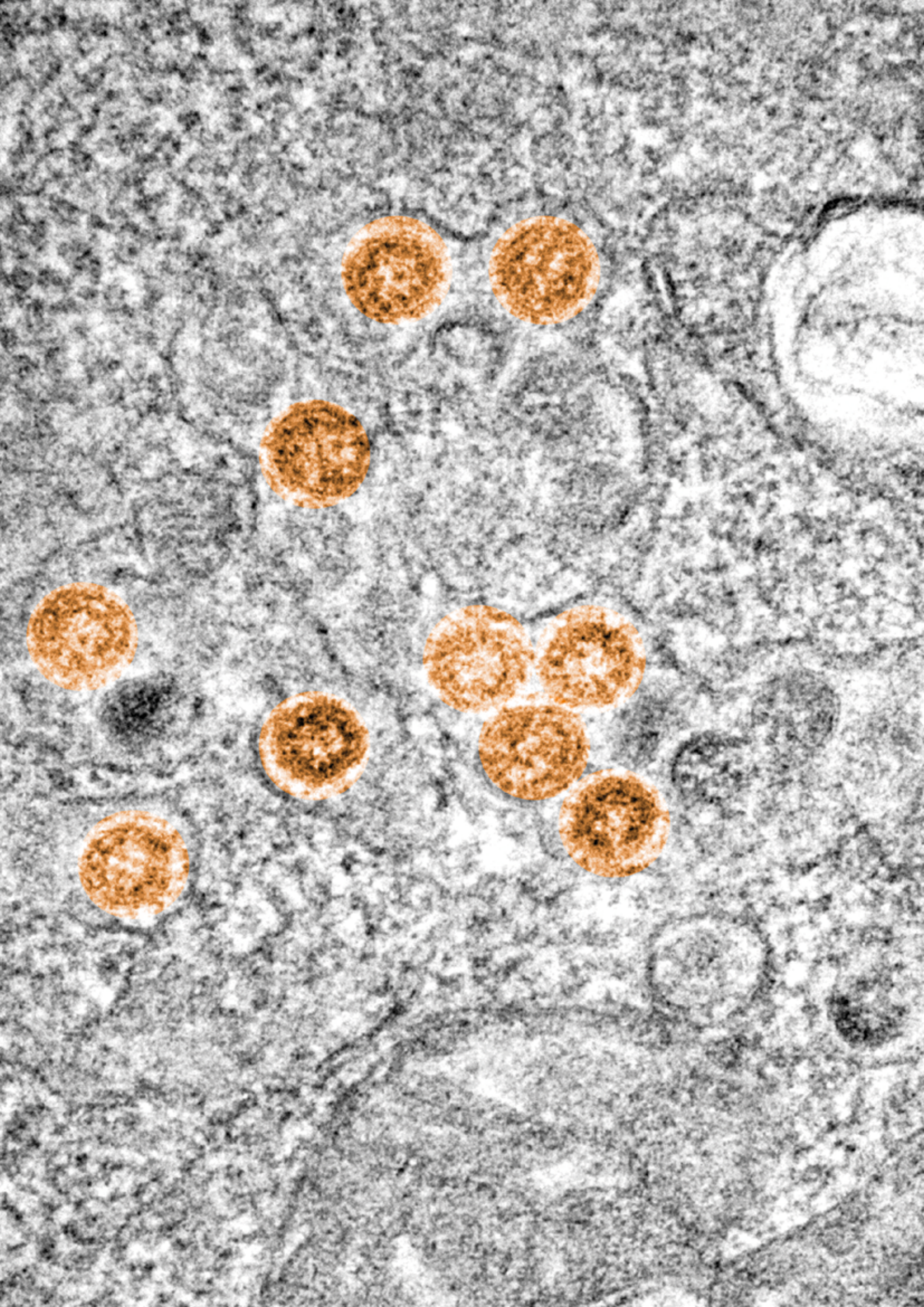


7

THE SARS-CoV-2 PANDEMIC CHALLENGES

The current COVID-19 pandemic represents one of the greatest challenges to humanity. Science and scientists all over the world have joined forces to provide responses to the society. Since March 2020, researchers at the CNB, a multidisciplinary research centre with a long-standing expertise in molecular and structural virology and immunology, have developed collaborative and interdisciplinary studies that exploit synergies between research groups and scientific services.

Our lines of action comprise more than fifteen projects led by researchers from the centre*. Many of these projects are included in the CSIC Global Health Platform**, that counts with more than 200 research groups addressing the scientific challenges posed by COVID-19 pandemic to provide short, medium and long term solutions.

Our contributions against SARS-CoV-2 include the development of vaccines and therapeutical approaches to tackle SARS-CoV-2 infection, structural and computational studies to identify potential therapeutic targets, the development of diagnostic kits to determine the presence of viral antigens or antibodies in biological samples and the development of computational models to evaluate the effect of the populations' behaviour in the spread of epidemics.

The excellent work of the CNB and the CSIC during the pandemic has been recognised by the Consejo General de la Abogacía Española through its Fundación Abogacía. Both the CSIC and the CNB have been awarded the XXII Human Rights Prize in the Institution category. These awards have been dedicated this year to the defense of universal access to health.

** CNB website: http://www.cnb.csic.es/index.php/en/research/sars-cov2-research*

***CSIC Global Health Platform Website: https://pti-saludglobal-covid19.corp.csic.es/*

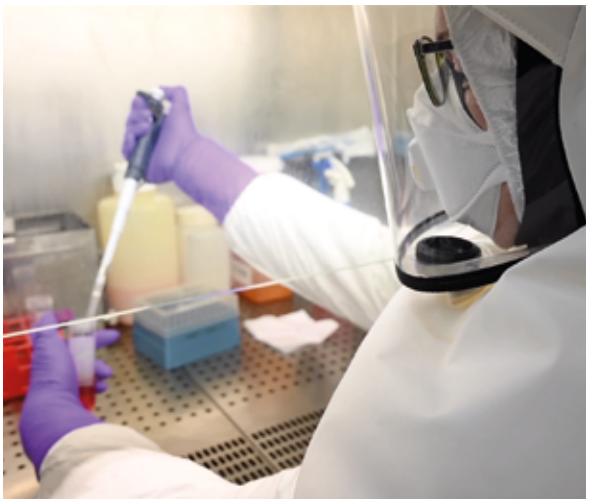
Section of a cell infected with SARS-CoV-2, showing newly formed viral particles (orange) between a double membrane vesicle where the virus genome is replicated and mitochondria (image from Gabriela Condezo, Carmen San Martín and Marta López de Diego).

