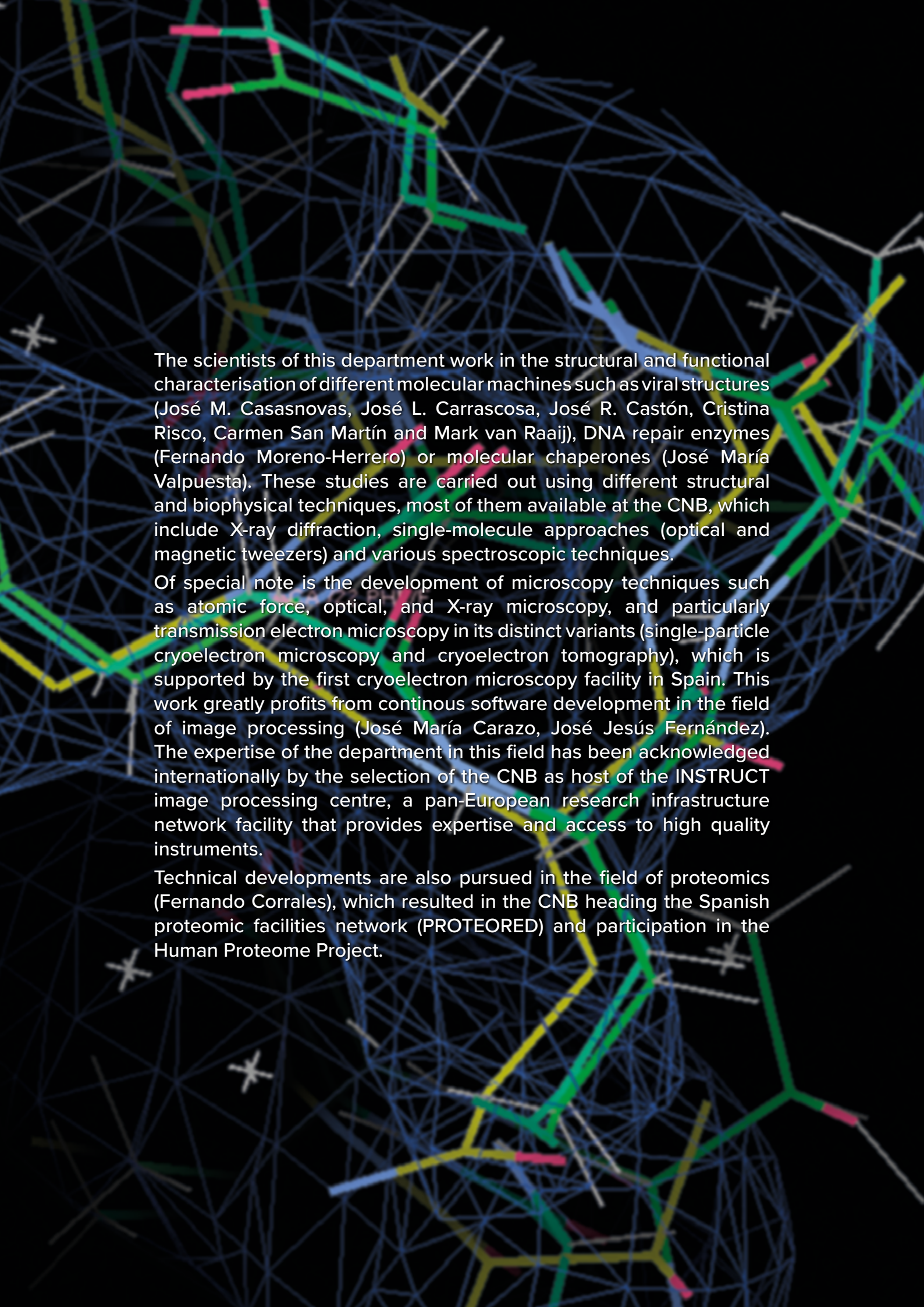

The scientists of this department work in the structural and functional characterisation of different molecular machines such as viral structures (José M. Casasnovas, José L. Carrascosa, José R. Castón, Cristina Risco, Carmen San Martín and Mark van Raaij), DNA repair enzymes (Fernando Moreno-Herrero) or molecular chaperones (José María Valpuesta). These studies are carried out using different structural and biophysical techniques, most of them available at the CNB, which include X-ray diffraction, single-molecule approaches (optical and magnetic tweezers) and various spectroscopic techniques.

Of special note is the development of microscopy techniques such as atomic force, optical, and X-ray microscopy, and particularly transmission electron microscopy in its distinct variants (single-particle cryoelectron microscopy and cryoelectron tomography), which is supported by the first cryoelectron microscopy facility in Spain. This work greatly profits from continous software development in the field of image processing (José María Carazo, José Jesús Fernández). The expertise of the department in this field has been acknowledged internationally by the selection of the CNB as host of the INSTRUCT image processing centre, 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 (Fernando Corrales), which resulted in the CNB heading the Spanish proteomic facilities network (PROTEORED) and participation in the Human Proteome Project.

Macromolecular Structures

HEAD OF DEPARTMENT
José María Valpuesta

RESEARCH GROUPS

1. **Biocomputing unit**
   José María Carazo & Carlos Óscar Sorzano Sánchez
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. **Functional proteomics**
   Fernando Corrales
6. **Electron tomography and image processing of cell structures**
   José Jesús Fernández
7. **Ultrastructure of viruses and molecular aggregates**
   Jaime Martín-Benito
8. **Molecular biophysics of DNA repair nanomachines**
   Fernando Moreno-Herrero
9. **Cell structure laboratory**
   Cristina Risco
10. **Structural and physical determinants of complex virus assembly**
    Carmen San Martín
11. **Structure and function of molecular chaperones**
    José María Valpuesta
12. **Structural biology of virus fibres**
    Mark J. van Raaij

Biocomputing unit

GROUP LEADERS
José María Carazo
Carlos Óscar Sorzano Sánchez

SENIOR SCIENTISTS
Joan Segura Mora
Marta Martínez
Roberto Melero del Río

POSTDOCTORAL SCIENTISTS
Amaya Jiménez
David Maluenda
Yunior Fonseca Reyna
Peter Horvath
Erney Ramírez

TECHNICIANS
Pablo Conesa Mingo
Laura del Caño Novales
Carmen Yaiza Rancel Gil

ADMINISTRATION
Blanca E Benítez

PhD STUDENTS
Javier Mota
José Luis Vilas
Rubén Sánchez García
David Strelak

MASTER'S STUDENT
Estrella Fernández Giménez

UNDERGRADUATE STUDENTS
Ana Sanmartín Domenech
Federico de Isidro Gómez
David Herreros Calero

VISITING SCIENTISTS
Roberto Marabini Ruiz
Lisandro Otero

SELECTED PUBLICATIONS

Ljubetič A, Lapenta F, *et al*. Design of coiled-coil protein-origami cages that self-assemble *in vitro* and *in vivo*. Nat Biotechnol 2017; 35: 1094-1101.

Segura J, Sanchez-Garcia R, *et al*. 3DBIONOTES v2.0: a web server for the automatic annotation of macromolecular structures. Bioinformatics 2017; 33: 3655-3657.

Sacristan C, Ahmad MUD, *et al*. Dynamic kinetochore size regulation promotes microtubule capture and chromosome biorientation in mitosis. Nat Cell Biol 2018; 20: 800-810.

Sorzano COS, Vargas J, *et al*. A new algorithm for high-resolution reconstruction of single particles by electron microscopy. J Struct Biol 2018; 204: 329-337.

Vilas JL, Gómez-Blanco J, *et al*. MonoRes: Automatic and Accurate Estimation of Local Resolution for Electron Microscopy Maps. Structure 2018; 26: 337-344.

