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Abstract

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Use of an interactomics pipeline to assess the potential of new antivirals against SARS-CoV-2.

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Background: In late 2019 SARS-CoV-2 infection appeared in China, becoming a pandemic in 2020. The scientific community reacted rapidly, characterizing the viral genome and its encoded proteins, aiming at interfering with viral spreading with vaccines and antivirals. The receptor binding domain (RBD) of the viral spike (S) protein plays a key role in cell entry of the virus. It interacts with the cellular receptor for SARS-CoV-2, the membrane-bound human Angiotensin Converting Ectoenzyme 2 (ACE2). With the goal of monitoring interference with this interaction by potential antiviral drugs, we have set up at the Institute for Biomedicine of Valencia (IBV-CSIC) an interactomics pipeline targeting the initial step of viral entry.

Methods: For the production part of the pipeline (pure RBD/Spike variants and soluble ACE2), see parallel poster. These proteins allowed monitoring of the RBD/Spike-ACE2 interaction in presence or absence of potential inhibitors. Thermal shift assays (thermofluor) were used for initial detection of compound binding at different ligand/protein ratios and media conditions (pH, buffers, chaotropic agents). Next, binding affinity and on/off kinetics were characterized using Biolayer interferometry (BLI), Surface plasmon resonance (SPR), Microscale Thermophoresis (MST) and/or Isothermal titration calorimetry (ITC). For protein-protein interactions, we mostly used BLI or SPR, whereas for protein-small compound analysis MST was generally best. Protein aggregation-dissociation was monitored by size exclusion chromatography with multiangle light scattering (SEC-MALS).

Results: Candidates proven by thermal shift assays to bind to RBD/spike protein without affecting the integrity of these proteins were subjected to quantitative affinity measurements. We successfully demonstrated that BLI, SPR and MST can be used to follow the interactions between SARS-CoV- 2 proteins and the putative drug candidates, as well as to monitor the interference with Spike-Ace2 binding of potential drug candidates. While BLI and SPR displayed reproducible results in the measurement of protein-protein interaction (applied to soluble ACE2 used as a decoy), they were less suitable for measuring the binding of small molecules. The fact that most small compounds were only soluble in organic solvents made difficult to obtain a low signal/noise while using BLI, necessary for the assessment of the binding. We overcame that problem by using MST. After dilution of the compounds to the final experimental concentrations, the technique could detect a significant binding signal enough to calculate binding parameters. MST also allowed to measure the degree of interference that each compound was having on RBD/Spike-ACE2 interaction. The pipeline has been customized and validated with compounds of very different nature provided by different groups belonging to the PTI and other external laboratories, as well as with different Ace2 decoys designed at the IBV.

Conclusions: The interactomics platform at the IBV has been used to successfully develop two different antiviral approaches in order to fight COVID-19. It has allowed technical specialization of the staff as well as the development, in a very short period of time, of two ambitious projects. We have demonstrated that we can perform interactomic characterization for challenging projects as well as provide information about binding of antivirals to potential new SARS-CoV-2 variants of concern.

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