

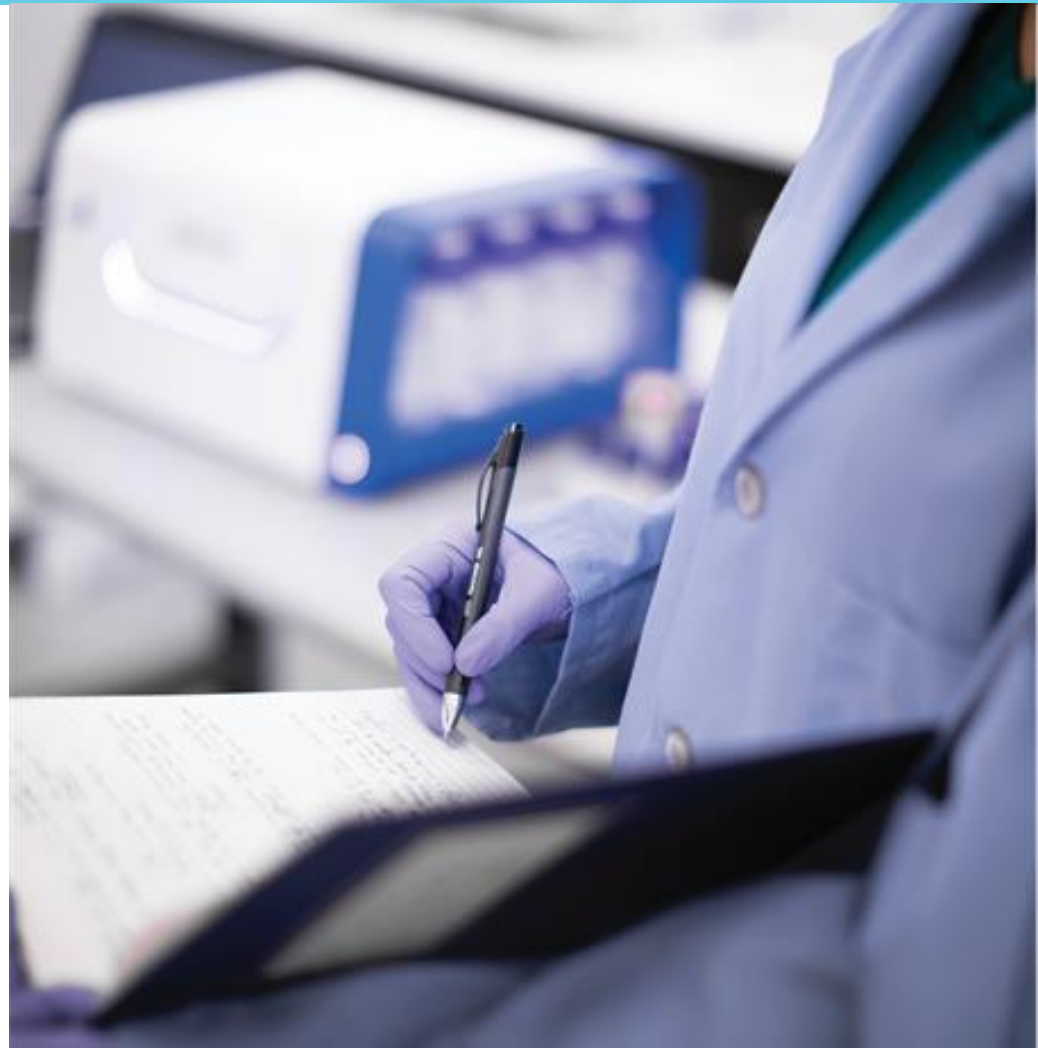
OpenSPR

Publish faster with real-time binding kinetics & affinity data

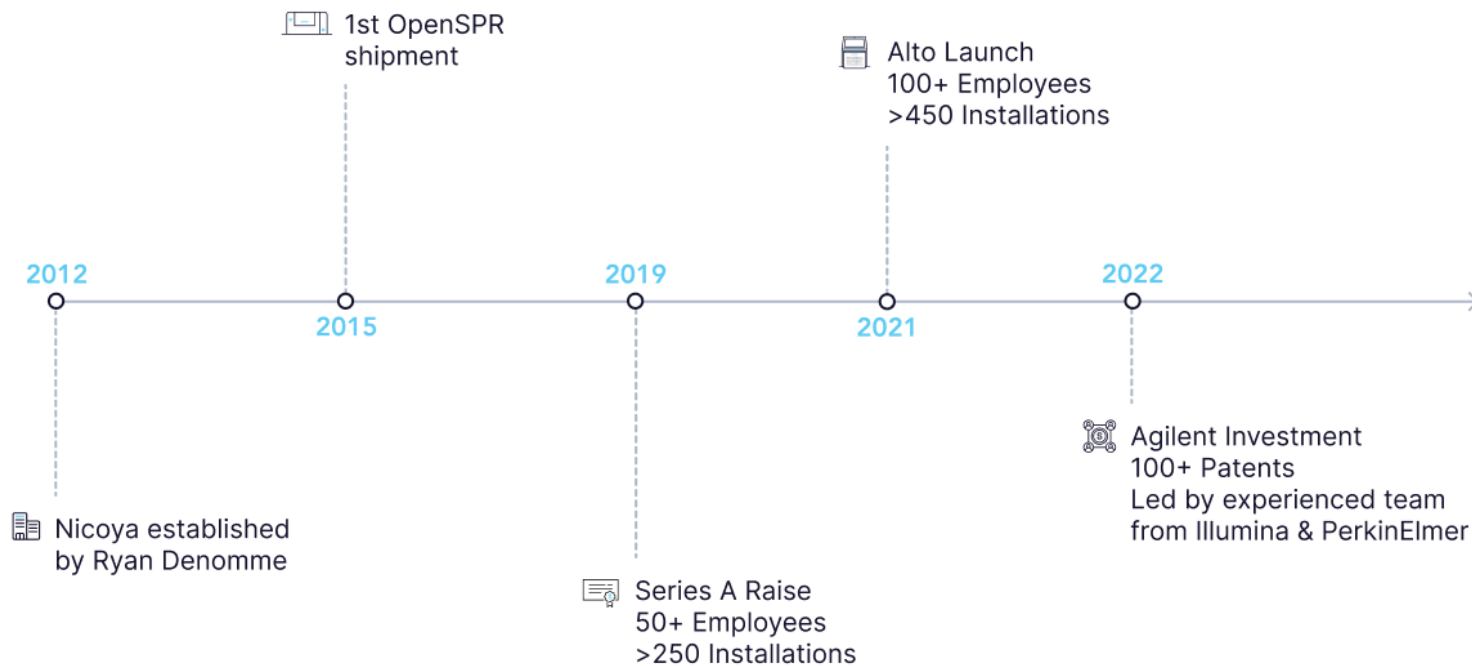


Agenda

1. Intro to Nicoya
