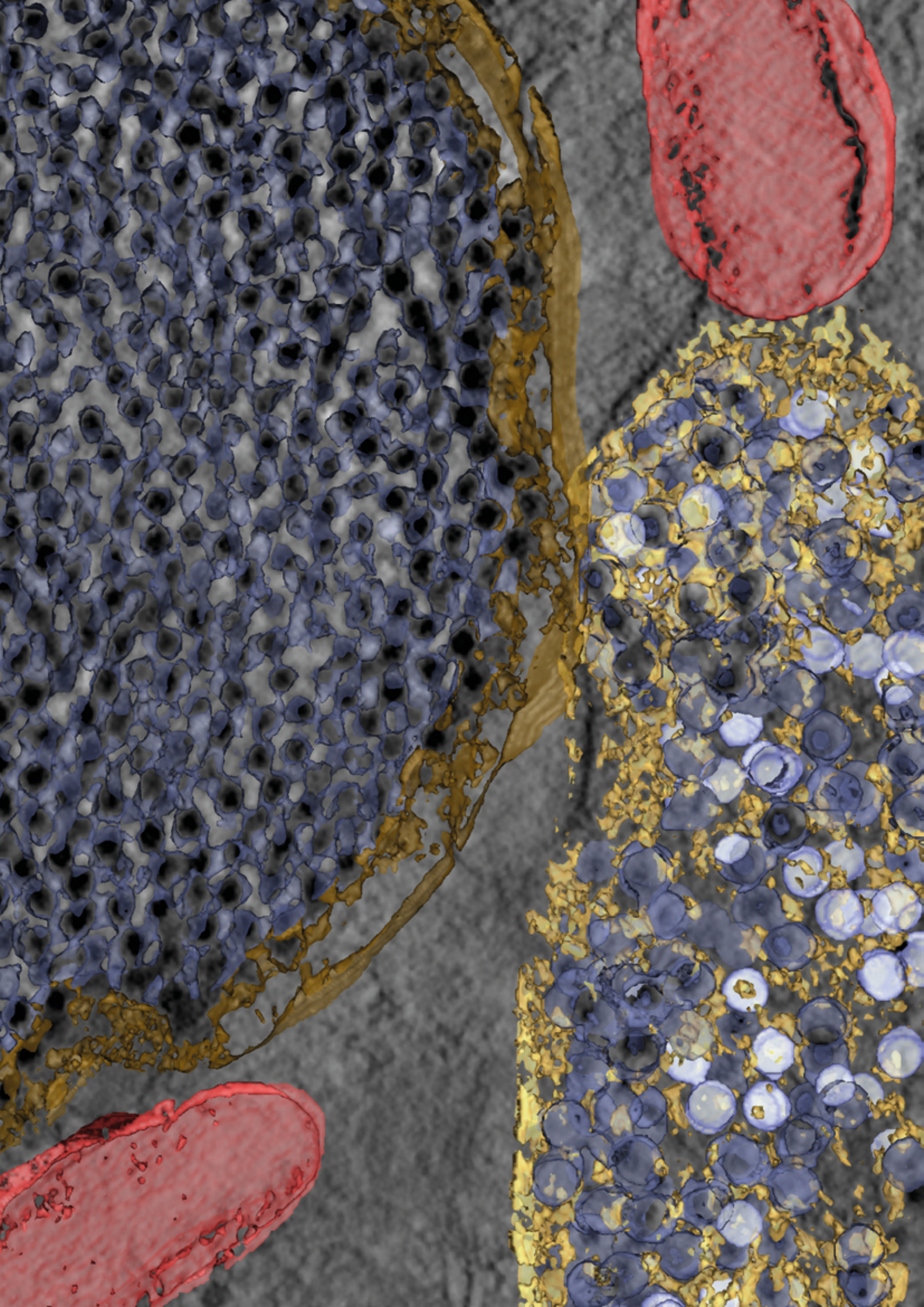

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MACROMOLECULAR STRUCTURES

Scientists in the department work in a large number of biological problems, in particular in the structural and functional characterisation of different molecular machines such as viral structures (Casasnovas, Carrascosa, Castón, Risco, San Martín and van Raaij), DNA repair enzymes (Moreno-Herrero) or molecular chaperones (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 techniques (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, cryoelectron tomography and very recently correlative cryomicroscopy), which is supported by the CNB's cryoelectron microscopy facility, the first one of this kind in Spain. This work is strongly supported by continuous software development in the field of image processing (Carazo, Fernández and Sánchez-Sorzano), which has led to the CNB hosting of the INSTRUCT image processing centre, a pan-European distributed research infrastructure that provides expertise and access to high quality instruments.

Technical developments are also pursued in the field of proteomics (Corrales), which resulted in the CNB being chosen to head the Spanish proteomic facilities network (PROTEORED) and participation in the Human Proteome Project.

Finally, it is important to stress the role played by scientists of this department in different aspects of the COVID19-related investigation carried out by the CNB, which has placed our centre at the forefront of the Spanish research in this field.

HEAD OF DEPARTMENT

José María Valpuesta

The image shows a reovirus-infected cell. Mature virions (blue) are collected by a modified lysosome (brown) from a viral inclusion (yellow), surrounded by mitochondria (red). (From Cristina Risco's lab).