Electron microscopy under cryogenic conditions (cryo EM) is nowadays one of the key technologies to unravel biological complexity, offering the possibility to analyse large and flexible macromolecules in close to their native state to quasi atomic resolution. Indeed, cryo EM was awarded the Nobel Prize in Chemistry in 2017.

Our group is focused on the development of new image processing algorithms capable of dealing with the low signal-to-noise ratios characteristic of these images and, at the same time, trying to extract all the information present in them. We are especially active in the domain of single particle analysis where we cover the whole image processing pipeline from the processing of the movies acquired by the microscope to the accurate 3D reconstruction of the macromolecular structural model under study. We have also proposed new ways of validating the results. All our algorithms are publicly available within the Xmipp software package. We actively collaborate with structural biologists participating in the image processing and structural elucidation of several biological macromolecules of high scientific interest. Additionally, we give structural analysis support to European scientists through the Instruct and iNext platforms, and we are the reference centre for image processing in the European Infrastructure for Structural Biology Instruct-ERIC.

Beside the development of new algorithms, we also develop software infrastructure for the community. In particular, an image processing workflow engine that allows the traceability and reproducibility of the data analysis steps taken from the raw data to the final structure. This workflow engine, called Scipion, is installed in several electron microscopy facilities worldwide, and it has served several thousands of projects around the world.

We also play a relevant role in Structural Bioinformatics by connecting the structural information obtained from the electron microscope with atomic models of these macromolecules and a whole range of genomic, proteomic and interactomic information publicly available in bioinformatics databases. This connection helps to better understand the biological properties of the reconstructed structures and has been recognised as one of the few Recommended Interoperability Resources of the European Infrastructure for Life Science Information, ELIXIR.

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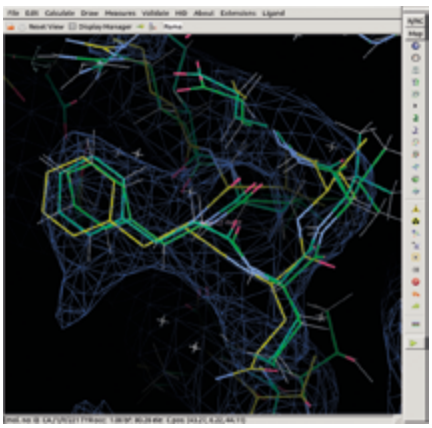
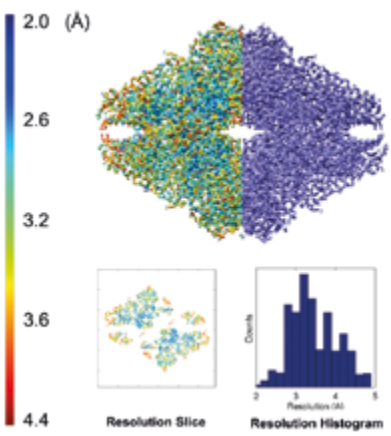


❶ *Detail of a macromolecular map and the variability of several atomic models fitted to it.*

❷



❷ *Example of local resolution result for a macromolecular map.*

Structure of macromolecular assemblies

GROUP LEADER
José L. Carrascosa

SENIOR SCIENTISTS
Ana Cuervo Gaspar
Rebeca Bocanegra Rojo
Francisco Javier Chichón
Mª Josefa Rodríguez Gómez

TECHNICIAN
Encarna Cebrián

PhD STUDENT
María del Mar Pérez Ruiz

VISITING SCIENTIST
Aleikar José Vásquez Suarez
(Universidad de Concepción, Chile)

We have continued our work on the analysis of the molecular bases of assembly and nanoscopic properties of different macromolecular complexes, using a combination of cryo-electron microscopy and other biophysical methods such as atomic force microscopy. Our studies have covered different resolution levels. We have carried out whole cell correlation of chemical characterisation and structural determination (introducing soft X-ray microscopy and spectroscopy) for nanoparticle interaction with eukaryotic cells with potential medical applications, as well as for the detailed analysis of the morphogenesis of complex eukaryotic viruses. At a more detailed level (molecular up to atomic resolution), we have studied different aspects of the molecular behaviour of viral-derived nanocontainers as potential vectors for site-directed delivery.

One of the main efforts of our work has been devoted to the study on how viral particles incorporate DNA inside the virus, how the DNA is stabilised, and which are the virus components involved in its ordered delivery upon infection. Extensive use of cryo-electron microscopy on different virus components of phage T7 have revealed the atomic structure of the machinery involved in DNA translocation (the connector and several tail components) which, in turn, has provided the bases for understanding how the DNA is securely packaged inside the viral shell, and how the different tail components assemble sequentially. Another main component of the phage head is the core, which is composed of several proteins that dissociate upon viral interaction with the bacterial receptor, and they reassemble again to build a conduit for DNA delivery to the cell cytoplasm. Different complexes derived from core proteins are currently being studied providing first insights on how this complex process is accomplished.

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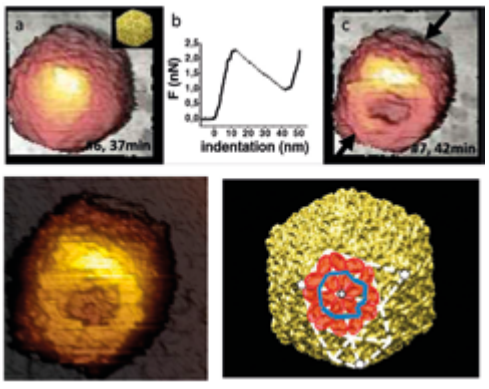

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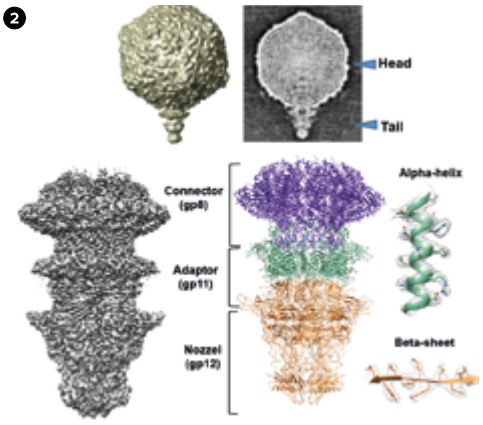

1 *Single indentation assay. Phage T7 particles (a) indented using AFM (b) produced the shell breakage following a discrete pattern (c). In the images below, the breakage area (darker) is interpreted on the bases of the T7 shell structure obtained by cryo-TEM. White: lattice lines. Red: capsid subunits. Blue: border of the breakage area following the subunits of one hexamer. Adapted from: De Pablo, Hernández-Pérez, Carrasco and Carrascosa. J Biol Phys 2018; 44: 225-235.*

2 *Cryo-EM structure of the tail of phage T7 at atomic resolution. Upper panel, left: CryoEM reconstruction of phage T7 particles. Upper panel, right: section along a longitudinal axis revealing the features of the head and the tail. Lower panel, left: Cryo-EM reconstruction of isolated tail particles. Secondary structure elements are clearly visible at 3.5 Å resolution. Lower panel, right: Atomic structure of the three components of the tail. As an example, the quality of the resolved data is shown in the case of one α-helix and one β-sheet.*

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Mingorance L, Castro V, Ávila-Pérez G, Calvo G, Rodriguez MJ, Carrascosa JL, Pérez-Del-Pulgar S, Forns X, Gastaminza P1. Host phosphatidic acid phosphatase lipin1 is rate limiting for functional hepatitis C virus replicase complex formation. PLoS Pathog 2018; 14: e1007284.

