

Positive and Negative Control Studies of Small Molecule Binding to Membrane Protein

SPR microscopy (SPRm) has emerged as a unique tool for measuring the affinity and kinetics of ligand binding to membrane proteins on the cells directly. The technology integrates the traditional SPR and optical microscopy into one platform, which resolves individual cells spatially and quantity ligand binding to each of them in real time and without labels.

In this application note, we present small molecule compounds binding to a membrane protein overexpressed on human embryonic kidney cells, HEK 293. 60k cells overexpressed with a target receptor were seeded on the SPRm sensor chip and grown using standard protocols. A negative control sensor chip was also prepared by seeding 60k not-overexpressed HEK cells on another SPRm sensor chip. 7 dilutions of two small molecule compounds (eg SM1 & SM2) were prepared from 20 μM in standard buffer (x3 dilutions) and injected into the system using the kinetic titration method. The experiments were performed using a buffer containing 10 mM PBS, pH 7.4, 0.1% BSA and 0.02% DMSO.

In the first run, compounds, SM1 and SM2, to the cells were sequentially injected into the system and the SPR signal of each cell to the compounds was measured. For SM1, the affinity (K_D) and binding kinetic constants (k_a and k_d) were determined to be $K_D = 865$ nM (372 – 2009 95% CI), $k_a = 2.0E4$ (95%CI: 0.9 to 4.5) and $k_d = 1.6E-2$ (95%CI: 1.3 to 2.0), respectively. These affinity and kinetic constants are consistent with functional assay results. SM2, a negative control compound, did not generate a SPR signal.

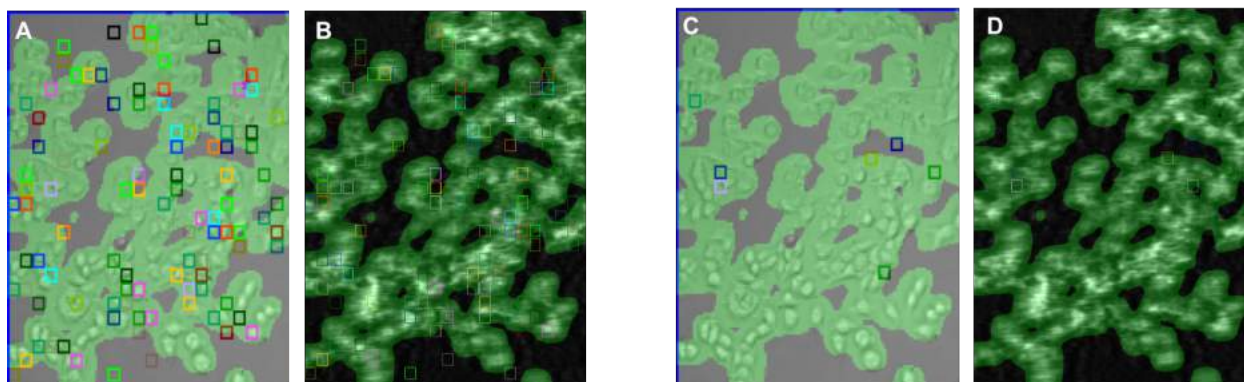


Fig 1. Bright field (A, C) and SPR (B, D) images of HEK 293 cells binding with small molecules. Figs A and B are results of SM1 binding to the cell membrane receptors. Figs. C and D are results of SM2, a negative control that is not expected to bind to the targeted receptor.

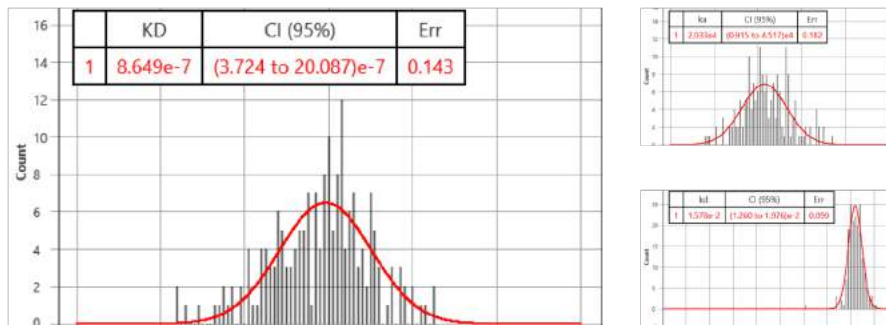


Fig 2. KD, ka, kd histograms for SM1

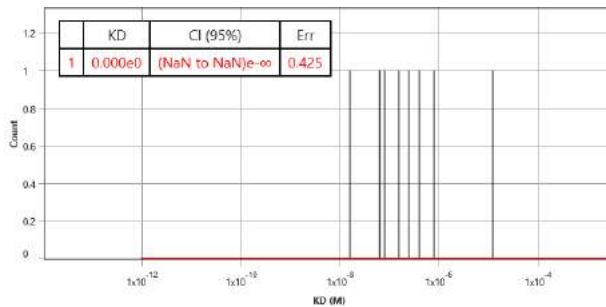


Fig 3. KD histogram for SM2, a negative control molecule. A few sites on the sensor surface have SPR signals, which is due to a small amount of NSA.

As a further control, binding of SM1 and SM2 to HEK cells that do not have the overexpression of the receptors. None of the compounds showed any SPR binding signals, which confirms the specific binding of SM1 to the target membrane receptor.

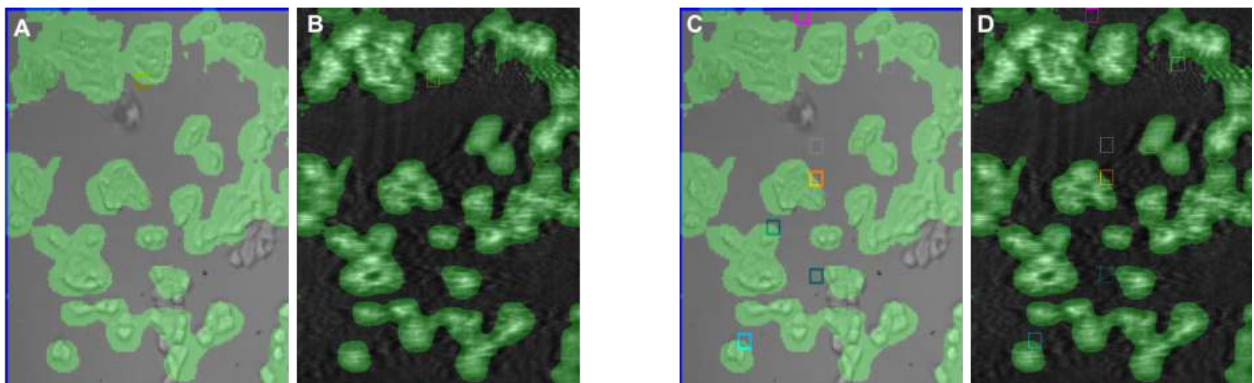


Fig 4. Bright field (A, C) and SPR (B, D) images of SM1 and SM2 on negative control HEK cell chip

SPRm 200 is a powerful system that measures binding of small molecule to GPCRs directly on single cells without the need of extracting and purifying these receptors. The system provides real time biophysical analysis of binding events in a physiological setting.

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