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Biocomputing unit

Electron microscopy of biological samples under cryogenic conditions (cryoEM) has established as a key player in structural biology. Starting from purified samples in the so-called single particle analysis approach, the technique allows elucidating the three dimensional structure of macromolecules up to atomic resolution. For cellular sections, the technique is known as Electron Tomography and it may provide information *in situ*, in the cell. Our group develops image processing algorithms that are able to deal with single particle and electron tomography data. Their goal is to extract the most information from the acquired data in a reliable and as automated as possible way. Particularly in Electron Tomography, our methodological advances are integrated in the context of an ambitious ERC Synergy project, targeting specifically the *in situ* analysis of the Epidermal Growth Factor Receptors family, together with the Medalia, Plueckthun and Olsen laboratories. Our algorithms are available through the Xmipp software package.

In addition to novel image processing algorithms, we also develop Scipion, a workflow engine for the execution of image processing pipelines integrating multiple software suites. Scipion guarantees the traceability and reproducibility of the results, as well as it solves an interoperability problem between the different software packages. Using Scipion, we give support for image processing in cryoEM through the European Infrastructure for Structural Biology Instruct-ERIC and the iNext-Discovery platforms.

Finally, we also have a Structural Bioinformatics role by developing an interface between the structural biology databases (EMDB, PDB) and the biomedical annotations databases containing genomic, proteomic and interactomic information. This connection helps to better understand the biomedical context of the reconstructed structures and has been recognised as one of the few Recommended Interoperability Resources of the European Infrastructure of Life Science Information, ELIXIR.

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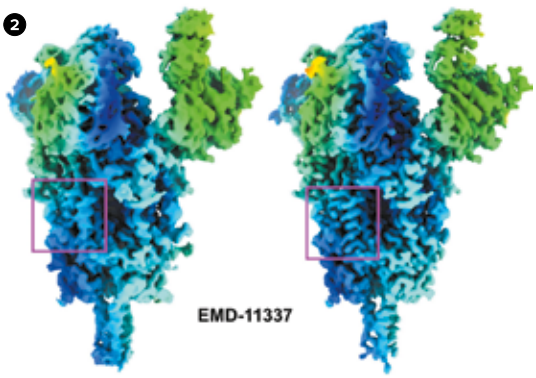

❶ *3DBionotes-Covid19. A Web-based interactive information integration environment (COVID-19 special edition).*

❷ *Effect of local sharpening algorithms (DeepEMhancer, Sanchez-García et al, BioRxiv.2020; LocalDeblurr, Ramirez-Aportela et al., ICUrJ, 2019). The results are shown on our reported data on SARS CoV-2 spike (Melero et al, IUCrJ, 2020).*

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Vilas JL, Tagare HD, Vargas J, Carazo JM, Sorzano COS. Measuring local-directional resolution and local anisotropy in cryoEM maps. Nat Comms 2020; 11: 55..

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Melero R, Sorzano COS, Foster B, Vilas JL, Martínez M, *et al.* Continuous flexibility analysis of SARS-CoV-2 spike prefusion structures. IUCrJ 2020; 7 (6): 1059-1069.

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Structure of macromolecular assemblies

Our group has worked for years in the analysis of the molecular bases of assembly and nanoscopic properties of different macromolecular complexes. We have used a combination of different microscopic approaches in a correlative way, with the idea to cover different resolution levels to provide structural information from the atomic to the cellular level.

We have continued the study on how viral particles incorporate DNA inside virus, how it is stabilised and which are the virus components involved in its ordered delivery upon infection. Using cryo-electron microscopy to solve the structure of different components of phage T7, we have revealed the full atomic structure of the machinery involved in DNA translocation (the connector and several tail components). The structures obtained using a combination of x-ray crystallography and cryo-electron microscopy, and the comparison of these structures in different viruses, have provided the bases for understanding how the DNA is released from the virus particle during viral infection. The cryo-electron microscopy study of the viral core, which is composed of several proteins that dissociate upon viral interaction with the bacterial receptor, has shown that these proteins reassemble to build a conduit for DNA delivery to the cell cytoplasm. The structures obtained at atomic resolution from these core complexes suggest how this process is accomplished.

We have also continued our studies integrating different microscopic approaches at increasing resolution levels. Correlative cryo-electron microscopy, light microscopy, soft –X ray microscopy and spectroscopy have been instrumental in a series of studies where we have defined the intracellular fate of anticancer compounds, the characterisation of different intracellular forms of a parasite, and the structural features of centrosomes in differentiated cells.

SELECTED PUBLICATIONS

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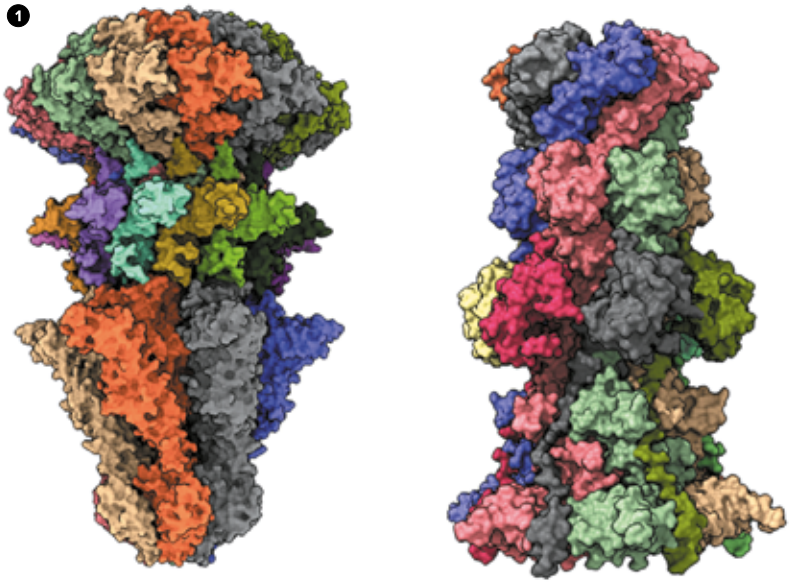