Mata CP, Mertens J, Fontana J, Luque D, Allende-Ballestero C, Reguera D, Trus BL, Steven AC, Carrascosa JL, Castón JR. The RNA-Binding Protein of a Double-Stranded RNA Virus Acts like a Scaffold Protein. J Virol 2018; 92: e00968-18.

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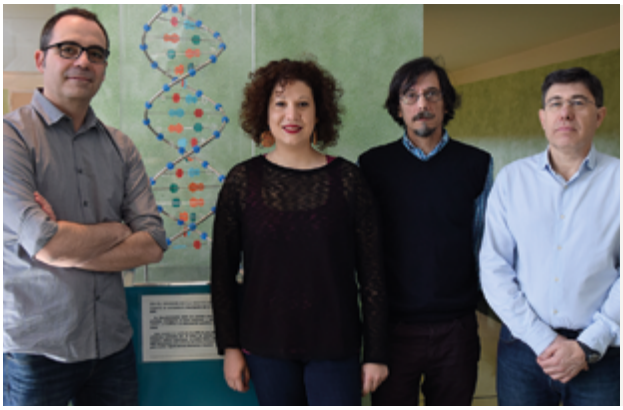

Cell-cell and virus-cell interactions

GROUP LEADER
José M. Casasnovas

SENIOR SCIENTIST
César Santiago

POSTDOCTORAL SCIENTISTS
José Luis Torán
Manuela Zonca

TECHNICIAN
David Almendral

UNDERGRADUATE STUDENT
Nuria Sanchez
Universidad de Alcalá de Henares, Spain

VISITING PhD STUDENT
Ana Belén Peñaherrera
Universidad Tecnológica Nacional, Nacional, Argentine
Sara Insausti
Universidad Politécnica Valencia, Spain

SUMMER STUDENTS
Ignacio Ramírez Gallego
Silvia Calvo Serrano

SELECTED PUBLICATIONS

Santiago C, Mudgal G, Reguera J, Recacha R, Albrecht S, Enjuanes L, Casasnovas JM. Allosteric inhibition of aminopeptidase N functions related to tumor growth and virus infection. Sci Rep 2017; 7: 46045.

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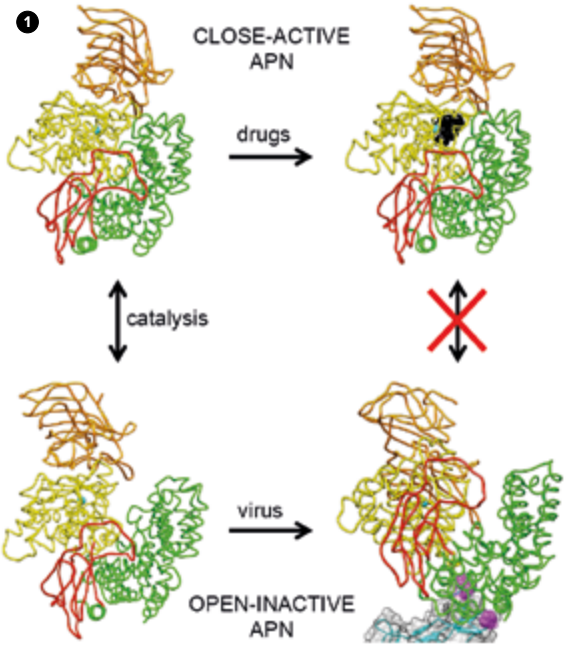
Our group studies the cell surface molecules that regulate the immune system and virus entry into host cells. We analyse receptor-ligand interactions related to immune processes, as well as virus binding to cells. In addition, we characterise virus neutralisation by humoral immune responses and its correlation 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 neutralising antibodies. Our multidisciplinary research applies structural (X-ray crystallography), biochemical and cell biology approaches.

Cell-surface ectoenzymes. Recently, we reported the dynamic conformation of aminopeptidase N (APN), a membrane-bound ectoenzyme associated to various physiological processes and diseases such as blood pressure control, angiogenesis, cell adhesion and motility, tumour cell growth and virus infections. Inhibitors of APN catalysis prevent tumour growth and invasion. We determined several crystal structures that defined the dynamic motion of the APN ectodomain and distinct functional forms (Figure 1), which are probably responsible for its "moonlighting" activity. Viral proteins bound to an inactive APN open form, prevented transition to a closed form and inhibited catalysis. In addition, drugs that target the active site and prevent tumour growth mediated allosteric inhibition of coronavirus cell infection. Blocking APN dynamics can thus be a valuable approach for the development of drugs that target this ectoenzyme.

Antibody neutralisation of viruses. We are analysing how antibodies prevent and neutralise virus infection. Presently we are characterising a human antibody that neutralise Human Immunodeficiency Virus (HIV) and designing bispecific molecules against HIV-1. In addition, we built human antibody libraries that comprise the immunoglobulin repertoire of convalescent patients of Ebola. We aim to identify human antibodies that neutralise Ebola virus.



1 *Allosteric inhibition of APN by preventing ectodomain motions. Structures of APN define the dynamic conformation of its ectodomain and different functional forms (closed to open). Peptide catalysis requires the transition between the closed and open APN forms. Coronavirus bind to the open form and prevent transition to the closed form and peptide hydrolysis. Drugs block formation of the open form and inhibit coronavirus infections.*

Viral molecular machines

GROUP LEADER
José R. Castón

SENIOR SCIENTIST
Javier María Rodríguez Martínez

POSTDOCTORAL SCIENTISTS
Alvaro Ortega Esteban
Carlos Pérez Mata

TECHNICIAN
Esther Marín Forero

PhD STUDENTS
Carolina Allende Ballestero
David Gil Cantero
Celia Gutiérrez García

MASTER'S STUDENT
Mª Jesús Rodríguez Espinosa

UNDERGRADUATE STUDENT
Diego Pérez Dones

VISITING SCIENTIST
Daniel Luque
(Instituto de Salud Carlos III, Spain)

Our studies aim to elucidate structure-function-evolution relationships of viral macromolecular complexes, also known as viral nanomachines, which control many fundamental processes in virus life cycle. Our model systems 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, defining different functional states, that participate in multiple processes: 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 has led to structural analysis by three-dimensional cryo-electron microscopy combined 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, focused on a number of double-stranded RNA viruses such as birnaviruses (infectious bursal disease virus, IBDV), human picobirnaviruses and several fungal viruses, as well as single-stranded RNA viruses such as rabbit hemorrhagic disease virus (RHDV). Some of these viruses cause serious diseases, and structural characterisation 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.

SELECTED PUBLICATIONS

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Mertens J, Bondia P, Allende-Ballestero C, Carrascosa JL, Flors C, Castón JR. Mechanics of Virus-like Particles Labeled with Green Fluorescent Protein. Biophys J 2018; 115: 1561-1568.

