2 / Macromolecular Structures

The department's interests cover a broad array of biological problems, from fundamental questions associated with protein folding (Muñoz and Valpuesta) to the functional and structural characterisation of various molecular machines, especially virus structures and their components (Casasnovas, Carrascosa, Castón, Risco, San Martín and van Raaij), and DNA repair nanomachines (Moreno-Herrero). These studies are carried out using 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. 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), of which the CNB hosts one of the largest communities in the world. This work is strongly supported by continuous software development in the fields of image processing (Carazo, Fernández) and transcriptomics (Pascual-Montano), which has led to the CNB hosting 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 (Albar), which resulted in the CNB being chosen to head the Spanish proteomic facilities network (PROTEORED) and participation in the Human Proteome Project.

Functional proteomics

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Virginia Pavón

Functional proteomics draws a complete map of protein dynamics, interactions and posttranslational 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 for 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 proteomics laboratories that study the protein expression profile of chromosome 16 in a broad panel of human cell lines. We design experimental approaches to detect and quantify both the "conspicuous" and the "hidden" proteome. This initiative is driven by the most advanced technology using standard unbiased shotgun as well as targeted (MRM) experimental approaches.

2. Deciphering signal transduction networks by phosphoproteomic analyses. TCR signalling and the specific role of diacylglycerol is being studied using a combination of phosphopeptide enrichment and SILAC labelling, allowing accurate phosphoprotein and phosphopeptide analysis and quantitation.

3. Interactomics. The project "Interactomics of the Centrosome", funded by the Madrid regional government, aims to characterise 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.

4. Computational proteomics

• Application of probability-based methods for large-scale peptide and protein identification and quantitation from tandem mass spectrometry data

• Implementation of strategies for data mining visualisation

• Development of proteomics data analysis workflow, from data integration, validation, inspection, deposition and reporting (MIAPE Extractor tool, part of the ProteoRed MIAPE Web ToolKit)

5. Quality control and experimental standardisation. 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.

6. Gluten allergen analysis. In the context of the study of coeliac diseases, our lab uses classical and proteomics experimental approaches to characterise wheat, barley and rye prolamins in foods.

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In memoriam

Juan Pablo Albar *(1953-2014), a lover of great challenges and an insatiable explorer, left us early in the morning of 19 July 2014. From the late 1990s on, he became one of the pioneers behind proteomics in Spain, and a key point of reference in leading national and international initiatives to understand human biology and the causes of illness. He was a scientific researcher for the Spanish Research Council, group leader of the Proteomics Facility of the CNB-CSIC, early leader and coordinator of the Carlos III Health Institute Networked Proteomics Platform (ProteoRed-ISCIII) as well as coordinator of the Biomolecular and Bio-computing Resources Platform PRB2-ISCIII. He was also a founder and a member of the executive board of the Spanish Society of Proteomics, a founder and coordinator of the Spanish network of Proteomics Laboratories ProteoRed-Genoma España, a driving force in founding the European Proteomics Association (EuPA), a member of the Human Proteomics Organisation (HUPO) Council, and a member of the Executive Committee of the Human Proteome Project (HPP). His work yielded over 160 scientific publications, inspiring many other researchers in the field of proteomics and biochemistry in general. In these few lines, we want to pay homage to someone who never set limits on his achievements in life and work, and who always wanted to go further.*

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

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Blanca E. Benítez
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Laura del Caño
José Gutiérrez Tabuenca

VISITING SCIENTISTS:
Roberto Marabini
(Universidad Autónoma de Madrid, Spain)

The 2013-2014 biennium was particularly important for the field of cryogenic electron microscopy (cryo EM). Thanks to key technological developments in instruments and software, it became obvious in 2013 that cryo EM had indeed matured as a technique, such that we can now systematically produce quasi-atomic structural information for a wide range of very challenging macromolecular complexes. The need to re-accommodate all image processing workflows to address the new challenges in the field consequently became the target of the group. This goal fitted very well with our focus on methods and software development in 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). We addressed a number of topics central to enhanced microscope performance and optimal image processing, such as accurate, parameter-free optical characterisation, automatic analysis of micrographs, or image-ranking procedures, all critical to render cryo EM a high throughput technique for structural biology. With regard to software, we introduced the beta version of our key development, Scipion, a graphic image-processing workflow integrator that assures full tracking of all analytic results, while offering an environment that integrates most of the best known software packages in the field.

With respect to X-microscopy, using the X-ray microscope at the Spanish synchrotron ALBA, we produced the first detailed optical characterisation with this type of instrument, while advancing in automation and image processing capabilities.

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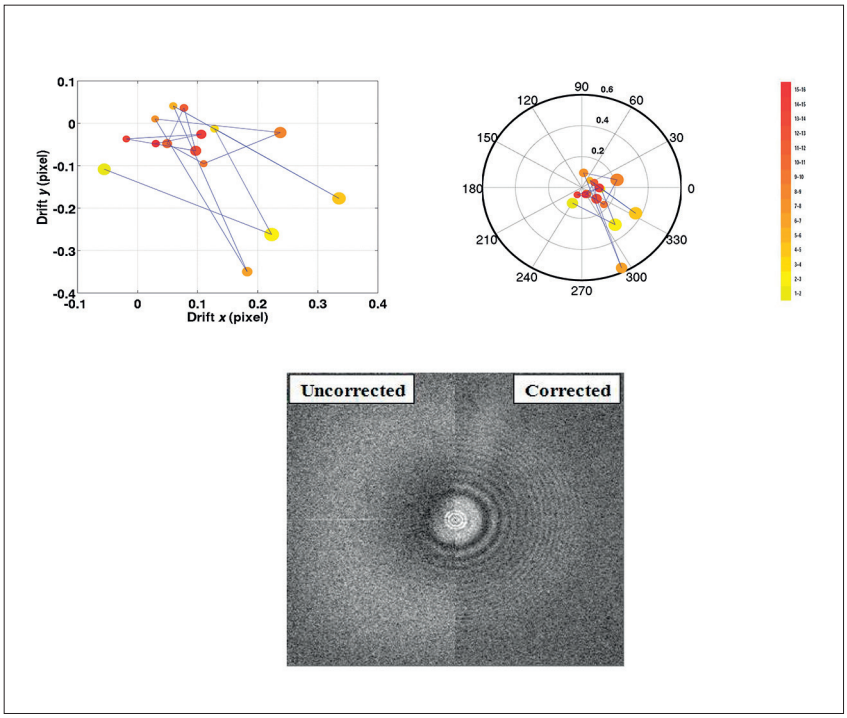