1 *T7 DNA translocation protein complexes involved in viral infection solved by cryo-electron microscopy. Left, three-dimensional reconstruction of T7 tail complex (~1.5 MDa) composed by the connector (gp8), gp11 and gp12 proteins. Right, three-dimensional reconstruction of T7 core complex (~1.4 MDa) composed by gp14 and gp15 proteins. Each protein monomer is colored in a different color.*

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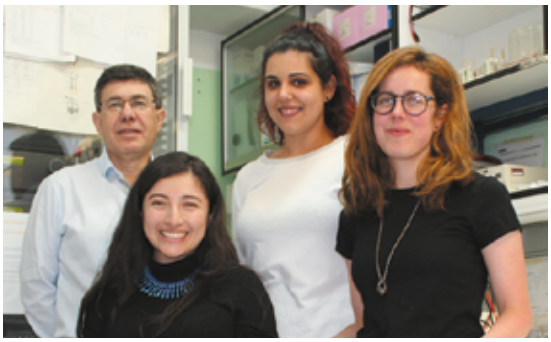

Cell-cell and virus-cell interactions

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 cell entry. 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.

We are characterising antibodies (Abs) that neutralise Human Immunodeficiency Virus (HIV), Ebolavirus (EBOV) or the SARS-CoV-2, responsible of the COVID-19 pandemic. Using electron microscopy, we found that a potent anti-HIV-1 Ab binds to the CD4-receptor binding site in the HIV env protein (Figure 1); this Ab likely neutralises HIV because it inhibits virus cell entry. Using several methodologies we are identifying anti-EBOV antibodies, which are potential therapeutics for the treatment of Ebola disease.

During this year, we have produced the SARS-CoV-2 envelope spike (S) antigen, which is being used in serological tests completed at the CNB-CSIC.

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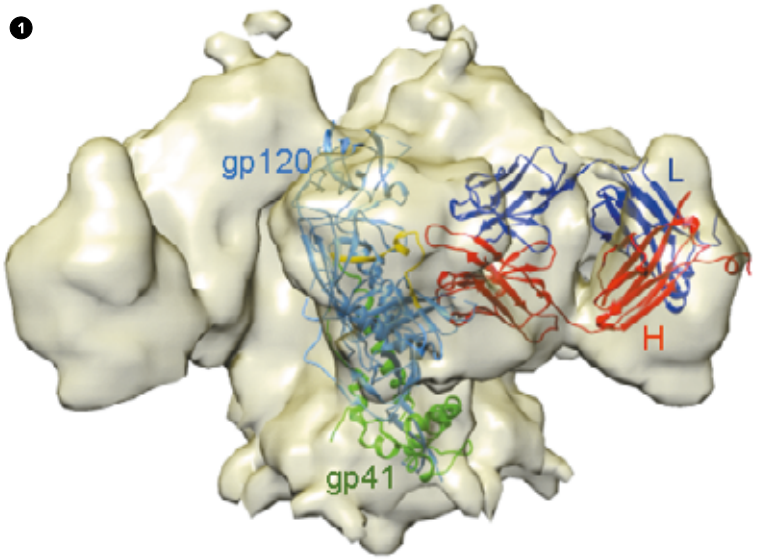
Martínez-Fleta P, Alfranca A, González-Álvaro I, Casasnovas JM, Fernández-Soto D, *et al.* SARS-CoV-2 cysteine-like protease antibodies can be detected in serum and saliva of COVID-19–seropositive individuals. J Immunol 2020; 205: 3130-3140.



1 *An antibody that neutralises HIV-1 and recognises the CD4 receptor binding site in the virus envelope glycoprotein. Electron density map of a trimeric HIV-1 gp140 protein in complex with a neutralising Ab. A monomer of the HIV-1 protein composed of the gp120 (blue) and the gp41 (green) subunits fitted in the map are shown as ribbons, together with the bound Ab (heavy chain in red y light chain in blue). The CD4 receptor-binding region in gp120 to which the Ab binds is in yellow.*

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Molecular machines laboratory

Our studies address to elucidate structure-function-evolution relationships of viral macromolecular complexes, also known as viral nanomachines, which control many fundamental processes in virus life cycle. For that, we have incorporated state-of-the-art approaches to obtaining high-resolution structures of many viral assemblies in near-native conditions, at resolutions better than 3 Å by combining data from several hundred or often thousands of electron microscope images.

Capsids are dynamic structures whose components have transient conformations associated with specific roles in the viral cycle. In addition, the capsid is a metastable assembly: it is robust enough to protect the genome, and labile enough to allow genome delivery into the host cell. Information on virus structures at the highest possible resolution is essential for identifying their molecular mechanisms and functions. Three-dimensional cryogenic electron microscopy (3D cryo-EM) of viruses and viral capsids is widely used to determine their structures at near-atomic resolution in near-native conditions. 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. Our basic structural research shows alternatives for interfering in their function, as well as clues for vaccine and/or antiviral drugs design. These studies are also central to establishing the basis for incorporation of heterologous proteins, nucleic acids, and/or chemicals into viral capsids (considered as nanocontainers), of potential use for future biotechnological applications.

We characterised the structure and conformational polymorphism of several viral systems, including double-stranded RNA (dsRNA) viruses such as birnaviruses (infectious bursal disease virus, IBDV), human picobirnavirus (HPBV) and several fungal viruses, as well as single-stranded RNA viruses such as rabbit haemorrhagic disease virus (RHDV) and human rhinovirus (HRV). We extend our studies to other bacterial and eukaryotic complexes in collaboration with several national and international groups.

