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

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Outline

- Introduction, background and aims
- Assay development and optimization
- Analysis and results
- Comparisons to literature and conclusions

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Peak Proteins

Contract research organisation (CRO) established in October 2014

Mark Abbott (CEO)

Based at Alderley Park research facility in Cheshire, UK

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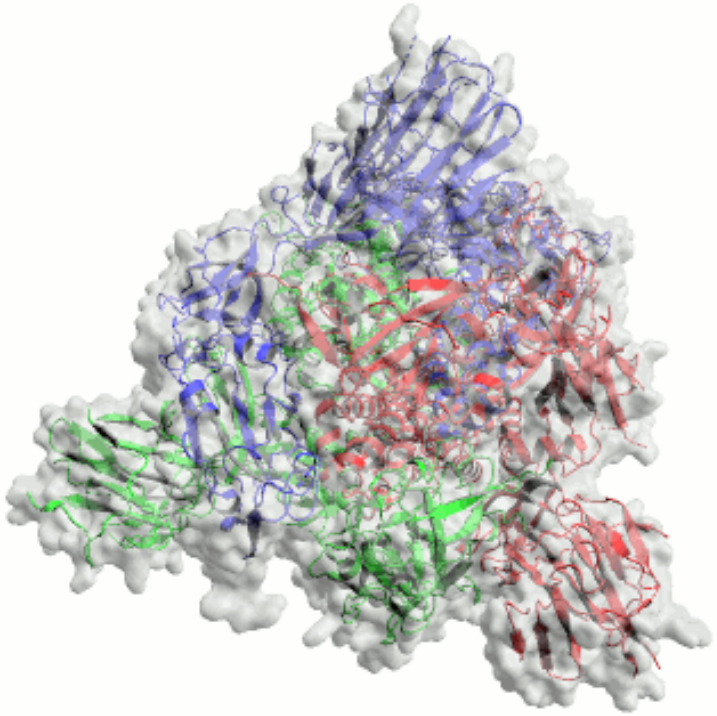
Background: coronavirus spike mediated invasion



2019-nCoV trimeric spike protein
(surface glycoprotein)

- Requests for various 2019-nCoV spike constructs and human angiotensin-converting enzyme (ACE2) started coming through in March

- Peak Proteins wanted to contribute to the scientific effort tackling the Covid-19 pandemic

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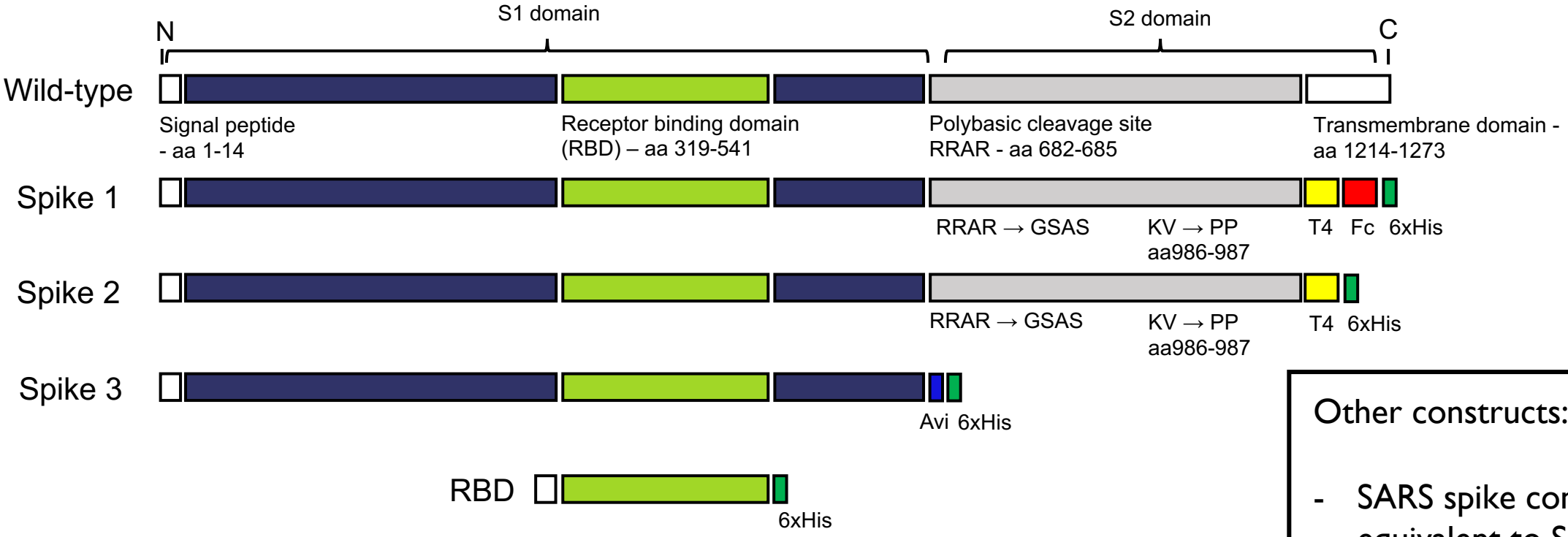
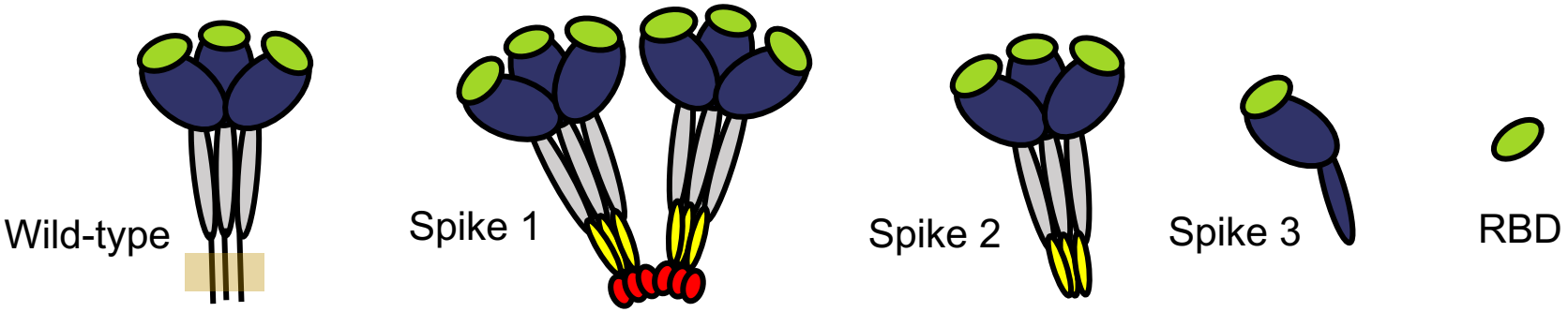
Background: 2019-nCoV spike constructs





