Measuring coronavirus Spike:ACE2 binding affinity and kinetics using the Biacore 8K

Raquel Faba-Rodriguez[a], Catherine Geh[a], Liz Flavell[a], Jon Renshaw[a], Claire Shepherd[b], Anette Person[b], Steven P. D. Harborne[a]*, and Mark Abbott[a]

[a]Peak Proteins, Alderley Park, Macclesfield, Cheshire SK10 4TG

[b]Cytiva, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA

* steven.harborne@peakproteins.com

1. Introduction

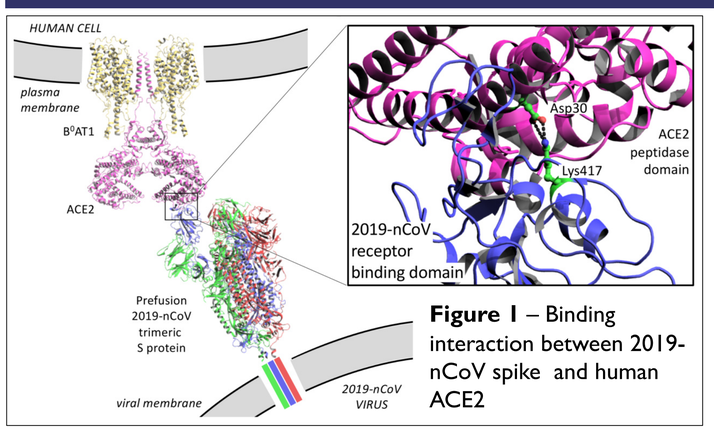

Figure 1 – Binding interaction between 2019-nCoV spike and human ACE2

- Peak Proteins wanted to contribute to the scientific effort tackling the Covid-19 pandemic.
- Customers were requesting purified 2019-nCoV spike and human angiotensin-converting enzyme (ACE2).
- We had access to a Biacore 8K to carry out SPR experiments through the Alderley Park open access lab.

Aims:

- Learn how to use the Biacore 8K, and bring the capability into Peak Proteins.
- Validate the spike and ACE2 constructs we produced were folded, functional and active.
- Measure the affinity and kinetics of the Spike:ACE2 interactions and compare with literature values.
- Choose an assay setup for validating other 2019-nCoV spike:human protein interactions.

2. 2019-nCoV spike constructs

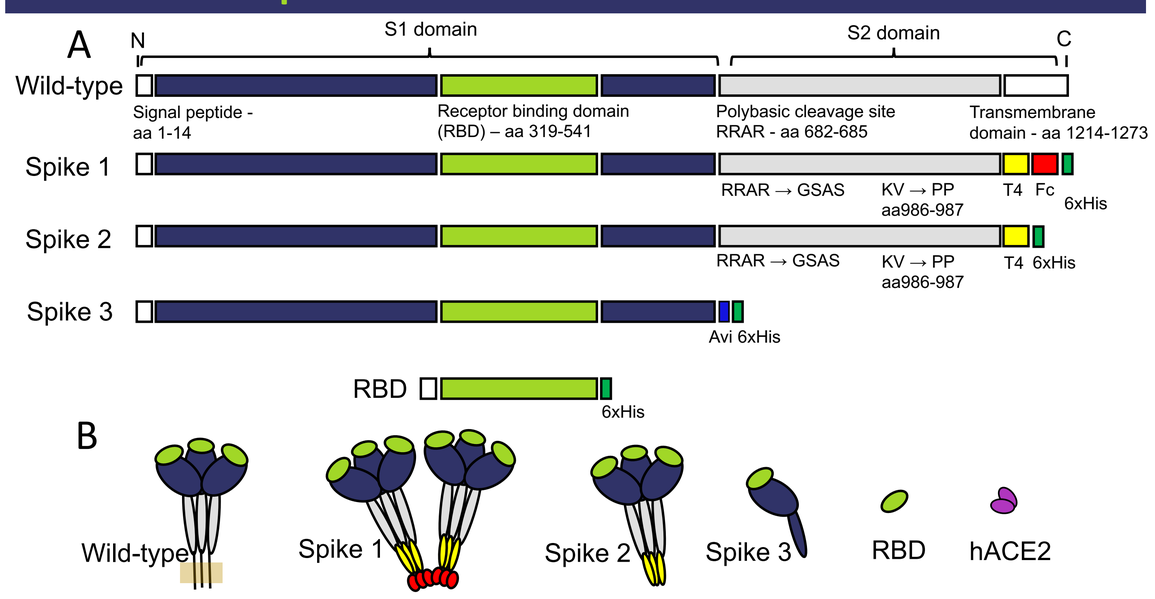

Figure 2 – A) Schematic representation of the spike gene constructs in comparison to wild-type to show key sequence features. B) Cartoon representations of the spike protein constructs. Other constructs: SARS spike construct equivalent to Spike1 and Avi tagged human ACE2 construct (aa19-615).