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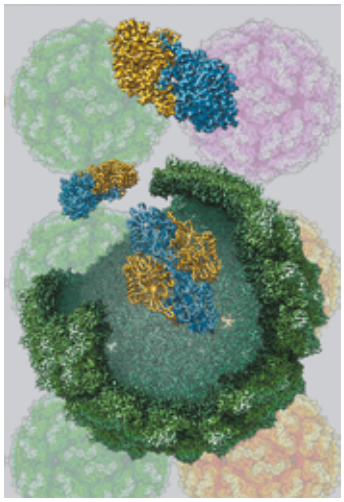

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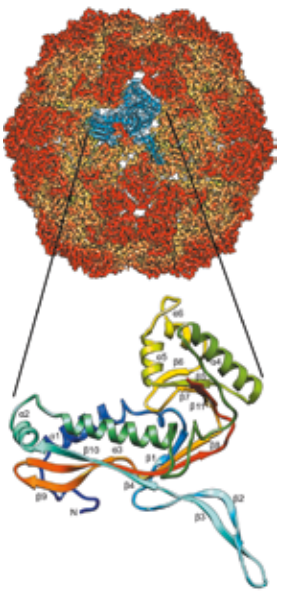

1 *Cryo-EM structure of human picobirnavirus (HPBV) at 2.6 Å resolution. HPBV is a dsRNA virus, broadly extended in the human population. The HPBV capsid is built of 60 capsid protein dimers (blue and yellow). Using an* in vitro *reversible assembly/disassembly system of HPBV, we isolated dimers and tetramers as possible assembly intermediates.*

2 *Cryo-EM Structure of* Brevibacterium linens *encapsulin (BlEnc) at 2.28 Å resolution. Structural comparison between BlEnc and the capsid proteins of the viral lineage HK97 shows high structural similarity, indicating that both compartments descend from a common ancestor.*

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Luque D, Castón JR. Cryo-electron microscopy for the study of virus assembly. Nat Chem Biol 2020; 16: 231-239.

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Functional proteomics

The Functional Proteomics laboratory of the CNB has two main research areas of interests: the study of mechanisms underlying the progression of liver diseases and the characterisation of HLA peptide repertoires to define the antigen presentation principles in the context of diseases, including COVID-19.

We have developed a sample processing method that provides a three-fold increased coverage of human bile proteome (Ciordia S *et al*, J Proteomics, 2020). This method allowed identification of a panel of bile proteins that might prove useful for the clinical management of cholangiocarcinoma patients (Urman J *et al*, Cancers, 2020). We have also developed a method for liquid biopsy analysis (Navajas R *et al*, Methods Mol Biol 2019) that is currently being used for stratification of pre-eclampsia patients.

We coordinate a cooperative project with 25 laboratories integrating the Spanish Proteomics Platform (ProteoRed) to investigate the interaction between SARS-CoV-2 and the host cell proteome aiming to discover the bases associated to the COVID-19 progression. We also aim to discover circulating proteins with prognostic value that may lead to an efficient stratification of COVID-19 patients (Calvo E *et al*, J Pers Med 2020). All this work is coordinated with international initiatives, including COVID-19 MS-Coalition (Struwe W *et al*, Lancet, 2020) and the Human Proteome Project (Adhikari S *et al* Nat Comm, 2020), where we are working to define the human proteome (Omenn GS *et al*, J Prot Res, 2020).

Finally, we have leadership in national and international initiatives such as ProteoRed, Spanish Proteomics Society, European Proteomics Association and the Human Proteome Project. The total number of publications in 2019 and 2020 were 23. (average IF 5.52) and 14 (average IF 8.96) respectively.

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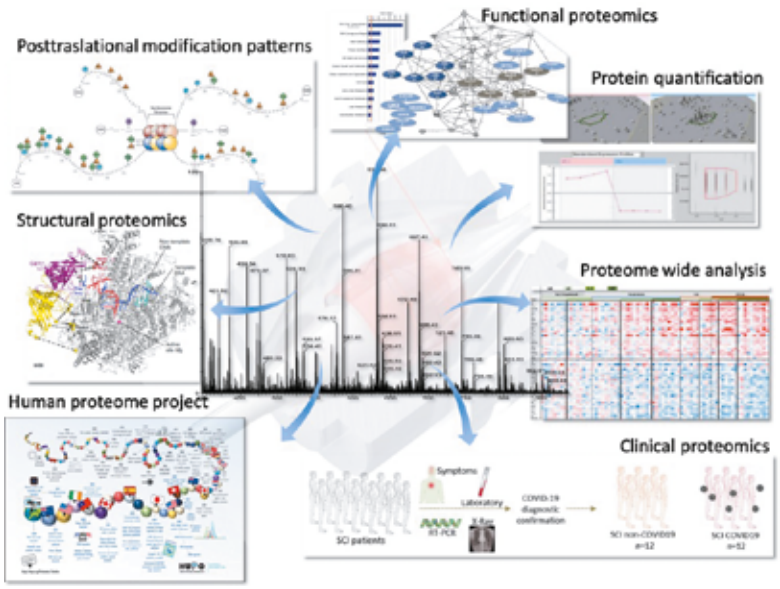

1 *Summary of the main research areas in the CNB Functional Proteomics Laboratory*

SELECTED PUBLICATIONS

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Adhikari S, Nice EC, Deutsch EW, ..., Corrales, *et al.* A high-stringency blueprint of the human proteome. Nat Commun 2020; 11(1): 5301.