Development of a SARS-CoV-2 vaccine based in non-infective replicons

PRINCIPAL INVESTIGATORS
Luis Enjuanes, Isabel Sola
Sonia Zúñiga (senior researcher)

The aim of this project has been to generate the SARS-CoV-2 virus by assembling synthetic DNA fragments. Using the full cDNA copy of the genome, and the reverse genetics system based on bacterial artificial chromosomes (BACs), genes responsible for virulence and propagation have been deleted to obtain propagation-deficient, highly immunogenic RNA replicons that can be used as specific SARS-CoV-2 vaccine candidates. In parallel, animal models (transgenic mice) have been developed for the validation of vaccines and other therapeutic agents to protect against COVID-19.

A patent to protect the development of vaccines based in self-replicative propagation-deficient RNAs that induce sterilising immunity has been presented in May 2020



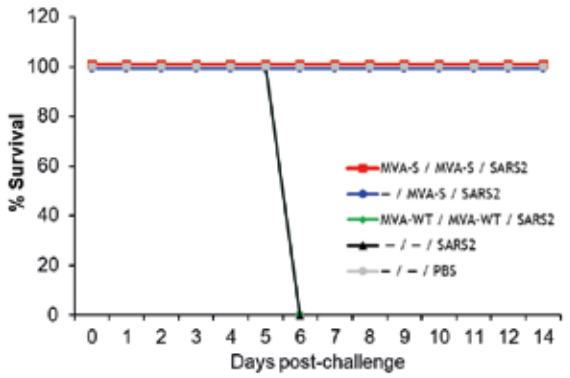
Development of vaccine(s) against SARS-CoV-2/ COVID-19 based on non-replicating viral vector (MVA)

PRINCIPAL INVESTIGATORS
Mariano Esteban, Juan García Arriaza, Carmen E. Gómez

EXTERNAL COLLABORATORS
David Sancho (CNIO), Susana Guerra (UAM)

An aim of the Poxvirus and Vaccines Group is to develop effective vaccines against the prevalent SARS-CoV-2 strain and its variants that might be applicable in humans. This is done using as platform the highly attenuated poxvirus strain MVA expressing different viral antigens of SARS-CoV-2, such as those corresponding to full-length proteins, virus-like particles (VLPs) and conserved multiepitope components.

We have developed a vaccine candidate MVA-CoV2-S expressing the complete S (Spike) protein that in mice triggers the induction of potent S-specific T-cell responses and high titers of neutralising antibodies. Remarkably, susceptible mice immunised with one or two doses of MVA-CoV2-S were 100% protected from SARS-CoV-2 lethality. Moreover, two doses of the vaccine prevented virus replication in lungs. Similar efficacy studies are ongoing with hamsters and macaques. The vaccine MVA-CoV2-S has been produced by a company and phase I/II clinical trials are planned along 2021.



The vaccine candidate MVA-CoV2-S administered in one or two doses in humanised mice protects 100% against lethality induced by SARS-CoV-2.

APPROACHES TO TACKLE SARS-COV-2 INFECTION

The CNB antiviral screening platform

PRINCIPAL INVESTIGATORS

Pablo Gastaminza, Urtzi Garaigorta

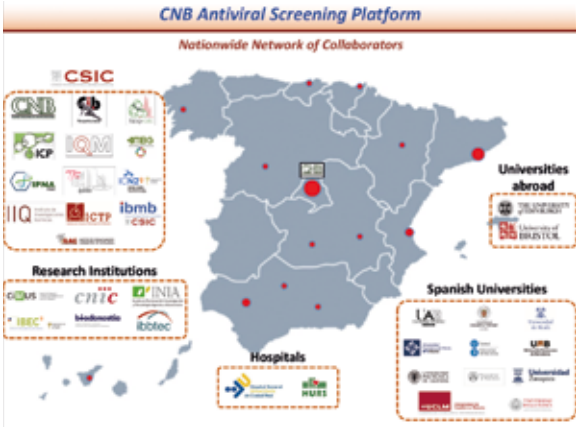
CNB COLLABORATORS

Mariano Esteban, Juan García Arriaza, Roberto Solano, Luis Ángel Fernández, José María Casasnovas, Fernando Corrales

EXTERNAL COLLABORATORS

18 Research Centres (12 from CSIC); 10 Spanish and 2 British Universities; 2 Spanish Hospitals

The objective of the CNB Antiviral Screening Platform is to provide a permanent structure dedicated to the identification and characterisation of antiviral compounds against human pathogenic viruses of biomedical relevance. The working model is based on the use of cell culture systems of viral infections including: SARS-CoV-2, mosquito-borne viruses (dengue, West Nile and Zika flaviviruses), hepatitis B and C viruses as well as influenza A virus. To do so, we have established a nationwide network of collaborators which provide collections of pure compounds and complex extracts to be tested as potential antivirals. So far, we have tested over 2400 repurposing drugs and around 3500 experimental compounds in the SARS-CoV-2 infection cell culture system. A handful of clinically approved drugs have shown antiviral activity in the absence of toxicity in the cell culture systems and they are being considered as potential candidates for clinical testing by the Global Health PTI platform at CSIC. Moreover, we have identified new families of experimental compounds and natural extracts with antiviral activity that have been protected by patent applications. These families of antivirals are being prioritised for further characterisation of their mode of action and preclinical animal studies. Lastly, we are giving support to other SARS-CoV-2-related projects at the CNB by providing our expertise and experimental systems to study the neutralisation capacity of therapeutic recombinant antibodies and sera from mice immunised with vaccine candidates. Finally, five patent applications have been filed in 2020.



Map of Collaborator Institutions.

