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Avidity vs Affinity: A Common Biosensor Problem or an Advantageous Biological Mechanism?

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Introduction

Pharmacologists traditionally model receptor-ligand interaction as single-site 1:1 binding. When multiple receptors are tethered close together in a cell membrane (or on a biosensor surface) and the ligand is multimeric and/or tethered on the surface of a particle (e.g. a virus), multiple linked binding interactions occur. Ligand dissociation is then dependent on probabilistic simultaneous dissociation of all binding interactions, resulting in apparently very high affinities - an effect called avidity. Bivalent antibodies have evolved to exploit avidity to remain tightly bound to their antigens. We demonstrate avidity for trimeric SARS-CoV2 spike protein binding to human lung cell ACE2 protein using two biosensor approaches: Surface Plasmon Resonance (SPR) and Bio-Layer Interferometry (BLI). We also show methods to avoid avidity. We then use the avidity of anti-spike antibody binding to spike trimer to measure antibody concentration in plasma samples.

Methods

- SPR was conducted on a Biacore T200; BLI was on a Sartorius Octet R8.
- Fc-ACE2, his-spike trimer and his-spike RBD (Ba.2/Omicron strain) were immobilised onto CM5 SPR sensors using amine coupling chemistry.
- Biotin-ACE2, his-spike trimer and his-spike RBD were captured onto SAX2, HISK1 and NiNTA BLI biosensors respectively.
- Spike trimer, spike RBD monomer or Fc-ACE2, over a range of concentrations up to 50 nM, were bound to immobilised ACE2 or spike proteins. The bound ligands were then dissociated in buffer alone.
- Binding responses were globally fitted to a single-site binding model to determine association-rate (k_a), dissociation rate (k_d) and affinity (K_D).

Results

Spike RBD monomer binding to high density ACE2 – Affinity only

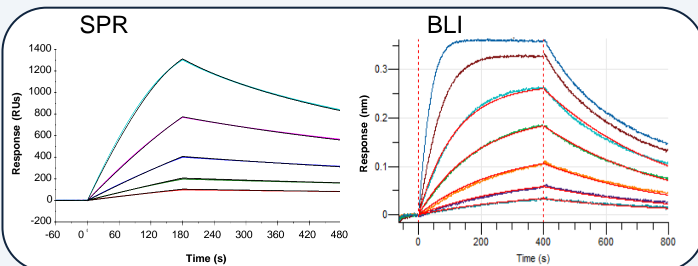

Figure 1 Spike RBD binding gave kinetic affinity constants (K_D) of 6.1 nM (SPR) and 4.8 nM (BLI).

Spike trimer binding to high density ACE2 – Avidity

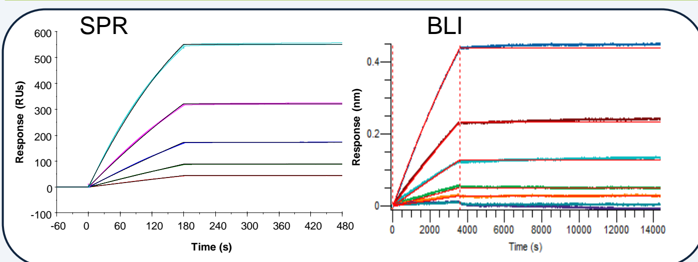

Figure 2. Spike trimer binding showed avidity, with an affinity constant (K_D) of < 1 pM (SPR and BLI).

ACE2 binding to spike trimer – Affinity only

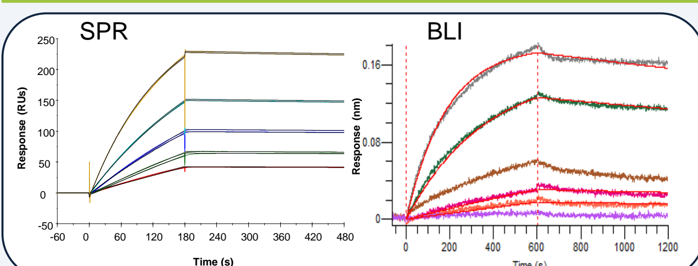

Figure 3 In the reverse orientation, with Spike trimer immobilised on the biosensor and ACE2 binding to it, avidity was avoided. ACE2 single-site binding gave K_D values of 0.3 nM (SPR) and 1.5 nM (BLI).