1 *Results of our 2D optical flow approach for frame alignment from movie data collected with the help of the new generation of director electron detectors. Note that 2D optical flow provides a unique characterisation of both local and global frame displacements, besides providing accurate and automatic (virtually parameter-free) image correction. We show Cartesian and polar displacement plots (left and right, respectively, top row), together with how a typical CTF (periodogram) looks before (left) and after (right) correction (bottom).*

Cell-cell and virus-cell interactions

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TECHNICIAN:
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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 such as cell adhesion and phagocytosis, as well as virus binding to cells. In addition, we characterise virus neutralisation by humoural 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-cell interactions

We studied how members of the TIM (T cell/transmembrane, immunoglobulin and mucin domain) and ICAM (intercellular cell adhesion molecules) subfamilies mediate cell-cell interactions. The TIM proteins are pattern recognition receptors, specialised in recognition of the PtdSer (phosphatidylserine) cell death signal. We helped to show that TIM-1 mediates cell adhesion interaction that regulates T cell trafficking during inflammation. The ICAM proteins have long been linked to cell adhesion processes. Our recent research focussed on ICAM-5, a protein expressed exclusively in telencephalic neurons. We used X-ray crystallography to determine how ICAM-5 mediate cell adhesion among neurons. ICAM-5 has an S-shaped configuration that supports formation of a cell adhesion complex built of multiple molecules on the neuron surface (Figure 1).

Virus-receptor interactions and virus neutralisation by antibodies

To analyse virus-receptor interactions in measles virus and coronavirus, we determined crystal structures of virus-receptor complexes. These structures defined the way in which measles virus and certain coronavirus bind to cell surface proteins; we identified the principal determinants of receptor recognition in those viruses. Moreover, we are analysing how antibodies prevent and neutralise virus infections. Potent neutralising antibodies of measles virus and coronavirus target virus residues engaged in binding to cell surface receptors, indicating that prevention of virus entry into host cells is a major mechanism of virus neutralisation by the immune system (Figure 2).

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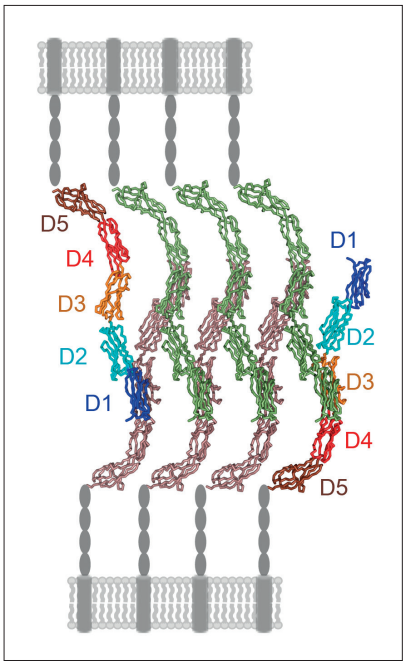

1 *Molecular model of the ICAM-5 homophilic cell adhesion complex in neurons.*

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

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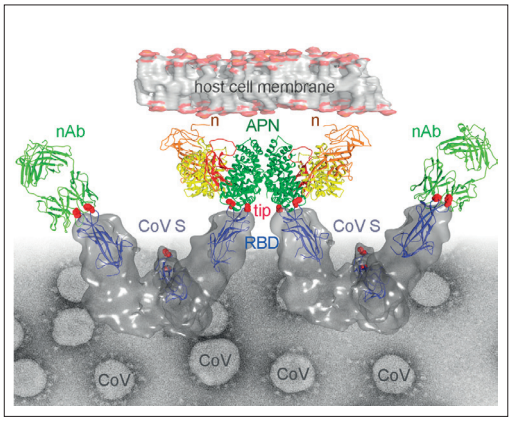

Structure of macromolecular assemblies

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Encarna Cebrián Talaya

The study of virus assembly allows us the molecular basis of macromolecular interactions with chemical and functional properties of the complexes assembled during the morphogenetic pathway of virus construction. Our group combines cryo-electron microscopy and computer three-dimensional image processing to obtain structures, at nanometric resolution, involved in bacteriophage T7 assembly. We solved two conformations of the DNA packaging motor (T7 large terminase), as well as several structures related to the DNA ejection complex (T7 tail), to obtain a detailed description of the DNA translocation process involved in the virus life cycle. Some components of these viruses (connector, terminase) are presently being used in synthetic environments to obtain delivery vehicles with improved specificity and efficiency.

Our structural studies are complemented with single-molecule analysis methods (atomic force microscopy, optical tweezers) to determine the mechanical properties of individual viral particles and subcomplexes at the nanoscopic scale. In this way, we associated the different mechanical behaviour of T7 intermediate maturation particles with their quasi-atomic structures. We also described macromolecular properties of various viral components and assemblies (dielectric constant of viral components, force development by molecular motors, differential properties of dsDNA and RNA).

We continued to extend the use of correlative microscopy by integration of electron tomographic methods with other microscopies, in particular with soft X-ray microscopy. The cryo-tomograms of whole, unperturbed cells infected with vaccinia virus obtained by X-ray microscopy showed previously unreported quantitative aspects of viral maturation and compartmentalisation. To exploit the possibilities of combined X-ray microscopy and analysis, we studied the interaction of magnetic nanoparticles and eukaryotic cells. Development of an approach that uses X-ray spectroscopy and tomography helped us to correlate the topological studies of cellular distribution of nanoparticles with the chemical quantitative information of metal uptake by cells.