Other constructs:

- SARS spike construct equivalent to Spike1
- His and Avi tagged human ACE2 construct (aa19-684)

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Background: Aims

- Learn how to use the Biacore 8K, and bring the capability into Peak Proteins!
- Validate the spike and ACE2 constructs we produced were 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

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Assay development

- Access to a Biacore 8K at the Alderley Park open access lab

- Many different options available for immobilisation and assay orientation:
  - Immobilised spike with ACE2 analyte:
    - Ni NTA chip
    - Anti-His Ab surface on CM5 chip
    - Protein A chip

  - Immobilised ACE2 with spike construct analytes:
    - SA chip

- Already had a good idea of binding affinity from literature (1-100 nM)

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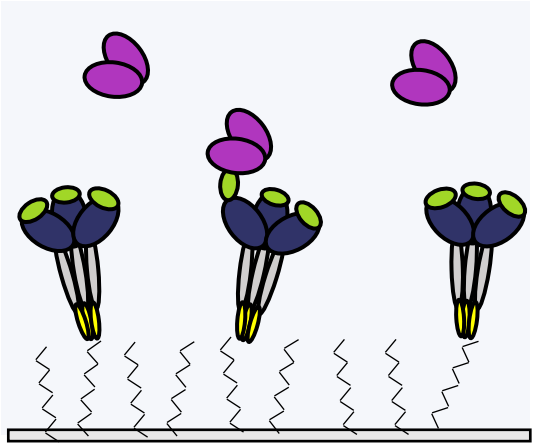
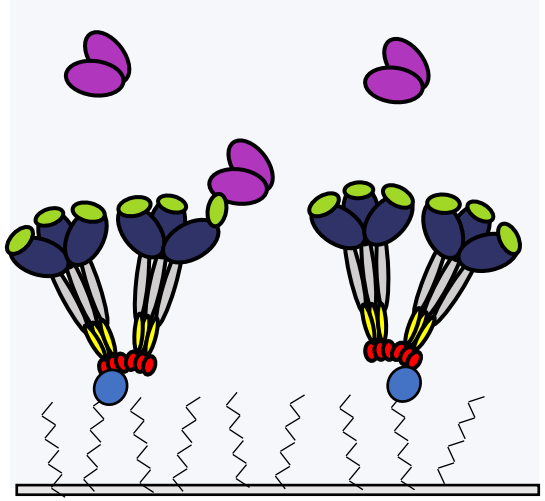
Assay development: Setups that didn't work!



Ni NTA chip

- Spike constructs immobilised onto Ni NTA surface
  - All spike constructs had a 6xHis tag
- ACE2 as analyte with varying concentration in multi-cycle kinetics (MCK)



Protein A chip

- Spike constructs immobilised onto Portein A surface
  - Constructs Spike1 and SARS1 had Fc tags
- ACE2 as analyte with varying concentration in multi-cycle kinetics (MCK)

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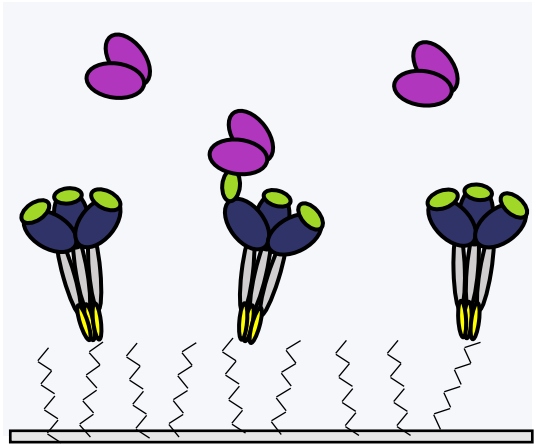
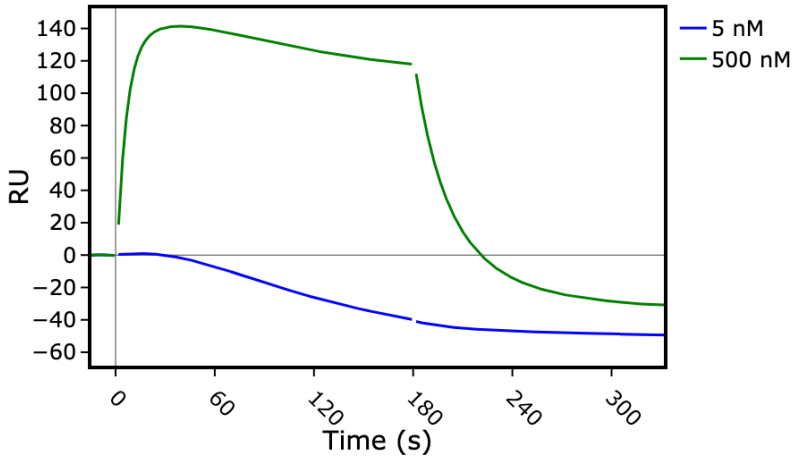
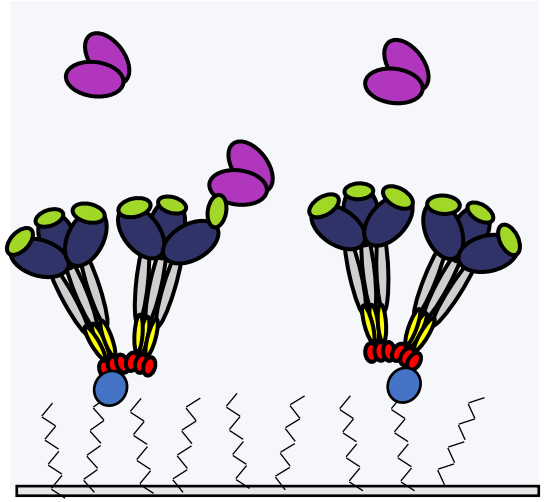
Assay development: Setups that didn't work!



