Spike Protein Assay

Biophysical Assay Services

➢ Approach

Two spike protein assays were developed at Reaction Biology based on the surface plasmon resonance (SPR) technology to investigate the binding interaction between the receptor binding domain of the viral Spike protein and ACE2. These assays can be used to measure the direct binding of potential therapeutics to the Spike protein receptor binding domain or ACE2 to determine the kinetics of the interaction or to measure whether the test molecule(s) disrupt the Spike protein/ACE2 interaction.

➢ Spike Protein Assay specifics

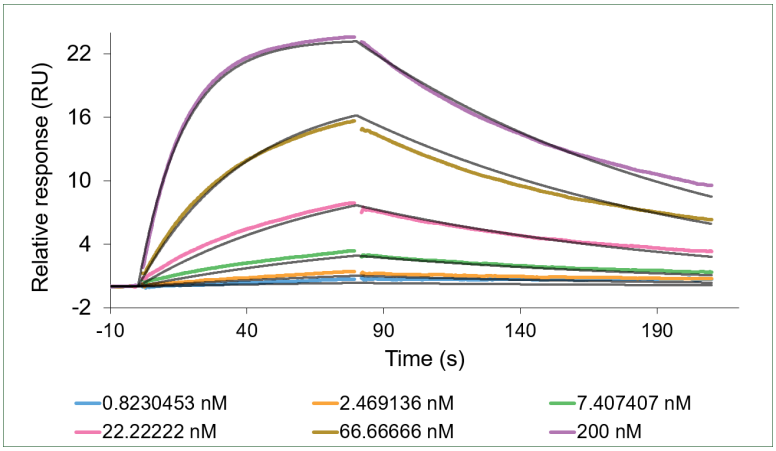
- SPR assays to study the interaction between ACE2 and SARS-CoV-2 Spike protein receptor binding domain
- Host protein: human ACE2 (MET1-SER740); Uniprot entry: Q9BYF1
- Viral protein: Spike glycoprotein receptor binding domain (Arg 319-Phe541); Uniprot entry: P0DTC2

➢ SPR allows evaluation of several parameters

1. Probe the direct binding kinetics (on/off-rate and KD), which can be used to optimize target engagement (on-rate) and residence time on the target (off-rate) to ensure a tight binding interaction.

2. New drug candidates can be screened for their potential to block the interaction of S protein receptor binding domain and ACE2 receptor with a throughput of up to 2,300 compounds per day.

➢ Spike protein binding to ACE2



kon (1/Ms) = 2.65E+05

koff (1/s) = 7.73E-03

KD (M) = 2.91E-08

ACE2 was captured to the sensor chip and soluble Spike protein receptor binding domain was used as the analyte (shown in various concentrations) to validate binding behavior. Using this assay setup, the binding of molecules to ACE2 can be measured to determine the binding kinetics of the molecule/ACE2 interaction and probe whether the molecules interfere with the Spike protein/ACE2 interaction.

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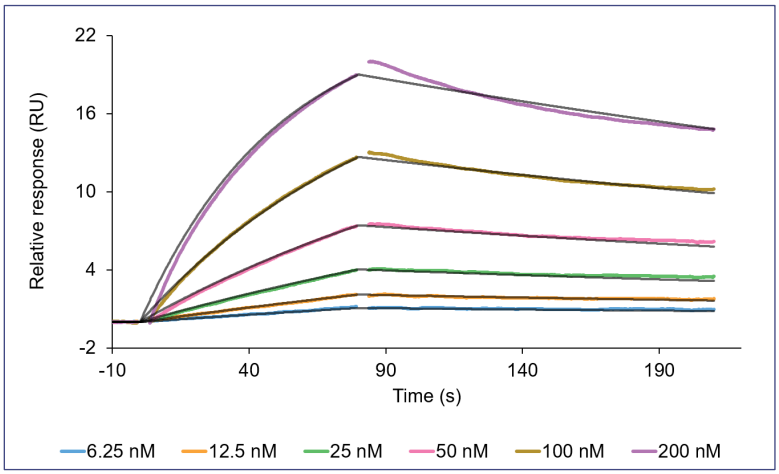
➢ ACE2 binding to Spike protein



kon (1/Ms) = 9.03E+04

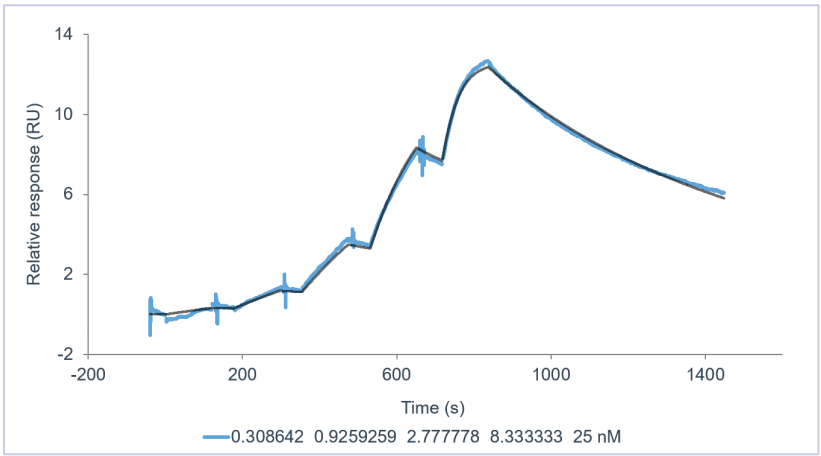
koff (1/s) = 2.50E-03

KD (M) = 2.76E-08

Spike protein receptor binding domain was captured to the sensor chip and soluble ACE2 was used as the analyte (shown in various concentrations) to validate the binding behavior. Using this assay setup, the binding of molecules to the receptor binding domain of the S protein can be measured to determine the binding kinetics of the interaction and probe whether the molecules interfere with the Spike protein / ACE2 interaction.

The data were generated with a Biacore 8K instrument and show similar binding affinity for the binding event in both directions, binding of Spike protein to ACE2 and binding of ACE2 to Spike protein. The on-rate is slightly faster for Spike protein (analyte) binding to ACE2 (target), while the off-rate is slightly slower for ACE2 (analyte) binding to Spike protein (target).

➢ ACE2 Inhibitor MLN-4760 Binding to ACE2



kon (1/Ms) = 1.13E+06

koff (1/s) = 1.51E-03

KD (M) = 1.34E-09

In order to directly probe the binding kinetics of small molecules to ACE2 relatively high levels of target on the sensor chip are needed (due to the large difference in mass between these molecules and the protein target). To achieve these higher levels, ACE2 was covalently attached to the sensor chip via amine coupling. To validate that the target is binding competent once immobilized to the sensor chip, the binding of ACE2 inhibitor MLN-4760 was measured using a kinetic titration (single-cycle kinetics). The KD value of ~1nM is consistent with the IC50 value of 440pM.