ESRF BAG report MX2369

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Proposal Reference Number	MX2369	**Date of Report**	February 25th 2022
Sessions Reported	MX2369		
Number of EMDB/PDB submissions since last report			
Number of publications since last report			

Use of beam time

This report covers the period since last report on February 2021 until now. This includes six sessions from MX2263 not reported in the previous document and 5 sessions from MX2269. The last three sessions scheduled for MX2269 (February/March 2022) will be reported in the next period as they are too recent or in the future.

Session	Beam-line	Date	User
MX2369-1	CM01	6 - 8 September 2021	Jose María Casasnovas
MX2369-2	CM01	29 - 1 October 2021	Jose María Casasnovas
MX2369-3	CM01	22 - 24 October 2021	Javier García Nafría
MX2369-4	CM01	03 - 05 December 2021	Jose Llácer
MX2369-5	CM01	24 - 26 January 2022	Nuria Verdaguer

Beam-time reports

MX2369-1 - *Structure of the SARS-CoV-2 envelope spike (S) protein in complex with a neutralizing nanobody (1.26)* (Jose María Casasnovas)

Session	Date	Micrographs	Ptcls	Fractions	Å/px	Images/hole
MX2269-1	6 - 8 September 2021	14000		40	0.42	1

Particle reconstructions was carried out throughout an INSTRUCT project 16168 in the Electron Microscopy Image Processing, I2PC, Madrid, which resulted in an EM map of the trimeric S with the bound Nb. We used Scipion 2.0 in order to easily combine different software suites in the analysis workflows of CryoEM data: Movie frames were aligned using MotionCor2; the contrast transfer function (CTF) of the micrographs was estimated using CTFFIND4; particles were automatically selected from the micrographs using autopicking from Gautomatch. Evaluation of the quality of particles and selection after 2D classifications, the initial volume for 3D image processing, the 3D-classification and the final refinement were calculated using cryoSPARC, whereas the sharpening was estimated by DeepEMhancer.

The final map showed a trimeric S with one receptor binding domain (RBD) in the closed conformation and two monomers with the RBD open. Local map resolution showed the S2 portion better defined than the S1 region and its RBD. Some density corresponding to the Nb was observed at the RBD top, and indicated that the Nb recognized the receptor-binding motif. Nonetheless, the inherent flexibility of the RBD and its poor EM density did not allow Nb fitting into the map. The relatively low number of lateral particle views collected limited the map resolution.

MX2369-2 - *Structure of the Makona V82 Ebola virus glycoprotein responsible of the virus transmission during the 2013-2016 epidemic.* (Jose María Casasnovas)

Session	Date	Micrographs	Ptcls	Fractions	Å/px	Images/hole
MX2369-2	29 - 1 October 2021	15177		45	0.839	1

We collected a dataset of the Ebola virus GP V82 sample. From this data collection we obtained more than 15000 Micrographs. 13744 were of good quality. After several cycles of 2d class averaging we keep a bit less than one million. This data set is still in the state of processing.

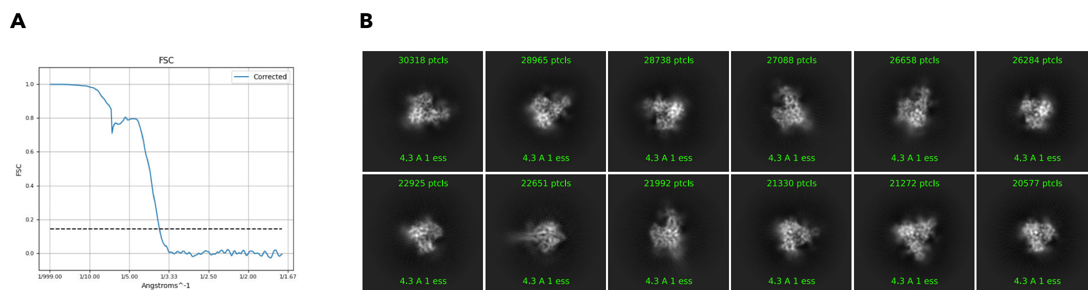

Fig. 7. MX2369-1/2. (A) Fourier Shell correlation (FSC) in the S-Nb particle reconstructions (MX2369-1). FSC versus resolution (1/Å), and resolution determination (~3.5Å) at the 0.143 value for the S-Nb complex reconstructions. **(B)** 2D class averages from MX2369-2.