Development of synthetic biology devises toward the screening of potential inhibitors of the SARS-CoV-2 3CL protease

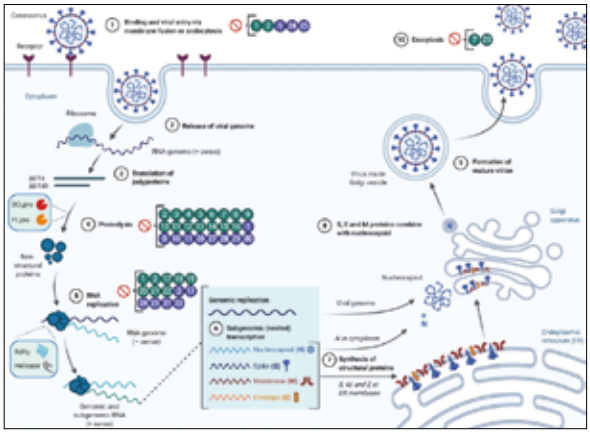
PRINCIPAL INVESTIGATOR

Juan Nogales Enrique

EXTERNAL COLLABORATORS

Felipe Lombó (Universidad de Oviedo); Tobias Goris (German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE); Uwe T. Bornscheuer (Greifswald University)

Different antivirals and natural compounds have been tested against coronaviruses, such as remdesivir, ribavirin or herbacetin. Of the natural anti-coronaviral compounds, flavonoids in particular have shown interesting inhibitory bioactivities. In our group we have evaluated the potential of flavonoids as antivirals by thoroughly reviewing experimental and computational evidences. We identified flavons and flavonols as more active flavonoids and the main viral protease, 3CL, as the more promising flavonoid target. Furthermore, we proposed a way forward for updating a classical industrial biotechnology Design, Built, Test and Lear (DBTL) cycle towards the rational design, construction and screening of combinatorial libraries of flavonoids with antiviral properties. Current efforts are focus on the implementation of synthetic biology devises toward the identification of potential inhibitors of the 3CL based on new-to-nature flavonoids.



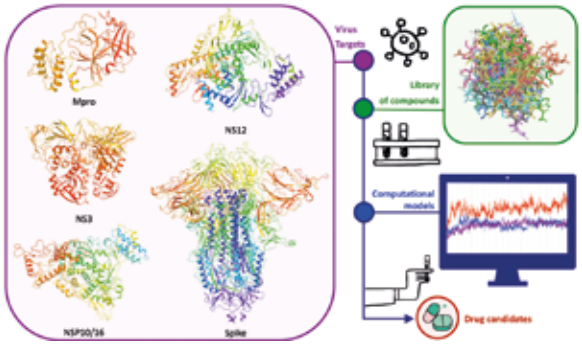
Overview of the coronavirus life cycle, indicating the attachment to the host cell membrane receptor, the translation of the viral (+)ssRNA genome in both polyproteins, the proteolysis carried out by 3CLpro and PLpro proteases, the viral genome replication steps and the virion maturation along endoplasmic reticulum and Golgi apparatus, with final exocytosis across the cell membrane. Numbers encircled in green represent flavonoids tested in vivo against SARS-CoV and/or MERS-CoV. Numbers encircled in purple represent flavonoids identified in silico as promising drugs against SARS-CoV-2 (Goris et al, 2020).

New drugs to combat Covid-19: from computational models to pre-clinical studies

PRINCIPAL INVESTIGATOR
Cristina Risco

EXTERNAL COLLABORATORS
José Pedro Cerón (Universidad Católica de Murcia, UCAM), Nuria Izquierdo-Useros (IrsiCaixa, Barcelona)

The project is a pre-clinical search for anti-SARS-CoV-2 drugs. Our list of potential antivirals includes inhibitors of SARS-CoV-2 MPro, Spike, MTase and RNApol identified by Dr. José Pedro Cerón at the UCAM, using state-of-the-art computational tools and databases of clinically approved drugs (Figure 1). Molecular modelling studies of the interaction of compounds with high-resolution crystal structures of SARS-CoV-2 proteins have produced a list with the best candidates. In addition, our library also includes inhibitors of cell factors used by other RNA viruses such as mitochondrial proteins, lipid transfer proteins, proteasome and protein kinases. These compounds are tested at the Cell Structure Lab (CSL) of the CNB using the human coronavirus 229E, a common cold virus that can be handled in BSL2 labs. The efficacy of compounds is studied by immunofluorescence and confocal microscopy of cell cultures infected in the presence and absence of the drugs. The most promising compounds of the library (10 out of 116 so far) are tested against SARS-CoV-2 in the BSL3 lab by Dr. Nuria Izquierdo-Useros (Irsi-Caixa), before studies with animal models and clinical trials. For those compounds with antiviral activity against SARS-CoV-2, electron microscopy studies are done at the CSL-CNB to obtain details about their mechanism of action.



Workflow for in-silico*, structure-based screening of our chemical library to identify inhibitors of SARS-CoV-2 proteins (José Pedro Cerón, UCAM).*

Identification of antivirals in plant extracts

PRINCIPAL INVESTIGATOR
Roberto Solano

CNB COLLABORATORS
Pablo Gastaminza, Urtzi Garaigorta

EXTERNAL COLLABORATORS
Alejandro Cifuentes (CIAL-CSIC)

SARS-CoV-2 pandemic is having devastating consequences, and has evidenced both a lack of effective treatments and the absence of a global plan to face future pandemics. Search for antivirals has had limited success so far. Therefore, there is an urgent need of new potent and safe antivirals against SARS-CoV-2 and other new viruses, expected to emerge in coming decades. Plants have an extremely rich specialised metabolism that provides them with a broad repertoire of chemicals of pharmaceutical interest. Different plant species use the same metabolic pathways with enzymatic variants to produce a unique blend of metabolites. Therefore, the identification of new plant sources of enzymatic variants and metabolites is key to discover new drugs. In our lab, we are generating suitable model plant systems that allow genetic manipulation and metabolic engineering to produce bioactive metabolites for their pharmacological exploitation.