3. Assay development: Setups that didn't work!

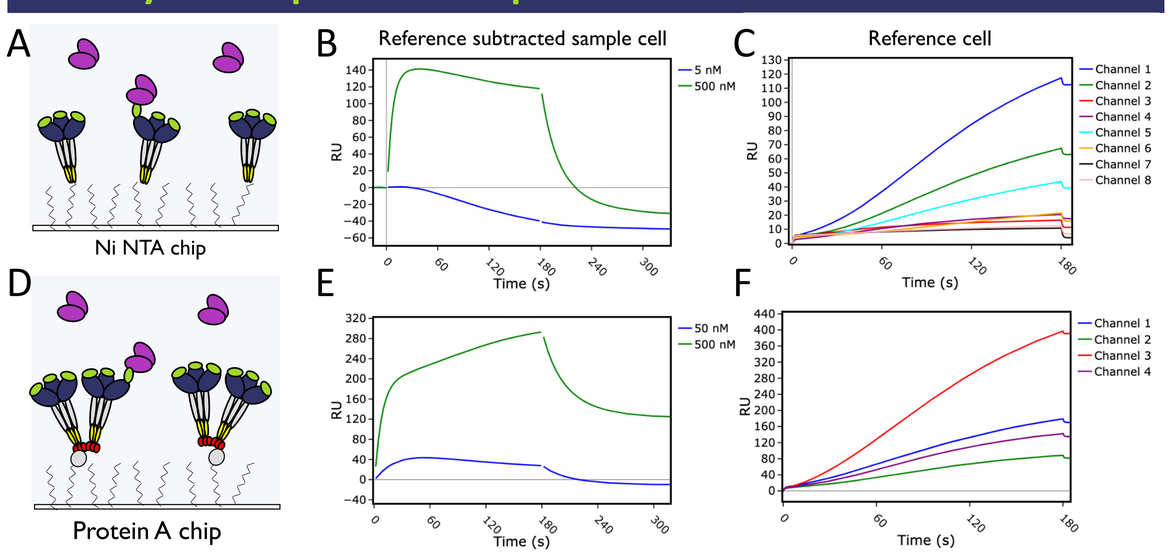

Figure 3 – A, B and C) Spike constructs immobilised onto Ni NTA surface and using ACE2 as analyte at 5 and 500 nM. D, E and F) Spike constructs immobilised onto Protein A surface and using ACE2 as analyte at 50 and 500 nM. A and D) show schematic representation of assay setup. B and E) show the reference cell subtracted data, which shows there is a specific interaction, but cannot be reliably fitted with a model. C and F) show sensorgrams from the reference cells during the experiment, demonstrating that there was significant non-specific binding of ACE2 in each case.

4. Immobilised ACE2 and spike analyte in single-cycle kinetics

In this setup, biotinylated ACE2 was immobilised onto streptavadin (SA) chip, and the different spike constructs used as analyte in a single-cycle kinetics (SCK) experiment (Fig 4A).

Specific binding was observed and data could be fitted with a 1:1 binding model. However, the off-rate did not fit well (red arrows in Fig. 4B). Even with a lengthened dissociation phase of 30 minutes, the signal did not return to baseline. Truncation of dissociation phase allowed data to be fitted, but these quoted binding affinities are unreliable.

There was a perfect agreement between predicted and observed Rmax for interaction between RBD and ACE2, showing ACE2 is completely folded as expected (Fig. 4C). However, for multimeric constructs with multiple binding sites, the percentage of binding increases as the sensor surface is less densely populated with ACE2, showing a classic avidity effect and explains the apparent slow dissociation rates.