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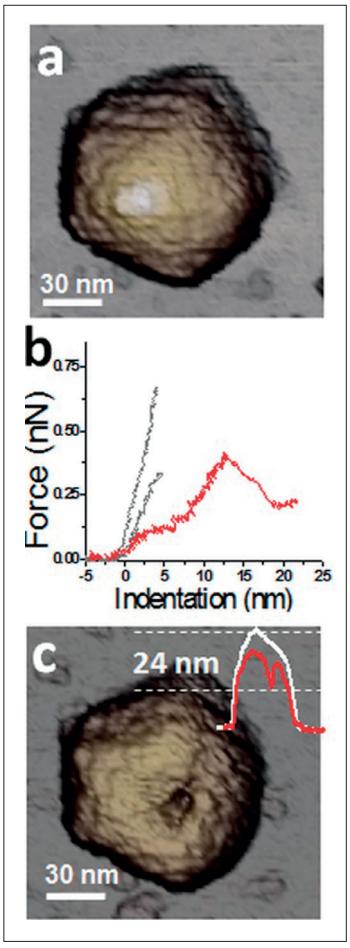

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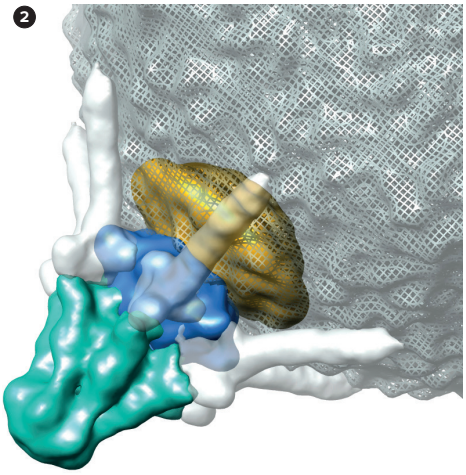

1 *Mechanical fatigue and fracture of T7 proheads. (a) AFM image before mechanical fatigue of a prohead. (b) Three nanoindentation cycles performed on (a). The red curve fractured the prohead. (c) Fractured prohead. The inset shows a comparison of the profiles along the damaged area before (white) and after (red) fracture.*

2 *Tail complex of bacteriophage T7 obtained by cryo-electron microscopy and computer reconstruction. Tail structural components are segmented in different colours. Capsid is outlined as a grey network.*

Viral molecular machines lab

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All live organisms assemble macromolecular nanomachines to carry out complex biological processes. These machines are proteins and nucleic acid-protein complexes that convert chemical energy into conformational changes; they are very dynamic and show conformational variability inherent to their mechanisms. Many fundamental cell biological and biochemical processes controlled by these macromolecular assemblies were discovered through the study of viruses, which alter cell functions and modulate cell behaviour.

As model systems of virus-derived molecular machines we study viral capsids, a paradigm of conformational flexibility, to analyse dynamic processes and macromolecular complexes that control fundamental processes in the virus life cycle. Three-dimensional (3D) structures of these macromolecular assemblies are crucial for advancing our understanding of key biological processes, i.e., how a molecular machine or protein complex works correctly or malfunctions.

We use a multidisciplinary approach that has led to structural analysis by 3D cryo-electron microscopy (cryo-EM) combined with atomic structures (hybrid approach). Our studies intend to establish the molecular interactions of the components in these complex assemblies, as well as the molecular basis of their functional implications. We have incorporated state-of-the-art approaches to obtain near-atomic resolution structure directly from two-dimensional micrographs. We are also using nanoscopic studies of these biomachines by single molecule manipulation techniques such as atomic force microscopy (AFM) to correlate structural features of capsomer interactions with their mechanical properties. Finally, virus-like particles can be used as nanocontainers to encapsulate different types of materials.

Our group studies several viral systems with varying levels of complexity, focussed on a number of double-stranded (ds)RNA viruses such as the birnavirus infectious bursal disease virus (IBDV; an avian pathogen), infectious pancreatic necrosis virus (IPNV; a fish pathogen) and several fungal viruses such as Rosellinia necatrix quadrivirus 1 (RnQV1) and Penicillium chrysogenum virus (PcV), as well as single-stranded (ss)RNA viruses such as rabbit hemorrhagic disease virus (RHDV) and human rhinovirus (HRV-2). Some of these viruses cause serious diseases, and structural characterisation of their macromolecular assemblies will offer new approaches to altering their function, as well as possible vaccination strategies. Modified capsids offer excellent opportunities in nanotechnology and nanomedicine.

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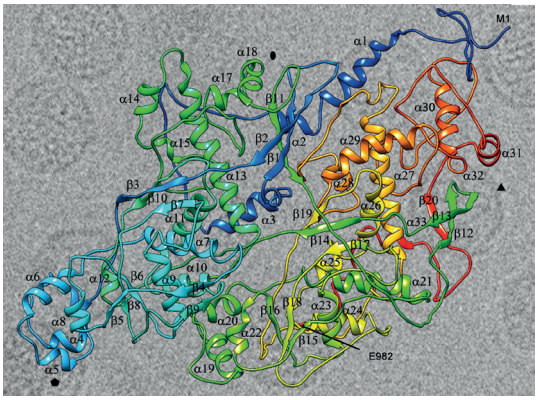
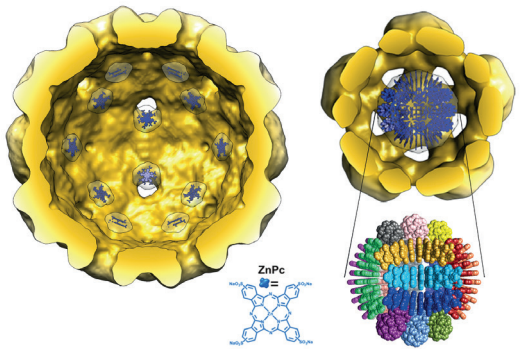


1 *Three-dimensional cryo-EM reconstruction of PcV virions at 4.1 Å resolution. Ribbon diagram of the PcV capsid protein, rainbow-coloured from blue (N terminus) to red (C terminus), the first (Met1; M1) and last (Glu982; E982) residues are shown. Symbols indicate icosahedral symmetry axis. The background image shows PcV particles frozen in vitreous ice.*