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Struwe W, Emmott E, Bailey M, Sharon M, Sinz A, Corrales FJ, *et al.* The COVID-19 MS Coalition-accelerating diagnostics, prognostics, and treatment. COVID-19 MS Coalition. Lancet 2020; 395 (10239): 1761-1762. d

Ciordia S, Alvarez-Sola G, Rullán M, Urman JM, Ávila MA, Corrales FJ. Digging deeper into bile proteome. J Proteomics 2021; 230:103984.

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SELECTED PUBLICATIONS

Li S, Fernandez JJ, Marshall W, Agard DA. Electron cryo-tomography provides insight into procentriole architecture and assembly mechanism. eLife 2019; 8: e43434

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Electron tomography and image processing of cell structures

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 subtomogram analysis.

In 2020 the group left the CNB to further focus on the biomedical field and health research.

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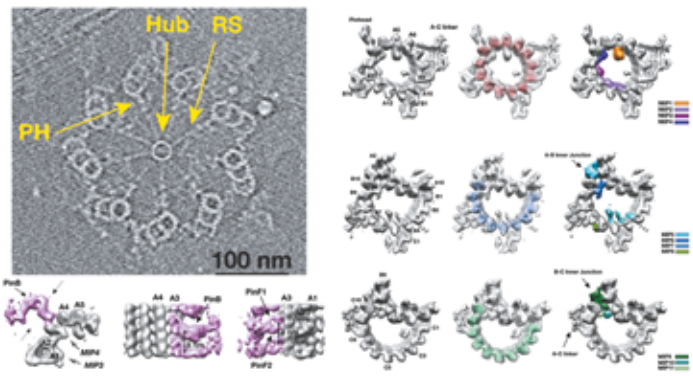

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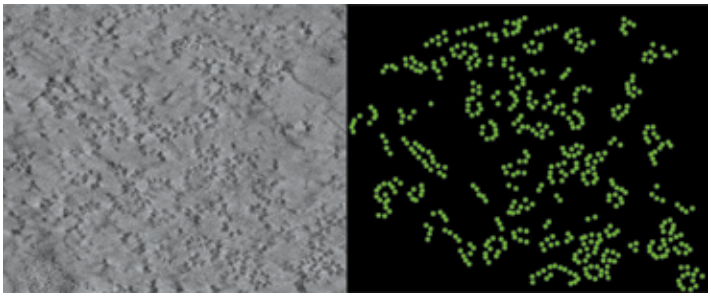

❶ *Molecular architecture of procentrioles revealed by electron cryo-tomography and image processing.*

❷ *Visualisation of the spatial distribution of ribosomes and polysomes in striatal medium-sized spiny neurons by electron tomography of brain tissue samples.*

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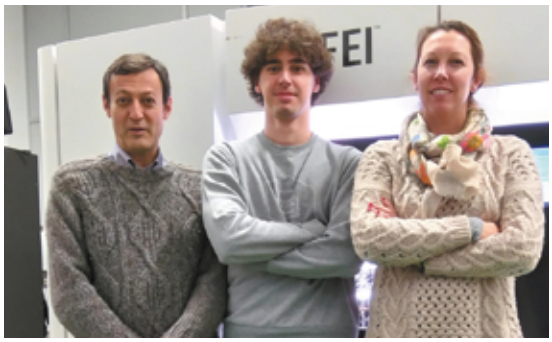

Ultrastructure of viruses and molecular aggregates

The main line of the group is focused on the study of the Influenza A ribonucleoproteins (RNPs) that conforms 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 the isolated RNPs at medium resolution and we have verified that this structure is present in native virions using cryoelectron tomography.

Currently our research on this topic is following two major subjects that will extend to the next years. The first one is the resolution improvement of the RNP structure, and for that we will make use of the state-of-the-art cryoelectron microscope equipped with direct electron detector, recently acquired by the CNB. We have already discovered the existence of an enormous conformational variability in the RNPs structure, and this has been possible through the design of a new protocol able to classify and reconstruct helical structures. We have demonstrated that this extreme conformational variability is closely related with the biological roles of the RNPs. With this idea we opened the second major line of our research, the elucidation of the structural basis of transcription and replication mechanisms and for that our plan is to complement structural data with biochemical assays that will allow us to establish the action mechanism. Our research also extends to the study of influenza virus polymerase at high resolution. Our goal is to solve the polymerase dimer structure that represents the functional complex for replication of RNPs.

SELECTED PUBLICATIONS

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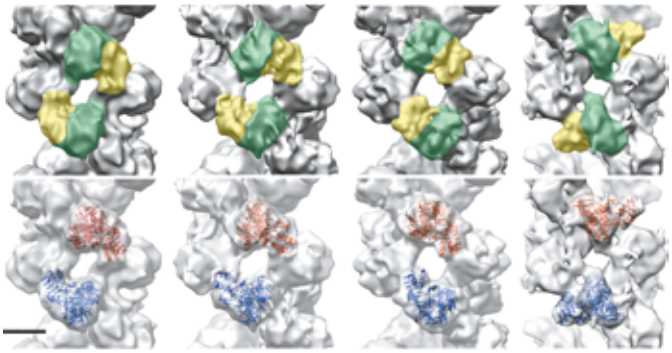

1 *3D reconstructions of different conformations of the helical part of the influenza virus ribonucleoproteins. Top row: the head and body domains of the nucleoproteins are outlined in yellow and green, respectively. Bottom row: the docking of the influenza A nucleoprotein atomic structure (pdb 2IQH) is shown in the opposite strands in red and blue. Scale bar, 50Å.*