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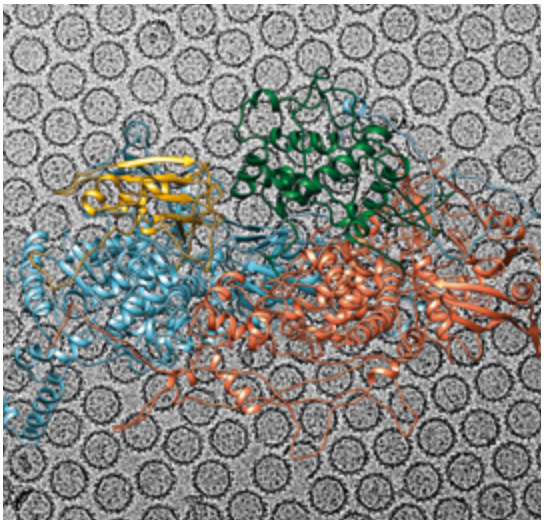

1 Rosellinia necatrix *quadrivirus 1 (RnQV1) is a fungal double-stranded RNA virus associated with latent infections of a pathogenic ascomycete. RnQV1 capsid is based on a single-shelled T=1 lattice built of heterodimers of P2 (blue) and P4 (orange). Domain insertions in P2 and P4 provide additional functions at the capsid outer surface. The P2 insertion has a fold similar to that of proteins related to cytoskeleton metabolism (yellow), and the P4 insertion suggests protease activity involved in P2 cleavage (green). The background shows a cryo-electron microscopy image of RnQV1.*

Functional proteomics

GROUP LEADER
Fernando J. Corrales

SENIOR SCIENTIST
Alberto Paradela

POSTDOCTORAL SCIENTISTS
Miguel Marcilla
Adán Alpízar
Lorena Carmona

PhD STUDENTS
Rosana Navajas
Antonio Ramos

UNDERGRADUATE STUDENT
José Ignacio Granero

SELECTED PUBLICATIONS

Álvarez-Sola G, Uriarte I, Latasa MU, Fernandez-Barrena MG, Urtasun R, Elizalde M, Barcena-Varela M, Jiménez M, Chang HC, Barbero R, Catalán V, Rodríguez A, Frühbeck G, Gallego-Escuredo JM, Gavaldà-Navarro A, Villarroya F, Rodríguez-Ortigosa CM, Corrales FJ, Prieto J, Berraondo P, Berasain C, Ávila MA. Fibroblast growth factor 15/19 (FGF15/19) protects from diet-induced hepatic steatosis: development of an FGF19-based chimeric molecule to promote fatty liver regeneration. Gut 2017; 66: 1818-1828.

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Clemente LF, Hernáez ML, Ramos-Fernández A, Ligero G, Gil C, Corrales FJ, Marcilla. Identification of the Missing Protein Hyaluronan Synthase 1 in Human Mesenchymal Stem Cells Derived from Adipose Tissue or Umbilical Cord. J Proteome Res 2018; 17: 4325-4328.

Mato JM, Elortza F, Lu SC, Brun V, Paradela A, Corrales FJ. Liver cancer-associated changes to the proteome: what deserves clinical focus? Expert Rev Proteomics 2018; 9: 749-756.

Our main interest is the investigation of the molecular mechanisms underlying the progression of chronic liver disorders. We have identified one carbon metabolism (1CM) as an essential process to preserve the differentiated phenotype of quiescent hepatocytes. 1CM is a master connection between the intermediate metabolism and epigenetic regulation that must be finely tuned since its unbalance triggers a progressive liver ailment leading to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC).

Our work has contributed to elucidate protein networks that explain the molecular pathogenesis of chronic liver diseases and HCC using a functional proteomics approach. Deficiencies on key 1CM enzymes, such as methionine adenosyltransferase (MAT1A) and methylthioadenosine phosphorylase (MTAP) lead to a deregulated methylation capacity of liver cells and a deep reshape of their protein methylation profile (including RNA binding proteins). Assuming the key role of 1CM remodelling in the progression of liver disorders, we have developed a multiplexed mass spectrometry-based method to quantify the enzymes catalysing 1CM reactions. This quantification kit is a straightforward method to follow up chronic liver disease patients.

All these studies are part of the Liver Initiative of the Biology and Disease-Driven Human Proteome Project (B/D-HPP), currently coordinated by our lab. We are also actively participating in the functional annotation of unknown proteins in the framework of the Chromosome Centric Human Proteome Project (C-HPP). Our research has been funded with grants for more than 20 years and our results have been published in more than 140 peer reviewed scientific articles.

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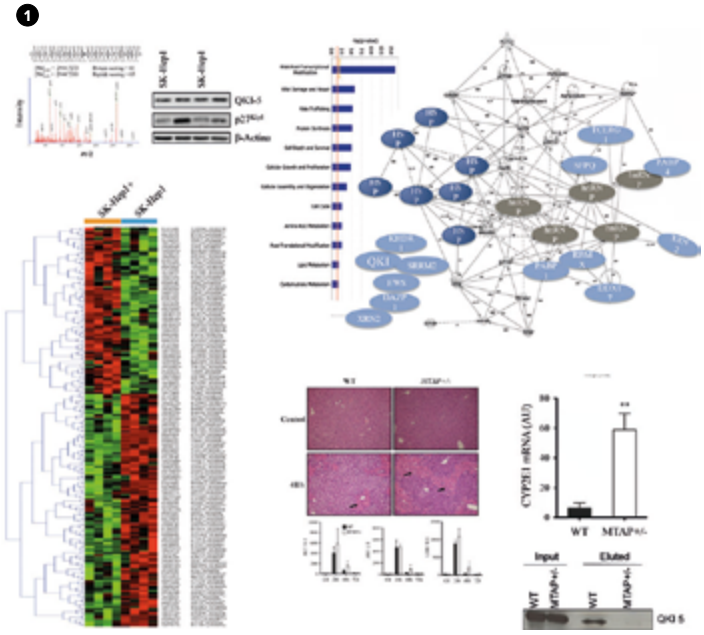
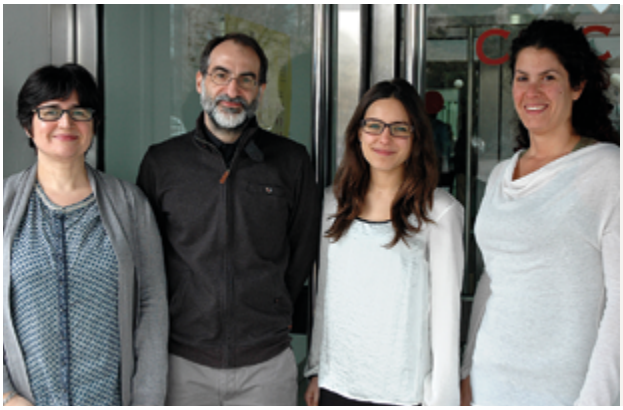


1 *Functional proteomics analysis reveals changes of protein abundance and methylation that explain mechanisms of liver disease progression.*



Electron tomography and image processing of cell structures

GROUP LEADER
José Jesús Fernández

SENIOR SCIENTIST
María del Rosario Fernández

PhD STUDENT
Eva Martín Solana

SELECTED PUBLICATIONS

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

Moreno JJ, Martínez-Sánchez A, Martínez JA, Garzón EM, Fernández JJ. TomoEED: fast edge-enhancing denoising of tomographic volumes. Bioinformatics 2018; 34: 3776-3778.

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Li S, Fernández JJ. Assembly of Yeast Spindle Pole Body and its Components Revealed by Electron Cryo-Tomography. BioRxiv 2018; 442574.

Li S, Fernández JJ, Marshall W, Agard DA. Electron Cryo-Tomography Provides Insight into Procentriole Architecture and Assembly Mechanism. BioRxiv 2018; 442590.

Our group is interested in the unique ability of electron tomography (ET) to visualise in three-dimensions the subcellular architecture and macromolecular organisation 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 is focused on the 3D analysis of the neuronal subcellular architecture. Here, ET and image processing are the central techniques along with protocols that ensure preservation of brain tissue samples in close-to-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-organising centre (MTOC). This is an important and complex cellular organelle whose dysfunction is linked to many diseases. In addition, we actively collaborate with other 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 sub-tomogram analysis.

