LABEL-FREE KINETIC MEASUREMENTS OF THE INTERACTION OF COVID-19 VIRAL S1 PROTEIN WITH CELLULAR AND RECOMBINANT ACE2 RECEPTORS

The pandemic due to the SARS-CoV-2 continues to evolve and remains an international concern. Covid infection is caused by the air-borne coronavirus 2 (SARS-CoV-2) and its mutants. The viral invasion of the host cells is mainly through the viral attachment to angiotensin (Ang)-converting enzyme 2 (ACE2) present in the epithelial cells of the host[1]. During a viral infection, the trimeric S protein is cleaved into S1 and S2 subunits, where the S1 subunit contains the receptor binding domain (RBD), which directly binds to the peptidase domain of ACE2[2]. Therefore, a better understanding of the binding interaction between S1 and ACE2 is key for the development of antiviral drugs.

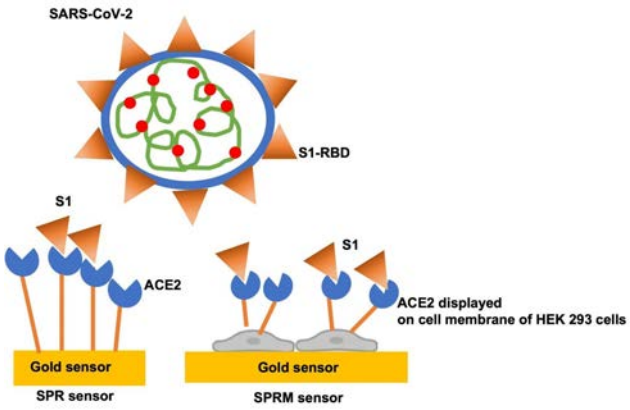

Figure 1: Schematic of ACE2-S1 interaction studies using SPR and SPRM

Kinetic interaction studies with membrane proteins using SPR typically require their isolation and purification from the host cell environment. In our prior works, isolated human ACE2 with a His-tag and an Avi-tag were immobilized for kinetic binding interaction studies with SARS-CoV-2 S1 protein. Both kinetic titration injection assay[3] and a quicker single injection assay[4] were implemented, using a five channel BI-4500A SPR system with the BI-DirectFlow™ technology to measure the binding kinetics of the interaction. The results were in good agreement with published literature.

S1 to ACE2 interaction	*k*a [1/M*s]	*k*d [1/s]	*K*D [nM]
Recombinant ACE2 Single-injection Technique	1.16×10^{5}	1.27×10^{-4}	1.10
Recombinant ACE2 Kinetic-Titration Technique	1.85×10^{5}	2.15×10^{-4}	1.10
Native ACE2 in HEK cell Kinetic-Titration Technique	5.46×10^{4}	2.43×10^{-4}	5.00

Table 1: Kinetic parameters measured for S1 and ACE2 binding.

However, the conformation of an extracted or recombinant receptor might be different than that of the same receptor in the membrane milieu. These alterations can influence the natural function and binding behavior of the receptors[5,6]. Therefore, measurements of the binding affinity and kinetic parameters of membrane proteins in their natural environment may be more accurate and biologically relevant for drug development and testing.

Surface plasmon resonance microscopy (SPRM) integrates label-free, high-resolution SPR binding kinetic measurements with bright-field optical microscopy for the study of biomolecular interactions on whole cells, including characterization of the binding heterogeneity of cell populations since the binding responses of each cell is individually observed.

The interaction of the S1 spike protein to the ACE2 receptor on HEK 293 cells was measured using the SPRm 200 instrument. To do this, HEK 293 cells were seeded and allowed to adhere to the sensor surface. After cell fixation, serial injections of six S1 spike protein solutions (12.00, 19.25, 38.50, 77.00, 154.00, and 192.50 nM) were exposed to the cells. The bright-field image in Figure 2a corresponds with the SPR image in Figure 2b.

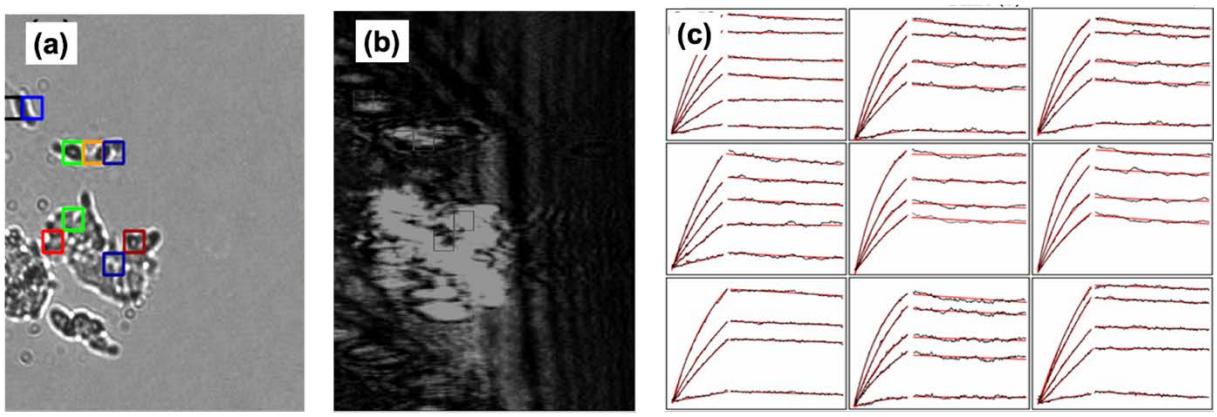

Figure 2: Kinetic interaction analysis was performed on HEK cells using SPRM: (a) bright-field image and (b) corresponding SPR image of the HEK cells with overlaid ROI regions (square outline), and (c) binding responses from each ROI (data shown in black). Each sensorgram was fitted to a 1:1 kinetic binding model with results shown in red.

