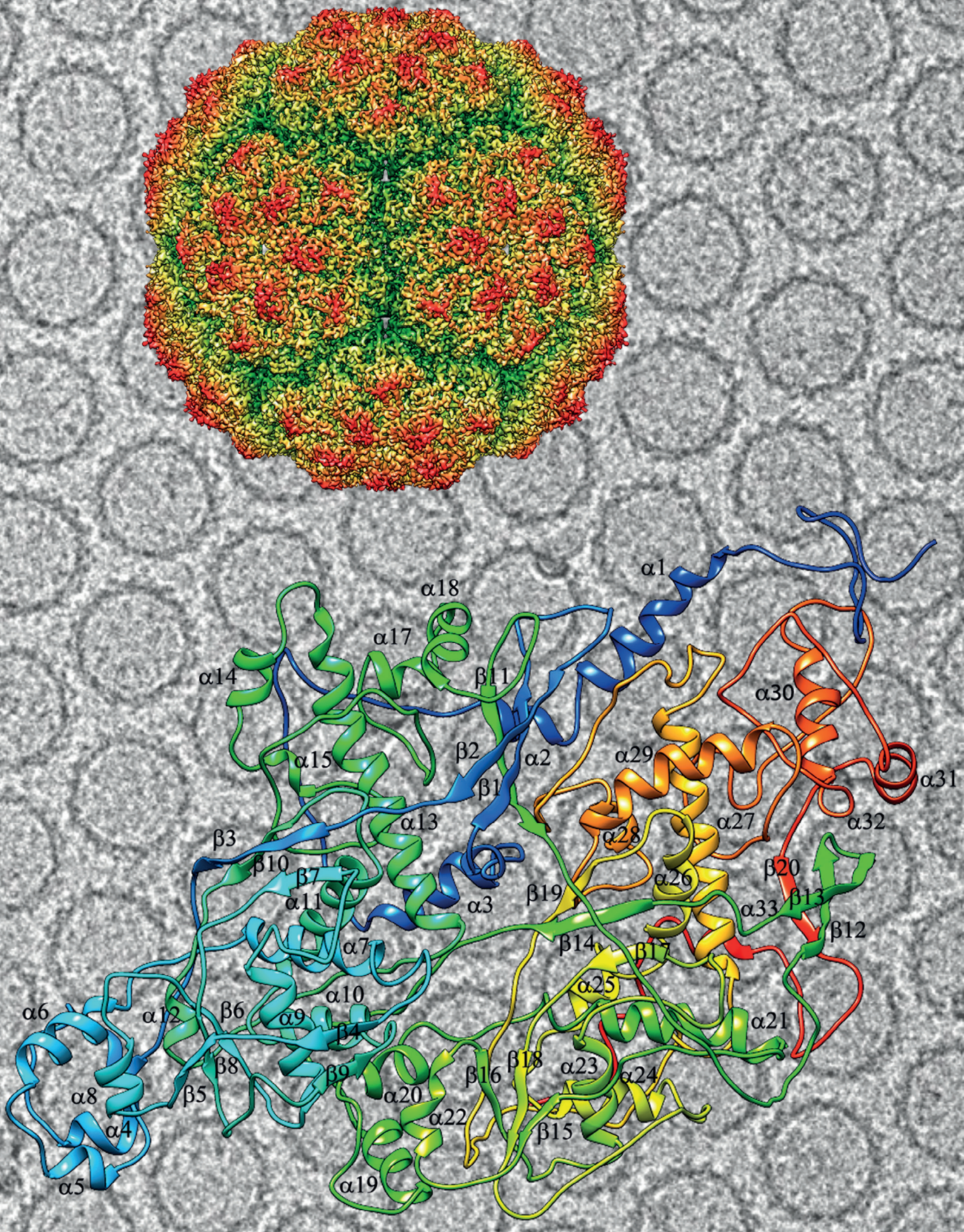
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MACROMOLECULAR STRUCTURES

The department's interests embrace a large number of biological problems, from fundamental questions associated with protein folding (Víctor Muñoz and José M. Valpuesta) to the functional and structural characterization of different molecular machines, especially virus structures and their components (José M. Casasnovas, José L. Carrascosa, José R. Castón, Cristina Risco, Carmen San Martín, Mark van Raaij) and DNA repair nanomachines (Fernando Moreno-Herrero). They use the numerous structural and biophysical techniques available in the department, including X-ray diffraction, nuclear magnetic resonance, single-molecule techniques (optical and magnetic tweezers) and various state-of-the-art spectroscopic techniques. A strength of our department is the development of microscopy techniques, such as atomic force, optical and X-ray microscopy and, in particular, transmission electron microscopy in its variants (single-particle cryoelectron microscopy and cryoelectron tomography), which received a hefty boost with the recent investment in a state-of-the-art 200 kV cryoelectron microscope, equipped with a direct electron detector and sample autoloader, unique in Spain. This acquisition, together with other equipment in the department, was used to create the first cryoelectron microscopy facility in Spain, open to users worldwide.

Electron microscopy work is strongly supported by continuous software development in the field of image processing (José M. Carazo, José J. Fernández), a major asset in the selection of the CNB as host of the image processing centre of INSTRUCT, a pan-European research infrastructure network facility that provides expertise and access to high quality instruments. Technical developments are also pursued in the field of proteomics, thanks to the work of the late Juan P. Albar, who remains alive in our memories. His continuous efforts led the CNB to head the Spanish proteomic facilities network (ProteoRed), and enabled its participation in the Human Proteome project. This effort will be continued by the incorporation of another excellent proteomics expert, Fernando Corrales.

HEAD OF DEPARTMENT

José María Valpuesta

OUR RESEARCH GROUPS

1. **Biocomputing unit**
   José María Carazo
2. **Structure of macromolecular assemblies**
   José L. Carrascosa
3. **Cell-cell and virus-cell interactions**
   José M. Casasnovas
4. **Viral molecular machines**
   José R. Castón
5. **Electron tomography and image processing of cell structures**
   José J. Fernández
6. **Ultrastructure of viruses and molecular aggregates**
   Jaime Martín-Benito
7. **Molecular biophysics of DNA repair nanomachines**
   Fernando Moreno-Herrero
8. **Conformational-functional behaviour of proteins**
   Víctor Muñoz
9. **Functional proteomics**
   Alberto Paradela
10. **Cell structure laboratory**
    Cristina Risco
11. **Structural and physical determinants of adenovirus assembly**
    Carmen San Martín
12. **Structure and function of molecular chaperones**
    José María Valpuesta
13. **Structural biology of virus fibres**
    Mark J. van Raaij

Near-atomic three-dimensional cryo-electron microscopy structure of a fungal double-stranded RNA virus (image from Luque et al. 2014. PNAS 111:7641)

Biocomputing unit

GROUP LEADER

José María Carazo

SENIOR SCIENTIST

Carlos Oscar Sorzano Sánchez

POSTDOCTORAL SCIENTISTS

Joaquín Otón
Roberto Melero del Río
Joan Segura Mora
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Josué Gómez Blanco
Mohsen Kazemi

PHD STUDENTS

José Luis Vilas Prieto
Rubén Sánchez Madrid

TECHNICIANS

Pablo Conesa Mingo
Laura del Caño Novales
Jesús Cuenca Alba
Blanca E. Benítez Silva

VISITING SCIENTIST

Roberto Marabini Ruiz
(Univ Autónoma de Madrid, Spain)

Electron microscopy in cryogenic conditions (cryo EM) is currently one of the key technologies used to unravel biological complexity, offering the possibility to analyse large, flexible macromolecules in near-native state at quasi-atomic resolution. Indeed, the journal Nature Methods chose cryo EM as method of the year in 2015. The need to re-accommodate all image processing workflows to address the new challenges in the field consequently became the target of our group. This goal fit very well with our focus on methods and software development, within our role as the image processing infrastructure providers for Instruct, the structural biology project of the European Strategic Forum for Research Infrastructures.