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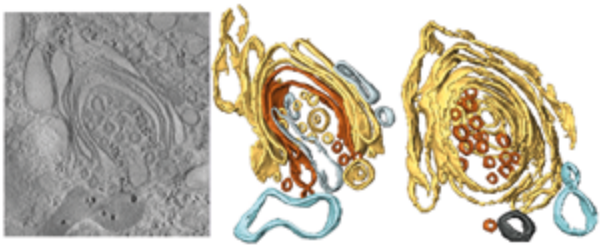

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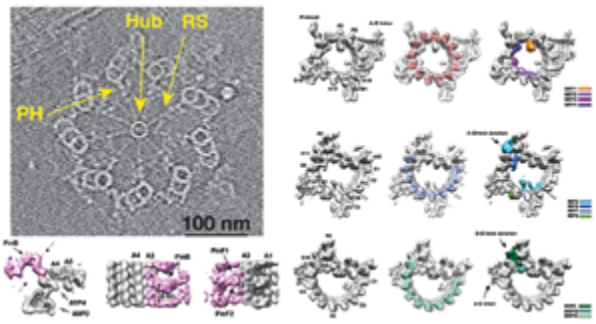

❶ *Three-dimensional visualisation of neuronal subcellular architecture with electron tomography and image processing. Distinct Golgi structures that sequester cytoplasmic contents for their potential degradation were unveiled by electron tomography of brain tissue. These structures are composed of several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

❷ *Molecular architecture of procentrioles revealed by electron cryo-tomography and image processing.*

Ultrastructure of viruses and molecular aggregates

GROUP LEADER
Jaime Martín-Benito

SENIOR SCIENTIST
Rocío Coloma Ciudad

PhD STUDENT
Diego Carlero Carnero

SELECTED PUBLICATIONS

Arranz R, Martín-Benito, Valpuesta JM. Structure and Function of the Cochaperone Prefoldin. Adv Exp Med Biol 2018; 1106: 119-131.

Scheffer MP, Gonzalez-Gonzalez L, Seybert A, Ratera M, Kunz M, Valpuesta JM, Fita I, Querol E, Piñol J, Martín-Benito J, Frangakis AS. Structural characterization of the NAP; the major adhesion complex of the human pathogen *Mycoplasma genitalium*. Mol Microbiol 2017; 105: 869-879.

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, that has been installed recently 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 hypothesised 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 mechanism. 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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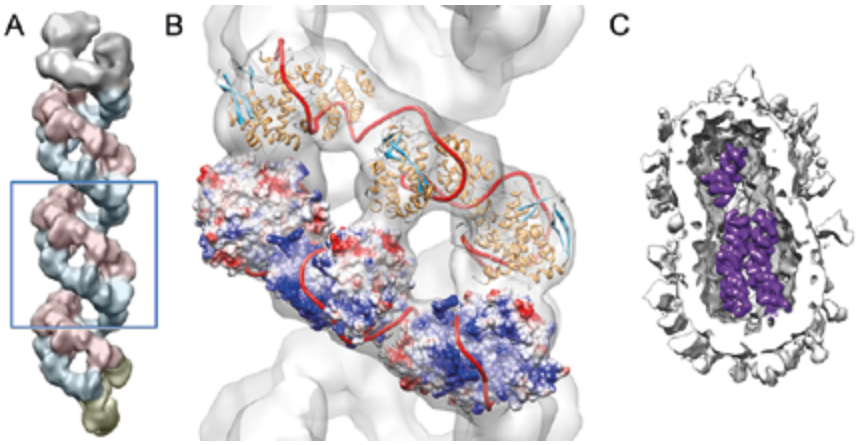

1 *A) Structure of the influenza virus ribonucleoprotein (RNP), the RNA polymerase is shown in grey. B) Detail of the helical part showing the nucleoprotein and the modelled RNA position (red thread). C) Tomogram of an influenza A virion showing the arrangement of the RNPs inside the virus.*

Molecular biophysics of DNA repair nanomachines

GROUP LEADER
Fernando Moreno-Herrero

SENIOR SCIENTISTS
Adriana Gil Gil
Carolina Carrasco Pulido
Silvia Hormeño Torres

COMPUTER SCIENTIST
María Teresa Arranz

POSTDOCTORAL SCIENTISTS
Clara Aicart Ramos
César López Pastrana

PhD STUDENTS
Julene Madariaga Marcos
Alejandro Martín González
Alberto Marín González

Our group develops and employs single-molecule techniques to study the inner workings of protein machines involved in the repair, replication and maintenance of chromosome structures. We use novel single-molecule approaches based on atomic force microscopy and magnetic tweezers, as well as molecular dynamics simulations.

In the last two years, we have developed and implemented a module to laterally stretch DNA molecules at a constant force, in our regular and combined magnetic tweezers (MT)–TIRF setups. The compatibility of the module with TIRF microscopy and the parallelisation of measurements was shown by characterising DNA binding by the ParB protein from *Bacillus subtilis*. Simultaneous MT pulling and fluorescence imaging demonstrated the non-specific binding of *Bs*ParB to DNA and its dynamic interaction under conditions restrictive to condensation. We also found that the central DNA binding domain of ParB is essential for anchoring at parS, but this interaction is not required for DNA condensation. Further work on the mechanism of generation of ParB networks unveiled a dual role for the C-terminal domain of ParB as both DNA binding and bridging interface.

We also investigated the architecture of the segrosome complex in Type III partition systems. We presented the particular features of the centromere site, *tubC*, of the model system encoded in *Clostridium botulinum* prophage c-st. Within the same project, we unravelled the molecular basis for TubZ filaments assembly and dynamics combining electron and atomic force microscopy and biochemical analyses.

Other line of work was the study of the mechanical properties of double-stranded DNA and double-stranded RNA subjected to force and torque by using constant-force, all-atom, microsecond-long molecular dynamics. The methodology and results have opened the field to explore larger forces to test experimental measurements, and to challenge the predictions given by our simulations.

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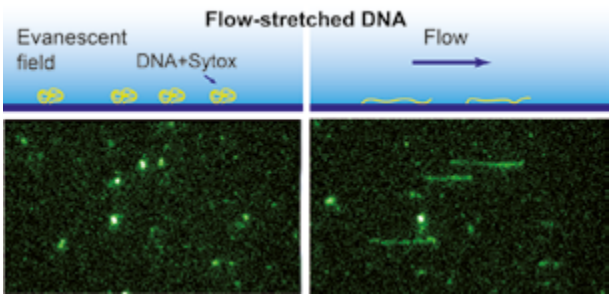
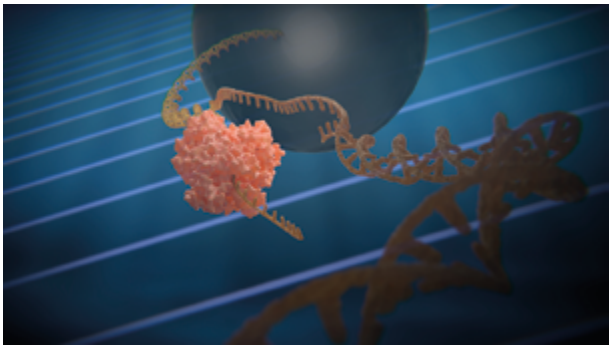


1 *Visualisation of fluorescence-labelled DNA molecules by stretching them with a flow in a combined Magnetic Tweezers TIRF setup.*

2



2 *Representation of an experiment using a Magnetic Tweezers setup where the action of individual proteins on DNA can be monitored in real time.*

SELECTED PUBLICATIONS

Fisher GL, Pastrana CL, Higman VA, Koh A, Taylor JA, Butterer A, Craggs T, Sobott F, Murray H, Crump MP, Moreno-Herrero F, Dillingham MS. The structural basis for dynamic DNA binding and bridging interactions which condense the bacterial centromere. Elife 2017; 6: e28086.