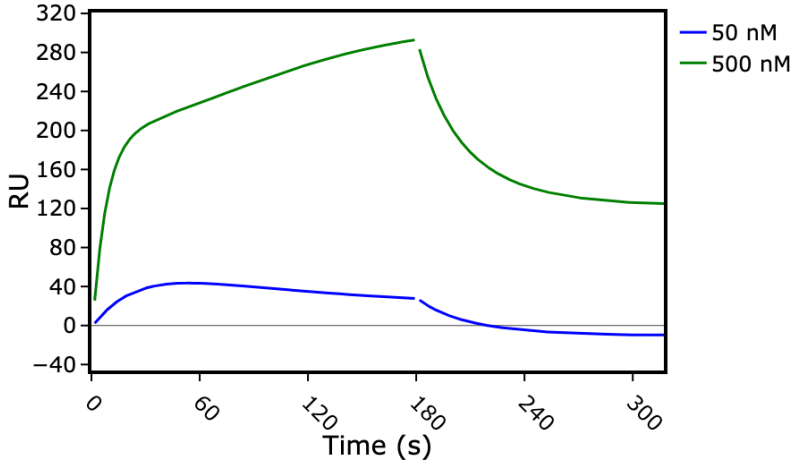
Ni NTA chip

Reference subtracted sample cell





Protein A chip



Representative data from SARS1

- Initial test run:
  - two different Spike concentrations
  - two different ACE2 analyte concentrations

Binding between Spike and ACE2 **YES**

Affinity measurements **Maybe**

Kinetics measurements **No**

ACE2 sticky?

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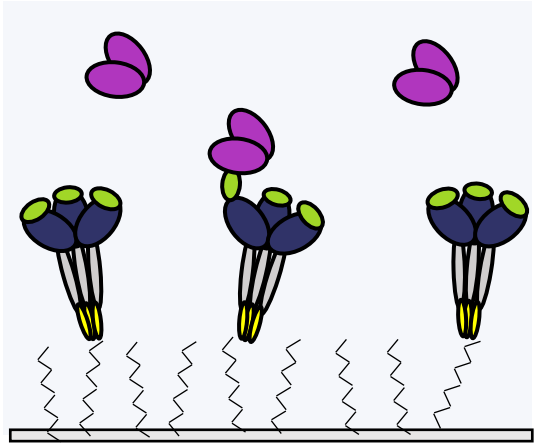
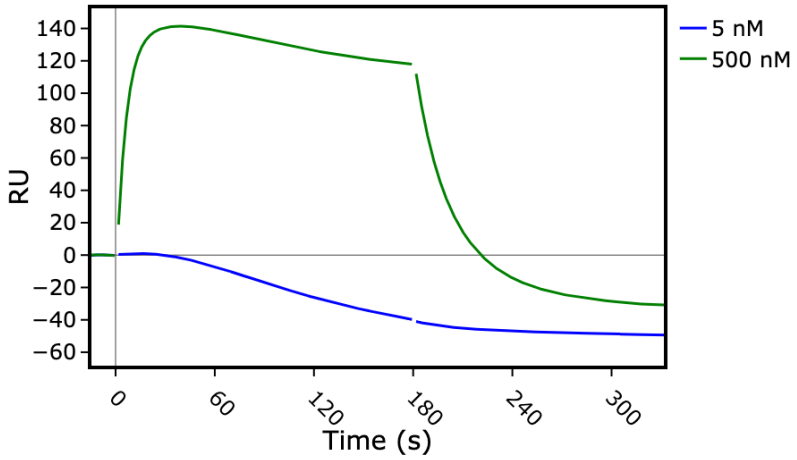
Assay development: Setups that didn't work!



Ni NTA chip

Reference subtracted sample cell



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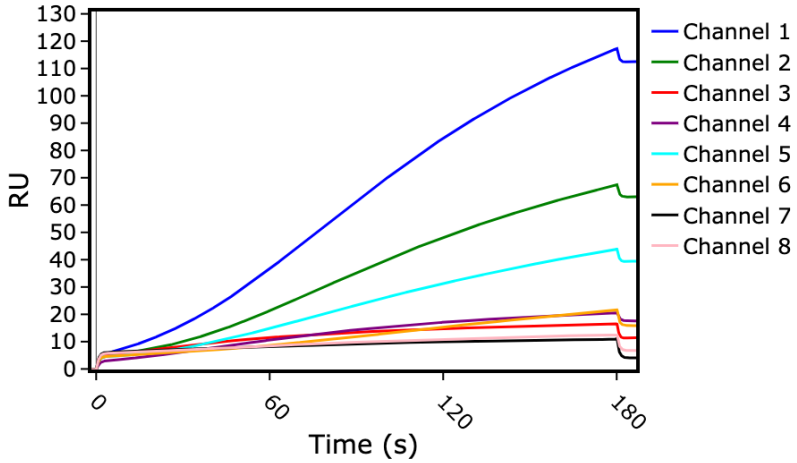
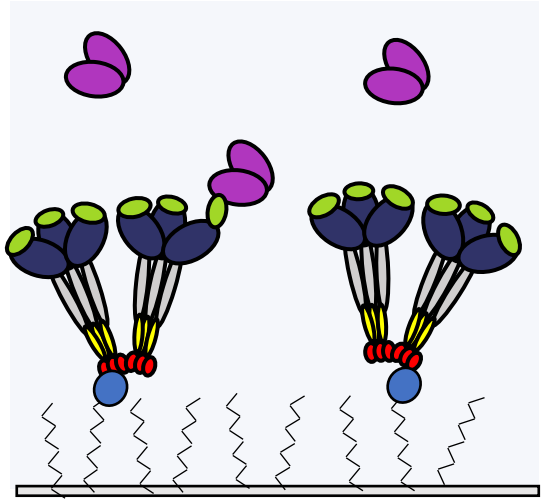
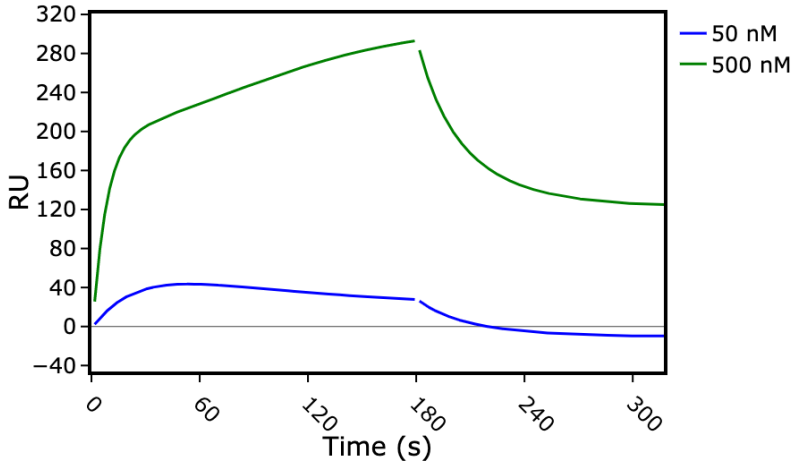
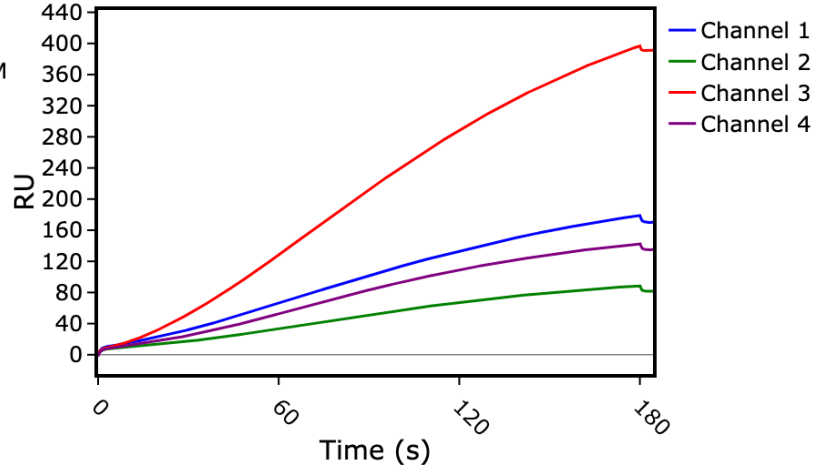