We have explored new areas of cryo EM image processing within our traditional software suite XMIPP (downloaded in recent years by thousands of researchers from all over the world), complemented by our newly developed software integration framework, named Scipion. Of particular importance are our novel approaches to soft validation of 3D maps and models, based on self-consistency of the reconstruction process and on a probabilistic view to protein domain-domain interactions, respectively.

Finally, in an effort to provide means to analyse cellular processes *in situ*, we have advanced in the field of cellular soft X-ray tomography, providing a detailed characterization of the instrument that has been used to enhance cellular 3D maps thanks to a combined reconstruction-restauration approach.

SELECTED PUBLICATIONS

Segura J, Sorzano CO, Cuenca-Alba J, Aloy P, Carazo JM. Using neighborhood cohesiveness to infer interactions between protein domains. Bioinformatics 2015; 31: 2545-52

Sorzano CO, Alcorlo M, de la Rosa-Trevín JM, Melero R, Foche I, Zaldívar-Peraza A, del Cano L, Vargas J, Abrishami V, Otón J, Marabini R, Carazo JM. Cryo-EM and the elucidation of new macromolecular structures: Random Conical Tilt revised. Sci Rep 2015; 5: 14290

Otón J, Pereiro E, Pérez-Berná AJ, Millach L, Sorzano CO, Marabini R, Carazo JM. Characterization of transfer function, resolution and depth of field of a soft X-ray microscope applied to tomography enhancement by Wiener deconvolution. Biomed Opt Express 2016; 7: 5092-5103

Vargas J, Otón J, Marabini R, Carazo JM, Sorzano CO. Particle alignment reliability in single particle electron cryomicroscopy: a general approach. Sci Rep 2016; 6: 21626

Koning RI, Gomez-Blanco J, Akopjana I, Vargas J, Kazaks A, Tars K, Carazo JM, Koster AJ. Asymmetric Cryo-Em Reconstruction of Phage Ms2 Reveals Genome Structure in Situ. Nat Commun 2016; 7: 12524

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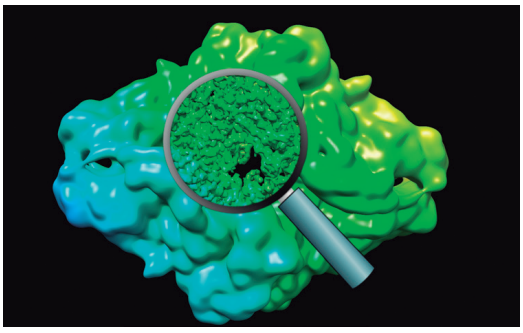

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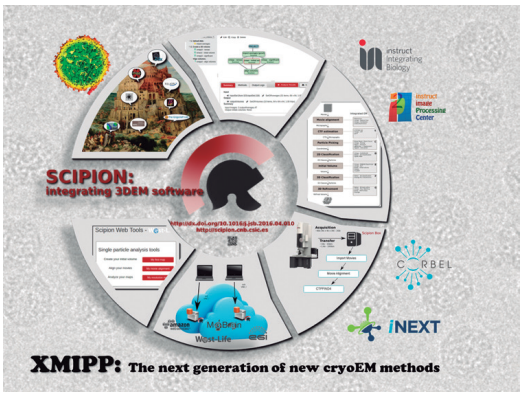

❶ CryoEM: probing the structure of large, flexible macromolecular complexes

❷ Image processing methods and software: at the leading edge

Structure of macromolecular assemblies

GROUP LEADER

José L. Carrascosa

POSTDOCTORAL SCIENTISTS

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Francisco Javier Chichón
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PHD STUDENTS

Jose Javier Conesa Muñoz
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TECHNICIANS

Encarna Cebrián

VISTING PHD STUDENTS

Aleikar Vásquez
(Universidad de Concepción, Chile)

Ramón Ramírez Orellana
(Pontificia Universidad Católica, Valparaíso, Chile)

The activity of the group is centred on the analysis of macromolecular nanomachines that carry out defined biological functions. We are presently studying the molecular basis of assembly and maturation in viral systems using cryo-electron microscopy and three-dimensional reconstruction at sub-nanometric resolution. We are also correlating structural data with other biophysical and nanomechanical measurements (AFM) to understand the molecular behaviour of protein containers designed for macromolecular delivery. Special emphasis is placed on DNA incorporation and ejection from viral particles, as well as on the use of viral assemblies in synthetic environments to obtain delivery vehicles with improved specificity and efficiency.

The interactions of viruses with target cells are studied using cryo-electron tomography of infected cells. To this end, we use correlative approaches with different microscopies; specifically, we are extending the use of tomographic methods by incorporation of new approaches such as soft X-rays microscopy, together with the use of absorption spectroscopy to correlate structural and chemical data at molecular resolution. These methods have been applied, besides our virus studies, to the interaction of super-paramagnetic nanoparticles with eukaryotic cells.

SELECTED PUBLICATIONS

Calero M, Chiappi M, Lazaro-Carrillo A, Rodríguez MJ, Chichón FJ, Crosbie-Staunton K, Prina-Mello A, Volkov Y, Villanueva A, Carrascosa JL. Characterization of interaction of magnetic nanoparticles with breast cancer cells. J Nanobiotechnol 2015; 13: 16

González-García VA, Pulido-Cid M, Garcia-Doval C, Bocanegra R, van Raaij MJ, Martín-Benito J, Cuervo A, Carrascosa JL. Conformational Changes Leading to T7 DNA Delivery upon Interaction with the Bacterial Receptor. J Biol Chem 2015; 290: 10038-10044

Mertens J, Casado S, Mata CP, Hernando-Pérez M, de Pablo PJ, Carrascosa JL, Castón JR. A protein with simultaneous capsid scaffolding and dsRNA-binding activities enhances the birnavirus capsid mechanical stability. Sci Rep 2015; 5: 13486

Chiappi M, Conesa JJ, Pereiro E, Sorzano CO, Rodríguez MJ, Henzler K, Schneider G, Chichón FJ, Carrascosa JL. Cryo-soft X-ray tomography as a quantitative three-dimensional tool to model nanoparticle:cell interaction. J Nanobiotechnol 2016; 14: 15

Conesa JJ, Otón J, Chiappi M, Carazo JM, Pereiro E, Chichón FJ, Carrascosa JL. Intracellular nanoparticles mass quantification by near-edge absorption soft X-ray nanotomography. Sci Rep 2016; 6: 22354

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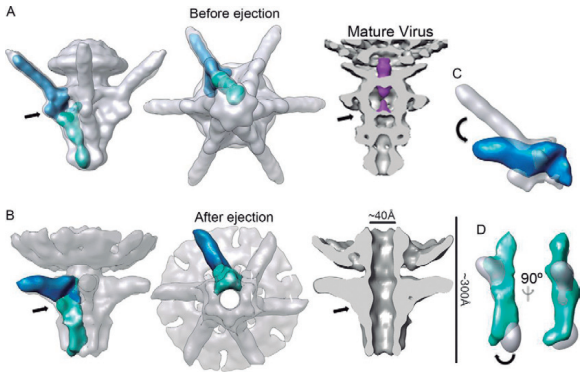

1 Conformational changes in the phage T7 tail complex related to DNA ejection from the viral capsid. A) Structural features of the tail in the DNA-containing mature phage. B) Changes undergone after DNA ejection. C, D) relative movements of tail structural components related to DNA ejection.