Marin-Gonzalez A, Vilhena JG, Perez R, Moreno-Herrero F. Understanding the mechanical response of double-stranded DNA and RNA under constant stretching forces using all-atom molecular dynamics. Proc Natl Acad Sci USA 2017; 114: 7049-7054.

Fuentes-Pérez ME, Núñez-Ramírez R, Martín-González A, Juan-Rodríguez D, Llorca O, Moreno-Herrero F, Oliva MA. TubZ filament assembly dynamics requires the flexible C-terminal tail. Sci Rep 2017; 7: 43342.

Martín-García B, Martín-González A, Carrasco C, Hernández-Arriaga AM, Ruíz-Quero R, Díaz-Orejas R, Aicart-Ramos C, Moreno-Herrero F, Oliva MA. The TubR–centromere complex adopts a double-ring segrosome structure in Type III partition systems. Nucleic Acids Res 2018; 46: 5704-5716.

Madariaga-Marcos J, Hormeño S, Pastrana CL, Fisher GLM, Dillingham MS, Moreno-Herrero F. Force determination in Lateral Magnetic Tweezers combined with TIRF microscopy. Nanoscale 2018; 10: 4579-4590.

Cell structure laboratory

GROUP LEADER
Cristina Risco

SENIOR SCIENTISTS
Isabel Fdez. de Castro Martín
Raquel Tenorio Vela
Beatriz Pacheco González

TECHNICIAN
Sara Yolanda Fdez. Sánchez

PhD STUDENTS
Moisés García Serradilla
Paula Ortega González
Alberto Fernández Oliva

SELECTED PUBLICATIONS

Fernández de Castro I, Barajas D, Fernández JJ, Nagy PD, Risco C. Three-dimensional imaging of the intracellular assembly of a functional viral RNA replicase complex. J Cell Sci 2017; 130: 260-268.

de Castro Martin IF, Fournier G, Sachse M, Pizarro-Cerda J, Risco C, Naffakh N. Influenza virus genome reaches the plasma membrane via a modified endoplasmic reticulum and Rab11-dependent vesicles. Nat Commun 2017; 8: 1396.

Tenorio R, Fernández de Castro I, Knowlton JJ, Zamora PF, Lee CH, Mainou BA, Dermody TS, Risco C. Reovirus σNS and μNS proteins remodel the endoplasmic reticulum to build replication neo-organelles. MBio 2018; 9: e01253-18.

Sachse, M, Fernández de Castro, I, Fournier, G, Naffakh, N, Risco, C. Electron microscopy imaging of influenza A virus ribonucleoproteins transport and packaging. Methods Mol Biol 2018; 1836: 281-301.

Knowlton J, Fernández de Castro I, Gestaut D, Zamora P, Ashbrook A, Frydman J, Risco C, Dermody T. The TRiC chaperonin controls reovirus replication through outer-capsid folding. Nat Microbiol 2018; 3: 481-493.

Antiviral drugs to treat many of the most troubling viruses have not been approved yet. One of the current strategies for antiviral drug development is the search for host cellular pathways used by many different viruses. Viruses alter lipid synthesis and flows to build replication neo-organelles or factories. Despite the importance of factories in viral infections, there are key gaps in knowledge about how they form and mediate their functions.

Our lab is pioneer in combining light and electron microscopy techniques to study viral infections. We have discovered that influenza virus builds a new organelle to transport its genome inside infected cells. This work received the European Microscopy Society 2017 Outstanding Paper Award in Life Sciences. With correlative light and electron microscopy (CLEM) we demonstrated that the replication neo-organelles of the human reovirus, also known as viral inclusions (VI), form by major remodelling of the endoplasmic reticulum and that ER tubulation and vesiculation are mediated by the reovirus σNS and μNS proteins, respectively.

High-throughput screening of clinically tested compounds provides a rapid means to identify undiscovered, antiviral functions for well-characterised therapeutics. We have selected 9 different drugs and studied their capacity to block bunyavirus infection. Four of these compounds are repurposed drugs validated originally to treat other pathologies. Our studies included CLEM, cell sorting and proteomics. One of these compounds has been selected for additional studies due to its low toxicity and high efficiency against bunyavirus infection.

We are currently studying how RNA viruses hijack lipid transfer proteins (LTPs) and mitochondrial proteins to build replication factories. Furthermore, we are looking for inhibitors of these LTPs and mitochondrial proteins using state-of-the-art computational tools and databases of clinically approved drugs. Our goal is to identify new targets for antiviral drug development and to validate broad-spectrum antivirals to treat many pathogenic viruses.

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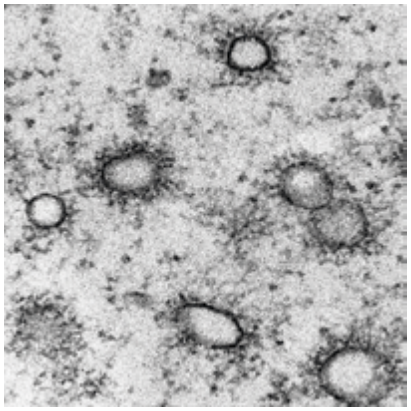

❶ *Transmission electron microscopy of ICVs (irregularly coated vesicles), a new organelle built by influenza virus to transport its genome.*

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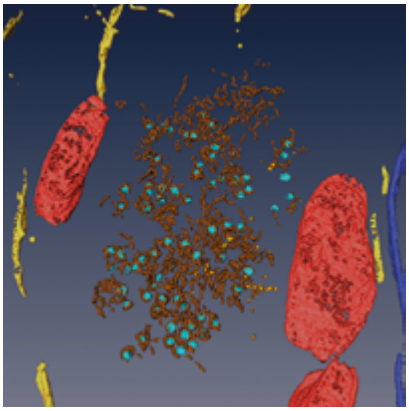

❷ *Double-tilt electron tomography of a reovirus inclusion. The VI is a collection of tubules and vesicles with viral particles (blue) attached to membranes (brown). Mitochondria are coloured in red.*

Structural and physical determinants of complex virus assembly

GROUP LEADER
Carmen San Martín

SENIOR SCIENTIST
Roberto Marabini Ruiz
(Visiting scientist, Universidad Autónoma de Madrid, Spain)

POSTDOCTORAL SCIENTISTS
Gabriela N. Condezo Castro
Mercedes Hernando Pérez

TECHNICIAN
Mara I. Laguna Castro

PhD STUDENTS
Marta Pérez Illana
Natalia Martín González
José Gallardo Hernanz

MASTER'S STUDENT
Eva Arriero País

UNDERGRADUATE STUDENTS
Marina Moreno Mayorga
Manuel Palacios Pérez
Adrián Plaza Pegueroles
Clara Rivero Rodríguez
Marina Botello Marabotto

SELECTED PUBLICATIONS

Condezo GN and San Martín C. Localization of adenovirus morphogenesis players, together with visualization of assembly intermediates and failed products, favor a model where assembly and packaging occur concurrently at the periphery of the replication center. PLoS Pathog 2017; 13: e1006320.

Marion S, San Martín C, Siber A. Role of Condensing Particles in Polymer Confinement: A Model for Virus-Packed "Minichromosomes". Biophys J 2017; 113: 1643-1653.

Menéndez-Conejero R, Nguyen TH, Singh AK, Condezo GN, Marschang RE, van Raaij MJ, San Martín, C. Structure of a Reptilian Adenovirus Reveals a Phage Tailspike Fold Stabilizing a Vertebrate Virus Capsid. Structure 2017; 25: 1562-1573.