2 *Models of phtalocyanine (ZnPc) organisation within ZnPc-loaded T=3 (left) and T=1 (right) VLP of cowpea chlorotic mottle virus (CCMV). ZnPc-loaded T=3: fitting of 20 ZnPc dimers into the internal density located beneath the hexameric capsomers in 28 nm capsids. ZnPc-loaded T=1: fitting of 18 ZnPc 10-mer stacks into the internal density of 20 nm cryo-EM 3D reconstructed particles.*

Electron tomography and image processing of cell structures

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VISITING SCIENTISTS:
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Our group is interested in the unique ability of electron tomography (ET) to visualise *in situ*, in three dimensions, the subcellular architecture and macromolecular organisation of cells and tissues, 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 the 3D analysis of neuronal subcellular architecture, for which ET and image processing are the central techniques, with protocols to 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 also work in close collaboration with Dr Sam Li (UCSF) on the structural elucidation of the microtubule-organising centre (MTOC). Dysfunction of this complex cell organelle is linked to many diseases. We also 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 methods for the distinct 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.

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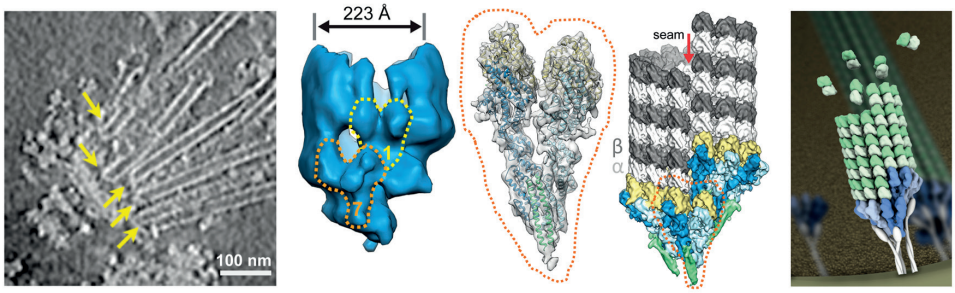

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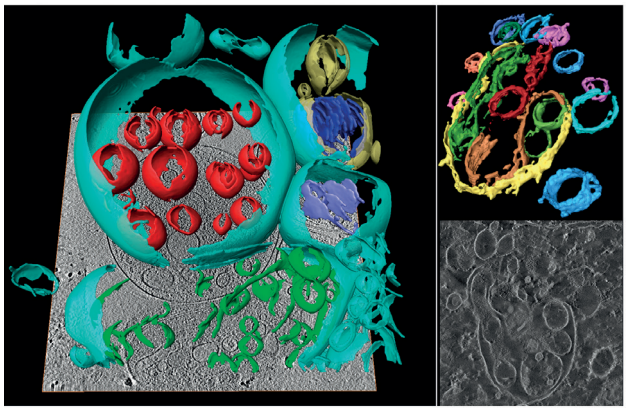

1 *Microtubule nucleation machinery as seen by electron cryomicroscopy. Electron cryotomography of isolated spindle pole bodies from yeast and subsequent subtomogram averaging revealed the in situ structure at 4 nm resolution, showing a ring of seven gTuSC subunits matching microtubule geometry. Electron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

2 *Three-dimensional visualisation of neuron subcellular architecture with electron tomography and image processing techniques. Left, Electron cryotomography of cerebrocortical synaptosomes from wild type rat. Right, Multivesicular body from a wild type mouse striatal sample prepared with high-pressure freezing and freeze-substitution.*

Molecular biophysics of DNA repair nanomachines

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Julene Madariaga Marcos
Alejandro Martín González

We develop and use single-molecule techniques to study the inner workings of protein machines involved in DNA repair processes and maintenance of chromosome structure. We are also interested in the mechanical properties of nucleic acids and their interaction with proteins using single-molecule approaches.

In the last two years, we reported the first use of the magnetic tweezers approach to monitor the dynamics of double-stranded (ds)DNA break resection at single molecule resolution. We defined the functional mechanism of the *Bacillus subtilis* AddAB complex by showing that the Chi recognition domain antagonises translocation activity, causing the complex to stall transiently at Chi sequences. We proposed a simple model for failed and successful Chi recognition, in which the battle between translocation- and sequence-specific binding activities selectively filters for *bona fide* Chi sequences. We also contrasted the mechanical properties of dsRNA and dsDNA at the single-molecule level using magnetic tweezers, optical tweezers and AFM, and showed the structural differences that arise from the A- and B-form arrangements of the polymers. dsRNA has two main elasticity regimes, an entropic regime ended at the A-form contour length and an intrinsic regime ended with a low-cooperative overstretching transition that extends 1.7 times its A-form contour length. We also studied Rep protein binding to single-stranded (ss)DNA in the AT-rich region of the plasmid replication origin. We found a tripartite structure composed of Rep proteins, dsDNA containing Rep-binding sites, and a strand-specific ssDNA of the DNA unwinding element. Finally, we completed construction of an optical tweezers machine (OT) that can manipulate and apply force to single DNA molecules.

Our single-molecule technology was also used in several collaborative projects that include the characterisation of nanometre-size DNA origami structures for potential nanopore devices (with Dr Ulrich F. Keyser, Univ. Cambridge), study of the interaction between a small condensing peptide and ssDNA and dsDNA (with Dr Felix Ritort, Univ. Barcelona), and imaging of bacterial prionoid RepA-WH1 filament structures (with Drs Óscar Llorca and Rafael Giraldo, CIB-CSIC).