Identification of antivirals inhibiting essential virus-host interaction during SARS-CoV-infection

PRINCIPAL INVESTIGATORS
Luis Enjuanes, José Manuel Honrubia, José Ramón Valverde

In order to select antivirals that inhibit cell-signalling pathways involved in CoV replication and pathology, our laboratory has previously identified the interaction of a viral motif (PBM) with a cellular protein (PDZ). The inhibition of this interaction prevents virus virulence. Structural studies led to the understanding of the residues involved in this binding, and are facilitating the inhibition of PBM-PDZ interaction, helping the selection of potent antivirals.

APPROACHES TO TACKLE SARS-COV-2 INFECTION

Monoclonal Antibodies against 2019-New Coronavirus (European Project MANCO)

PRINCIPAL INVESTIGATORS
Luis Enjuanes, Isabel Sola
Sonia Zúñiga (Senior researcher)

This project, in collaboration with research groups from Germany and The Netherlands, aims to obtain IgG neutralising antibodies specific for SARS-CoV-2 that elicited full protection, both in experimental models (mice and hamster) and humans. The project includes Phase I and II clinical trials to evaluate protection in persons. The aim is to administer a combination of two neutralising antibodies selected from the pool of more than 70 monoclonal antibodies obtained.

Development of therapeutic antibodies against SARS-CoV-2

PRINCIPAL INVESTIGATORS
Luis Ángel Fernández, José M. Casasnovas

CNB COLLABORATORS
Víctor de Lorenzo, Pablo Gastamiza, Urtzi Garaigorta, Isabel Sola, Luis Enjuanes

EXTERNAL COLLABORATORS
Juan Alberto Corbera Sánchez (Universidad de Las Palmas de Gran Canaria, Spain)

This project aims for the generation of therapeutic antibodies able to block the entry of SARS-CoV-2 into human cells with the final goal of being administered to symptomatic COVID-19 patients, to reduce the risk of progression to severe forms of the disease. To this end, we have focused on the generation of camel-derived nanobodies (Nbs) binding to the receptor binding domain (RBD) of the SARS-CoV-2 envelope Spike (S) protein. Candidates will be expressed and proved in a humanised transgenic mice SARS-CoV-2 infection model to identify neutralising clones with therapeutic potential. In parallel, structural studies will be conducted to define the interaction of Nbs to RBD and S proteins at the molecular level. In addition we are developing antibody engineering technologies to improve Nbs that respond to new and challenging virus variants. Lastly, the Nbs will be also tested in diagnostic applications in collaboration with other Spanish Research Institutions: Institut Català de Nanociència i Nanotecnologia (ICN2), Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Centro de Astrobiología (CAB, CSIC-INTA), and Centro de Investigación Biomédica (CINBIO, Universidad de Vigo).

Preclinical validation of therapeutical agents for SARS-CoV-2 treatment based in monoclonal antibodies

PRINCIPAL INVESTIGATORS
Luis Enjuanes, Isabel Sola, Leonor Kremer

This research group has obtained neutralising monoclonal antibodies against SARS-CoV-2. Their protection efficiency against SARS-CoV-2 infections is being tested in humanised transgenic mice models.

Control of SARS-CoV-2 infection through the modulation of the energy metabolism of the cell

PRINCIPAL INVESTIGATORS
Fernando Almazán Toral, Francisco José Iborra Rodríguez (IBV-CSIC)

The development of effective therapies against COVID19 disease necessarily involves the knowledge of the fundamental mechanisms of the pathogenesis of SARS-CoV-2. A recurrent mechanism of viral pathogenesis is the metabolic reprogramming. Viruses alter cellular energy metabolism for their own benefit, making it especially attractive to identify these alterations to intervene pharmacologically and prevent or cancel the progression of the viral infection. Each virus uses unique metabolic strategies, so it is sometimes difficult to obtain general treatments, even for viruses of the same family. In this project we are studying the metabolic alterations induced by SARS-CoV-2 infection in cell cultures in order to identify the metabolic pathway affected and explore how the use of drugs targeting the routes identified interfere with SARS-CoV-2 infection. To date, we have identified several proteins of the cellular metabolism that are downregulated during SARS-CoV-2 infection in several cell lines and we are analysing the effect of different pharmacological treatments against the identified proteins on the course of SARS-CoV-2 infection.

Immunosuppressive nanoparticles with lung tropism to stop the cytokine storm and viral replication

PRINCIPAL INVESTIGATOR
Domingo F. Barber

CNB COLLABORATOR
Marta López de Diego

EXTERNAL COLLABORATOR
María del Puerto Morales (ICMM-CSIC)

It has been recently described that iron oxide nanoparticles (IONPs) are capable of inhibiting the replication of the influenza virus. In this project we aim to understand how IONPs, which are already used in clinic for magnetic resonances or for the treatment of anemia, interfere with the replication and infective capacity of different viruses such as influenza and SARS-CoV-2. We also intend to design immunosuppressive nanoparticles that can be used to reduce lung inflammation caused by the cytokine storm generated in the most severe cases of respiratory viral infections, such as those caused by SARS-CoV-2 and the influenza virus.

Immune evasion and immunopathology caused by COVID-19

PRINCIPAL INVESTIGATORS
Isabel Mérida, Margarita del Val (CBMSO)

CNB COLLABORATORS
José María Casasnovas, J. Francisco Rodríguez Aguirre

EXTERNAL COLLABORATORS
Teresa Santos Mendoza (Instituto Nacional de Enfermedades Respiratorias, Mexico)

The SARS-CoV-2 is a betacoronavirus of animal origin closely related to other zoonotic coronaviruses like SARS-CoV and the Middle East Respiratory coronavirus (MERS-CoV). COVID-19 disease comprises two phases: an early period after infection where an adequate and rapid immune response limits virus replication and a second phase where viral-induced inflammatory responses results in immunosuppression and acute respiratory distress syndrome (ARDS). The severe immunopathological features associated to COVID-19 include acute cytokine release syndrome (CRS), characterised by elevated serum levels of inflammatory cytokines. Our team, with a long experience in the study of the mechanism that trigger immune evasion in cancer aims through different approaches to identify the mechanisms by which SARS-CoV-2 triggers immune evasion and inflammatory responses.