Protein A chip





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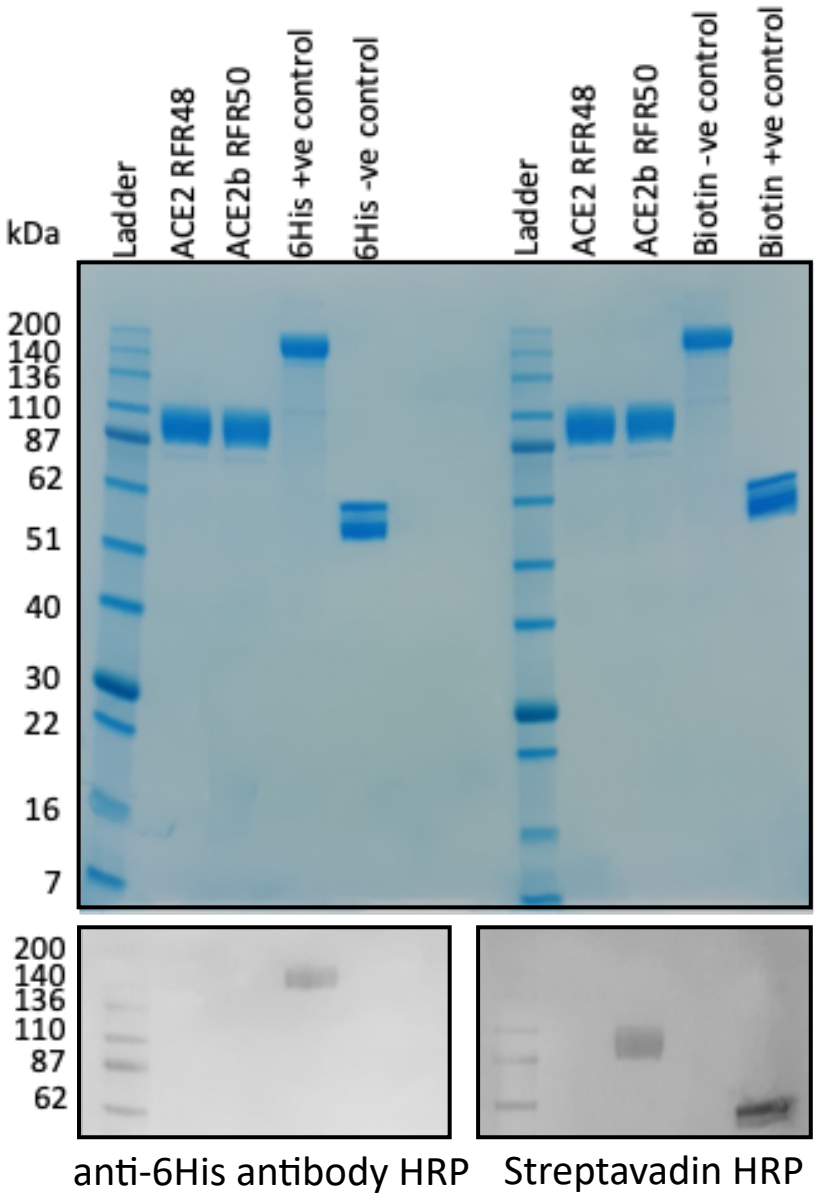
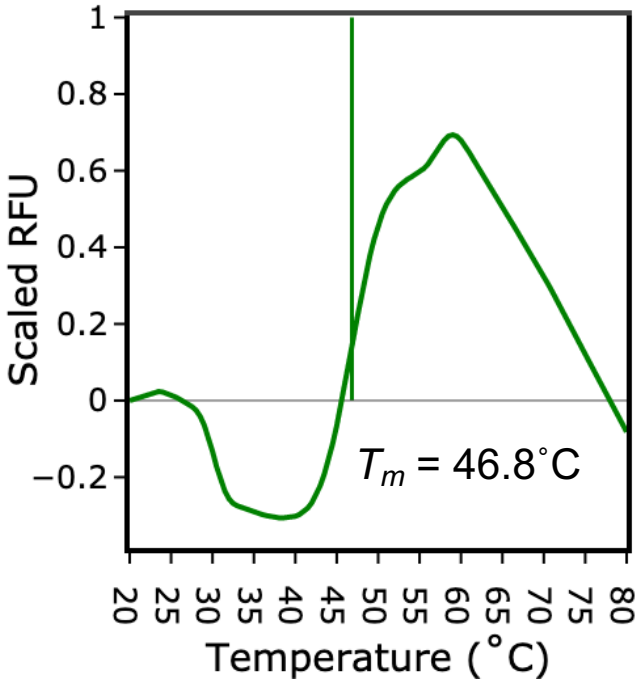
Assay development: due diligence on ACE2 construct





