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Abstract

Comunicaciones Orales y Pósters

Página	Pósters
127	Discovery of novel Pikfyve inhibitors as potential antiviral agents #61
128	New thiophenederivates as potential drugs against ebola virus #62
129	CryoEM structures of the SARS-CoV-2 spike bound to antivirals #63
130	Proteolytic chimeras targeting purine biosynthesis: at the crossroads of antiproliferative and antiviral effects #64
131	Scipion-Chem: a traversal tool for the development of antiviral drugs #65
132	Small-Molecule Inhibitors against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) #66
133	Discovery and characterization of broad-spectrum antivirals against RNA viruses with dual mode of action against SARS-CoV-2 #67
134	Photopolymerizable Glyconanomicelles as Inhibitors of SARS-CoV- 2 Entry #68
135	Characterization of the in vitro polymerization activities of different SARS-CoV-2 nsp12 variants from patients' Isolates #69
136	Drug combination studies of cell-targeted antivirals against SARS- CoV-2 #70
137	Broad-range antiviral activity against coronaviruses exerted by non-coding RNAs derived from a viral genome #71
138	In vivo experimental platform for the evaluation of the efficacy of novel therapies against SARS-CoV-2 #72
139	SARS-CoV-2 pathogenesis mediated by E protein PBM was prevented by modulators of CFTR function #73
140	Genomics and NGS (GENGS) Core Facility at the CBM Severo Ochoa, and its role with the PTI+ Global Health #74
141	Micro Electron Diffraction CSIC Facility: New tools for structure determination #75
142	Tryptophan trimers efficiently inhibit SARS-CoV-2 entry into the host cells #76
143	Synergistic inhibitory effect of remdesivir and ribavirin against SARS-CoV-2 and human coronavirus 229E #77
144	Natural products as antivirals. High-throughput screening of the MEDINA collection of microbial natural products. #78
145	Pharmacological modulation of the interaction between tubulin and a structural protein of SARS-CoV-2 #79
146	Use of an interactomics pipeline to assess the potential of new antivirals against SARS-CoV-2. #80

CryoEM structures of the SARS-CoV-2 spike bound to antivirals

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Background: Single-particle cryoelectron microscopy (cryoEM) has played a key role in the fight against COVID-19. The molecular mechanisms for the action of some of the currently approved drugs targeting the SARS-CoV-2 RNA-dependent RNA polymerase, the fast developments of the current available vaccines and antibody therapies are examples of the impact of the knowledge gained from the cryoEM structures of SARS-CoV-2 proteins in complex with proteins (ACE2 or antibodies/nanobodies) or small compounds. Our aim is to use this technology to understand structurally how certain antiviral compounds and proteins targeting the spike may inhibit viral entry.

Methods: 1) Production of wild-type and mutated spike and ACE2 proteins using baculovirus/insect cells. 2) Spike binding kinetics: protein-protein and protein-small compound interactions measured by BLI Biolayer interferometry (BLI) and/or microscale Thermophoresis (MST). 3) Buffer optimization for cryoEM grid preparation of spike variants by thermal shift assays and negative-staining electron microscopy (NSEM). These techniques are also used to adjust the molar ratio of spike:ACE2 and spike:small-compound complexes. 4) Structural characterization by cryoEM.

Results: At IBV-CSIC we have created a pipeline for the production and characterization of several spike variants and ACE2 decoys. While this pipeline is described in detail in other oral/poster communications, this communication is centered around one of the pillars within this pipeline; the structural characterization of possible drug candidates bound to the SARS-CoV-2 spike by cryoEM. In this way, we have successfully solved structures of the spike bound to: A) protein inhibitors as ACE2 decoys; B) a small inhibitory compound; C) mixtures of proteins and small-compound (nanobody-heparan derivative) working cooperatively as inhibitors. These protein/drug candidates were previously selected based on the results obtained in our interactomics platform, whereas their concentration and the buffer conditions for cryoEM grids preparation were established based on thermal shift assays and NSEM.

Conclusion: CryoEM is a powerful tool to directly visualize the effect caused by a potential drug on a protein target. In a short period of time we have developed this technique in our institute to be applied to the SARS-CoV-2 spike protein, not only to obtain high-resolution structures of SARS- CoV-2 spike variants of concern (see WP4) but also to obtain the structures of complexes of the spike with various inhibitory compounds of very different nature.

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