2. Basics of SPR
3. Applications
4. OpenSPR



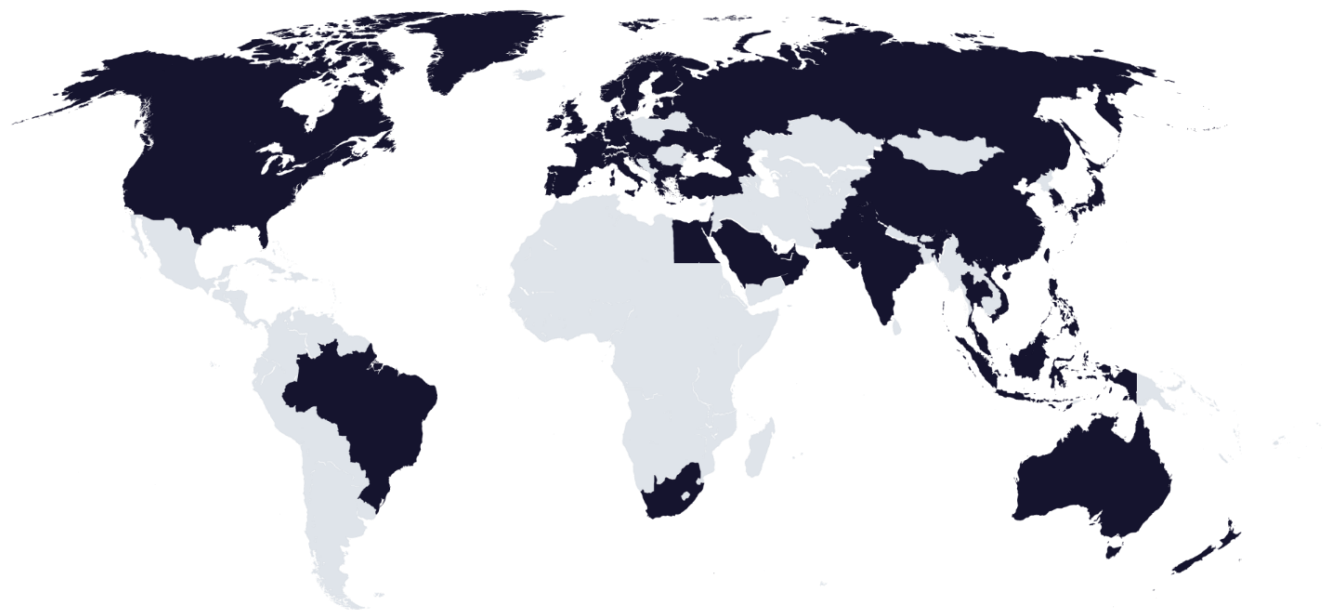
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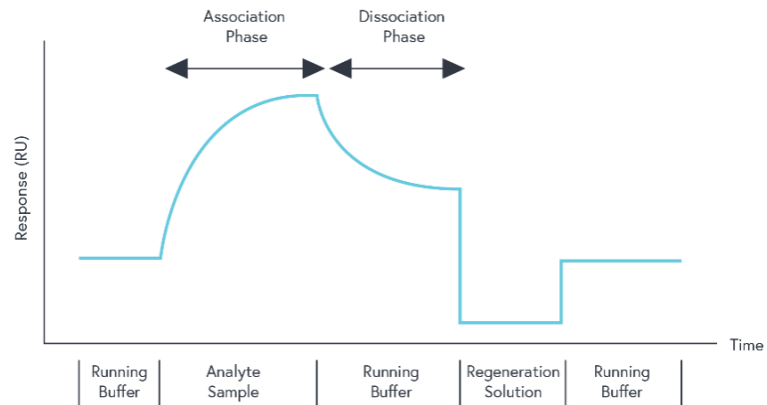
Basics of SPR