- ACE2 his tag has been completely removed by protease cleavage
- ACE2 has successfully been Biotinylated
- ACE2 shows a clear unfolding curve centered around a temperature of 47˚C – evidence that the protein is folded

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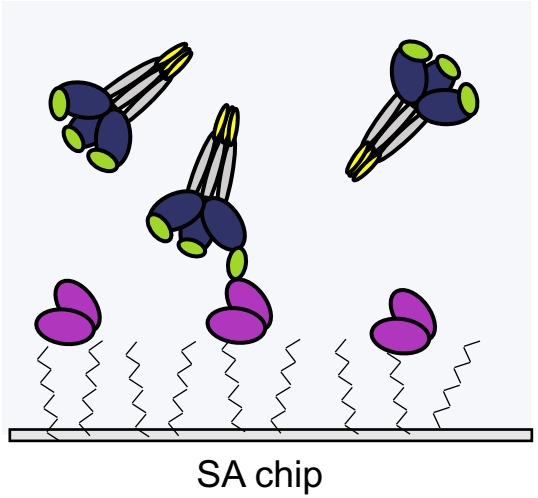
Analysis and results: immobilised ACE2 and spike analyte in SCK



- Biotinylated ACE2 immobilised onto streptavadin (SA) chip

- Spike constructs as analyte with varying concentration in single-cycle kinetics (SCK)

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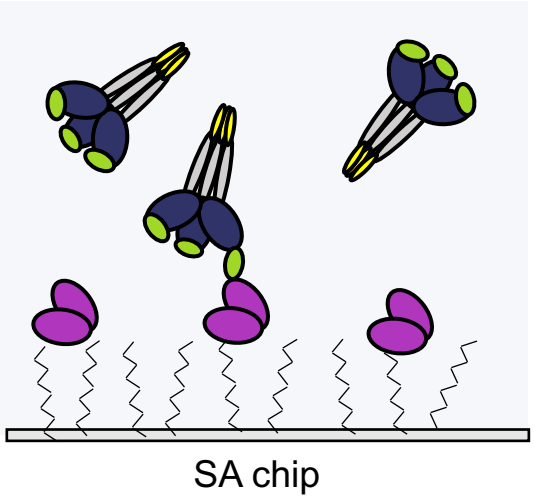
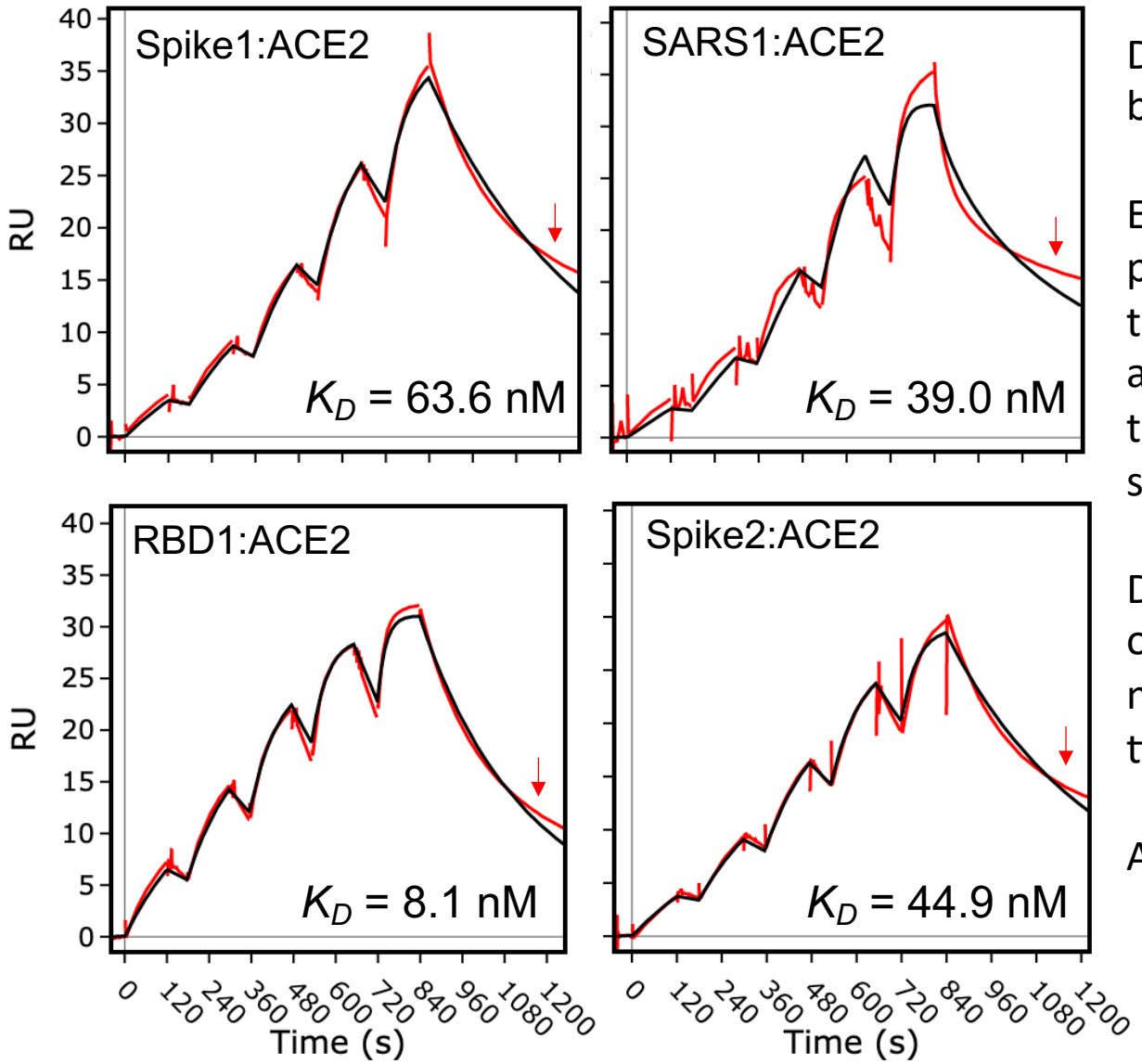
Analysis and results: immobilised ACE2 and spike analyte in SCK





Data were fitted using a 1:1 binding model

Even with local Rmax and drift parameters set to improve fits, the off-rate did not fit well (red arrows) – dissociation phase had to be truncated to allow any sensible fit of the data

Dissociation phase was extended out to 30 minutes, but signal did not return to baseline in this time

Avidity effects??