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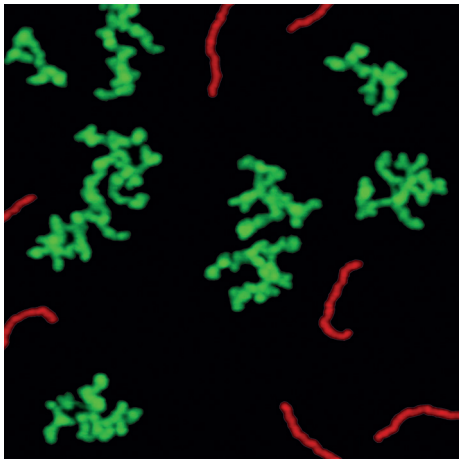

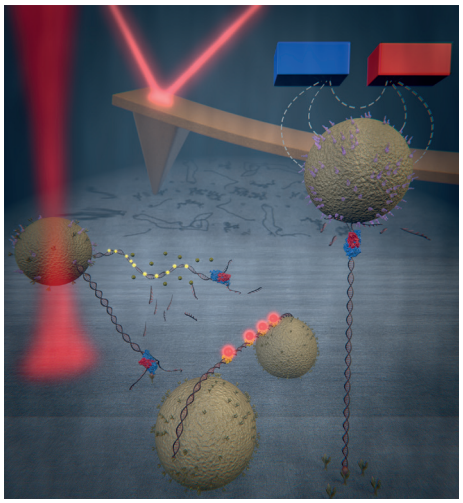

1 *AFM imaging of single- and double-stranded DNA. DNA molecules in ss and ds form were imaged simultaneously with atomic force microscopy (AFM). The difference in their secondary structures is readily visible with AFM, and allowed us to colour each population of molecules. Using an AFM volumetric method with dsDNA fragment as a fiducial marker, we show that ssDNA molecule length can be accurately related to their AFM volume.*

2 *Single-molecule methods for watching DNA repair one molecule at a time. AFM (background) for characterisation of SMC-like proteins involved in DNA repair. Optical tweezers (centre) assays used to monitor DNA resection. Tightrope DNA assay (front left) to monitor the dynamics of DNA excision repair proteins using quantum dots. Magnetic tweezers (front right) method to follow resection of a DNA end by the B. subtilis AddAB helicase-nuclease.*

Conformational-functional behaviour of proteins

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Ignacio Torres Rodríguez

Our group studies the conformational and functional behaviour of proteins using a multidisciplinary approach that combines physical chemistry, advanced molecular spectroscopy (ultrafast and single-molecule), structural biology, computer science, protein engineering, and molecular biology. Our efforts in the last two years have centered on three major areas -- experimental and theoretical analysis of protein folding ensembles, investigation of the molecular rheostat hypothesis, and engineering of macromolecular assemblies.

In reference to analysis of protein folding ensembles, we continued our efforts from previous years in developing a catalogue of folding archetypes corresponding to small single-domain proteins with elementary combinations of secondary structure elements.

Our second area of interest emerged from the experimental discovery of downhill folding, which led us to hypothesise that downhill folding modules can exploit their special behaviour, by which they change structure gradually rather than cooperatively, to implement molecular rheostats. Molecular rheostats are proteins able to produce analogue signals at the single-molecule level rather than the binary outputs of conventional molecular switches. These rheostats could offer a new variety of functional and regulatory properties for processes that depend on quantitative signals at the nanoscale; such devices might be used in nature. They also offer tremendous potential for nanobiotechnological applications. In this area, we recently showed proof of concept of a rheostatic biosensor based on the downhill folding module BBL. In parallel, we analyse the biological roles of conformational rheostats in coordinating protein networks via conformational selection, in the phenomenon of DNA sliding and homing-to-target by transcription factors during gene expression, and as molecular springs in macromolecular assemblies.

In a third research area, we recently embarked on a new project that involves engineering of macromolecular devices from monomeric globular proteins. This effort borrows ideas from molecular evolution to implement a design strategy that would facilitate domain swapping between otherwise monomeric proteins by engineering their folding behaviour in ways that reduce their intrinsic folding cooperativity.

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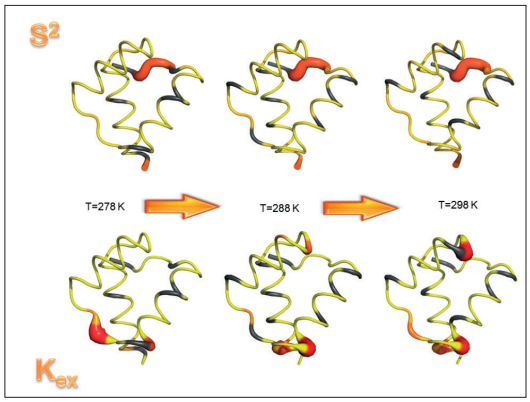

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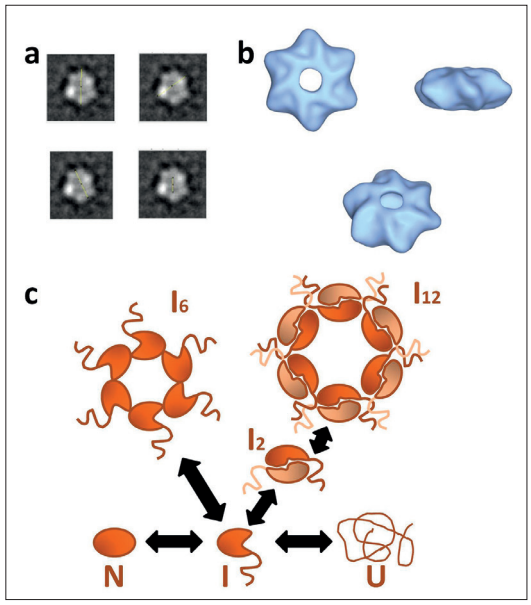

1 *Representation of amide backbone dynamics of the protein structure (pdb code 1ENH) showing mobility in the piconanosecond time scale, according to model-free Lipari Szabo analysis. Order parameter S2 and exchange constant K_{ex} are shown. Colour intensity and tube thickness are inversely proportional to the entity of the parameter in the case of S2, as a lower value indicates greater mobility. Amides for which data are absent are shown in grey.*

2 *Controlled oligomerisation of CI2 induced by mutation. (a) Electron microscopy of individual particles for the dodecamer. (b) 3D reconstruction of the dodecamer after averaging individual particles. (c) Scheme for the oligomerisation process in CI2 mutants.*

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Functional bioinformatics

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To understand the biology underlying experimental settings, we develop new methods and analytical techniques to solve clearly oriented, specific biological questions. We concentrate on functional bioinformatics, on new methodologies for analysis and interpretation of biological data in two main areas, transcriptomics and functional analysis.