Spike trimer binding to low density ACE2

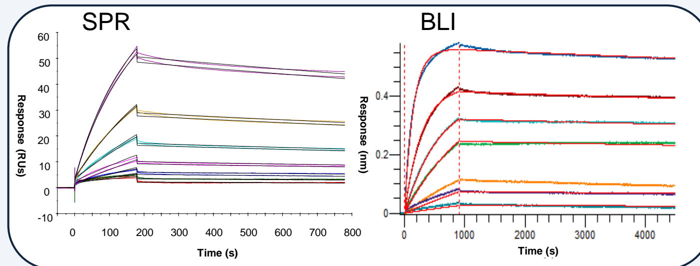

Figure 4 Reducing the density of ACE2 on the sensor surface at least 10-fold avoids spike trimer interacting with multiple ACE2 proteins. Binding interactions become single site and avidity is reduced or avoided, resulting in K_D values of 1.8 nM (SPR) and 0.1 nM (BLI).

Calibration and quantitation of anti-spike Mab in biological samples

- Rabbit anti-spike was diluted 1:1000 to 1.9 ng/mL in HBS-EP ± 1 mg/mL BSA or HBS-EP with 10% foetal calf serum (FCS). Binding to spike RBD used HBS-EP ± BSA or HBS-EP + 10% FCS as the running/wash buffer.

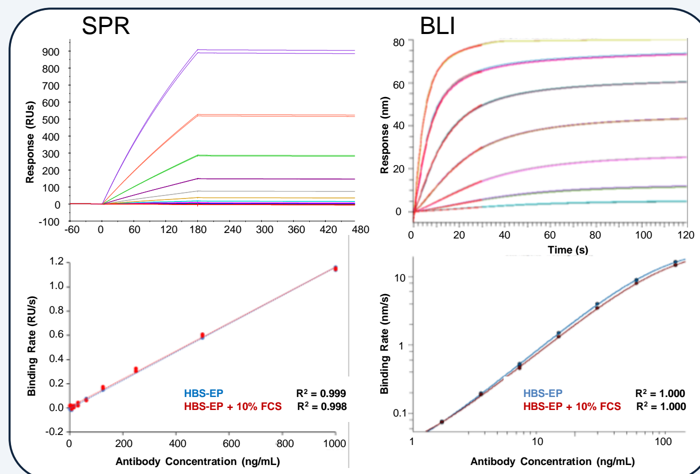

Figure 5 Binding responses of rabbit anti-spike Mab binding to spike RBD in HBS-EP buffer and HBS-EP with 10% serum by direct detection (SPR) or after signal amplification with goat anti-rabbit-HRP conjugate and Ni-DAB precipitation (BLI). Calibration curves were constructed from binding rates over the initial 30 seconds. SPR (without amplification): lower limit of detection (LLD): 3.9 ng/mL; lower limit of quantitation (LLQ): 15.6 ng/mL. BLI with amplification: LLD = 0.1 ng/mL; LLQ = 3 ng/mL.

Conclusions

- High ligand density on biosensor surfaces can allow multivalent binding analytes to form linked interactions with adjacent immobilised proteins, leading to measurement of multi-site avidity, not single-site affinity. We have demonstrated this effect using the interaction between trimeric SARS–CoV2 spike protein and its membrane-anchored target, ACE2.
- The avidity effect of trimeric binding is exploited by the virus to ensure it remains tightly bound to lung cells for long enough to ensure infection.
- Using monovalent binding domains (e.g. spike RBD), or configuring the assay with the multivalent partner (spike trimer) as the immobilised ligand and the monovalent partner (ACE2) as the analyte, helps prevent avidity.
- Immobilising low levels of ligand on the sensor surface also prevents multivalent interactions. This reveals that spike protein has single-site binding affinity at ACE2 of approximately 2 nM.
- We also demonstrate the utility of immobilised spike RBD to determine the titre of anti-COVID-19 antibodies in serum.

Materials

Biotinylated human Angiotensin Converting Enzyme 2 (biotin-ACE2), Fc–tagged ACE2, his-tagged SARS-CoV2 spike trimer (BA.2/Omicron variant) and his–tagged spike RBD (BA.2/Omicron variant) were from Acro Biosystems. Rabbit anti-spike MAb was from Sino Biological. Goat anti-rabbit-HRP conjugate and goat normal serum were from Invitrogen. Ni-enhanced DAB HRP staining kit was from Sigma/Merck. Foetal calf serum was from Gibco.

Acknowledgements

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