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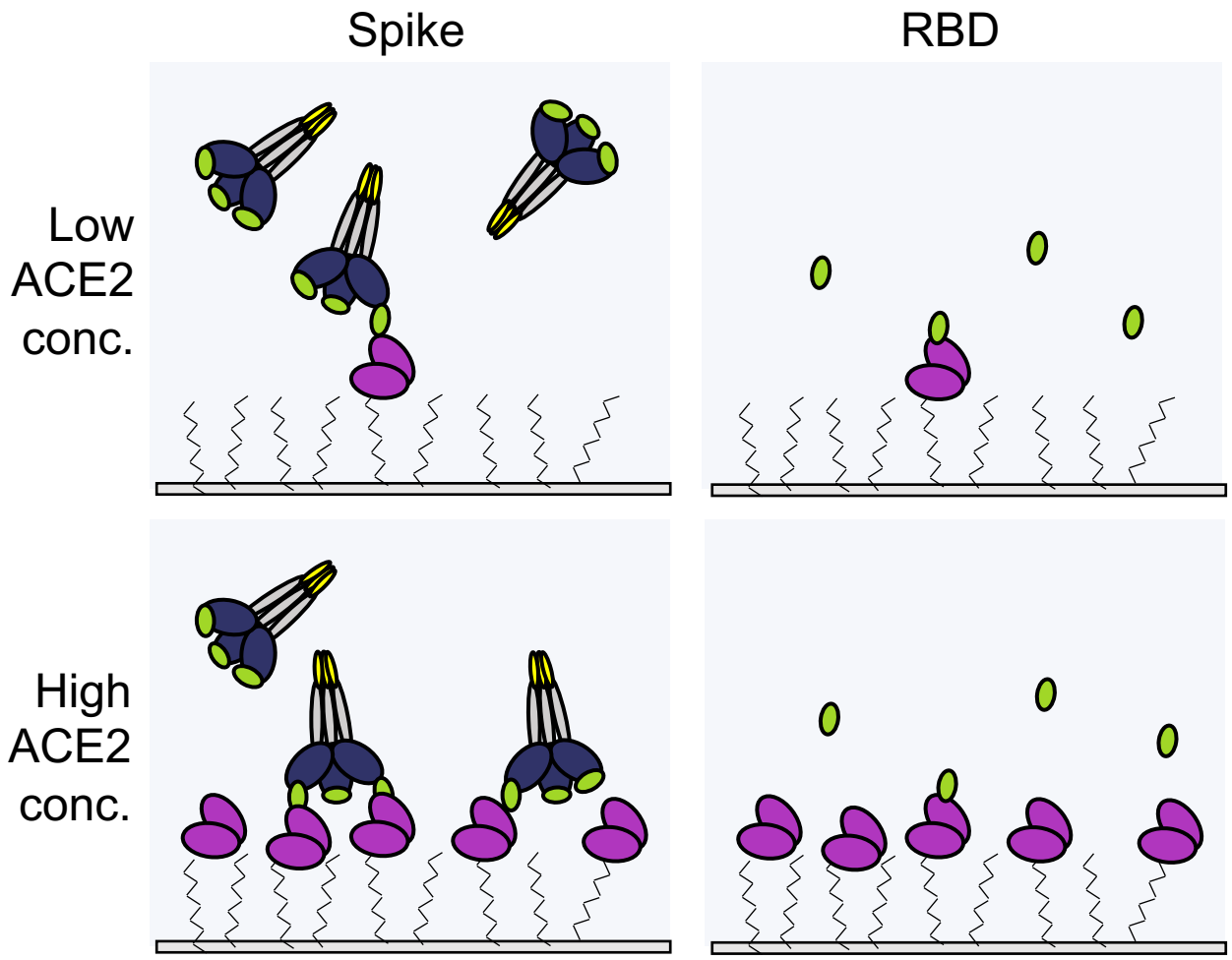
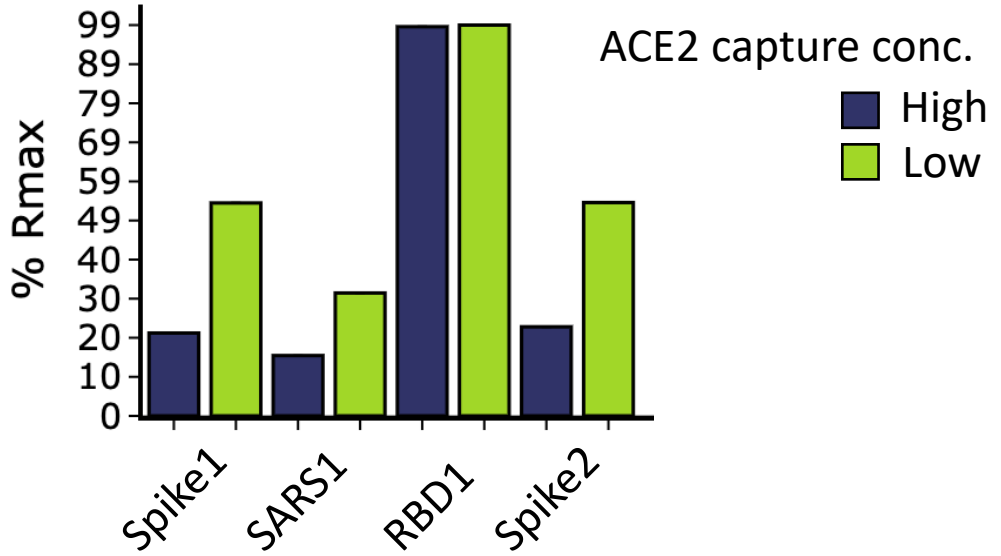
Analysis and results: immobilised ACE2 and spike analyte in SCK





- Almost perfect agreement between predicted and observed Rmax with RBD1 showing ACE2 is almost completely active