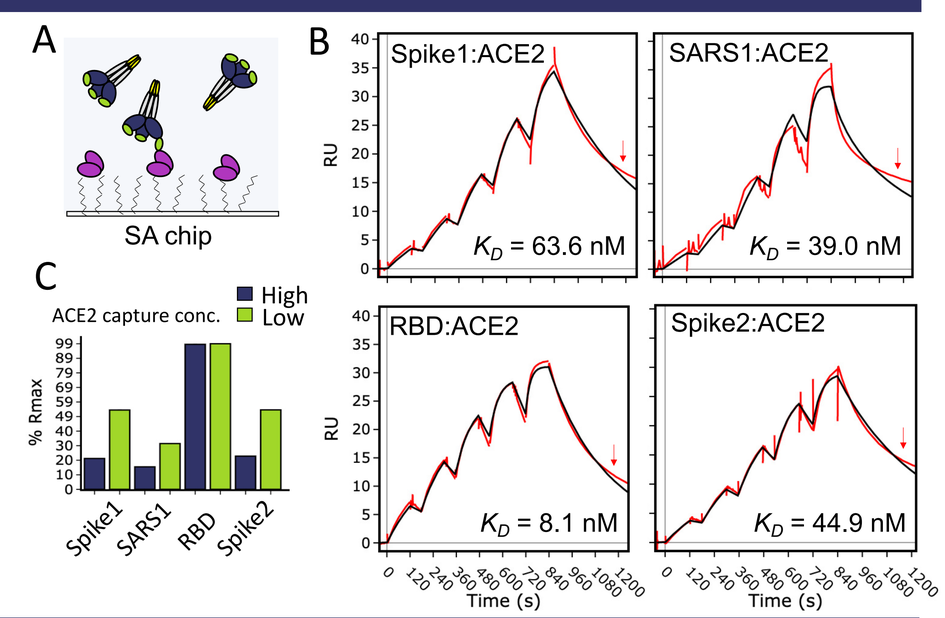

Figure 4 – Experiment using Biotinylated ACE2 immobilised onto streptavadin (SA) chip. A) Schematic representation of assay setup. B) Sensorgrams for the interaction of four different spike constructs with hACE2. Raw data is displayed (red), fitted using a 1:1 binding model (black). C) Bar graph of the observed maximal signal (Rmax) as a percentage of theoretical maximal signal. The experiment was repeated at both a high (60 s, 10 µl/min, 100 nM capture) and low (60 s, 10 µl/min, 10 nM capture) ACE2 surface density.

5. Immobilised spike and ACE2 analyte in multi-cycle kinetics

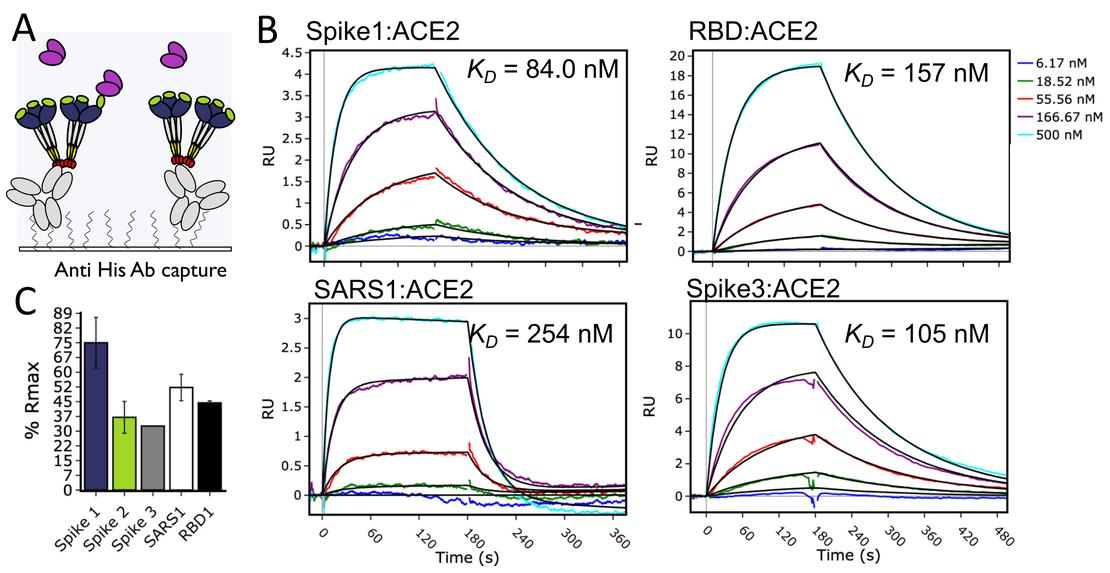

Figure 5 – Experiment using spike constructs immobilised onto an anti His Ab surface. A) Schematic representation of assay setup. B) Sensorgrams for the interaction of four different spike constructs with hACE2. Raw data is displayed (coloured traces), fitted using a 1:1 binding model (black). C) Bar graph of the observed maximal signal (Rmax) as a percentage of theoretical maximal signal.

In this setup, the sensor surface was prepared by the amine coupling of an anti 6xHis antibody to the surface of a CM5 chip. The different spike constructs were then immobilised onto this surface. ACE2 was used as an analyte at varying concentration in a multi-cycle kinetics (MCK) experiment (Fig. 5A).

Specific binding was observed and data could be fitted with a 1:1 binding model. Unlike the previous setup, there were no observable avidity effects. Furthermore, there was good agreement in measured binding constants between matched samples run on independent days. Our binding constants agree well with those reported in the literature (Table 1).

There was excellent agreement between theoretical Rmax and observed Rmax, particularly for Spike1 construct, indicating functionally active spike constructs.

6. Conclusions

- **SARS-CoV spike constructs generally have a 3 to 10-fold weaker interaction with human ACE2 compared with 2019-nCoV spike constructs.**
- **2019-nCoV spike:hACE2 has a slow dissociation rate.**
- **SARS-CoV spike:hACE2 interaction has faster association, but also has a faster dissociation rate, resulting in a net weaker K_D.**

Table 1

Interaction	Reported K_D range (nM)	Peak Proteins SCK measurement (nM)	Peak Proteins MCK measurement (nM)
Trimeric 2019-nCoV spike:hACE2	14.7 - 86.7	63.6*	90.6
Trimeric SARS-CoV spike:hACE2	-	39.0*	225
2019-nCoV RBD:hACE2	1.2 - 133	8.1	197
SARS-CoV RBD:hACE2	5.0 - 409	ND	ND

* Values likely to be affected by avidity

References

[1] Walls AC, et al. (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*:1–12., [2] Wrapp D, et al. (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367(6483):1260–1263., [3] Wang Q, et al. (2020) Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell* 181(4):894-904.e9.