Our recent results are related to transcriptomics regulation. We determined potential interactions between microRNAs and their target transcripts. These interactions are predicted using our methods, then quantified using expression information from mRNA and miRNA in the same samples. We also designed techniques to compare expression profiles with public databases of experiments, allowing association of new information regarding diseases and drugs. A web-based application can be accessed at http://m3rna.cnb.csic.es

The group studies new ways of drug repurposing, using a known drug to treat conditions different from those for which it was designed. We developed NFFinder, a bioinformatics tool to identify potentially useful drugs in the context of orphan diseases. NFFinder uses transcriptomic data to find relationships between drugs, diseases and a phenotype of interest. On a dashboard, the application shows a series of graphics and tables to help researchers formulate repositioning hypotheses and identify potential biological relationships between drugs and diseases. NFFinder is freely available at http://nffinder.cnb.csic.es

Finally, we coordinated and developed dasHPPboard, a proteomics-based dashboard that collects and reports experiments from the Spanish Human Proteome Project consortium (SpHPP), following the strategy of analogue genomics projects such as the Encyclopedia of DNA Elements (ENCODE), which provides a vast amount of data on human cell line experiments. The dashboard includes results of Shotgun and Selected Reaction Monitoring proteomics experiments, post-translational modification information and proteogenomics studies. We processed transcriptomics data from the ENCODE and Human Body Map (HBM) projects for identification of specific gene expression patterns in different cell lines and tissues, with special interest in expressed genes with little available proteomic evidence (missing proteins). Peptide databases were built using single nucleotide variants and novel junctions derived from RNA-Seq data that can be used in search engines for sample-specific protein identification on the same cell lines or tissues. dasHPPboard is freely accessed at http://sphppdashboard.cnb.csic.es

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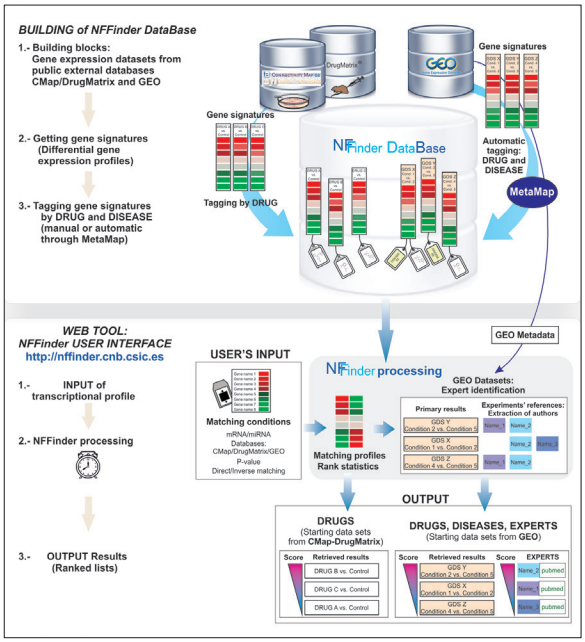

1 *NFFinder, a tool for searching similar transcriptomics experiments in the context of drug repositioning.*

Cell structure lab

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The project of the Cell Structure Laboratory makes use of current developments in imaging technology to study the biogenesis of viral factories and the structures that support virus replication, morphogenesis and egress. Recent developments in sample preparation and visualisation by light and electron microscopy are facilitating our understanding of the complex interactions that viruses trigger inside cells (Fernández de Castro *et al.* Cell Microbiol 2013). Among these new technologies, three-dimensional (3D) imaging is making a notable impact in the field of virology, as it helps us understand how viruses control cells (Risco *et al.* Annu Rev Virol 2014).

In the last two years, our group has characterised the architecture of the virus factory assembled by the human reovirus (Fernández de Castro *et al.* mBio 2014) and the role of the ESCRT machinery and oxysterol-binding proteins in the formation of the Tombusvirus replicase in intracellular membranes (Barajas *et al.* PLoS Pathog 2014a; Barajas *et al.* PLoS Pathog 2014b). This work was done in collaboration with Drs Terry Dermody (Valderbilt University, TN, USA) and Dr. Peter D. Nagy (Kentucky University, USA). The assembly of the Tombusvirus replicase complex was visualised by metal-tagging transmission electron microscopy, a new, highly-sensitive nanotechnology developed in our lab that reveals proteins in cells at molecular-scale resolution (Fernández de Castro *et al.* Methods Cell Biol 2014).

For the first time, we have 3D images that show how the viral replicase molecules change their organisation when incorporated into the active domains of the intracellular replication compartment. Several steps in the bunyavirus life cycle were also characterised, including the organisation of the viral ribonucleoproteins in the infectious virions (Li *et al.* Proc Natl Acad Sci USA 2013), the assembly of viral factories and replication organelles in cells (Risco & Fernández de Castro Subcell Biochem 2013; Fernández de Castro & Risco Future Virol 2014) and the structures that facilitate virus egress and propagation (Sanz-Sánchez & Risco PLoS One 2013). In 2013, our group created the microscopy consulting service BioGrid Solutions to provide expert advice to scientists and companies (http://www.cnb.csic.es/~Riscolab/).