San Martín C and van Raaij MJ. The so far farthest reaches of the double jelly roll capsid protein fold. Virol J 2018; 15: 181.

Ortega-Esteban A, Martín-Gonzalez N, Moreno-Madrid F, Llauro A, Hernando-Pérez M, San Martín C, de Pablo PJ. Structural and Mechanical Characterization of Viruses with AFM. Methods Mol Biol 2019; 1886: 259-278.

We are interested in the principles governing complex virus assembly. Our main model system is adenovirus, a specimen of interest in both basic virology and nanobiomedicine. Adenoviruses are human pathogens but can be engineered as therapeutic tools. 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 characterised, and there are still considerable open questions regarding the infectious particle architecture and assembly.

Our research lines focus on answering some of these questions, such as: how the adenovirus capsid is assembled and the genome packaged within the capsid; what are the key elements modulating virion stability; and what are the physicochemical properties of uncharacterised adenoviruses, with potential uses as alternative vectors. We use a multidisciplinary approach to this problem that combines biophysics, structural and molecular biology techniques. Within the past two years, we have used immunofluorescence and immunoelectron microscopy to define the location of the human adenovirus assembly factory in the infected cell. We have also provided evidence indicating that adenovirus capsid assembly and genome packaging occur in a concerted manner, rather than sequential like in other dsDNA viruses. As a consequence of these studies, we have proposed a model for the mechanism of adenovirus assembly (Figure 1). Using a combination of cryo-electron microscopy and protein crystallography (in collaboration with Mark J. van Raaij at the CNB), we have discovered that the capsids of adenoviruses infecting reptiles are stabilised by a cementing protein with a trimeric beta-helix fold that, in viruses, had only been previously found in receptor binding proteins of bacteriophages (Figure 2).

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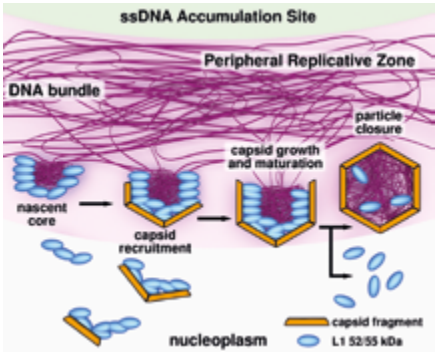

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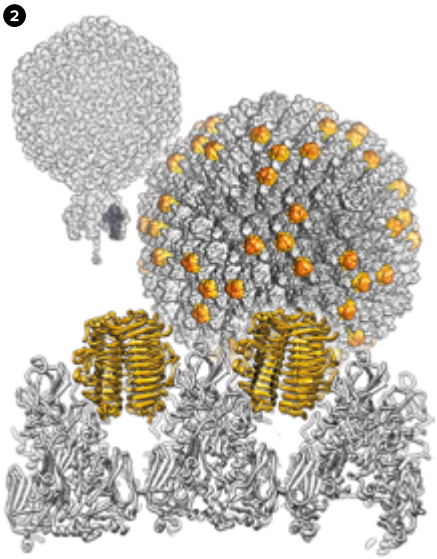
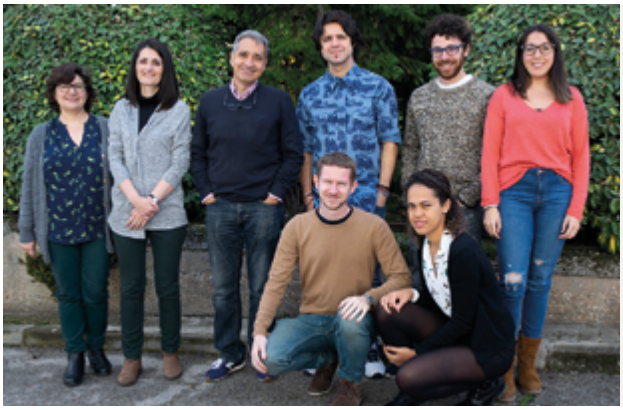


1 *A model for the adenovirus assembly mechanism (modified from Condezo & San Martín, PLoS Pathog 2017; 13: e1006320).*

2 *Structure of snake adenovirus type 1. The cementing protein LH3 (highlighted in gold), has a fold typical of bacteriophage tailspikes, and makes multiple contacts with the major coat protein (hexon, in grey) to stabilise the capsid. Bacteriophage P22 capsid (EMDB-8005) with the tailspike protein in blue (PDB-1TSP) is shown in the top left corner for comparison.*



Structure and function of molecular chaperones

GROUP LEADER
José María Valpuesta

SENIOR SCIENTISTS
Jorge Cuéllar
Rosario Fernández

POSTDOCTORAL SCIENTIST
Lucía Quintana

PhD STUDENTS
Teresa Bueno
Marcos Gragera
Moisés Maestro
Lissette Ochoa

MASTER'S STUDENTS
Miguel Cantero
José Gallardo
Juan García-Bernalt

SELECTED PUBLICATIONS

Fernández-Fernández MR, Gragera M, Ochoa-Ibarrola L, Quintana-Gallardo L, Valpuesta JM. Hsp70 - a master regulator in protein degradation. FEBS Lett 2017; 591: 2648-2660.

Sot B, Rubio-Muñoz A, Leal-Quintero A, Martínez-Sabando J, Marcilla M, Roodveldt C, Valpuesta JM. The chaperonin CCT inhibits assembly of α-synuclein amyloid fibrils by a specific, conformation-dependent interaction. Sci Rep 2017; 7: 40859.

Cuellar J, Valpuesta JM, Wittinghofer A, Sot B. Domain Topology of Human Rasal. Biol Chem 2017; 399: 63-72.

Fernández-Fernández MR, Valpuesta JM. Hsp70 chaperone: a master player in protein homeostasis. F1000Res 2018; 7: 1497.

Pouchucq L, Lobos-Ruiz P, Araya G, Valpuesta JM, Monasterio O. The chaperonin CCT promotes the formation of fibrillar aggregates of γ-tubulin. Biochim Biophys Acta Proteins Proteom 2018; 1866: 519-526.

Most of the cellular processes are executed by sets of proteins that work like molecular machines in a coordinated manner, thus acting like an assembly line and making the process a more efficient one. One of such assembly lines is the one formed by molecular chaperones, a group of proteins involved in cell homeostasis through two opposite functions, protein folding and degradation. Over the last years it has been found that chaperones are not only devoted to assist the folding of other proteins but also, given the right conditions and the presence of specific cochaperones, they can be active players in protein degradation. The two processes are carried out through the transient formation of complexes between different chaperones and cochaperones. Our goal is the structural characterisation, at the highest possible resolution, of some of these complexes, using as a main tool state-of-the-art cryoelectron microscopy and image processing techniques.