Surface plasmon resonance

LSPR Sensor Layer
Sensor Chip



Glass Substrate



Why SPR?

Data Obtained with SPR:

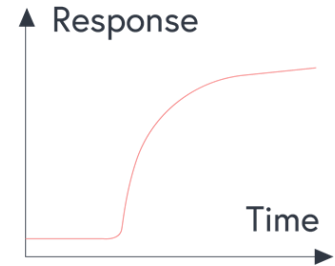
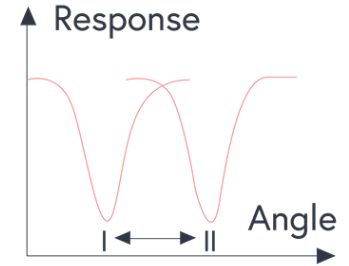
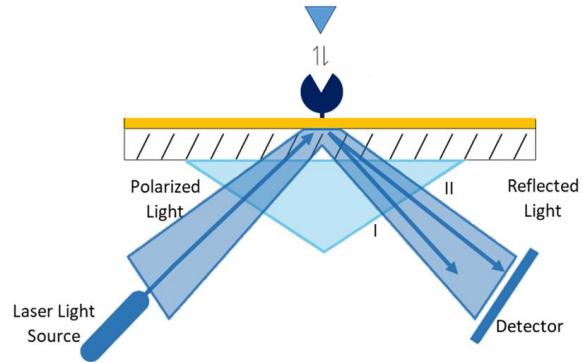
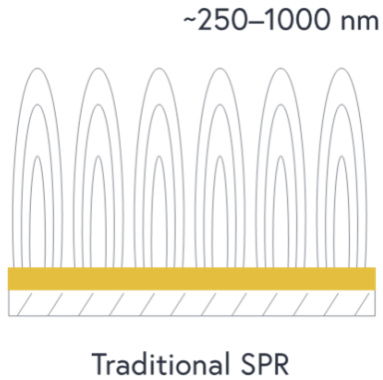
- Kinetics Data
 - Association rate constant (k_a)
 - Dissociation rate constant (k_d)
 - Equilibrium dissociation constant (K_D)
- Quantitation
- Epitope mapping

Benefits of SPR

- ✓ Label-free
- ✓ Specificity
- ✓ Yes/No binding
- ✓ Real-time data
- ✓ Low sample consumption
- ✓ Repeatable