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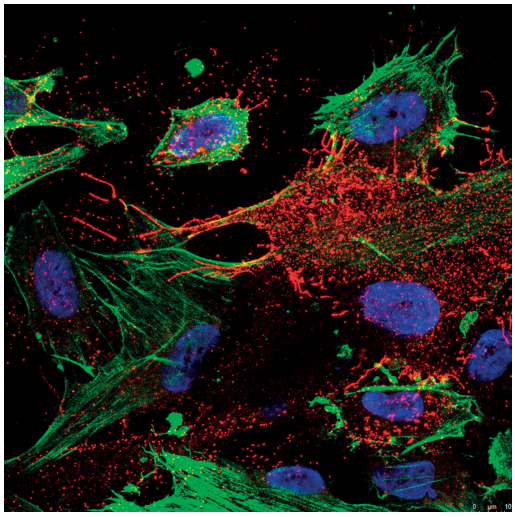

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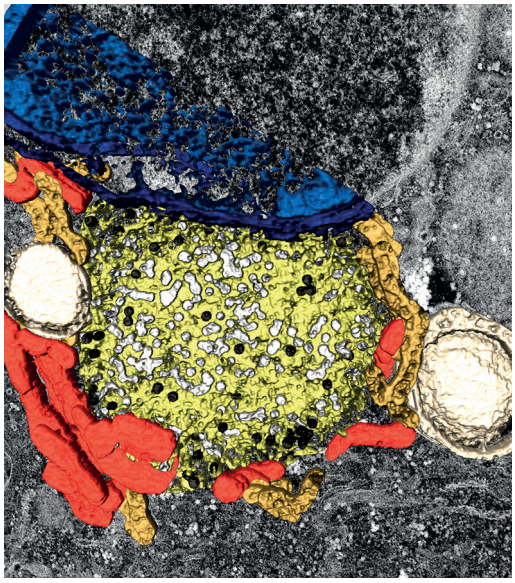

1 *Confocal microscopy of bunyavirus egress and propagation*

2 *Reovirus factory in three dimensions*

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Structural and physical determinants of adenovirus assembly

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

Adenoviruses (AdV) are pathogens with particular clinical relevance in the immunocompromised population. They are widely used as vectors for gene therapy, vaccination and oncolysis. Adenoviruses have one of the most complex icosahedral, non-enveloped capsids known. Their genome, a dsDNA molecule, is bound to large amounts of positively charged proteins that help condense it and form the core, which is confined within an icosahedral capsid composed of multiple copies of seven viral proteins. The final stage of adenovirus morphogenesis consists of proteolytic processing of several capsid and core proteins. The immature virus, which contains all precursor proteins, is not infectious due to an uncoating defect.

Using atomic force microscopy in physiological conditions, we showed in real time how individual AdV mature and immature particles disassemble sequentially due to mechanical fatigue. We also identified a previously unreported substrate for the viral protease, the putative scaffolding protein L1 52/55k. Cleavages in L1 52/55k disrupt its interactions with other virion components, providing a mechanism for its removal during maturation. Although more than 200 types of AdV have been isolated from a variety of vertebrates, most current knowledge of their biology is derived from a few human AdV. We provided the first molecular description for a lizard AdV with novel architecture of proteins involved in binding to the virus receptor in the cell membrane. This AdV also has favourable characteristics as an alternative therapeutic vector.

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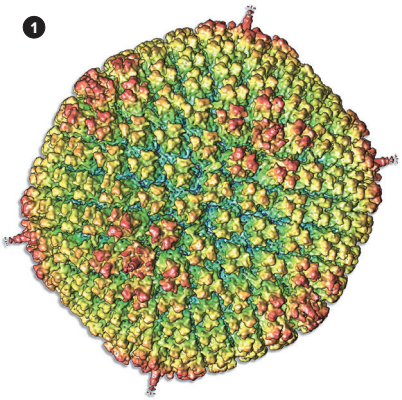

1 *General architecture of human adenovirus as determined by cryo-electron microscopy. This complex capsid has a maximum diameter of 95 nm, not including the long fibres that protrude from the vertices, whose flexibility renders them invisible in cryo-EM studies. The capsid is composed of multiple copies of seven proteins, and contains the dsDNA genome, condensed with the help of three viral proteins similar to histones. Protein L1 52/55k is needed for genome packaging but is not present in the final virion; it is released after packaging with the help of the viral protease.*

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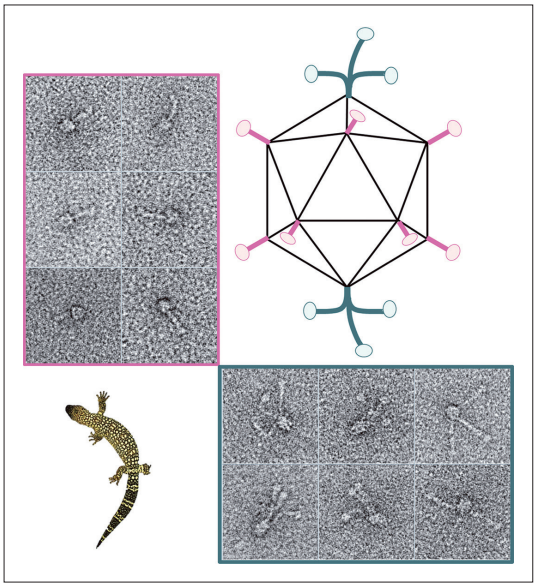

2 *The unusual vertex organisation of a lizard adenovirus. Lizard adenovirus type 2 (LAdV-2) was isolated from Heloderma horridum (bottom left). It is the first adenovirus with either one short (pink) or three long (green) fibres per vertex. Our studies indicate that only one or two vertices per particle harbour the long fibre triplet. Because fibres bind the virus receptor at the cell surface, this previously unobserved fibre arrangement suggests an alternative form of cell entry for this reptilian adenovirus.*

Structure and function of molecular chaperones

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Molecular chaperones are proteins that assist the folding and degradation of other proteins. Our main line of work deals with the structural and functional characterisation of different molecular chaperones and their interaction in the protein folding and degradation assembly pathways. Using various techniques, principally electron microscopy and image processing, we work with chaperones like CCT, Hsp110, Hsp90, Hsp70, Hsp40, nucleoplasmin, and with some of their cochaperones, including Hop, Hip and CHIP. We have characterised several complexes formed by these chaperones and their co-chaperones that form part of the various assembly lines involved in protein homeostasis with substrates such as p53, VHL, Gb transducin. The same techniques have been used to study other proteins and macromolecular complexes such as amyloids and RNA processing proteins.