MX2369-3 - *Dopamine D3 Receptor* (Javier García Nafría)

Session	Date	Micrographs	Ptcls	Fractions	Å/px	Images/hole
MX2369-3	22-24/10/2021	14741	1592845	50	0.839	2

With >800 genes, G protein-coupled receptors (GPCRs) represent the largest family of receptors in the human body. They also represent the target for ~34% of approved FDA drugs. Drug promiscuity occurs when a drug binds additional targets apart from the one that is designed to modulate, with the usual consequence being undesired side effects. Drug promiscuity is a severe problem when targeting aminergic GPCRs (which include muscarinic, adrenergic, dopaminergic, histaminergic, serotonergic and trace amine receptors) since there is high sequence similarity at the orthosteric pocket, especially between close-homologues. Since aminergic GPCRs are the target for ~25% of the drugs in the clinic, improving drug specificity has the potential to improve treatments for a wide variety of diseases. One of the strategies proposed to achieve subtype-selectivity are bitopic ligands, which result from chemically linking a drug that binds to the orthosteric pocket and a drug that binds to a secondary pocket (or allosteric site) that is divergent in sequence between receptor subtypes. Although bitopic ligands present improved selectivity profiles, they are poorly available due to the lack of structural information that can accelerate rational design. On this project we used a bitopic ligand with specificity for the human dopamine D3 receptor to obtain a high-resolution structure of the D3 receptor bound to a G protein trimer and a bitopic ligand.

The sample containing the receptor and three G proteins together with the bitopic ligand showed high biochemical stability and homogeneity, and previous data collections had achieved 4.7 Å resolution. The sample collected on this session at ESRF had further improvements including the addition of an scFv molecule to lock previously observed intra-molecular flexibility. After obtaining >14.000 movies we obtained a 3.8 Å resolution map, however the ligand had unusually por density for the achieved resolution and quality in the rest of the map (Fig. 8). We are trying to understand whether this might be due to the nature of the ligand which can bind independently on two sites. The dataset has yielded a "side-chain resolution" structure and it is useful in the progress of the project. Further experiments will be carried out and additional data collections with other bitopic ligands (readily available to us) will inform on the properties of these ligands.

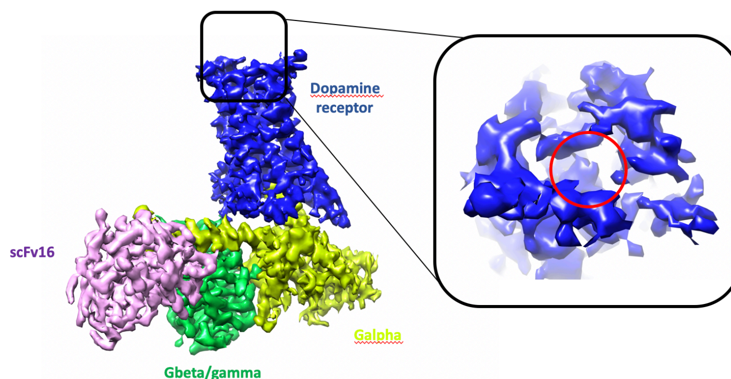

Fig. 8. MX2369-3. (A) Cryo-EM map of the dopamine 3 receptor in complex with G proteins and bound to a bitopic ligand (left) and inset into the ligand binding site where there is poor ligand density (almost absent) (red circle) (right).

MX2369-4 - *CryoEM grids of SARS-CoV-2 Spike in complex with a nanobody and an antiviral compound (S-dp-N)* (José Luis Llácer)

Session	Date	Micrographs	Ptcls	Fractions	Å/px	Images/hole
MX2369-4	03-05/10/2021	15,458	656,542	40	1.05	3

The protein S (spike) of SARS-CoV-2 is responsible of the entry of the virus into human cells by binding to the angiotensin-converting enzyme 2 (ACE2) receptor. Hence the spike protein is the main target for development of vaccines and antiviral therapies. Here we try to address the structural mechanism behind the synergic neutralizing action of nanobodies and certain heparin derivatives by obtaining the cryoEM structure of the protein S (D614G variant) in complex with a nanobody and an heparin derivative. Our preliminary data shows that the present nanobody binds to the RBD of the spike with a K_D of ~300 nM, but in the presence of the heparin derivative under study that K_D value is better than ~100 nM.

The complex was prepared using molar excess of 2 and 5 times over the protein S for the nanobody and the heparin derivative, respectively. The spike protein was at around 0.5 mg/ml. CryoEM grids were prepared in a Leica GP2 EM cryoplunge and screened in a FEI Talos Microscope at the CNB-CSIC (Spain). Best grids were selected for high-resolution data collection at ESRF CM01 beamline (Grenoble). More than 15,000 images were collected and after several rounds of 2D-classification, we selected ~655.000 particles (Fig.9). These 2D-classes of the spike show an even distribution on different orientations, and therefore we expect to have high-resolution 3D structures of the spike protein. A preliminary 3D-classification (using C1 symmetry) has already shown some particles containing extra densities non-attributable to the spike protein that may correspond to 1 molecule of nanobody plus 1 molecule of antiviral bound to a trimer of protein S. We are currently performing different 3D-classification approaches in order to isolate all the particles of protein S trimers showing distinct densities for both the nanobody and the antiviral compound.