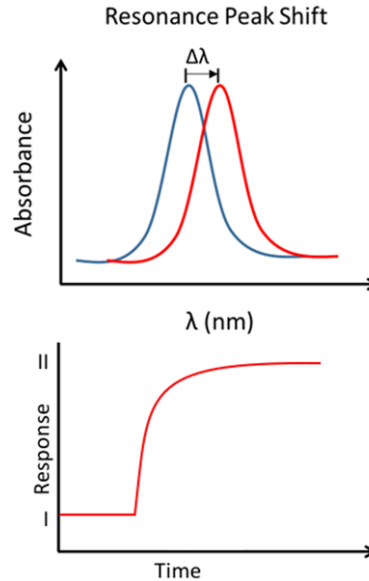
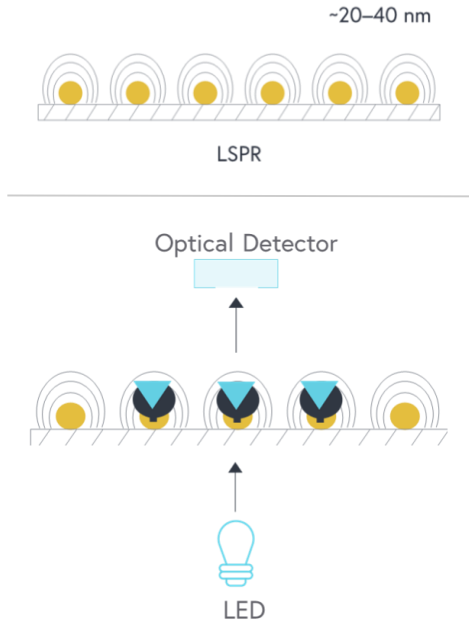


Traditional SPR instruments



Basics of SPR

OpenSPR: Localized SPR



Benefits of LSPR

- ✓ Benchtop
- ✓ Affordable
- ✓ Easy to use
- ✓ Low maintenance
- ✓ Minimal background interference
- ✓ Simple and robust optics



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Applications

Compatible with:

- Proteins/peptides
- Antibodies
- Nucleic acids
- Lipids
- Small molecules*
- Adeno-associated viruses (AAV)
- Virus-like particles (VLPs)
- Hormones/cytokines
- Crude samples

**application dependent*

Supported assays

Kinetics/affinity characterization

Kinetics/affinity screening

Yes/no binding

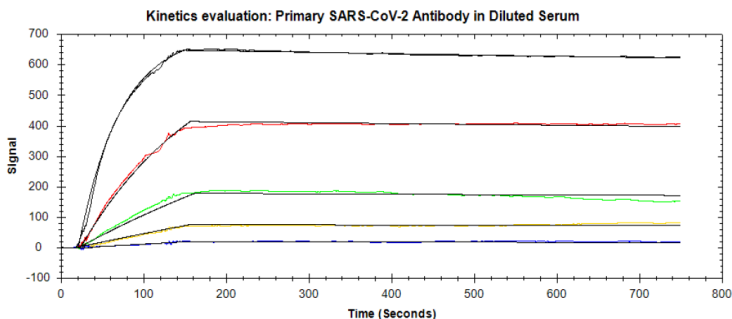
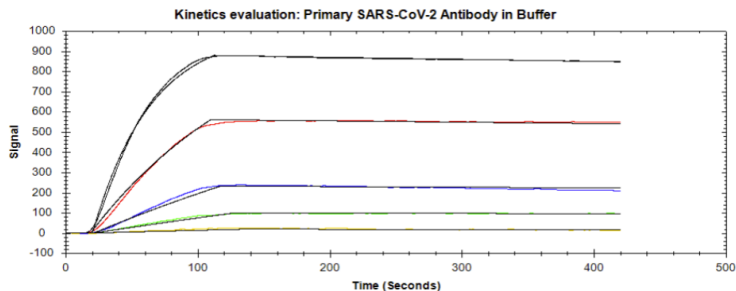
Competition assays

Concentration analysis

Epitope mapping



Direct binding kinetics of SARS-CoV-2 mAb in serum

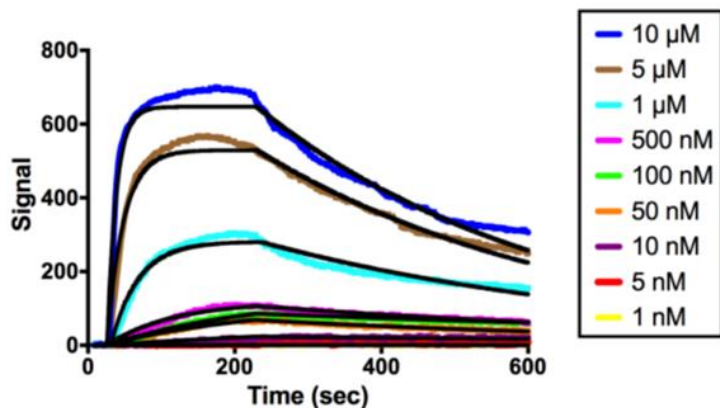


- Direct detection of SARS-CoV-2 mAb in 50% diluted serum, with RBD of the spike protein and fast screening method.
- Ability to measure viral-antigen and antibody interaction in serological sample is key to developing sensitive diagnostic assay.