Targeting coronavirus RNA genome with CRISPR-Cas13d

PRINCIPAL INVESTIGATORS
Dolores Rodríguez, Lluís Montoliu, Miguel Ángel Moreno Mateos (CABD-UPO/CSIC, Sevilla)

CNB COLLABORATORS
Almudena Fernández (CIBER-ISCIII, Madrid), Fernando Almazán

EXTERNAL COLLABORATORS
Manuel Collado (SERGAS, Santiago de Compostela), Pablo Alfonso del Pino (Universidad de Santiago de Compostela)

In this scientific proposal we will use a new variant of the CRISPR gene-editing tools, Cas13d, with an RNA-guided RNAse specific activity, to target and destroy the RNA genome of the SARS-CoV-2 inside infected cells. This is a direct treatment aiming to inactivate the SARS-CoV-2 genetic material with one of the newest programmable endonucleases. This proposal will proceed stepwise, securing every technological advance, before moving onto the next phase. We want this strategy to be effective but, above all and most importantly, safe. We will first assess the potential toxicity and efficacy of CRISPR Cas13d reagents in zebrafish embryos as an in vivo model. Thereafter, the proof-of-concept of this project will be validated in two related cellular and viral experimental systems. Eventually, upon confirming all previous steps, this strategy will be tested under appropriate BSL3 conditions directly on human epithelial cells infected with SARS-CoV-2 and, next, using adequate mouse models susceptible to this coronavirus. This consortium encompasses proved expertise in CRISPR technology, in Cas13d, in animal models, in cell biology and virology, and in nanobiotechnology.

APPROACHES TO TACKLE SARS-COV-2 INFECTION

SARS-CoV2-host proteomic interactions

PRINCIPAL INVESTIGATORS
Fernando J Corrales, Alberto Paradela

CNB COLLABORATORS
Pablo Gastaminza, Urtzi Garaigorta, Francisco Rodríguez, César Santiago, Hugh Reyburn, Leonor Kremer, Luis Ángel Fernández

EXTERNAL COLLABORATORS
Spanish ProteoRed

Our project aims to consolidate a mass spectrometry-based platform to characterise a) recombinant proteins produced for COVID-19 research and applications, b) serum proteome of COVID-19 patients to define methods for stratification, prognosis and follow-up, c) the immunological response of COVID-19 patients (immunopeptidomics and immunoproteomics) and d) the SARS-CoV-2 host cell interaction at the proteome and phosphoproteome levels.

We have analysed 72 SARS-CoV-2 recombinant protein preparations by MALDI TOF and ESI-MS/MS. Products were identified, the Mr accurately measured and further optimization of purification strategies were implemented to reduce contaminants.

We have identified serum proteins that recapitulate the response of spinal cord injury patients to SARS-CoV-2 infection and suggest treatment strategies to prevent severe symptoms. Additionally, we have identified a 62 serum protein panel that allows stratification of COVID-19 patients by severity and age. Moreover, we have developed a protein array (105 target proteins involved in inflammation, cell adhesion and coagulation) with NAPPA technology to detect complementary blood autoantibody profiles that may help patient stratification

We have designed, synthesised, HPLC purified and characterised by mass spectrometry 70 SARS-CoV-2 peptides containing putative immunogenic epitopes with capacity to activate CD4+ y CD8+ lymphocytes. These peptides have been chosen for their ability to bind to the predominant class I and class II HLA alleles in the Spanish population.

We produced a dodeca-peptide array covering the sequence of the SARS-CoV-2 S protein to characterise the antibody profile raised against this protein by COVID-19 patients.

We set up an immunopeptidomics workflow to identify viral epitopes presented by HLA-I molecules in SARS-CoV-2 infected cells. Up to 13000 sequences were identified. Analysis of cells expressing N, M or E proteins is in process.

Development and experimental validation of sterilisation and decontamination systems for SARS-CoV-2 inactivation

PRINCIPAL INVESTIGATOR
Fernando Usera

EXTERNAL COLLABORATORS
ICV-CSIC, ISCIII, Several public and private entities

The laboratory of Biological safety level 3 (BSL-3) is the key infrastructure of the CNB for carrying out experiments with the SARS-CoV-2, other high-risk coronaviruses and other viruses and bacteria belonging to risk group 3 of human pathogens. The Biosafety Service participates in a series of studies in collaboration with public entities and companies that aim to develop and validate sterilisation and decontamination methods for the inactivation of SARS-CoV-2, other virus, different bacteria, bacterial spores and fungi in different environments and contaminated surfaces.

In addition, we are studying the dynamics of evaporation and persistence of aerosol droplets and the evolution of the viral titer in these droplets and in droplets deposited on different surfaces.

STRUCTURAL STUDIES

Structural analysis of the spike protein of SARS-CoV-2

PRINCIPAL INVESTIGATORS

José María Carazo, Carlos Óscar Sorzano

CNB COLLABORATORS

Mariano Esteban, César Santiago

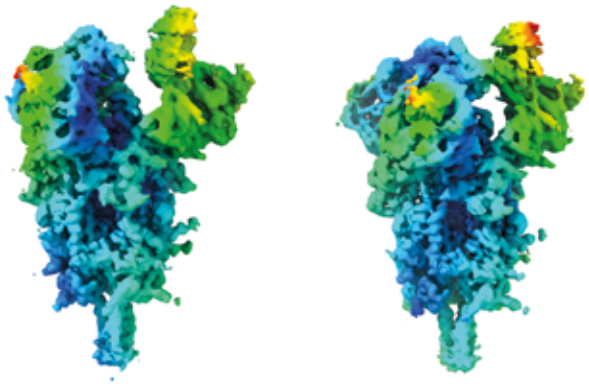
EXTERNAL COLLABORATORS

Pablo Chacón (Instituto Rocasolano, CSIC); Iñaki Comas and José Luis Llacer (Instituto de Biomedicina de Valencia, CSIC); Modesto Orozco (IRB, Barcelona); Heman Tagare (Yale University); Jason S McLellan (University of Texas)

The aim of this project is to describe the structure and dynamics of the spike protein of SARS-CoV2, a macromolecular complex that plays a central role in the infection process of the virus. To achieve this goal, we employ Single Particle Analysis by Cryo-Electron Microscopy (CryoEM) using stablished and new image processing tools, where the emphasis is on analysing the spike continuous flexibility at high resolution. We have studied the wild type virus, in collaboration with McLellan laboratory, continuing now with the analysis of mutants, especially those with a high prevalence in Spain (in collaboration with Comas and Llacer groups`). These fruitful collaborations are being stablished in the context of CSIC internal projects running for the next two years. This knowledge is key to understand how the virus gets into our cells, how the different drugs and vaccines work and how the different mutants of the virus may acquire novel characteristics, potentially impacting therapies.