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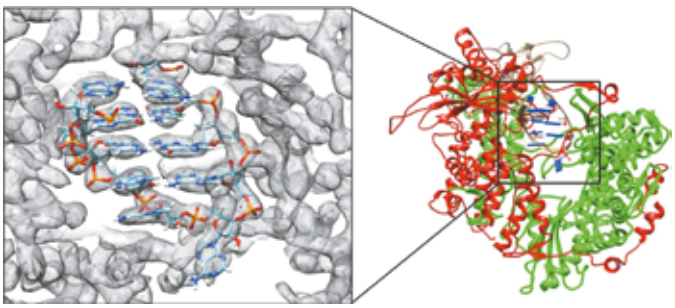

2 *Structure of the polymerase of the influenza A virus at 3.0 Å resolution bound to the cRNA promoter. On the right, the atomic structure of the polymerase obtained by electron cryomicroscopy is shown and on the left, a detail of the atomic density map corresponding to cRNA bound to protein.*

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Molecular biophysics of DNA repair nanomachines

Our group is interested in the development and use of single-molecule techniques to study the mechanical properties of nucleic acids and the mode of action of protein machines involved in the repair, replication and maintenance of chromosome structures. We use novel single-molecule approaches based on atomic force microscopy (AFM) optical and magnetic tweezers, as well as molecular dynamics simulations.

Currently, we are developing and implementing a combined TIRF-AFM system. The combination of high resolution AFM imaging with single-molecule fluorescence will allow us to correlate morphology with the presence of a particular protein. We also manage a hybrid system combining optical tweezers and confocal microscopy (C-Trap™ from Lumicks), which enables manipulating single DNA/RNA molecules while simultaneously imaging the fluorescence between the beads in real time.

Apart from instrument development, in the last two years we have focused on the study of the physical properties of DNA sequences, which has led to the understanding of how they regulate genome folding. In two recent studies, we showed that the crookedness and flexibility of DNA is regulated by nucleotide sequence at short length scales (Marín-González *et al* 2019 and 2020).

We also investigated different molecular motors and DNA-interacting proteins involved in the rolling-circle replication of plasmids (RepC and PcrA) (Carrasco *et al* 2020) and identified a novel DNA2-like helicase-nuclease as a single-stranded DNA looping motor (Wilkinson *et al* 2020).

Lastly, we have used single-molecule assays to investigate the functional and dynamic characteristics of yeast Smc5/6 holocomplex bound to DNA. We showed that the third and less studied eukaryotic SMC complex locks plectonemes and can compact DNAs in an ATP-dependent manner (Gutiérrez-Escribano *et al* 2020). Our results demonstrate that the Smc5/6 complex recognises DNA tertiary structures involving juxtaposed helices, and might modulate DNA topology by plectoneme-stabilisation and compaction.

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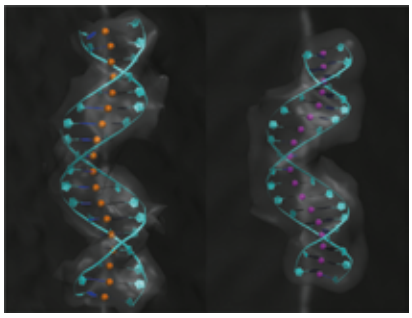

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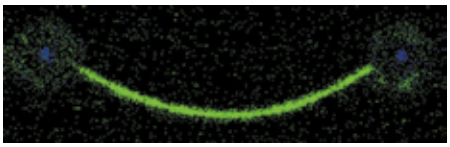

❶ *Molecular dynamics simulation of two DNA molecules showing how their nucleotide sequences affect their crookedness (path given by yellow and pink dots).*

❷ *C-trap fluorescence image of AlexaFluor 488-labelled RPA bound to a single-stranded DNA molecule captured between two beads.*

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Cell structure laboratory

Emerging and re-emerging viruses are a global threat because there are no vaccines nor specific drugs for many of them. Our lab works on the cell biology of viral infections to identify new targets for antiviral therapies. We are currently studying Bunyaviruses, Reoviruses and Coronaviruses. Bunyaviruses are a large group of RNA viruses, many of them transmitted by mosquitoes or ticks, that includes important pathogens for humans, animals and plants. Reoviruses are common pathogens of mammals that have been linked to celiac disease. Coronaviruses cause lethal pathologies such as SARS, MERS and COVID-19.

During the last two years, we have used live cell imaging, correlative light and electron microscopy and electron tomography to study the biogenesis of viral replication factories and virus egress pathways. We have discovered that human reoviruses hijack cell lysosomes. On the periphery of the viral factory, these lysosomes collect mature virions and transport them to the cell surface. This is a new, nonlytic virus egress mechanism and a potential new target for therapeutic intervention.

With state-of-the-art computational tools and databases of clinically approved drugs, we have completed a pre-clinical search for Bunyavirus and Coronavirus inhibitors and we now count with a list of more than one hundred potential antivirals. These include inhibitors of mitochondrial proteins, lipid transfer proteins, proteasome and protein kinases, together with inhibitors of Bunyavirus RNA polymerases, and SARS-CoV-2 MPro, Spike, MTase and RNApol. Ten of these compounds have been selected for subsequent steps of pre-clinical studies. Our research on new antivirals to combat COVID-19 has been supported by almost 3,000 citizens and 9 companies, through the Precipita crowdfunding platform of the Fecyt (Fundación Española para la Ciencia y la Tecnología). The main goal of this project is to validate broad-spectrum antivirals to combat SARS-CoV-2 and many other pathogenic viruses.