Parameter	SARS-CoV-2 mAb in buffer	SARS-CoV-2 mAb in Serum
k_a [1/M*s]	1.51e5 ($\pm 2.17e0$)	1.30e5 ($\pm 3.94e0$)
k_d [1/s]	1.20e-4 ($\pm 1.60e-5$)	6.75e-5 ($\pm 2.00e-5$)
K_D [M]	7.93e-10 ($\pm 1.06e-10$)	5.18e-10 ($\pm 1.53e-10$)



Discovering novel therapeutic peptides



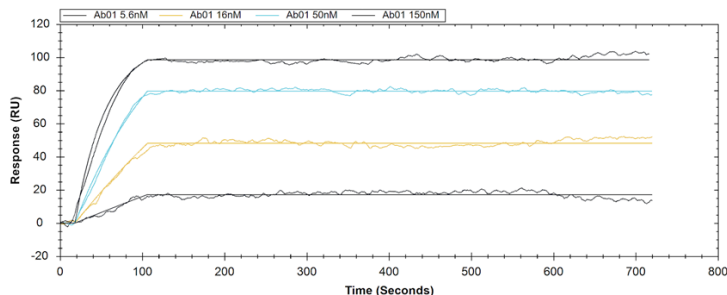
- HA-RHAMM interaction is pro-angiogenic and inflammatory.
- Full length RHAMM production and isolation is difficult, thus a mini-peptide (7 kDa RHAMM) was synthesized.
- SPR showed 7 kDa RHAMM has similar affinity for HA and tubulin-derived peptide as that of the full length RHAMM.
- 7 kDa RHAMM is an efficient and effective replacement of full length RHAMM, and can be used for screening and discovering novel ligands.



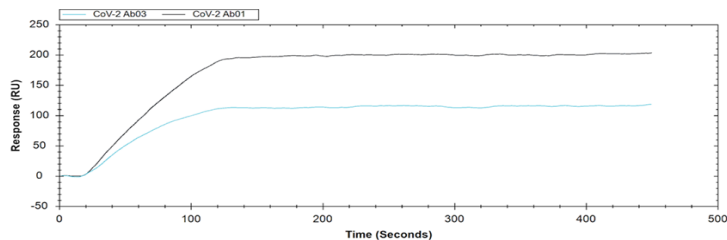
Applications

Characterizing SARS-CoV-2 antibodies

Capture antibody



Detector antibody



Affinity Ranking

Antibody	kon (1/M*s)	koff (1/s)	KD
CoV-2 Ab01	8.92e4	5.93e-4	6.65 nM
CoV-2 Ab02	1.07e5	5.69e-5	534 pM
CoV-2 Ab03	1.26e6	7.74e-5	60.9 pM
CoV-2 Ab04	2.69e5	3.81e-5	141 pM

Epitope binning

		Capture Antibody							
		CoV-2 Ab01	CoV-2 Ab02	CoV-2 Ab03	CoV-2 Ab04	CoV-2 Ab05	CoV-2 Ab06	CoV-2 Ab07	CoV-2 Ab08
Detector Antibody	CoV-2 Ab01	Black	Black	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue
	CoV-2 Ab02	Black	Black	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue	Light Blue
	CoV-2 Ab03	Black	Black	Black	Black	Black	Black	Black	Black
	CoV-2 Ab04	Black	Black	Black	Black	Black	Black	Black	Black
	CoV-2 Ab05	Black	Black	Black	Black	Black	Black	Black	Black
	CoV-2 Ab06	Black	Black	Black	Black	Black	Black	Black	Black
	CoV-2 Ab07	Black	Black	Black	Black	Black	Black	Black	Black
	CoV-2 Ab08	Black	Black	Black	Black	Black	Black	Black	Black