In addition, we have expanded our information integration portal 3DBionotes, focusing on SARS-CoV2 and making emphasis on quality modeling together with genomic information. It should be noted that 3DBionotes is one of the few Recommended Interoperability Resources of the European Research Infrastructure (RI) on Life Science information (ELIXIR), and that in mid-2020 it was the topic of a joint press release between ELIXIR and the RI for Structural Biology, Instruct.

3D Bionotes-WS: http://3dbionotes.cnb.csic.es/ws/covid19



SARS-CoV-2 Spike protein structural changes

Structural determination of the SARS-CoV-2 nucleocapsid

PRINCIPAL INVESTIGATOR

Jaime Martín-Benito Romero

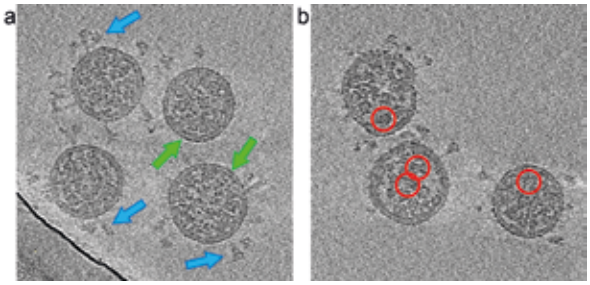
CNB COLLABORATOR

Cryoelectron microscopy service

EXTERNAL COLLABORATORS

Dr. Beata Turoňová (EMBL, Heidelberg, Germany)

The SARS-CoV-2 nucleocapsid is the structure formed by the viral genome bound to multiple copies of a protein called Nucleoprotein (NP). This structure stabilises the genome inside the virion and plays a crucial role in the virus life cycle, participating as a key element in the processes of viral transcription and replication, i.e., in the proliferation of the virus. Nevertheless, and despite its importance, little is known about the nucleocapsid structure and its arrangement inside the virion. Our project aims to determine the nucleocapsid structure using transmission electron cryomicroscopy and image processing techniques. From the acquisition of tilted serial images of SARS-CoV-2 virion samples followed by a reconstruction process, we could determine the 3D structure of individual viruses and how the NP is arranged within the virion. This electron tomography studies are further complemented by other structural techniques and molecular biology studies.



Sections of two three-dimensional reconstructions of SARS-CoV-2 virions obtained by electron tomography. In panel (a) the blue arrows point to the virus spike protein and the green arrows to the viral membrane visualized as a double black line. In panel (b) the red circles mark some details of the nucleocapsid that will be used to determine its structure. The tomographic data have been provided by Dr. Beata Turoňová (EMBL, Heidelberg) and reconstructed at the CNB-CSIC.

STRUCTURAL STUDIES

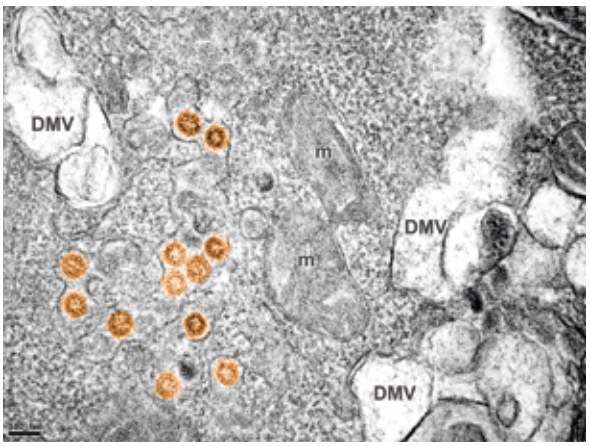
Structural characterisation of SARS-CoV-2 assembly

PRINCIPAL INVESTIGATOR
Carmen San Martín

CNB COLLABORATORS
Marta López de Diego, Mark J. van Raaij

EXTERNAL COLLABORATORS
Marçal Vilar (IBV-CSIC), Daniel Luque (ISCIII)

The global aim of this project is to understand the structural basis of SARS-CoV-2 morphogenesis, to interfere with virus propagation. We are using a combination of fluorescence microscopy, conventional and advanced electron microscopy to analyse key aspects regulating the formation of the SARS-CoV-2 infectious particle.



Section of a cell infected with SARS-CoV-2, showing newly formed viral particles (orange) between a double membrane vesicle (DMV) where the virus genome is replicated and mitochondria (m).

Production and crystallography of COVID-19 related proteins

PRINCIPAL INVESTIGATOR
Mark J van Raaij

CNB COLLABORATORS
Jaime Martín-Benito, Carmen San Martín

EXTERNAL COLLABORATORS
Jorge Pérez Juste (Universidade de Vigo); Maribel Botana Rial (Servicio Galego de Saúde)

In this project, we express COVID-19 related proteins in bacteria for structural biology purposes and for the development of sensors for SARS-CoV-2. The project is financed by a CSIC intramural project and by the Supera COVID fund (SERSforSARS: SERS-based Lateral flow point-of-care immunoassay for ultrasensitive detection of SARS-CoV-2).

Development of chimeric IBDV capsids with SARS-CoV-2 epitopes for therapeutical use

PRINCIPAL INVESTIGATOR
José R. Castón

CNB COLLABORATORS
Luis Enjuanes, Isabel Sola, Sonia Zúñiga, Pablo Gastaminza, Urtzi Garaigorta

Our lab analyses the potential of infectious bursal disease virus (IBDV) capsid to accommodate heterogeneous proteins and peptides fused to the capsid protein. We aim to develop an efficient assembly system of chimeric, IDBV-based virus-like particles where epitopes of different SARS-CoV-2 structural proteins can be inserted to engineer chimeric capsids able to induce protective immunity against SARS-CoV-2.