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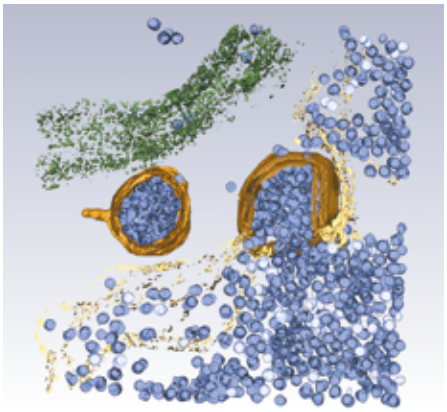

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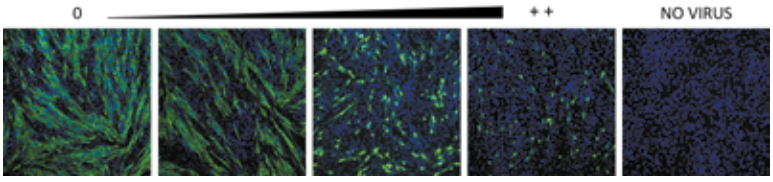

❶ *Egress machinery of human reovirus as visualized by 3D electron tomography. Mature virions (purple) are collected by lysosomes (brown) for their transport to the plasma membrane (green).*

❷ *Testing antivirals for coronaviruses. From left to right, cells infected with the human coronavirus HCoV229E (green) were incubated with increasing amounts of drugs to determine the non-toxic concentration that inhibits viral growth. Nuclei are stained with DAPI (blue).*

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Structural and physical determinants of complex virus assembly

We are interested in the principles governing complex virus assembly. Our main model system is adenovirus, a large non-enveloped icosahedral virus with a 95 nm capsid composed of more than 10 different proteins. Adenoviruses are human pathogens, but can be engineered as therapeutic tools. More than 60 years after their discovery, there are still considerable open questions regarding adenovirus morphogenesis. To address these questions, we use a multidisciplinary approach that combines Biophysics, Structural and Molecular Biology techniques.

Most recently, we have analysed the role of core protein VII, a histone-like protein, in adenovirus assembly. Protein VII was thought to condense the dsDNA genome for packaging within the capsid. Surprisingly, our collaborator P. Hearing (Stony Brook University) showed that protein VII is not required for genome encapsidation, but particles assembled in its absence (Ad5-VII-) are deficient in maturation of minor coat protein VI. Protein VI binds to the inner surface of hexon capsomers, and contains a lytic peptide which must be released during entry to ensure endosome escape. In collaboration with P. Hearing, P. de Pablo (IFIMAC-UAM) and U. Greber (U. Zurich), we have contributed to clarify the role of protein VII and its interplay with protein VI in adenovirus assembly and entry.

We observed that Ad5-VII- particles cannot leave the endosomes during entry due to failure to expose protein VI. A cryo-electron microscopy map (Fig. 1A) showed that, in the absence of core protein VII, the lytic peptide remains trapped inside the hexon cavity (Fig. 1B), unavailable for cleavage by the maturation protease and for endosome membrane disruption. Difference maps between Ad5-VII- and complete particles indicate that proteins VI and VII can interact with the same pocket in hexons. Based on these results, we have proposed a model where the competition between proteins VI and VII for hexon binding during assembly is responsible for releasing the lytic protein from the hexon cavity, facilitating its complete maturation and exposure during uncoating in the endosome (Fig. 1C).

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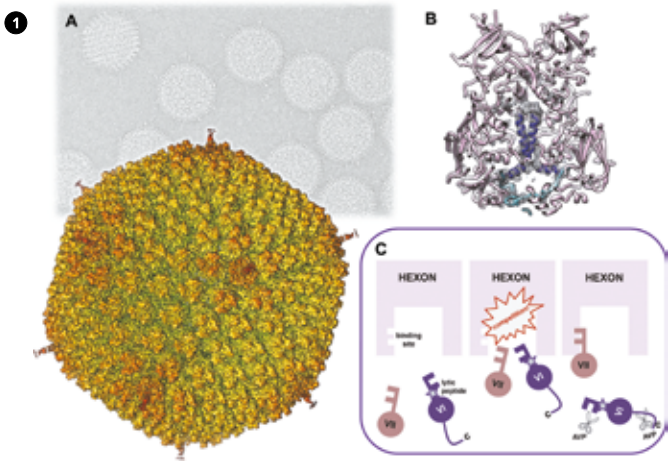

1 *(A) A cryo-electron microscopy map of human adenovirus type 5 lacking core protein VII. (B) Localisation of the coat protein VI lytic peptide (purple) trapped inside a cavity in the major coat protein (hexon, light pink) when protein VII is not present. (C) The information gathered from cryo-EM and other molecular and biophysical techniques indicates that proteins VI and VII compete for binding to the same site in the hexon cavity, and this competition is crucial for the lytic peptide to be exposed during entry to facilitate adenovirus escape from the endosome.*