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

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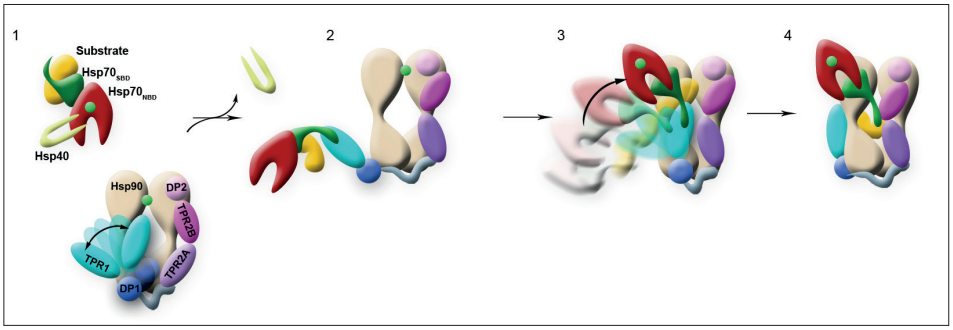

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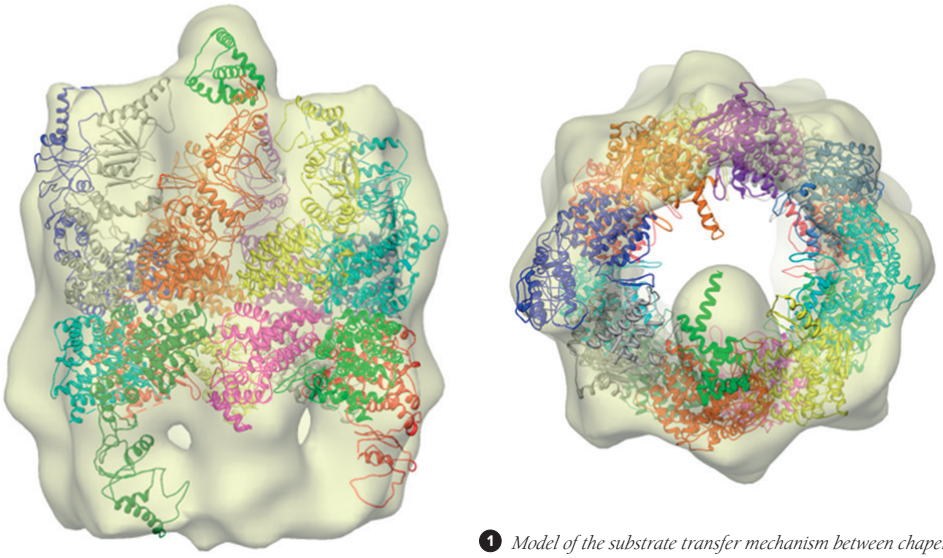

1 *Model of the substrate transfer mechanism between chaperones Hsp70 and Hsp90 mediated by the co-chaperone Hop.*

2 *Two orthogonal views of the complex between the chaperonin CCT and the protein PDC5, with the docking of the crystal structure of the open form of CCT coloured by subunit (Protein Data Bank code 2XSM) and the atomic structure of PDCD5 in green (Protein Data Bank code 2K6B).*

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Structural biology of viral fibres

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Some viruses and bacteriophages attach to their host cell via proteins integral to their capsids, for example poliovirus, coxsackievirus and rhinovirus (common cold virus). Other viruses bind to their host cell receptors via specialised spike proteins (for example HIV, the AIDS virus), or via specialised fibre proteins, like adenovirus, reovirus and bacteriophages T4, T5 and T7. These fibres all have the same basic architecture; they are trimeric and have an N-terminal virus or bacteriophage attachment domain, a long, thin but stable shaft domain, and a globular C-terminal cell attachment domain. These trimeric fibrous proteins are very resistant to temperature- and detergent- induced denaturation.

In 2013 and 2014, we determined the structures of the L-shaped tail fibre of bacteriophage T5 (the pb1 protein) with and without its C-terminal intramolecular chaperone, of the bacteriophage T4 fibre protein gp34, and of several animal adenovirus fibre proteins (snake atadenovirus 1, turkey siadenovirus 3, bovine atadenovirus 4, goose aviadenovirus 4 and mouse mastadenovirus 2).

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

We also collaborated with other research groups in crystallisation and structure solutions for the proteins and peptides they produce, and determined structures of cyclic antibiotic peptides, bacterial dehydroquinases and shikimate kinases in complex with inhibitors, and of the bacteriophage K endolysin domain CHAPK.

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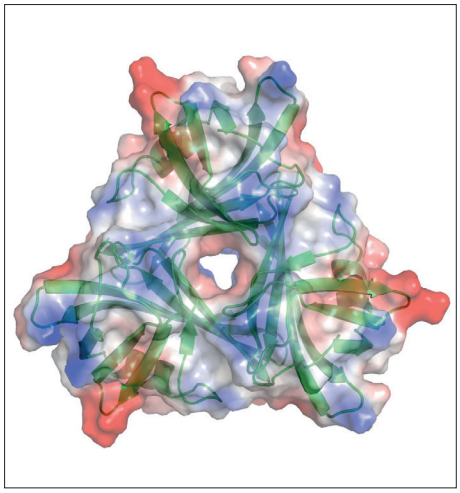
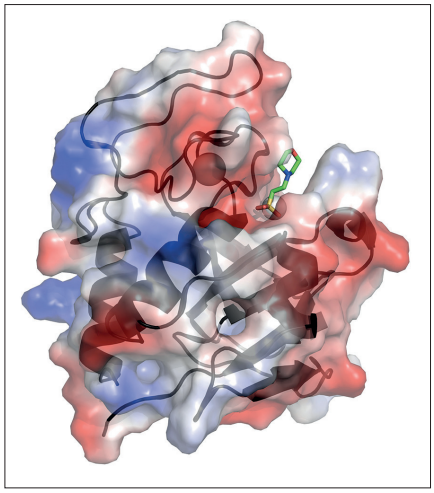


1 *Structure of the atadenovirus Snake Adenovirus 1 fibre head.*



2 *Structure of the staphylococcal endolysin CHAPK domain.*