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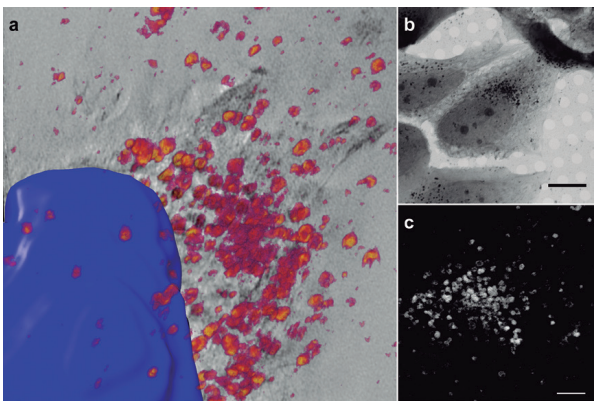
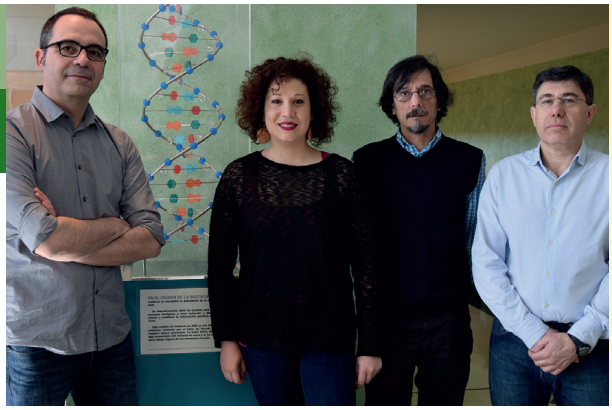


2 NEASXT analysis of iron oxide particles in cultured cells. a) Volumetric representation of iron oxide densities within a three-dimensional x-ray tomographic reconstructed cell. Blue is a segmented nucleus and iron is orange/red-coded. b) X-ray projection images of cells at 709 eV. c) Differential projection image containing specific iron signal.



Cell-cell and virus-cell interactions

GROUP LEADER

José M. Casasnovas

SENIOR SCIENTIST

César Santiago

POSTDOCTORAL SCIENTISTS

José Luis Torán
Manuela Zonca

MASTER'S STUDENT

Nerea Bernardo

SELECTED PUBLICATIONS

Muñoz-Alía MA, Fernández-Muñoz R, Casasnovas JM, Porras-Mansilla R, Serrano-Pardo A, Pagán I, Ordobás M, Ramírez R, Celma ML. Measles virus genetic evolution throughout an imported epidemic outbreak in a highly vaccinated population. Virus Res 2015; 196: 122-127

Echbarthi M, Zonca M, Mellwig R, Schwab Y, Kaplan G, DeKruyff RH, Roda-Navarro P, Casasnovas JM. Distinct trafficking of cell surface and endosomal TIM-1 to the immune synapse. Traffic 2015; 16: 1193-1207

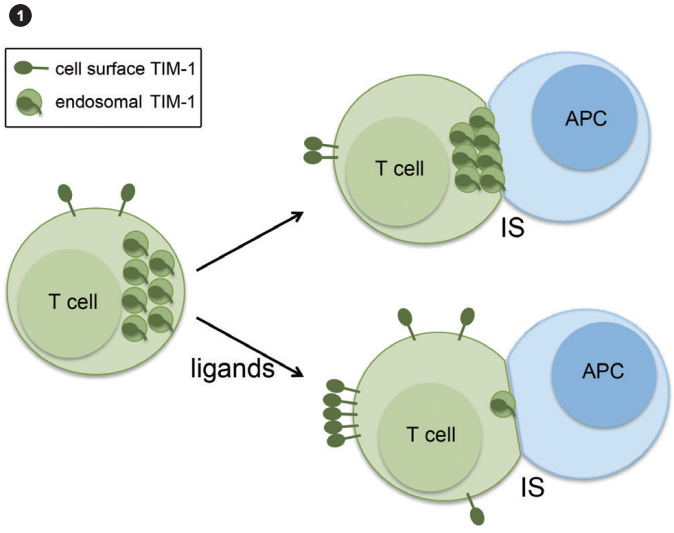
Our group studies the cell surface molecules that regulate the immune system and virus entry into host cells. We analyse receptor-ligand interactions related to immune processes, as well as virus binding to cells. In addition, we characterize virus neutralization by humoral immune responses and its correlation with virus entry into cells. Our research has provided key observations regarding immune receptor function, and has identified viral epitopes essential for virus infection, some of which are targeted by neutralizing antibodies. We apply structural (X-ray crystallography), biochemical and cell biology approaches.

Cell-cell interactions

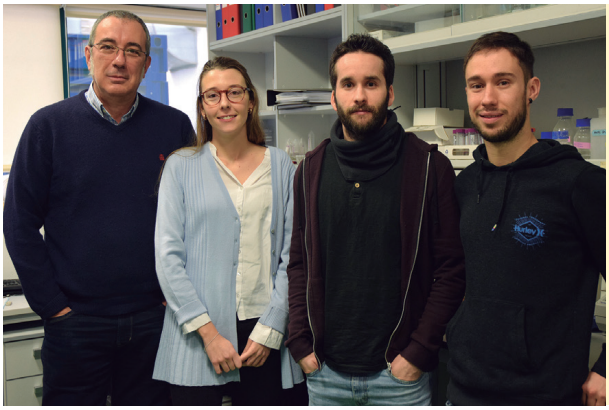
We studied how members of the TIM (T cell/transmembrane, immunoglobulin and mucin domain) family regulate immune responses. We recently analysed the subcellular distribution of TIM-1, a T cell costimulatory molecule linked to the development of atopic diseases. We determined that TIM-1 sorts mainly to endosomes in lymphoid cells. At difference from the cell surface protein, endosomal TIM-1 translocates to the immune synapse (IS), where it can contribute to antigen-dependent T cell co-stimulation. TIM-1 ligands increase the amount of cell surface protein, preventing its traffic to the IS. The bipolar sorting of TIM-1 observed during IS formation is determined by differences in its subcellular location, and probably modulates antigen-driven immune responses (Figure 1).

Antibody neutralization of viruses

Our group analysed virus-receptor interactions in measles virus and coronavirus. We determined crystal structures of virus-receptor complexes that define the mode used by measles virus and certain coronaviruses to bind to cell surface proteins to penetrate cells. Moreover, we are analysing how antibodies prevent and neutralize virus infection. We are presently identifying and characterizing human antibodies that neutralize human immunodeficiency virus, Ebola and measles viruses.



1 Subcellular TIM-1 location determines its trafficking to the immune synapse. In T cells, TIM-1 sorted to endosomal vesicles traffics to the immune synapse (IS) formed with antigen-presenting cells (APC), whereas cell surface TIM-1 does not traffic to the IS and can cluster opposite the IS.



Viral molecular machines

GROUP LEADER

José R. Castón

POSTDOCTORAL SCIENTISTS

Alvaro Ortega-Esteban
Carlos P. Mata

PHD STUDENTS

Carolina Allende-Ballestero

VISITING SCIENTIST

Daniel Luque
(Instituto de Salud Carlos III, Spain)

SELECTED PUBLICATIONS

Mertens J, Casado S, Mata CP, Hernando-Pérez M, de Pablo PJ, Carrascosa JL, Castón JR. A protein with simultaneous capsid scaffolding and dsRNA-binding activities enhances the birnavirus capsid mechanical stability. Sci Rep 2015; 5: 13485

Pascual E, Mata CP, Gómez-Blanco J, Moreno N, Bárcena J, Blanco E, Rodríguez-Frandsen A, Nieto A, Carrascosa JL, Castón JR. Structural basis for development of avian virus capsids to display influenza virus proteins for induction of protective immunity. J Virol 2015; 89: 2563-2574

Garcia-Ferrer I, Arêde P, Gómez-Blanco J, Luque D, Duquerroy S, Castón JR, Goulas T, Gomis-Rüth FX. Structural and functional insights into *Escherichia coli* α2-macroglobulin endopeptidase snap-trap inhibition. Proc Natl Acad Sci USA 2015; 112: 8290-8295

Luque D, Mata CP, González-Camacho F, González JM, Gómez-Blanco J, Alfonso C, Rivas G, Havens WM, Kanematsu S, Suzuki N, Ghabrial SA, Trus BL, Castón JR. Heterodimers as the structural unit of the T=1 capsid of the fungal doublestranded RNA Rosellinia necatrix quadrivirus 1. J Virol 2016; 90: 11220-11230

Llauró A, Luque D, Edwards E, Trus BL, Avera J, Reguera D, Douglas T, de Pablo PJ and Castón JR. Cargo-shell and cargo-cargo couplings govern the mechanics of artificially-loaded virus-derived cages. Nanoscale 2016; 8: 8328-9336

Our studies aim to elucidate structure-function-evolution relationships of viral macromolecular complexes, also known as viral nanomachines, which control many fundamental processes in the virus life cycle. Our model systems of viral molecular machines are the viral capsid and other viral macromolecular complexes, such as helical tubular structures and replication and ribonucleoprotein complexes.