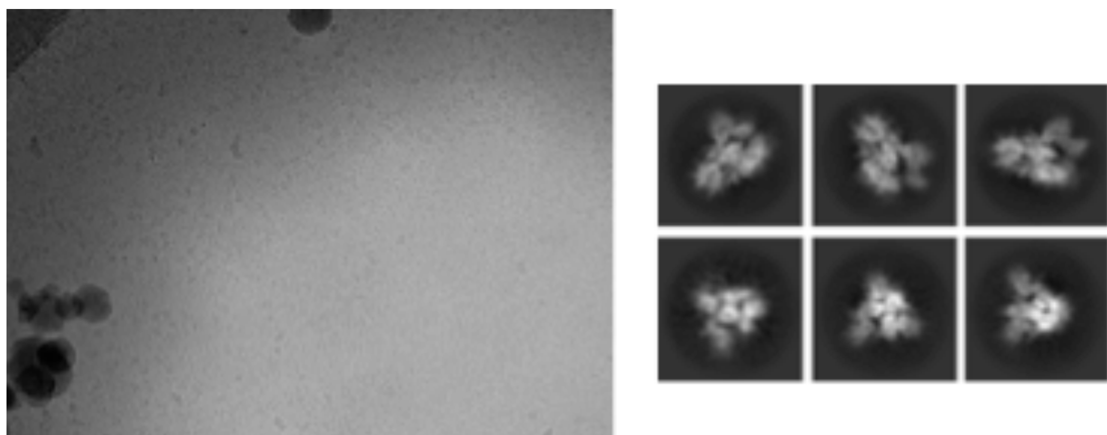

Fig. 9. MX2369-4. (A) Typical micrograph. **(B)**, Representative 2-D classes after 2-D classification of SARS-CoV-2 complexed to a nanobody and an antiviral compound.

MX2369-5 - Deciphering MVP monomers distribution in *Dictyostelium discoideum* vaults assembly (Núria Verdaguer Massana)

Session	Date	Micrographs	Ptcls	Fractions	Å/px	Images/hole
MX2369-5	24-26/01/2021	15,150	132,765	40	1.06	2

With our previous data collection of D. discoideum vaults, we obtained a map of the vault particle of 3.7 Å resolution usign D39 symmetry processing in Relion 3.0. We were able to trace the main chain of the MVP monomers and side chains. Unfortunately, we were not able to distinguish between the two MVP monomers that form this particle. For this dataset, we have fused a peptide that is recognized by a commercial nanobody to one of the MVP monomers.

We have recently collected 15,150 movies of recombinant vaults from *Dictyostelium discoideum* in complex with a nanobody in gold grids using the FEI Titan Krios electron microscope in super resolution counting mode with EPU at x81000 (pixel size of 1.06 Å/pixel). We used an electron dose rate of 20.0 electrons/pixel/second and each movie contains 40 frames recorded in 2.2 seconds.

The movies collected show always regions with several particles. However, the particle itself presents heterogeneity due to the presence of half- and full-particles or opening intermediates in the same area (Fig. 10). The quality of the images is considerably high, reaching the 2.7Å resolution in the CTF estimation. With this data collection, we think we have enough movies to decipher the complex nanobody-vault particle structure. Currently, we are on the first steps of the data processing. Once we obtain the complex nanobody- vault particle structure, we will be able to identify the distribution of the monomers in the assembly of the vault particle.

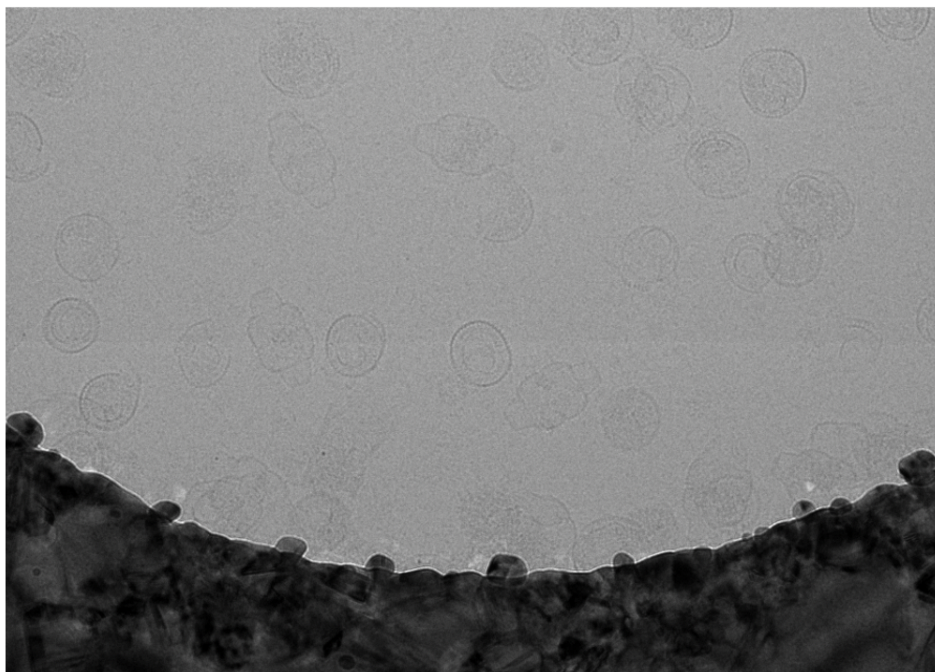

Fig. 10. MX2263-5. (A) Representative aligned micrograph of the dataset.