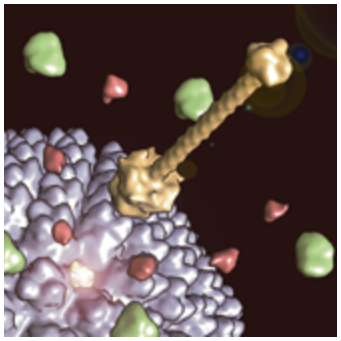

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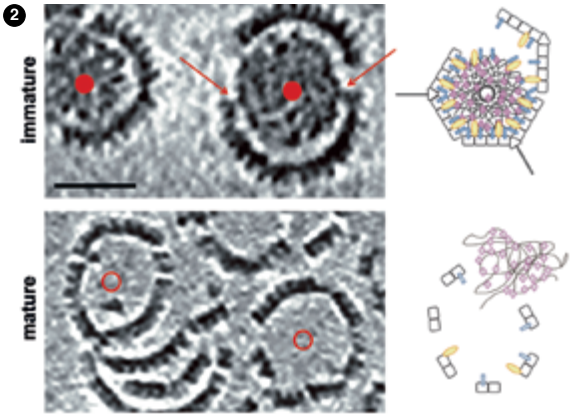

❶ *The first stages of mature adenovirus uncoating. In mildly acidic conditions that mimic those of the early endosome, adenovirus virions (purple) release a few pentons (orange) and internal proteins located at the core periphery (green and red)*

❷ *The final stages of adenovirus uncoating. Cryo-electron tomography data (left panel). In the immature virion, even under high stress conditions, the genome (filled red circles) remains a compact spherical particle attached to capsid fragments (red arrows). In the mature virion, the capsid cracks open and the genome is completely released, leaving an empty shell (empty red circles)*

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SELECTED PUBLICATIONS

Knijnenburg AD, Tuin AW, Spalburg E, de Neeling AJ, Mars-Groenendijk RH, Noort D, Otero JM, Llamas-Saiz AL, van Raaij MJ, van der Marel GA, Overkleeft HS, Overhand M. Exploring the conformational and biological versatility of β-turn-modified gramicidin S by using sugar amino acid homologues that vary in ring size. Chemistry. 2011 Mar 28;17(14):3995-4004

Tizón L, Otero JM, Prazeres VF, Llamas-Saiz AL, Fox GC, van Raaij MJ, Lamb H, Hawkins AR, Ainsa JA, Castedo L, González-Bello C. A prodrug approach for improving antituberculosis activity of potent *Mycobacterium tuberculosis* type II dehydroquinase inhibitors. J Med Chem. 2011 Sep 8;54(17):6063-84

Garcia-Doval C, van Raaij MJ. Crystallization of the C-terminal domain of the bacteriophage T7 fibre protein gp17. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2012 Feb 1;68(Pt 2):166-71

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Structural biology of viral fibres

Some viruses and bacteriophages attach to their host cell via proteins integral to their capsids, for example poliovirus, coxsackievirus and rhinovirus ('common cold virus'). Other viruses bind to their host cell receptors via specialised spike proteins (for example HIV, the AIDS virus), or via specialised fibre proteins, like adenovirus, reovirus and bacteriophages such us T4, T5, T7 and lambda (Ur). It is these fibre proteins that form the main research interest of our research group. The fibres all have the same basic architecture: they are trimeric and contain an N-terminal virus or bacteriophage attachment domain, a long, thin, but stable shaft domain and a more globular C-terminal cell attachment domain. They are very stable to denaturation by temperature or detergents.

In 2011, we determined the structure of gp17, the fibre of the *Escherichia coli* bacteriophage T7. The structure revealed a pyramid domain of unknown fold and a tip domain with a novel structural topology. Amino acid residues important for determining the host specificity of bacteriophage T7 and related phages are located on the top of this tip domain, facing the bacterium in early stages of bacteriophage infection. Knowledge of the structures of bacteriophage fibre proteins may lead to different biotechnological applications. Modification of the bacteriophage fibre receptor binding specificities may lead to improved detection and elimination of specific bacteria.

As adenovirus is used in experimental gene therapy, modification of its fibre should allow targeting to specific cellular receptors. In 2012 we determined the structures of adenovirus fibre receptor domains from two families of adenovirus for which no structures were known. We also collaborate with other research groups in crystallisation and structure solution of the proteins and peptides they produce. In 2011-2012, we have determined structures of several cyclic antibiotic peptides and bacterial dehydroquinases complexed with inhibitors. We also determined the structure of bacterial (*Thermus thermophilus*) enoyl-acyl carrier protein reductase in the apo-form, in complex with NAD+, and in complex with NAD+ and the antibacterial agent triclosan.