Capsids should be considered dynamic structures that define different functional states and participate in multiple 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 is therefore essential to understand their properties. To reveal the three-dimensional structure of such complex assemblies, we use a multidisciplinary approach that combines structural analysis by three-dimensional cryo-electron microscopy with atomic structures (hybrid approach). We have incorporated state-of-the-art approaches to obtain near-atomic resolution structure directly from two-dimensional micrographs. Structural analysis of viruses is complemented by study of mechanical properties by atomic force microscopy (AFM), to examine the relationship between physical properties, such as rigidity and mechanical resilience, and virus biological function. Finally, our research establishes the basis for incorporation of heterologous proteins and/or chemicals into viral capsids (considered as nanocontainers), of potential use for future biotechnological applications.

Our group studies several viral systems with varying levels of complexity, focusing on a number of double-stranded RNA viruses such as birnaviruses (infectious bursal disease virus, IBDV) and several fungal viruses, as well as single-stranded RNA viruses such as rabbit haemorrhagic disease virus (RHDV). Some of these viruses cause serious diseases, and structural characterization of their macromolecular assemblies will offer new alternatives for altering their function, as well as possible vaccination strategies. We extend our studies to other viruses and eukaryotic complexes in collaboration with several national and international groups.

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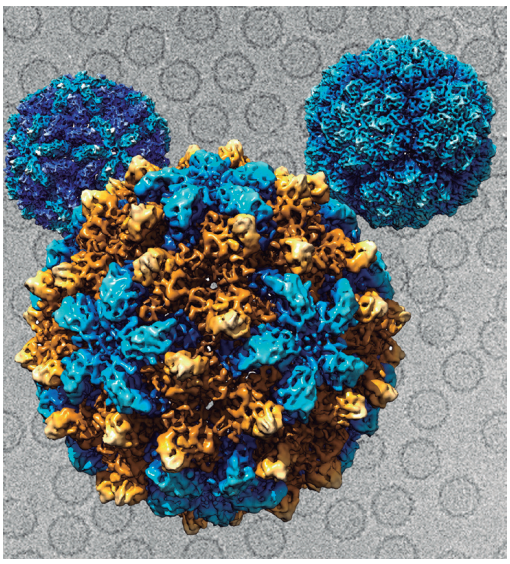

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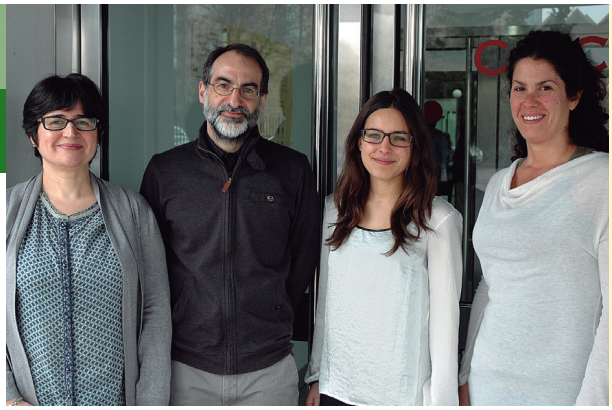
Most dsRNA viruses have a 120-subunit T=1 icosahedral capsid a with an asymmetric unit that includes two repeating units; these are two different proteins in the case of the dsRNA fungal virus Rosellinia necatrix quadrivirus 1 (front), whereas for reoviruses and the L-A virus of the yeast *Saccharomyces cerevisiae*, they are two copies of the same protein (left), and for Penicillium chrysogenum virus, two similar domains of a single protein (right). The background shows RnQV1 particles frozen in vitreous ice.



Electron tomography and image processing of cell structures

GROUP LEADER

José J. Fernández

POSTDOCTORAL SCIENTISTS

María del Rosario Fernández Fernández

MASTER'S STUDENT

Eva Martín Solana

UNDERGRADUATE STUDENT

Aira Merayo Mera

Our group is interested in the unique ability of electron tomography (ET) to visualize, in three dimensions, the subcellular architecture and macromolecular organization of cells and tissues *in situ* at a resolution of a few nanometres. Combined with image processing, ET has emerged as a powerful technique to address fundamental questions in molecular and cellular biology.

One of our research interests focuses on 3D analysis of the neuronal subcellular architecture. Here, ET and image processing are the central techniques, together with protocols that ensure preservation of brain tissue samples in near-native conditions. With this approach, we are exploring the structural alterations that underlie neurodegenerative diseases, particularly Huntington's disease.

We are also working in close collaboration with Dr. Sam Li (UCSF) in structural elucidation of the microtubule-organizing centre (MTOC). This is an important, complex cellular organelle whose dysfunction is linked to many diseases. In addition, we collaborate with teams at the CNB and other international groups in experimental structural studies.

Another important focus of our research is the development of new image processing techniques and tools for the advancement of ET. We are working on new methods for the different computational stages involved in structural studies by ET: image alignment, correction for the transfer function of the microscope, tomographic reconstruction, noise reduction, automated segmentation and subtomogram analysis.

SELECTED PUBLICATIONS

Kollman JM, Greenberg CH, Li S, Moritz M, Zelter A, Fong KK, Fernandez JJ, Sali A, Kilmartin J, Davis TN, Agard DA. Ring closure activates yeast γTuRC for species-specific microtubule nucleation. Nat Struct Mol Biol 2015; 22: 132-137

Agulleiro JI, Fernandez JJ. Tomo3D 2.0 - exploitation of advanced vector extensions (AVX) for 3D reconstruction. J Struct Biol 2015; 189: 147-152

Bernabe-Rubio M, Andres G, Casares-Arias J, Fernandez-Barrera J, Rangel L, Reglero-Real N, Gershlick DC, Fernandez JJ, Millán J, Correas I, Miguez DG, Alonso MA. Novel role for the midbody in primary ciliogenesis by polarized epithelial cells. J Cell Biol 2016; 214: 259-273

Fernandez JJ, Laugks U, Schaffer M, Bauerlein FJB, Khoshouei M, Baumeister W, Lucic V. Removing contamination-induced reconstruction artefacts from cryoelectron tomograms. Biophys J 2016; 110: 850-859

Fernandez-Fernandez MR, Ruiz-Garcia D, Martin-Solana E, Chichon FJ, Carrascosa JL, Fernandez JJ. 3D Electron Tomography of brain tissue unveils distinct Golgi structures that sequester cytoplasmic contents in neurons. J Cell Sci 2017; 130: 83-89

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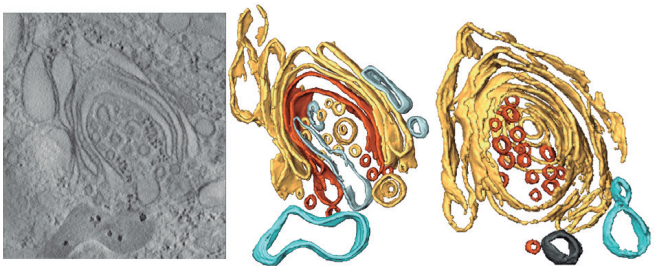

1 Three-dimensional visualization of neuronal subcellular architecture with electron tomography and image processing. Distinct Golgi structures that sequester cytoplasmic contents for their potential degradation were unveiled by electron tomography of brain tissue. These structures are composed of several concentric double-membrane layers that appear to be formed simultaneously by the direct bending and sealing of discrete Golgi stacks.