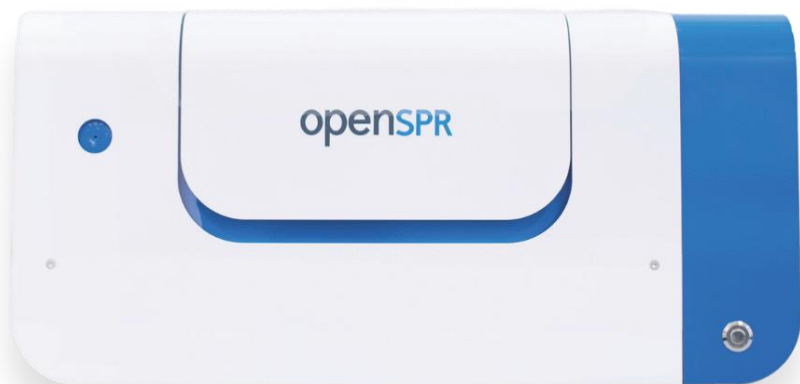
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OpenSPR

Features



Channels	2
Injection	Semi-Automated
Sensor docking	Automated
Buffer selection	Automated 3 buffer selection
Temperature control	4 - 40°C (lower limit 10°C below ambient)
Flow rate	5 - 200 $\mu\text{l}/\text{min}$
Sample volume	150 μL (100 μL sample loop + 50 μL)
Affinity range	mM - pM



OpenSPR

Sensors

Direct



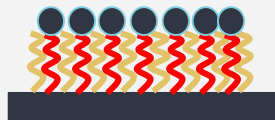
Gold
Carboxyl
Amine

Capture



NTA
Biotin
Streptavidin
Protein A

Hydrophobic



Liposome Binding
Hydrophobic

Standard sensors



High sensitivity sensors



OpenSPR-XT

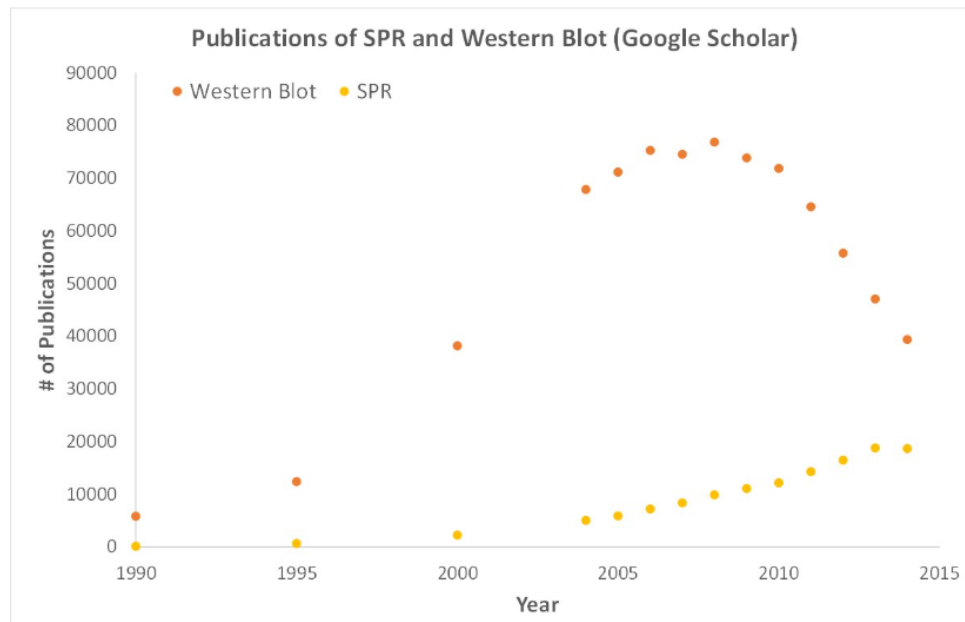
- 2 channels
- Sample area cooled to 4°C
- 2 x 96-well plate capacity
- Automated injections
- 24 hours unattended operation