- However, 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

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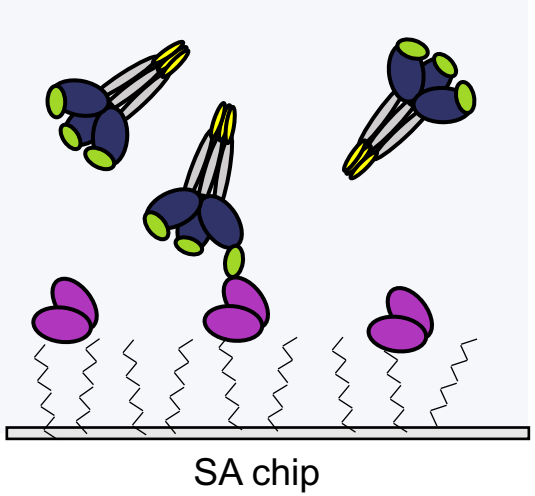
Analysis and results: immobilised ACE2 and spike analyte in SCK



Binding between Spike and ACE2 **YES**

Affinity measurements **YES**

Kinetics measurements **Maybe**

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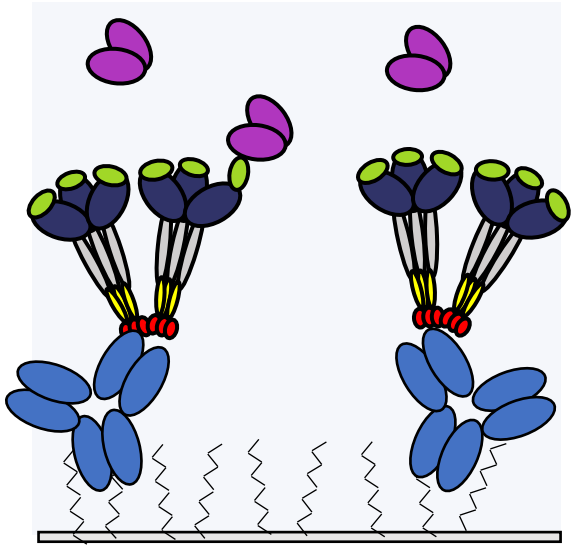
Analysis and results: immobilised spike and ACE2 analyte in MCK



Anti His Ab capture

- Amine coupling of anti 6xHis antibody to CM5 chip
  - Used His capture kit, but 10-fold less antibody than recommended in the kit
- Spike constructs immobilised onto Anti His Ab surface
  - All spike constructs had a 6xHis tag
- ACE2 as analyte with varying concentration in multi-cycle kinetics (MCK)

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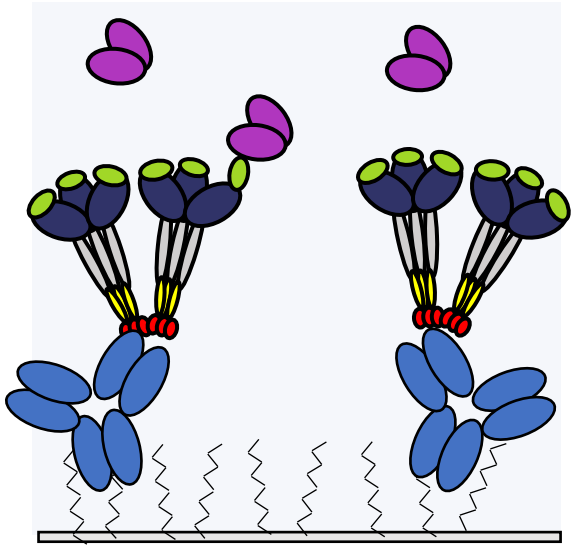
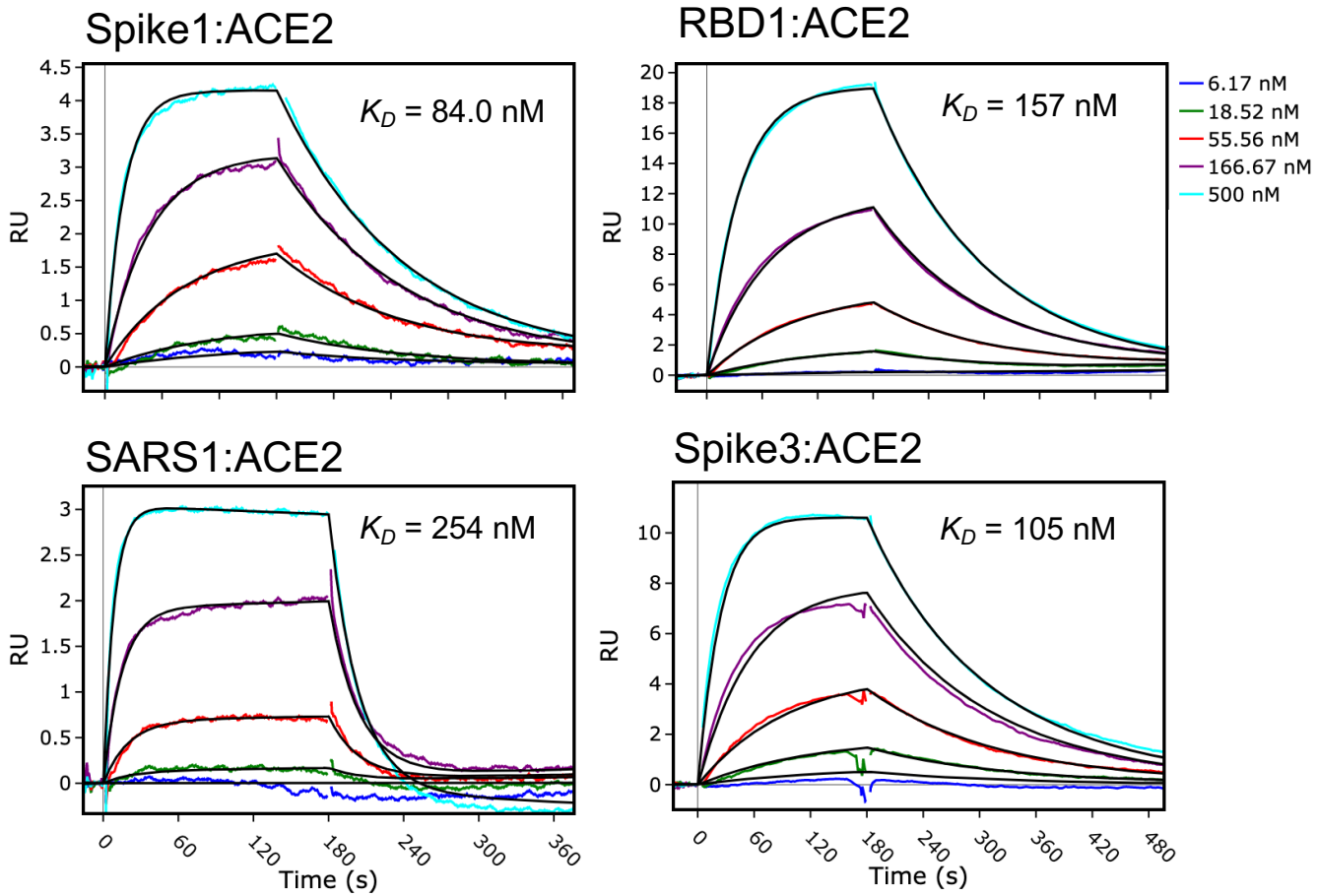
Analysis and results: immobilised spike and ACE2 analyte in MCK