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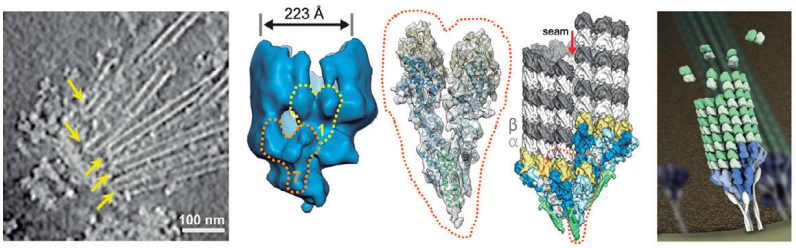
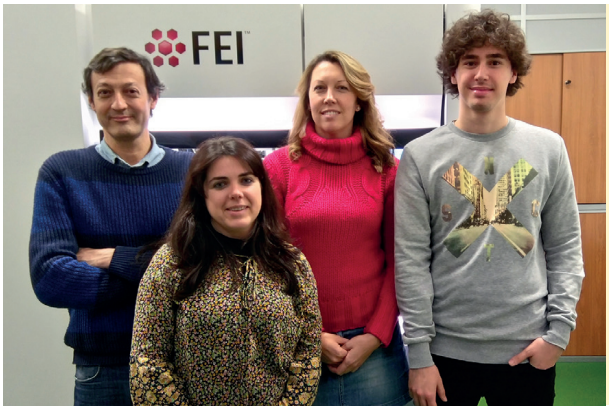


2 Microtubule nucleation machinery determined by electron cryomicroscopy. Electron cryotomography of isolated spindle pole bodies from yeast and subsequent subtomogram averaging revealed the *in situ* structure at 4 nm resolution, showing a ring of seven γTuSC subunits matching microtubule geometry. Electron cryomicroscopy and iterative helical real-space reconstruction allowed determination of the *in vitro* γTuSC structure at high resolution (7 Å) and modelling in pseudo-atomic detail.



Ultrastructure of viruses and molecular aggregates

GROUP LEADER

Jaime Martín-Benito

SENIOR SCIENTISTS

Rocío Coloma Ciudad
Rocío Arranz Ávila

PHD STUDENT

Diego Carlero Carnero

SELECTED PUBLICATIONS

Serna M, Carranza G, Martín-Benito J, Janowski R, Canals A, Coll M, Zabala JC, Valpuesta JM. The structure of the complex between α-tubulin, TBCE and TBCB reveals a tubulin dimer dissociation mechanism J Cell Sci 2015; 128: 1824-34

Ortín J, Martín-Benito J. The RNA synthesis machinery of negative-stranded RNA viruses. Virology 2015; 479-480: 532-44

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Torreira E, Moreno-Del Álamo M, Fuentes-Perez ME, Fernández C, Martín-Benito J, Moreno-Herrero F, Giraldo R, Llorca O. Amyloidogenesis of bacterial prionoid RepA-WH1 recapitulates dimer to monomer transitions of RepA in DNA replication initiation. Structure 2015; 23: 183-9

Sorzano CO, Martín-Ramos A, Prieto F, Melero R, Martín-Benito J, Jonic S, Navas-Calvente J, Vargas J, Otón J, Abrishami V, de la Rosa-Trevín JM, Gómez-Blanco J, Vilas JL, Marabini R, Carazo JM. Local analysis of strains and rotations for macromolecular electron microscopy maps. J Struct Biol 2016; 195: 123-8

The main research line of our group is the study of the influenza A ribonucleoproteins (RNPs) that conform the virus nucleocapsid. RNPs are macromolecular complexes, composed of the genomic RNA bound to multiple monomers of a nucleoprotein, and a single copy of the polymerase. In recent years, our laboratory has determined the structure of isolated RNPs at medium resolution, and by cryoelectron tomography we have verified that this structure is present in native virions. We are currently pursuing two major lines of research in this field that will extend into coming years. The first is improving the resolution of the RNP structure. For this purpose, we will use the state-of-the-art cryoelectron microscope, equipped with a direct electron detector, which was recently installed at the CNB. We have already discovered enormous conformational variability in RNP structure, made possible by the design of a new protocol able to classify and reconstruct helical structures. We hypothesized that this extreme conformational variability is closely related to the biological roles of the RNP. With this idea in mind, we opened the second major line of our research, elucidation of the transcription and replication mechanisms. We plan to complement structural data with biochemical assays that will allow us to establish the mechanism of action that underlies the biological function of RNPs.

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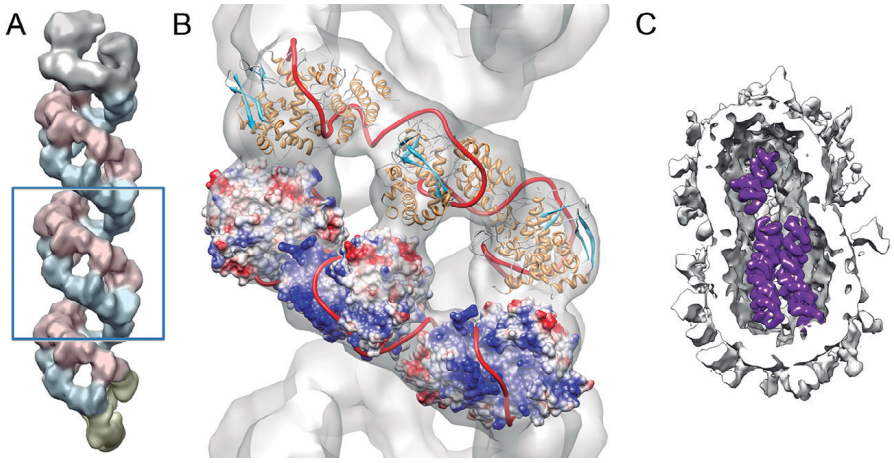

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A) Structure of the influenza virus ribonucleoprotein (RNP), the RNA polymerase is shown in grey.
B) Detail of the helical part showing the nucleoprotein and the modelled RNA position (red thread).
C) Tomogram of an influenza A virion showing the arrangement of the RNPs inside the virus.

Molecular biophysics of DNA repair nanomachines

GROUP LEADER

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We develop and employ single-molecule techniques to study the inner workings of protein machines involved in DNA repair processes and maintenance of chromosome structures. We are also interested in studying the mechanical properties of nucleic acids and their interaction with proteins, using novel single-molecule approaches based on atomic force microscopy (AFM) and magnetic tweezers.

In the last two years, we developed temperature-controlled magnetic tweezers to study the kinetics of double-stranded DNA processing by the protein complex AddAB, a helicase nuclease involved in dsDNA break repair. This method allowed us to determine the kinetic barrier of the enzymatic reaction. During this research, we also deepened our knowledge of the mechanism of Chi regulatory sequence recognition by AddAB, using a combination of biophysical and biochemical techniques. AddAB has a molecular latch that enables part of the DNA substrate to evade degradation beyond Chi. Our data suggest a model in which allosteric communication between Chi binding and the latch ensures quality control during recombination hotspot recognition.

We also investigated the ParB protein interaction with centromere-like DNA sequences, called parS, located near the origin of replication. We determined that ParB binds parS as a homodimer and displays positive co-operativity associated with formation of larger, poorly defined nucleoprotein complexes. Our data demonstrated that non-specific ParB binding leads to DNA condensation that is reversible by protein unbinding or force. These findings have consequences for chromosome condensation and segregation. Other ongoing projects using biophysical methods include study of the initiation of replication by the rolling circle mechanism and use of fluorescence microscopy and force spectroscopy in a combined magnetic-tweezers TIRF setup developed in-house. In addition, we use molecular dynamics methods to understand the effect of force in the mechanical properties of DNA and RNA. Finally, we have pushed the resolution limits of our AFM to resolve the A-form subhelical pitch periodicity of double-stranded RNA in near-physiological conditions.