For kinetic analysis, a virtual grid containing regions of interest (ROIs) was applied to the surface. ROIs defined by the colored squares in both the bright-field and SPRM images produced the binding responses observed in the sampling of sensorgrams in Figure 2c (response data shown in black). A 1:1 kinetic interaction model was fitted to the binding data for kinetics analysis, producing the simulated sensorgrams that overlay the data shown in red, which closely fit the experimental data shown in black.
The kinetic analysis results from all ROIs were gathered to form histograms of the kinetic parameters shown in Figure 3. A Gaussian distribution was fit to the histograms to determine the mean and 95% confidence interval for each kinetic parameter. From the statistical analysis, the kinetic values for the entire cell population were determined to be ka of 5.46×10^{4} $M^{-1}s^{-1}$, kd of 2.43×10^{-4} s, and KD of 5.00×10^{-9} M (see Table 1).

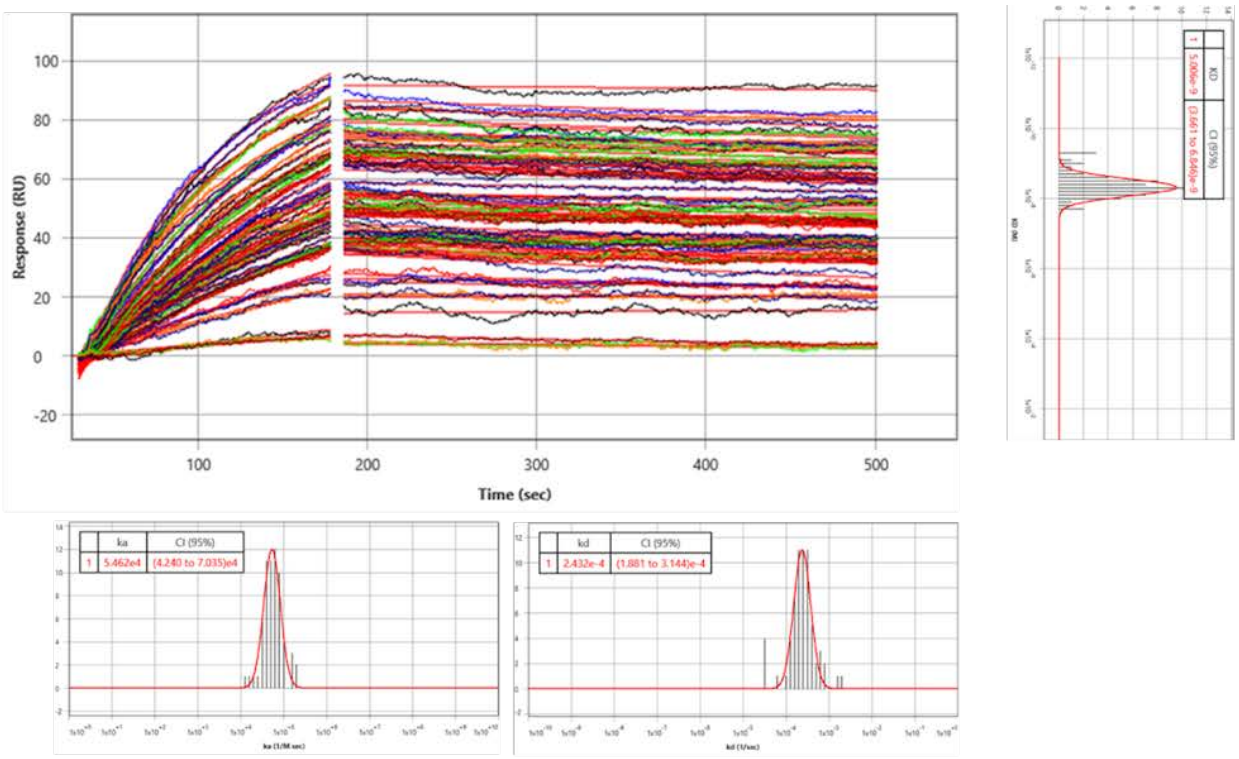

Figure 3 The SPRM kinetic analysis results of all ROIs were aggregated for statistical analysis. Histograms of the kinetic parameters were fitted with Gaussian distributions to extract the mean and 95% confidence interval of the cell population.

As previously described, the kinetic interaction of S1 to the recombinant ACE2 receptor exhibits an affinity of 1.10 nM. In contrast, the native cell-based environment exhibits a weaker 5.0 nM affinity with broader distribution, having a 95%CI of 3.6 to 6.8 nM. Furthermore, we note that the association rate constant appears to be more influenced by the extraction and purification process than the dissociation rate constant. These effects on the kinetic behavior are attributed to the heterogeneity and complexity of the cellular environment. This work highlights the need for cell-based kinetic binding studies for extracting more biologically relevant information.

References:

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