Injection	Automated
Sample cooling	4 - 22°C
Sample volume	200 µL

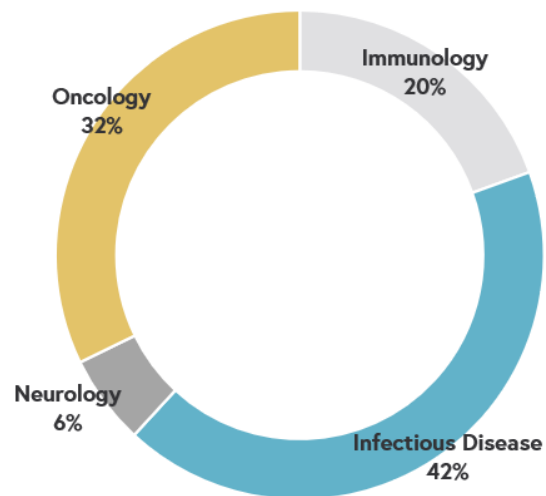
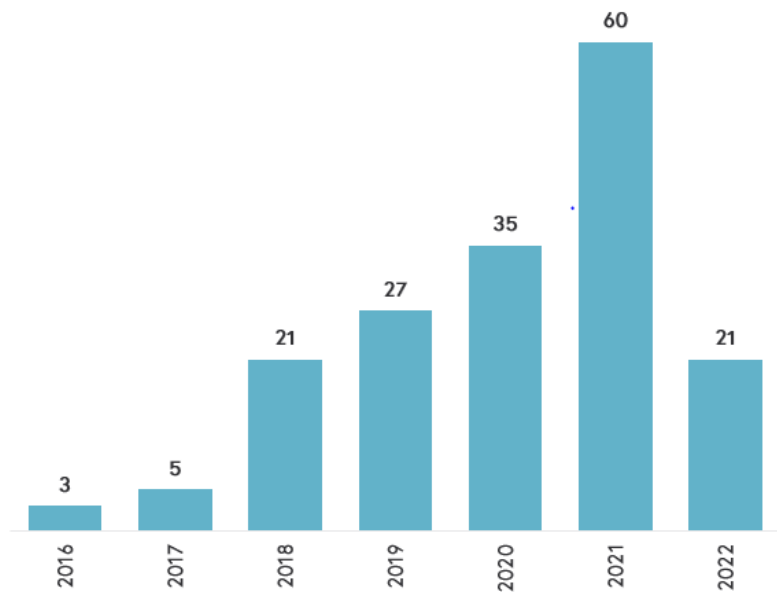


Why consider SPR for your research?

- SPR data are now essential in presenting binding kinetics
- More and more researchers are using SPR data to compliment their publications
- SPR field has been growing rapidly in the last 10 years and has become a stable instrument in research environments



OpenSPR cited in 170+ publications



Accelerate your research with **OpenSPR**



Benchtop



User-Friendly



Real-Time Data



Low Maintenance



Thank you!

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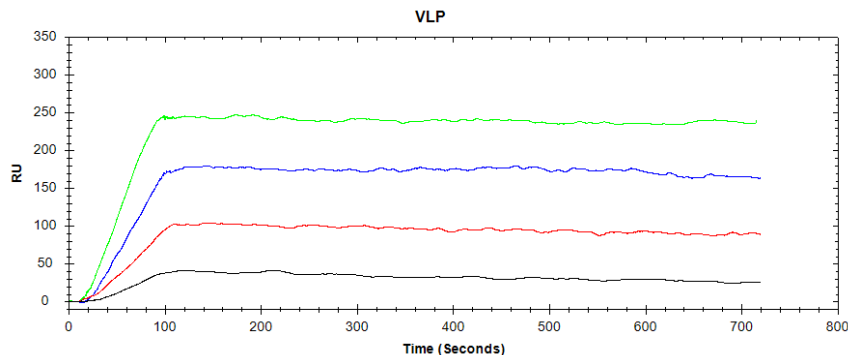


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Additional Applications Slides



Studying binding of protein and virus-like particles

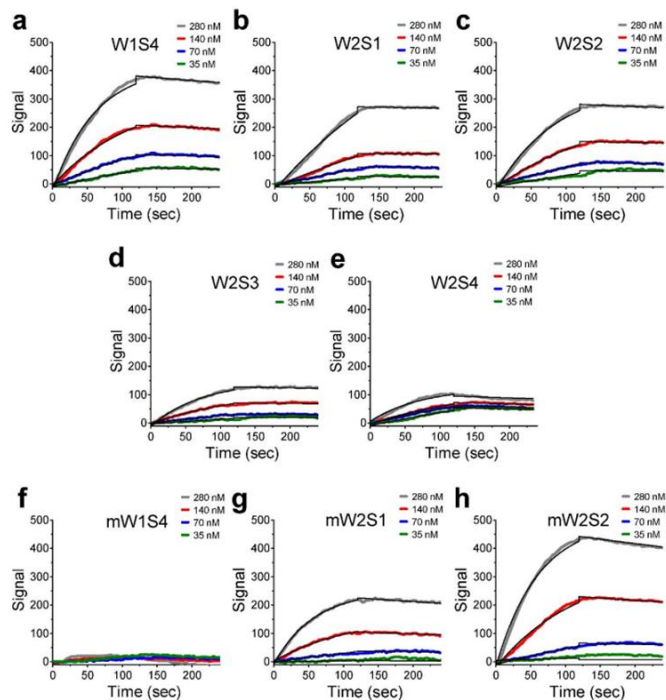


[Full Application Note](#)

- Virus-like particles (VLPs) are complex molecules composed of multiple subunits that resemble a specific virus without its genetic material.
- Their applications include the development of therapies and vaccines against viral diseases, as well as the identification of viral protein components.
- OpenSPR-XT instrument was used to detect a VLP with an approximate molecular weight of 10 MDa to an immobilized antibody. Binding of the VLP could be detected at sub-nanomolar concentrations.