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Ares P, Fuentes-Perez ME, Herrero-Galán E, Valpuesta JM, Gil A, Gomez-Herrero J, Moreno-Herrero F. High resolution atomic force microscopy of double-stranded RNA. Nanoscale 2016; 8: 11818-11826

Pastrana CL, Carrasco C, Akhtar P, Leuba SH, Khan SA, Moreno-Herrero F. Force and twist dependence of RepC nicking activity on torsionally-constrained DNA molecule. Nucleic Acids Res 2016; 44: 8885-8896

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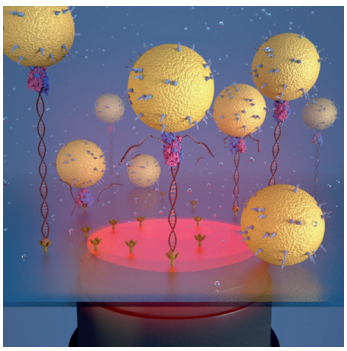

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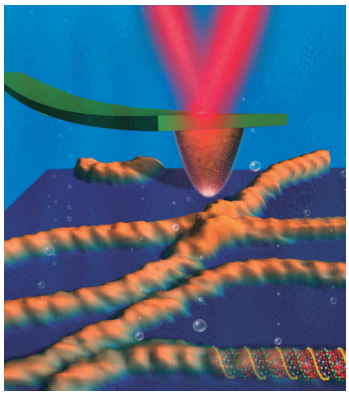

❶ Temperature-controlled magnetic tweezers. The kinetics of double-stranded DNA processing is captured at single-molecule resolution with a thermally stabilized magnetic tweezers microscope. An individual protein complex termed AddAB links one end of a DNA molecule to a superparamagnetic bead that is subjected to an upwards-directed external force. The other DNA end is specifically attached to a glass slide. The thermal conditions inside the flow cell are controlled at a precision of 0.1°C by modulating the temperatures of glass slide holder and objective simultaneously.

❷ High resolution atomic force microscopy of double-stranded RNA. Imaging of the pitch periodicity of double-stranded RNA under liquid using high-resolution atomic force microscopy (AFM). High-resolution images were reproduced with different high sensitivity AFM imaging modes. The experiments highlighted the two critical aspects to obtain such resolution, firstly, the interacting force, which can be minimized with appropriate tuning of each imaging mode parameter, and secondly, the sharpness of the tip.

Conformational-functional behaviour of proteins

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Our group focuses on the biophysical study of protein folding mechanisms, with special emphasis on the ultrafast folding regime, including our pioneering work on the downhill and one-state scenarios. We use a divide-and-conquer strategy, in which we extract mechanism-structure relationships by investigating a catalogue of fold archetypes (a collection of small, ultrafast folding domains representing all elementary folds observed in nature). We study the folding behaviour of such archetypes at the structural dynamic, thermodynamic, kinetic and single-molecule stochastic levels using (or developing) a panoply of top-notch experimental techniques including ultrafast kinetics, single-molecule fluorescence and single-molecule force spectroscopy, multidimensional nuclear magnetic resonance (NMR) and NMR relaxation, and differential scanning calorimetry (DSC), in conjunction with theoretical modelling and computer simulations. Our current catalogue comprises 16 characterized folds that provide unparalleled insights into folding mechanisms. In addition, we have continued developing improved methods for folding analysis, such as microsecond-resolution single-molecule fluorescence, analysis of protein folding at atomic resolution, and the reversible mechanical (un)folding of fast-folding proteins.

A second research focus targets the roles of folding mechanisms in protein function with an emphasis on conformational rheostats, a novel allosteric mechanism that exploits the conformational heterogeneity of downhill folding modules to produce analogue signals at the single-molecule level (in contrast to the binary response of allosteric switches). Here we are pursuing four main avenues: 1) development of protein-based biosensors based on downhill folding modules, 2) investigating the role of conformational rheostats in coordinating protein-protein interaction networks and 3) in the homing mechanism transcription factors use to search efficiently for and bind to their target DNA sequence, and 4) engineering of controllable symmetric macromolecular complexes from monomeric globular proteins using the principle of partial unfolding coupled to assembly.

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Schönfelder J, Perez-Jimenez R, Muñoz V. A simple two-state protein unfolds mechanically via multiple heterogeneous pathways at single-molecule resolution. Nat Commun 2016; 7: 11777

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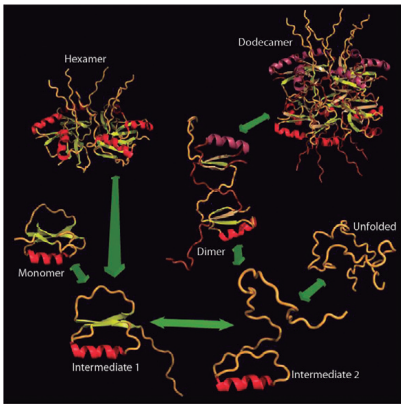
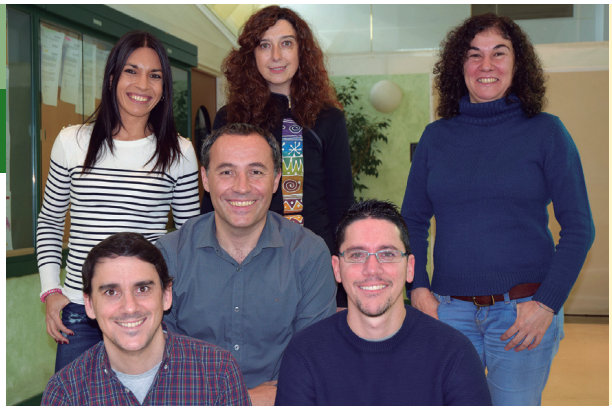
2



2 **Agg-Model:** General scheme for the assembly process created in CI2 after a mutational procedure developed to produce oligomerization. In this process the native state suffers two partial openings to generate intermediate 1 and intermediate 2, which can oligomerize to form hexameric and dodecameric toroidal rings. These assemblies can be isolated and they are very stable in solution.

1 **EnHDalongDNA:** Engrailed homeodomain, a Drosophila transcription factor, specifically binding to its DNA target sites to carry out its biological function. The multiple binding modes in the DNA searching process were addressed by coarse-grained molecular simulations. The dynamic picture facilitates DNA co-localization as well as specific DNA binding (function-on) and release (function-off) during EnHD-DNA recognition.



Functional proteomics

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Functional proteomics draws a complete map of protein dynamics, interactions and post-translational modifications that take place in the cell. Our group monitors proteins involved in molecular interactions and pathways relevant to pathologies in a variety of tissues, cell types and organisms following various experimental treatments/conditions. We are also incorporating the latest technologies to specific functional proteomic projects:

1) Human Proteome Project: This HUPO-sponsored project intends a systematic mapping of the human proteome by constructing a protein catalogue on a chromosome-to-chromosome basis. We coordinate a national initiative consisting of 15 proteomic laboratories that study the protein expression profile of chromosome 16 in a broad panel of human cell lines and samples, using shotgun as well as targeted (MRM) experimental approaches.

2) Interactomics: The project "Interactomics of the Centrosome", funded by the Madrid Regional Government, aims to characterize interactions between centrosomal proteins and to identify macromolecular complex components through cell map proteomics approaches based on affinity tags, stable isotopic labelling, mass spectrometry and peptide arrays.

3) Immunopeptidomics: We are characterizing the immunopeptidome bound to major histocompatibility complex (MHC) class I molecules, emphasizing disease-associated differences and designing methods for the analysis of post-translational modifications (phosphorylation) in the MHC class I-bound peptide set.

4) Development of advanced qualitative and quantitative proteomic strategies to study a model of human glioblastoma.

5) Computational proteomics: Application of probability-based methods for large-scale peptide and protein identification and quantitation from tandem mass spectrometry data.

6) Quality control and experimental standardization: Reproducibility and robustness of proteomics workflows are key issues being addressed by our laboratory through participation in multicentre studies within the ProteoRed-ISCIII project led by our group.