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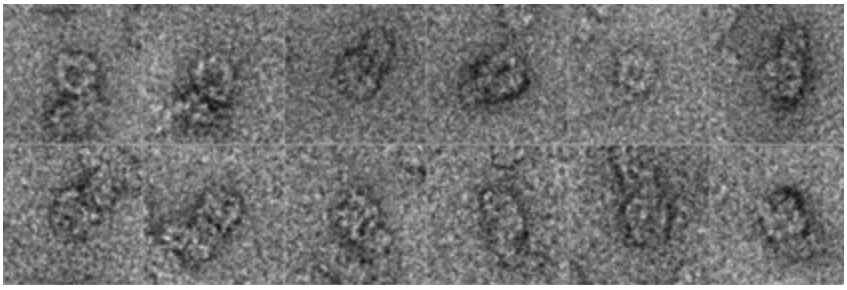

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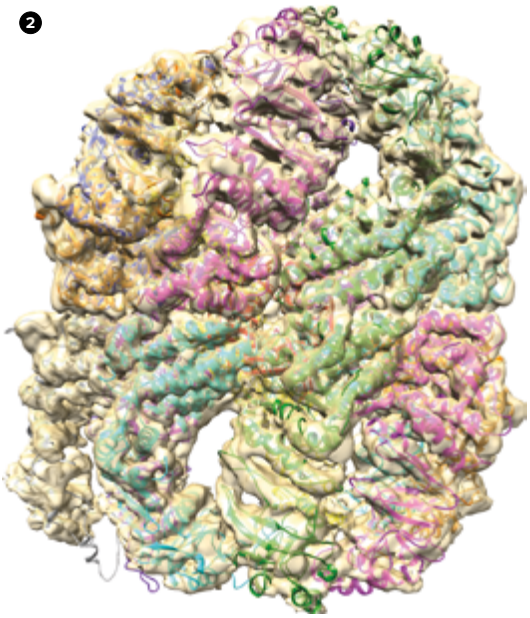
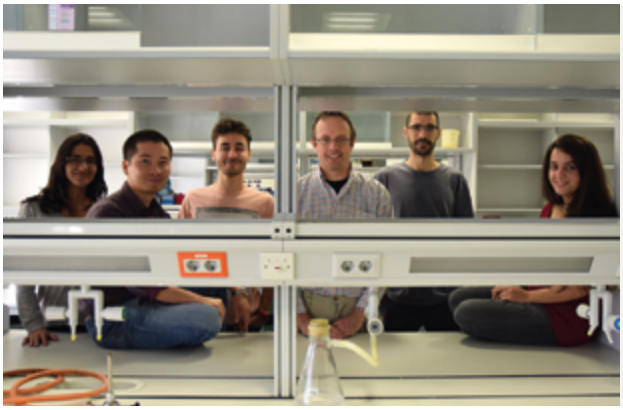


❶ *Gallery of images, obtained by transmission electron microscopy, of complexes between the eukaryotic chaperonin CCT and oligomers of the α-synuclein A53T mutant. The chaperonin has been shown to inhibit the formation of α-synuclein fibres.*

❷ *Image of a high-resolution map (3.9 Å) of the eukaryotic chaperonin CCT obtained by cryoelectron microscopy.*



Structural biology of virus fibres

GROUP LEADER
Mark J. van Raaij

POSTDOCTORAL SCIENTISTS
Pablo Soriano Maldonado
Thanh Hong Nguyen

TECHNICIAN
Mara Inmaculada Laguna Castro

PhD STUDENTS
Marta Sanz Gaitero
Mateo Seoane Blanco
Antonio Pichel Beleiro

MASTER'S STUDENTS
Daniel Salas Lloret
Laida Pagalday Arrizabalaga

UNDERGRADUATE STUDENTS
Daniel Gutiérrez Tejedor
David Nuñez Nepomuceno

SELECTED PUBLICATIONS

Granell M, Namura M, Alvira S, Kanamaru S, van Raaij MJ. Crystal structure of the carboxy-terminal region of the bacteriophage T4 proximal long tail fiber protein gp34. Viruses 2017; 9: E168.

Menéndez-Conejero R, Nguyen TH, Singh AK, Condezo GN, Marschang RE, van Raaij MJ, San Martín C. Structure of a reptilian adenovirus reveals a phage tailspike fold stabilizing a vertebrate virus capsid. Structure 2017; 25: 1562-1573.

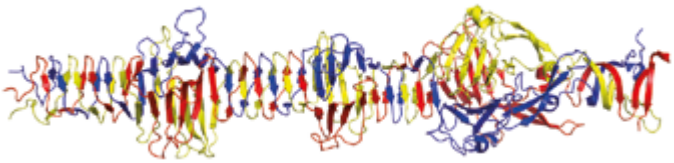
Taylor NMI, van Raaij MJ, Leiman PG. Contractile injection systems of bacteriophages and related systems. Mol Microbiol 2018; 108: 6-15.

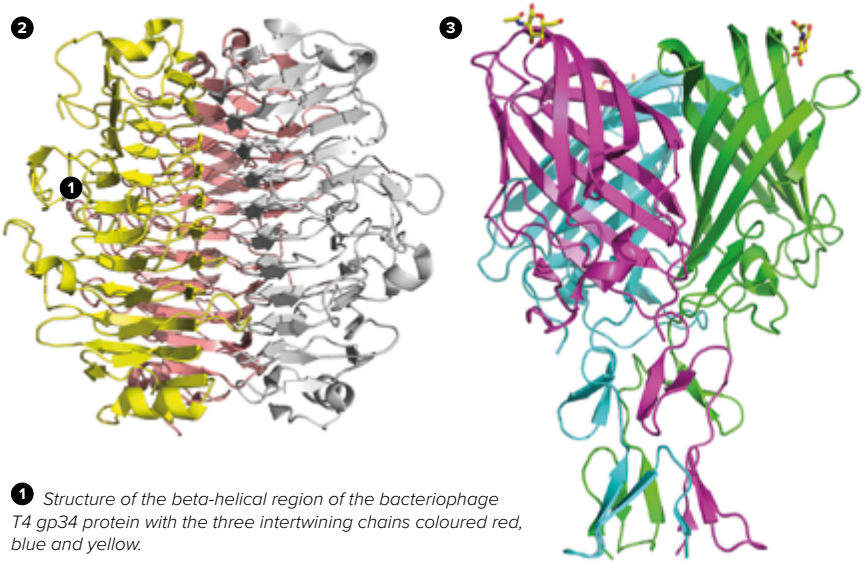
Kokotidou C, Jonnalagadda SVR, Orr AA, Seoane-Blanco M, Apostolidou CP, van Raaij MJ, Kotzabasaki M, Chatzoudis A, Jakubowski JM, Mossou E, Forsyth VT, Mitchell EP, Bowler MW, Llamas-Saiz AL, Tamamis P, Mitraki A. A novel amyloid designable scaffold and potential inhibitor inspired by GAIIG of amyloid beta and the HIV-1 V3 loop. FEBS Lett 2018; 592: 1777-1788.

Singh AK, Nguyen TH, Vidovszky MZ, Harrach B, Benkő M, Kirwan A, Joshi L, Kilcoyne M, Berbis MÁ, Cañada FJ, Jiménez-Barbero J, Menéndez M, Wilson SS, Bromme BA, Smith JG, van Raaij MJ. Structure and N-acetylglucosamine binding of the distal domain of mouse adenovirus 2 fibre. J Gen Virol 2018; 99: 1494-1508.

Recognition of the correct host cell to infect is of crucial importance to a virus. Many viruses bind to their host cell receptors via specialised spike proteins or via specialised fibre proteins, like adenoviruses and bacteriophages. These 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 the years 2017 and 2018, we have determined the structures of three receptor-binding proteins of Staphylococcal bacteriophage K and of two endolysin proteins. In addition, we collaborated with other research groups in crystallisation and structure solution of the proteins and peptides they produce. Knowledge of the structures of bacteriophage receptor-binding and endolysin proteins may lead to different biotechnological applications. Modification of the bacteriophage fibre receptor binding specificities may lead to improved detection of specific bacteria, while a better understanding of endolysin structure, stability and specificity may lead to better elimination of pathogenic or otherwise unwanted bacteria.





❶ *Structure of the beta-helical region of the bacteriophage T4 gp34 protein with the three intertwining chains coloured red, blue and yellow.*

❷ *Structure of the snake adenovirus 1 LH3 protein.*

❸ *Structure of the mouse adenovirus 2 fibre receptor-binding domain bound to N-acetyl-glucosamine. The three protein chains are coloured green, cyan and magenta.*