Receptor-based peptides for leukotoxin inhibition

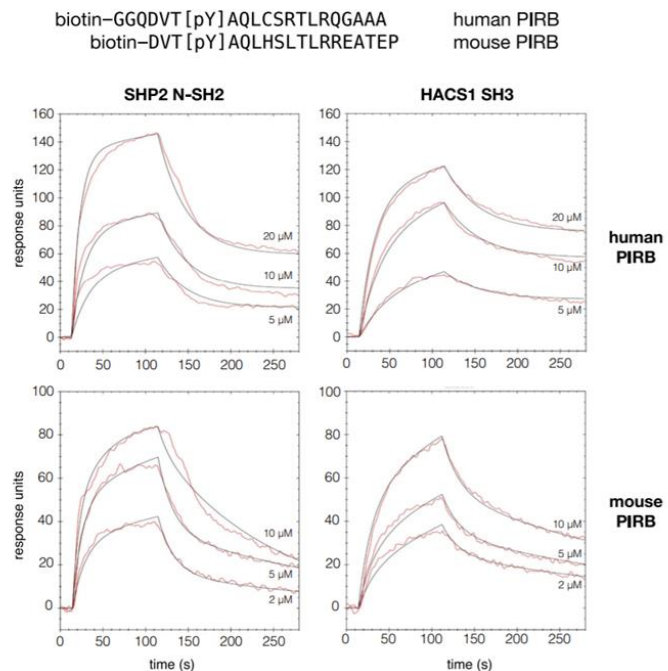


Application: Protein – Peptide

- Goal of study: inhibition of leukotoxin (LtxA) - lymphocyte function-associated antigen-1 (LFA-1) binding.
- SPR data revealed the binding kinetics between each of the designed peptides and LtxA, with peptide W2S4 having a reduced affinity for the toxin.
- Target-based peptides were used to inhibit LtxA activity, and a similar approach could be used to hinder the activity of other RTX toxins.



Elucidating immune signaling pathways

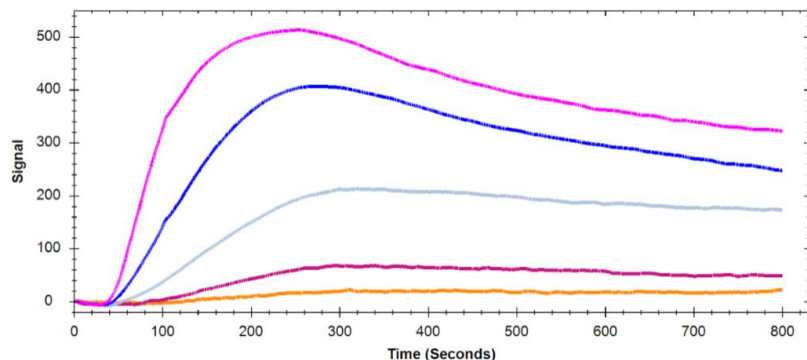


Application: Protein – Nanobody

- HACS1 is a signaling adaptor protein involved in angiogenesis, while PIRB contributes to both innate and adaptive immune responses.
- Inactivation leads to hypersensitivity, enhanced T-cell response and cytokine signaling.
- SPR revealed HACS1 SH3 domain has varying affinities for both mouse (15.9 μM) and human PIRB (8.7 μM).
- These interactions may influence immune and neuronal cell fate.



Rapid detection of SARS-CoV-2 variants



Application: Protein – Antibody

- Goal of study: identification of SARS-CoV-2 variants.
- SPR data showed strong interaction between the monoclonal antibody (2E8) and SARS-CoV-2 Spike protein ($K_D = 7.38$ nM). In conjunction with another mAb, in a sandwich ELISA was able to distinguish between delta, alpha/gamma, and beta variants.
- Rapid detection of SARS-CoV-2-variants is critical to develop point-of-care-diagnostics and prevent spread of unique and deadly variants.