SELECTED PUBLICATIONS

Horvatovich P, Lundberg EK, Chen YJ, *et al.* Quest for Missing Proteins: Update 2015 on Chromosome-Centric Human Proteome Project. J Proteome Res 2015; 14: 3415-31

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Sánchez-López ÁM, Bahaji A, De Diego N, Baslam M, Li J, Muñoz FJ, Almagro G, García-Gómez P, Ameztoy K, Ricarte-Bermejo A, Novák O, Humplík JF, Spíchal L, Doležal K, Ciordia S, Mena MC, Navajas R, Baroja-Fernández E, Pozueta-Romero J. Arabidopsis Responds to *Alternaria alternata* Volatiles by Triggering Plastid Phosphoglucose Isomerase-Independent Mechanisms. Plant Physiol 2016; 172: 1989-2001

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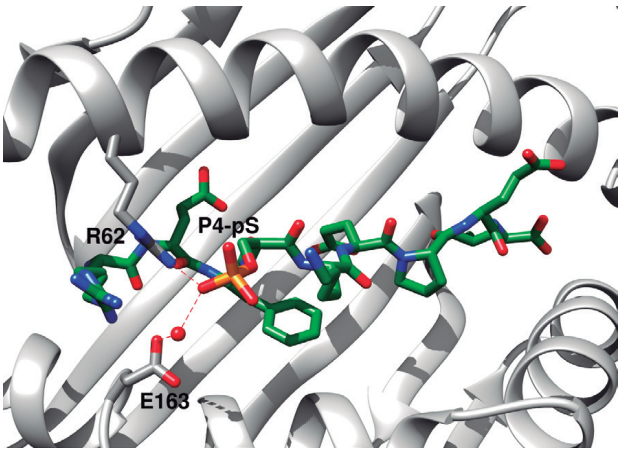

1 HLA-B*40 (grey) in complex with a phosphorylated ligand (green). The phosphopeptides displayed by HLA-B molecules are preferentially phosphorylated at peptide position 4. This is explained by the conservation of residue Arg62 in the heavy chain, which interacts with the phosphate group of the peptide ligand.

Cell structure laboratory

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SELECTED PUBLICATIONS

Fernández de Castro I, Tenorio R, Risco C. Virus assembly factories in a lipid world. Curr Opin Virol 2016; 18: 20-26

Nawaz-ul-Rehman MS, Prasanth KR, Xu K, Sasvari S, Kovalev N, Fernández de Castro I, Barajas D, Risco C, Nagy PD. Viral replication protein inhibits cellular cofilin actin depolymerization factor to regulate the actin network and promote viral replicase assembly. PLoS Pathog 2016; 12: e1005440

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RNA viruses, which are devastating pathogens for humans, animals and plants, replicate their genomes on intracellular membranes of their hosts. These viruses recruit numerous cell factors to build their membranous replication platforms, also known as viral factories. Due to the complex interactions detected in these structures, factories could be considered as the actual living state of viruses.

In our laboratory, we study how viruses manipulate cell organization to build their replication factories. In 2015 and 2016, our team characterized the factories assembled by several pathogenic RNA viruses. We discovered that human Reovirus triggers a complex process of endomembrane remodelling to build the replication platform, and that the human influenza virus assembles a new organelle to transport the viral ribonucleoproteins from the nuclear envelope to the plasma membrane. Other studies identified several cell factors that are critical for Tombusvirus replication.

During the last two years, we also undertook three new projects with the following aims: 1) to identify the lipid transfer proteins subverted by Reoviruses and Bunyaviruses to build their factories. Viruses were recently found to subvert cellular proteins involved in lipid synthesis and transport to build replication organelles, but details of mechanisms involved in these processes remain unknown. 2) To understand how resistance to antivirals is generated in cells. The results of these studies might identify potential targets for developing new therapeutic antiviral drugs and for drug repurposing. 3) To apply new protocols of correlative light and electron microscopy (CLEM) to determine how PALM/STORM super-resolution microscopy works in whole cells and use this new approach to study virus-cell interactions.

Finally, we developed a new website (www.cellstructurelab.es), submitted a project to the FECYT crowdfunding platform "Precipita" (www.precipita.es/proyecto/estudio-de-farmacos-contra-virus-con-nuevas-tecnicas-de-imagen.html), and presented our research on national radio stations and in news media.

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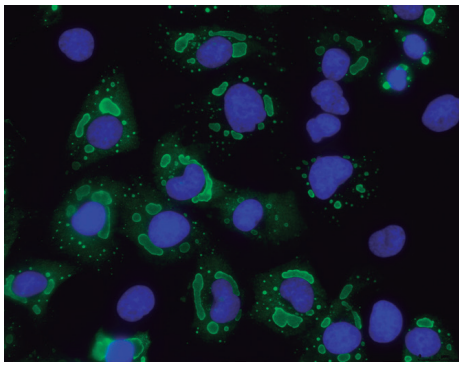

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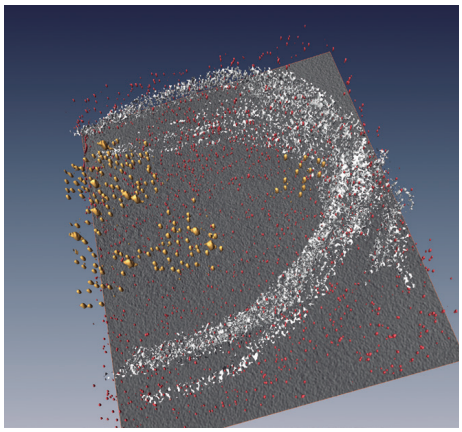
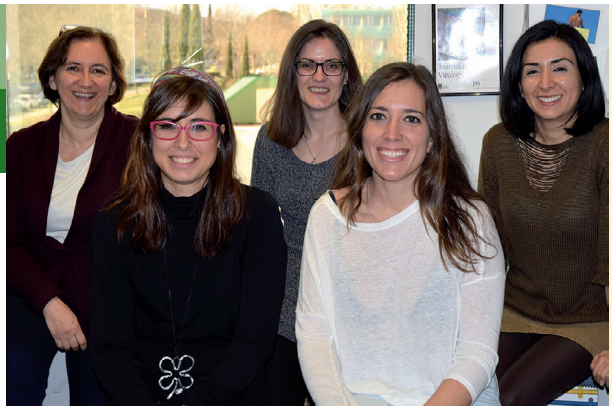


1 Confocal microscopy of Reovirus factories in human cells

2 Electron tomogram of Tombusvirus replicase molecules in yeast cells



Structural and physical determinants of adenovirus assembly

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We are interested in the principles that govern complex virus assembly. Our model system is adenovirus, a specimen of interest in both basic virology and nanobiomedicine. Adenoviruses are human pathogens and potential tools for vaccination and oncolysis. With a 95 nm capsid, composed of more than 10 different proteins, adenovirus is among the most complex non-enveloped icosahedral viruses. Of the approximately 200 adenovirus types found so far in nature, only a few have been characterized, and there are still considerable open questions regarding the architecture and assembly of the infectious particle.

Our research lines focus on answering some of these questions, such as how the adenovirus genome is packaged and condensed to fit the tiny space within the capsid, what are the key elements that modulate virion stability, and what are the physicochemical properties of uncharacterized adenoviruses, with potential uses as alternative vectors. We use a multidisciplinary approach to address these issues, combining biophysics, structural and molecular biology techniques. We have described the structural and physical changes experienced by adenovirus during maturation, how they modulate its stability and hence, its infectivity. Using atomic force microscopy, we have shown that maturation pressurizes adenovirus to prepare it for uncoating after entering the host cell (Figure 1). We determined the location of a genome-capsid linking protein in the empty particle, and how this link is removed during maturation to allow genome release in the host cell (Figure 2). Using cryo-electron tomography, we reported the first 3D images of the adenovirus mini-chromosome in its physiological environment, that is, within the virion.

We collaborate to develop efficient adenovirus vectors for therapeutic purposes, and coordinate the Network of Excellence AdenoNet, bringing together Spanish groups dedicated to the study of adenoviruses, from their detection in nature to their clinical impact and uses in virotherapy.