Anti His Ab capture



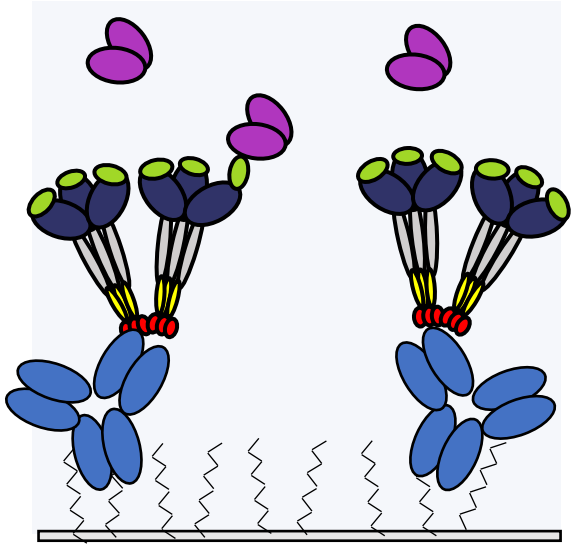
No binding observed in the refence cell

Data were fitted using a 1:1 binding model

Local Rmax and drift parameters set to improve fits

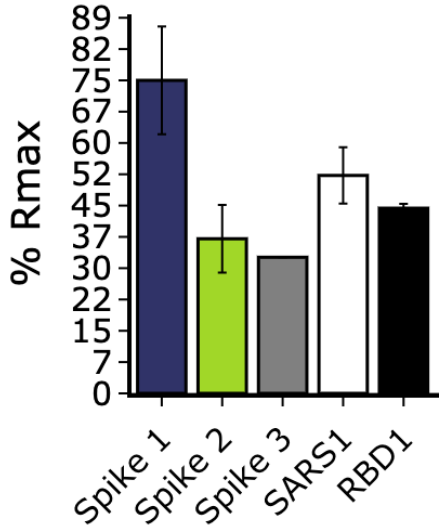
PEAK PROTEINS

Analysis and results: immobilised spike and ACE2 analyte in MCK



Anti His Ab capture

	15th May			17th September		
	ka (1/Ms)	kd (1/s)	KD (nM)	ka (1/Ms)	kd (1/s)	KD (nM)
Spike 1	$8.3x10^4$	$7.0x10^{-3}$	84.0	$6.2x10^4$	$6.1x10^{-3}$	97.2
SARS1	$1.7x10^5$	$4.3x10^{-2}$	254.0	$1.9x10^5$	$3.8x10^{-2}$	195.0
Spike 2	-	-	-	-	-	-
Spike 3	-	-	-	$8.1x10^4$	$8.6x10^{-3}$	105.2
RBD	$8.9x10^4$	$1.4x10^{-2}$	157.1	-	-	-



- Good agreement between matched samples run on independent days
- Avidity effects not observed
- Good agreement between theoretical Rmax and observed Rmax, particularly for Spike1 construct, indicating functionally active spike constructs

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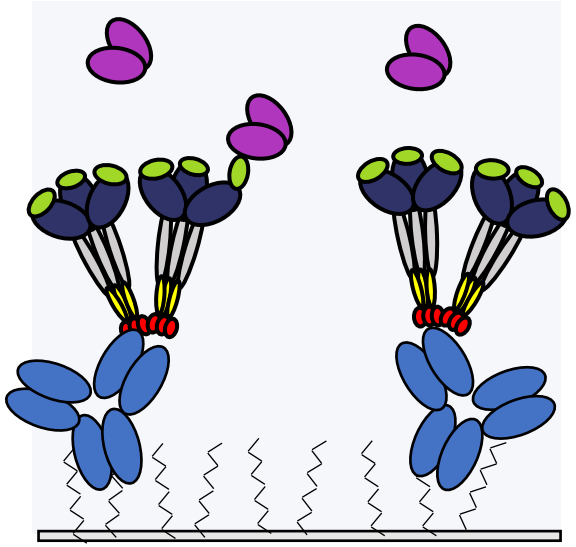
Analysis and results: immobilised spike and ACE2 analyte in MCK



Anti His Ab capture

Binding between Spike and ACE2 **YES**

Affinity measurements **YES**

Kinetics measurements **YES**

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Comparisons to literature and conclusions

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

* Affected by avidity

- 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

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

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Comparisons to literature and conclusions

- Learn how to use the Biacore 8K, and bring the capability into Peak Proteins! ✓
- Validate the spike and ACE2 constructs we produced were 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
  - Spike construct immobilised on an Anti His Ab surface and candidate protein as analyte using multi cycle kinetics ✓

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Proteins Available

Proteins available for immediate dispatch:

- SARs CoV-2 FL Spike trimer (aa14-1213)-6His
- SARS-CoV-2 Spike RBD (aa319-541)-Avi-6His
- ACE2 receptor ECD (aa19-685)-Avi-6His
- ACE2 receptor ECD (aa19-685)-Avi-6His, biotinylated
- SARS-CoV-2 Spike S1 domain (aa14-685)-Avi-6His
- SARS-CoV-2 FL Nucleocapsid-Avi-6His

Prices:

£850 for 0.5 mg
£1500 for 1 mg
£2500 for 2 mg
Discounted prices for > 2 mg

Find out more at www.peakproteins.com or contact us info@peakproteins.com +44 (0)1625 238892

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Acknowledgments

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Cytiva Team

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Anette Persson

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THANKS!

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