DIAGNOSTIC SOLUTIONS

Development of an antibody test to asses humoral immunity against Covid-19

PRINCIPAL INVESTIGATORS

José María Casasnovas, Hugh Reyburn, José Miguel Rodríguez Frade, Mar Valés

CNB COLLABORATOR

Salomé Prat

EXTERNAL COLLABORATORS

Francisco Sánchez-Madrid (Hospital de La Princesa), Eduardo López Granados (Hospital La Paz); Immunostep S.L.

Serological tests detect specific antibodies and allow recognition of individuals that have been in contact with the SARS-CoV-2. This technology has been validated in collaboration with La Princesa and La Paz Hospitals in Madrid. The tests are now being manufactured by the Spanish company Immunostep S.L. in ELISA kit format and are already available to the whole country, distributed by Eurofins Megalab. The development of this test in record time demonstrates the benefits of good medical-scientific collaboration.

The test is based on several viral proteins, including some that have not been used in diagnostics previously, that stimulate a strong production of antibodies. Specifically, we have found that the cysteine-like protease, an enzyme produced by the virus during infection, can act as an antigen to generate antibodies that can be detected in patient blood samples.

The tests detect different types of antibodies: IgM, generated usually five or six days after the onset of symptoms; IgG, produced at a slightly later stage of infection, but that persists over time; and IgA, which is produced in early stages, but can also be detected in later phases and which is more localised on mucosal surfaces, such as the respiratory tract, although it is also detected in patient serum.

Commercial ELISA kit developed by CNB-CSIC researchers and manufactured by Immunostep S.L.

Generation of an ELISA test for the detection of SARS-CoV-2 seropositive individuals

PRINCIPAL INVESTIGATORS

José F. Rodríguez, Juan R. Rodríguez

CNB COLLABORATORS

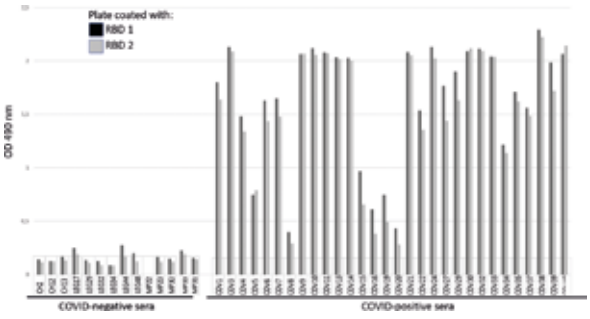
Dolores Rodríguez, Fernando Almazán, César Santiago

EXTERNAL COLLABORATORS

Esther Blanco (CISA-INIA), María Teresa Pérez (CNM-ISCIII)

Our initial aim was to produce recombinant SARS-CoV-2 polypeptides to collaborate in the development and production of serological tests using recombinant versions of the SARS-CoV-2 Spike protein, the Spike's receptor binding domain (RBD) and the cellular receptor ACE2. Recombinant proteins were produced using the baculovirus/insect cell expression system and purified by immobilised metal affinity followed by gel filtration chromatography. Purified polypeptides were initially used to determine optimal conditions to develop highly sensitive ELISA tests (Fig.1). Thereafter, our ELISA test was distributed to different hospitals during the first pandemic wave, before commercial serological tests were available.

Our group has also provided large protein quantities to laboratories of different Spanish institutions working on SARS-CoV-2 related projects. Results obtained with several versions of the RBD polypeptide were the subject of a CNB-CSIC European patent application. Finally, we are interested in applying the protein expression/purification technology developed by our group for the production of novel subunit vaccine candidates against SARS-CoV-2.



Recombinant proteins RBD1 (black bars) or RBD2 (grey bars), were used to determine by ELISA the presence or absence of SARS-CoV-2 in human erum samples.

COMPUTATIONAL MODELS

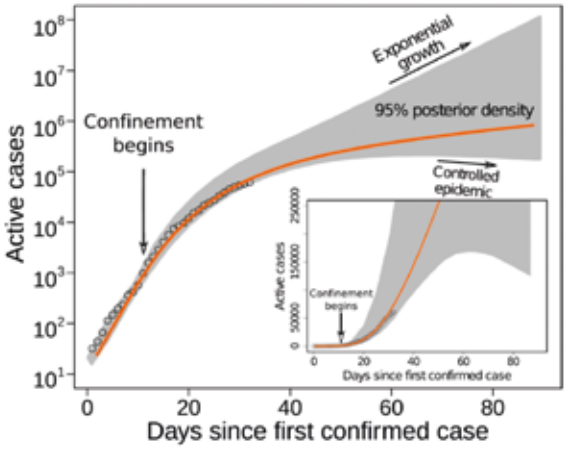
Prediction of the COVID-19 epidemic dynamics

PRINCIPAL INVESTIGATORS
Susanna Manrubia, Saúl Ares

EXTERNAL COLLABORATORS
Mario Castro (Universidad Pontificia Comillas); José A. Cuesta (Universidad Carlos III de Madrid)

The focus of this project is the development of tools to manage, through predictive models, the appropriate social distancing measures to contain or prevent the expansion of COVID-19. The first phase of the project focused on the development of predictive and consensual models. We proposed a new model including reversible confinement of susceptible population. An analytical solution shows that slowing down of epidemic expansion does not guarantee the "flattening of the curve" and could be a transient behavior leading to continuing growth. The theory accurately describes the propagation of COVID-19 in Spain and shows that predictions for its subsequent evolution are disparate, even contradictory. The future of ongoing epidemics is so sensitive to parameter values that predictions are only meaningful within a narrow time window and in probabilistic terms, much as what we are used to in weather forecasts. This work was presented in a PNAS publication.