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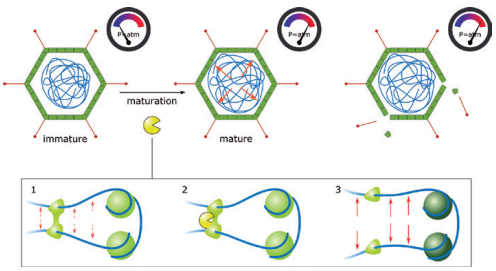

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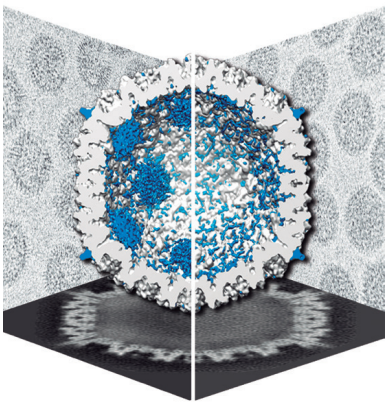

1

Adenovirus encodes histone-like proteins that associate with its genome, resulting in a confined DNA–protein condensate (core). Cleavage of these proteins during maturation decreases core condensation and increases the pressure inside the icosahedral capsid. This pressurization destabilizes the vertex proteins, facilitating the first steps of adenovirus uncoating in the cell.

2

Packaging protein L1 52/55 kDa links the Adenovirus genome and capsid during assembly. Difference maps (blue surfaces) between cryo-EM structures of the mature adenovirus particle and two types of genome-lacking particles show that full-length L1 52/55k interacts preferentially with the inner vertex components (left). After partial processing by the maturation protease, this interaction is lost (right), removing the capsid-core link to facilitate genome release in the last virus uncoating steps.

SELECTED PUBLICATIONS

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Structure and function of molecular chaperones

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SELECTED PUBLICATIONS

Plimpton RL, Cuéllar J, Lai CW..., Prince JT, Carrascosa JL, Valpuesta JM, Willardson BM. Structures of the Gβ-CCT and PhLP1–Gβ-CCT complexes reveal a mechanism for G-protein β-subunit folding and Gβγ dimer assembly. Proc Natl Acad Sci USA 2015; 112: 2413-2418

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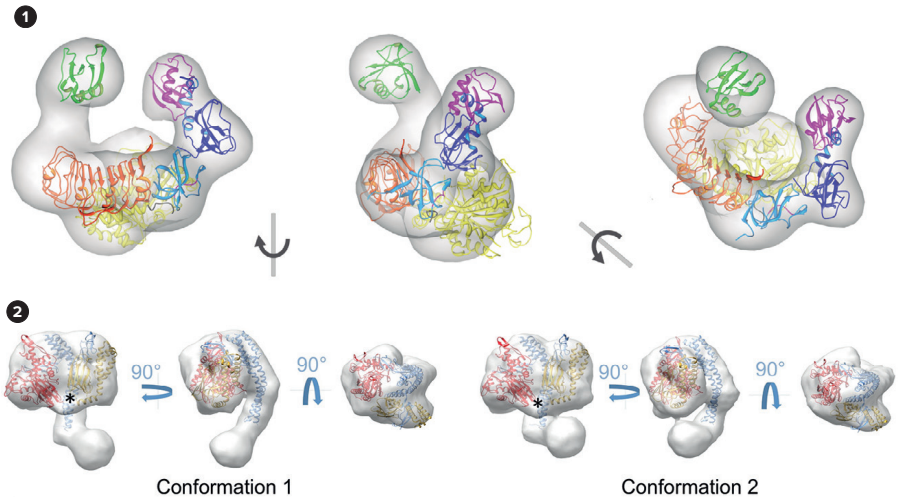
Most cell processes are executed by sets of proteins that work like molecular machines in a coordinated manner, thus acting as an assembly line and making the process more efficient. One such assembly line is that formed by molecular chaperones, a group of proteins involved in cell homeostasis through two opposite functions, protein folding and degradation. In recent years, it was found that chaperones are not only devoted to assisting the folding of other proteins but also, in certain conditions, that they can be active players in protein degradation. The two processes are carried out through the transient formation of complexes between different chaperones and co-chaperones. Our goal is the structural characterization of some of these complexes, with the aim of understanding the structural mechanisms by which they function. To this end, we are using electron microscopy and image processing techniques as our main tools, and combining the information obtained with these techniques with the available atomic structures of some of these chaperones and co-chaperones.



1

Three views of a complex between the chaperones TBCE (green, orange and blue domains) and TBCB (pink and navy blue domains), and the substrate α-tubulin (yellow). The two chaperones are involved in the folding and degradation pathways of α-tubulin (from Serna *et al.*, 2015).

2

Three orthogonal views of the two main conformations obtained for the complex between the chaperone DnaK (Hsp70) and its cochaperone, the nucleotide exchange factor GrpE

Structural biology of virus fibres

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SELECTED PUBLICATIONS

Nguyen TH, Vidovsky MZ, Ballmann MZ, Sanz-Gaitero M, Singh AK, Harrach B, Benko M, van Raaij MJ. Crystal structure of the fibre head domain of bovine adenovirus 4, a ruminant adenovirus. Virol J 2015; 12: 81

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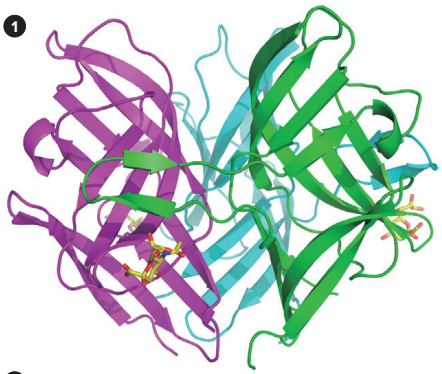
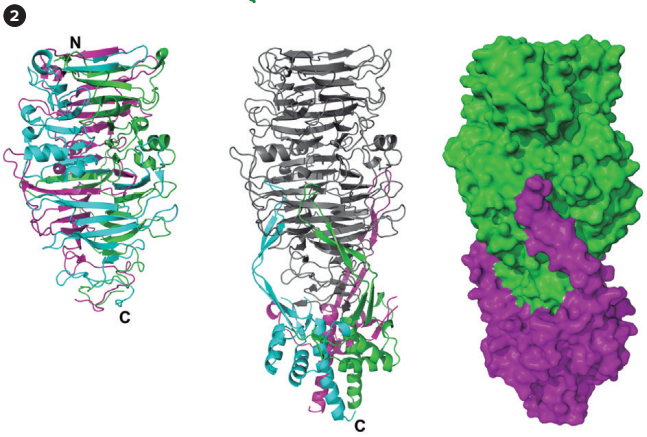
Recognition of the correct host cell to infect is of crucial importance to a virus. Many viruses bind to their host cell receptors via specialized spike proteins or fibre proteins, like adenoviruses and bacterial viruses such as T4, T5 and T7. The fibres all have the same basic architecture: they are 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. These trimeric fibrous proteins are very stable to denaturation by temperature or detergents.

In 2015 and 2016, we determined the structures of a domain of the fibre of the Salmonella phage epsilon15, of the fibres of Staphylococcal phages S24-1 and K, of several animal adenovirus fibre head domains (raptor adenovirus and lizard adenovirus 2), and of the atadenovirus LH3 capsid protein, which showed a surprising similarity to beta-helical bacteriophage tailspikes. We also solved co-crystal structures of a bacteriophage fibre protein and several adenovirus fibre proteins with glycans, revealing the atomic basis of their interaction with their putative receptors. In addition, we collaborated with other research groups in crystallization and structure solution of the proteins and peptides they produce, such as that of the *Fasciola hepatica* calcium-binding protein FhCaBP2.

Knowledge of the structures of viral and bacteriophage fibre proteins could give rise to various biotechnological applications. As adenovirus is used in experimental gene therapy, modification of its fibre should allow targeting to specific cell receptors. Modification of bacteriophage fibre receptor-binding specificities might lead to improved detection and elimination of specific bacteria.



1 Structure of the turkey adenovirus 3 fibre head (monomers in green, cyan and magenta) bound to sialyllactose (yellow, red and blue).



2 Structure of the receptor-binding domain of the T5 L-shaped tail fibre without its intramolecular chaperone (left) and with its intramolecular chaperone (centre and right).