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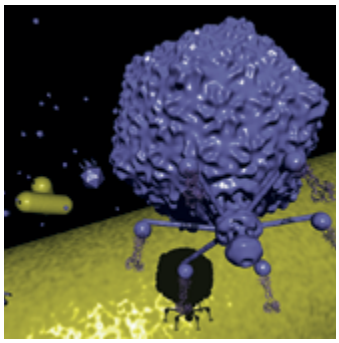

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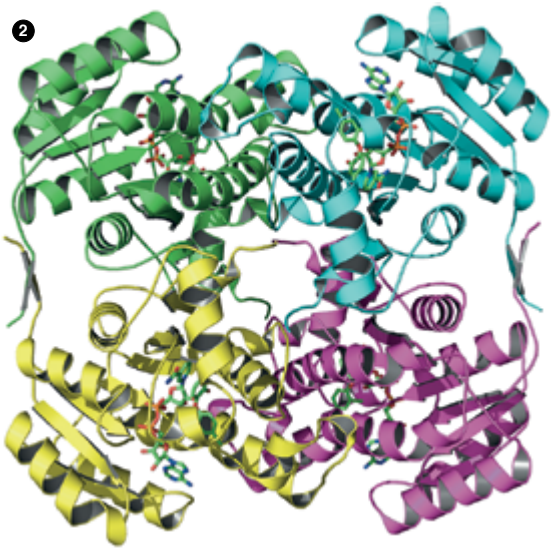

❶ *T7 bacteriophages (purple) at the point of recognising and infecting Escherichia coli bacteria (yellow). At the end of the six fibres, the crystal structure of the lipo-polysaccharide domains is represented.*

❷ *Structure of the tetrameric Thermus thermophilus enoyl-acyl carrier protein reductase in complex with NAD+ and the antibacterial agent triclosan*

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Fernando Lledías

SELECTED PUBLICATIONS

Muñoz IG, Yébenes H, Zhou M, Mesa P, Serna M, Park AY, Bragado-Nilsson E, Beloso A, de Cárcer G, Malumbres M, Robinson CV, Valpuesta JM, Montoya G. Crystal structure of the mammalian cytosolic chaperonin CCT in complex with tubulin. Nat Struct Mol Biol. 2011 Jan;18(1):14-9

Yébenes H, Mesa P, Muñoz IG, Montoya G, Valpuesta JM. Chaperonins: two rings for folding. Trends Biochem Sci. 2011 Aug;36(8):424-32

Peña A, Gewartowski K, Mroczek S, Cuéllar J, Szykowska A, Prokop A, Czarnocki-Cieciura M, Piwowarski J, Tous C, Aguilera A, Carrascosa JL, Valpuesta JM, Dziembowski A. Architecture and nucleic acids recognition mechanism of the THO complex, an mRNP assembly factor. EMBO J. 2012 Feb 7;31(6):1605-16

Arranz R, Mercado G, Martín-Benito J, Giraldo R, Monasterio O, Lagos R, Valpuesta JM. Structural characterisation of microcin E492 amyloid formation: Identification of the precursors. J Struct Biol. 2012 Apr;178(1):54-60

Arranz R, Coloma R, Chichón FJ, Conesa JJ, Carrascosa JL, Valpuesta JM, Ortín J, Martín-Benito J. The structure of native influenza virion ribonucleoproteins. Science. 2012 Dec 21;338(6114):1634-7

Structure and function of molecular chaperones

Molecular chaperones are proteins that assist the folding of other proteins, although they were also recently found to be involved in protein degradation. Our main line of work deals with the structural and functional characterisation of molecular chaperones and their interaction in the protein folding and degradation assembly pathways. Using various techniques, principally electron microscopy and image processing, we have been working with chaperones such as CCT, Hsp110, Hsp90, Hsp70, Hsp40 and nucleoplasmin, as well as with some of their co-chaperones like Hop, Hip and CHIP. We have characterised several complexes formed by these chaperones and their co-chaperones that constitute part of the various assembly lines involved in protein homeostasis. These techniques were used to study other proteins and macromolecular complexes including various amyloids, RNA processing proteins and centrosomal proteins.

We also studied centrosomes and ciliary components using microscopy techniques such as electron and X-ray tomography.

Finally, we are developing single-molecule techniques such as optical tweezers, collaborating with other groups in the characterisation of the mechanical properties of long polymers like DNA and RNA, and in the mechanochemistry of the phage Φ29 DNA polymerase.