In a second phase, the refinement of predictive models and potentially useful reports for a future pandemic and the study of explanatory models are being tackled. We have shown that the parameters of a large class of compartmental models are related if properly renormalised, while the effective dynamics do not change. We are also working on stochastic extensions of our results. Finally, we publish analysis of public health data on Twitter, and are in contact with regional and national administrations to discuss data and share our expertise.

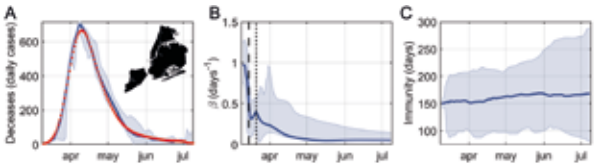


Fit to data obtained in real time for the daily number of active cases in Spain (from March 1st to March 29th) and peak forecast. The shaded area represents the 95% predictive posterior interval: Its increasing width implies that predictability decays exponentially fast. In fact, opposite predictions for the future number of active cases can be derived.

Characterisation of the duration of immunity to SARS-CoV-2 from epidemiological data series

PRINCIPAL INVESTIGATOR
Juan F Poyatos

Among the many open problems associated with the present 2019 coronavirus outbreak, the question about the duration of immunity to SARS-CoV-2 is arguably one of the most significant. This question is traditionally determined through longitudinal serological studies that track antibody prevalence in the same cohort for an extended time. But this method can demand a very long time and requires ample human and technical resources. In this project, we examine an alternative approach to estimate the duration of immunity. This is grounded on the condition that the dynamics of an epidemic where recovered patients become immune for any period should differ significantly from those of one where the recovered promptly become susceptible. We exploit this difference to provide a reliable protocol that can estimate immunity early in an epidemic. We examine this protocol with synthetic data to then apply it to evaluate human immunity to SARS-CoV-2 in mortality data series from New York City. Our results indicate that New York's mortality figures are incompatible with immunity lasting anything below 105 or above 211 days (90% CI.) and set an example on how to assess immune memory in emerging pandemics before serological studies can be deployed. Therefore, we demonstrate that epidemiological models together with state-of-the-art numerical methods are complementary to traditional approaches in providing estimates of the duration of immunity during the COVID-19 pandemic after only four months since the declaration of the pandemic.



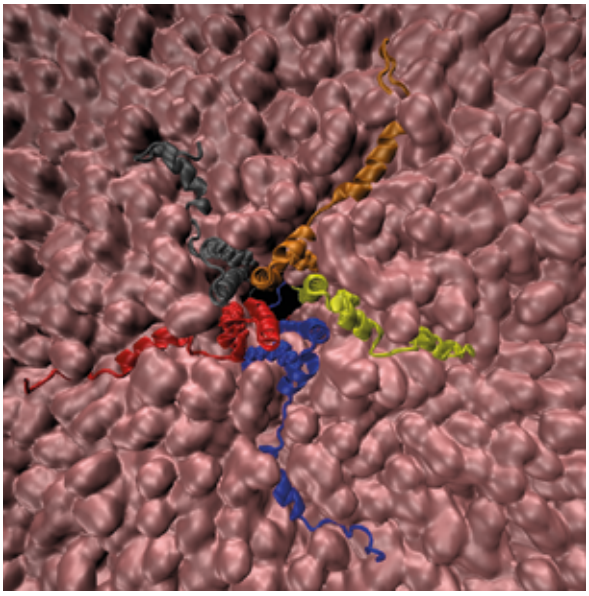
(A) Data (red dots) and algorithm estimate (blue solid, median and 95% CI) of New York City's daily deceases of COVID-19. Data and prediction are in good agreement (ρ => 0.99). (B) Estimate of the infection rate, β, dynamics (median and 95% CI). Drops in β are well aligned with the days on which social distancing measures took place: school closings (black dashed) and the pause order (black dotted). (C) Estimate of the immune memory duration τ (median and 95% CI). The distribution of τ becomes significantly different from that of a control variable δ (two-sample Kolmogorov-Smirnov test p= 0.017) and sets the lower and upper bounds to $\tau \in [80, 288]$ days with (95% CI).

COMPUTATIONAL MODELS

Computer models for the design of therapeutic interventions against SARS-CoV-2

PRINCIPAL INVESTIGATORS
José Ramón Valverde, Luis Enjuanes, J. Manuel Honrubia

This project applies computational methods to several key aspects of SARS-CoV-2 research, including the modelling of mechanisms by which the virus could trigger the different symptoms of COVID-19, the analysis of antibody interaction with SARS-CoV-2 for the design of neutralising antibodies, as well as *in silico* studies of the dynamics of interaction between surface proteins of the virus and its receptor in order to generate a humanised mouse model for pre-clinical studies.



Representation of SARS-CoV-2 E protein pore

Scientific publications

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Patents

In the context of COVID-19 pandemic, there has been an important increment in the number of patents filed by CNB researchers. More information on the knowledge transfer outcome can be found in the Innovation section of this Report.

CSIC Press Conference held on the 7-7-2020 to announce the development of the serological diagnostic kit. Rosa Menéndez, CSIC President (right) and Mar Valés (left), CNB-CSIC researcher.

Communications to Society

During the pandemic, interest in keeping abreast of scientific developments and understanding the fundamentals of virology and immunology has grown among the general public. CNB research projects have been in the spotlight in national and international media and there have been more than 1,200 appearances in the media related to COVID-19, with interviews and reports featuring the progress of the CNB researchers.

Revista Abogacía Española, diciembre 2020

XL Semanal 18-10-2020

XL Semanal 12-5-2020

El País, 27-1-2020

ABC, 10-5-2020

EuropaPress, 4-5-2020

SINC, 19-5-2020

El País, 29-5-2020

TVE 7-7-2020

El Correo 1-12-2020

EFE 4-6-2020

SINC, 21-12-2020

La Vanguardia, 22-10-2020

COVID-19 funding and sponsorship acknowledgements

CNB COVID-19 research projects have received funding in competitive calls from the European Union, the Spanish Government or private institutions. In addition, the unprecedent social impact of COVID-19 pandemic has motivated private companies, citizens' associations, groups and anonymous individuals to sponsor the research projects carried out in our institute. We would like to express our gratitude to all of them for their support to our research.

European Commission

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