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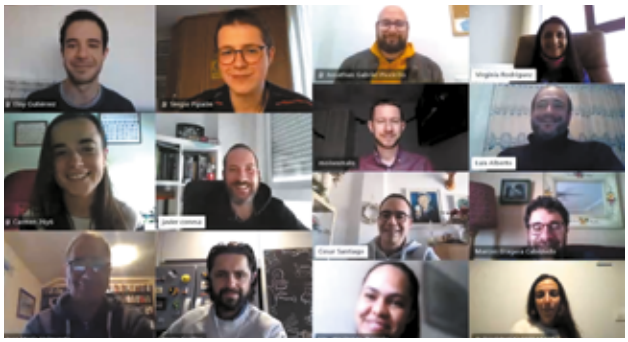
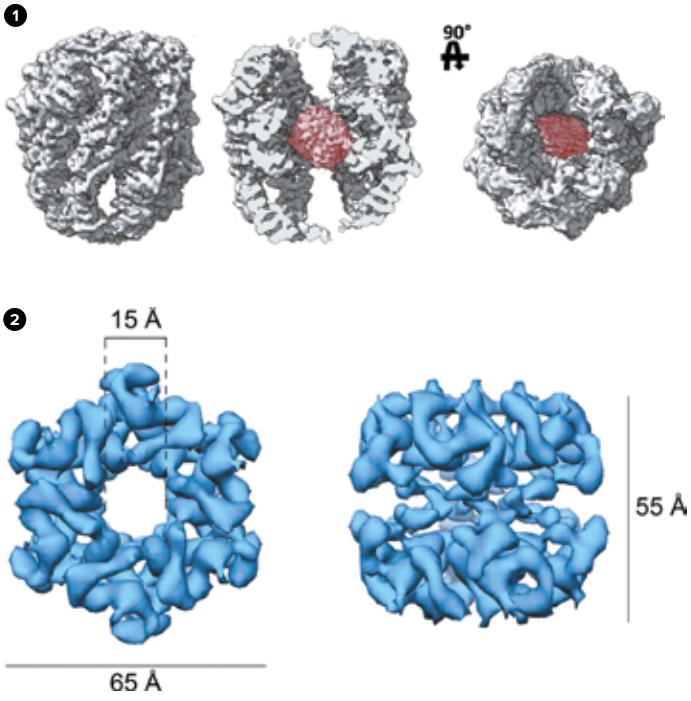


Structure and function of molecular chaperones

We use different various biophysical techniques, chiefly cryoelectron microscopy, to study the structure and function of different macromolecular complexes, in particular those formed by molecular chaperones, a group of proteins involved in cell homeostasis through two opposite functions, protein folding and degradation. These two cellular processes are carried out through the transient formation of complexes between different chaperones and cochaperones, acting like an assembly line and making the process a more efficient one. The two processes are carried out through the transient formation of complexes between different chaperones and cochaperones. Our main 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. We also aim to study from a structural point of view the implication of different chaperones in the regulation of complex cellular events as the immune synapse. For that we are implementing correlative approaches to locate and resolve molecular events in a native cellular context.



1 *Three images of the three-dimensional reconstruction (by cryoelectron microscopy) of a complex between the chaperone CCT and its substrate, the protein mLST8 (red mass) (1000 kDa; 3.9 Å resolution). Left, side view of the outer surface. Centre, a view of the interior of the CCT cavity. Right, end-on view showing mLST8 in the CCT cavity.*

2 *Two orthogonal images of the three-dimensional reconstruction (by cryoelectron microscopy) of the engineered protein CI2, which forms a double hexamer in solution (88 kDa; 8.5 Å resolution).*

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Structural biology of viral fibres

Correct recognition of the bacterial cell wall is of crucial importance to the life cycle of a bacteriophage, both in deciding which bacterium to infect as in lysing the host after phage multiplication.

Many bacteriophages bind to their host cell receptors via specialised spike proteins or via specialised fibre proteins. These proteins tend to 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. By careful analysis of their domain structure and adapted expression and purification protocols, we obtain suitable quantities of these proteins for crystallisation and structures solution by crystallography.

After infection and generation of multiple infective particles, the bacteriophage needs to lyse the host cell to disperse the daughter phages so they can encounter new bacteria to infect. To this end, the phage produces endolysins that digest the bacterial peptidoglycan layer. In many cases, biotechnologically produced endolysins can also be applied “from the outside” to lyse bacteria.

In the years 2019 and 2020, we have determined the high-resolution structures of receptor-binding proteins of a *Salmonella* and a *Campylobacter* phage and of a *Pseudomonas* phage endolysin protein in complex with a peptidoglycan fragment. In addition, we collaborated with other research groups in crystallisation and structure solution of the proteins and peptides they produce.

Deep knowledge of the structures of bacteriophage receptor-binding and endolysin proteins may lead to different applications. Modification of the bacteriophage fibre receptor binding specificities may lead to improved detection of specific bacteria and to mutant phages with improved host ranges. A better understanding of endolysin structure, stability and specificity may similarly lead to better elimination of pathogenic or otherwise unwanted bacteria, and to mutant endolysins with a different or wider range of target bacteria.

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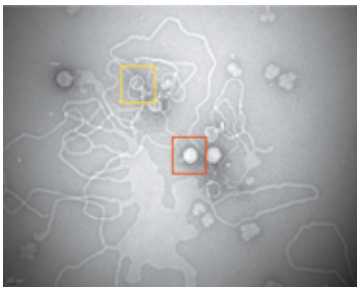

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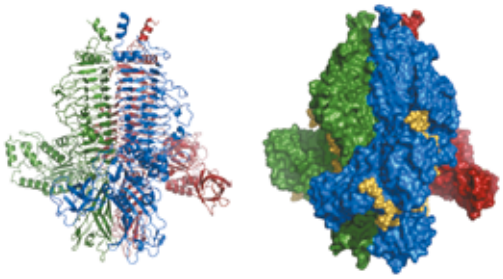

❶ *Electron microscopy image of* Salmonella enterica *subspecies* Enterica, *serovar Anatum A1 bacteriophage epsilon15 in the presence of bacterial lipo-polysaccharide (LPS). The red square highlights a mature phage with a DNA-filled head. The short phage tail and spikes are just visible on the bottom left of the phage head. The yellow square highlights a phage that has transferred the DNA from its head into an LPS vesicle. The size bar in the bottom left corner is 50 nm long.*

❷ *Structure of bacteriophage epsilon15 tailspike gene product 20 (gp20). On the left, a ribbon representation of the trimer is shown, with each monomer coloured differently. On the right, gp20 is shown in space-filled representation, with bound ligands (*Salmonella *O-antigen fragments) shown in yellow.*