A Simple SPR Solution for Complex Samples

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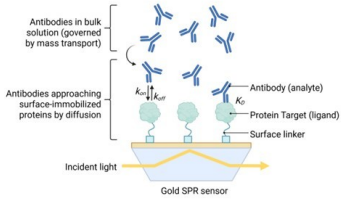
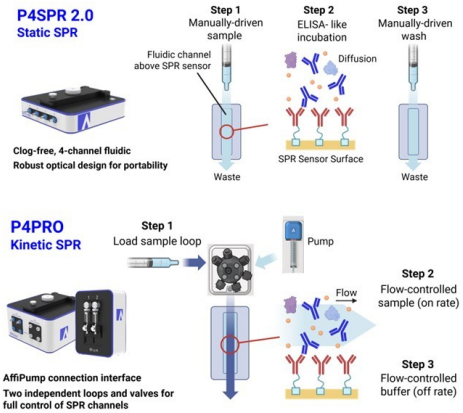
INTRODUCTION



Surface Plasmon Resonance (SPR) detects the interaction between an analyte and a ligand, displaying kinetic and affinity information on a sensorgram. Our **groundbreaking Lensless SPR technology**, with patented features such as the integrated Dove prism and broad-spectrum LEDs, streamlines the traditional complexities of SPR making it easier than ever to take quick, accurate measurements for even the most complex samples.

P4 SERIES: PORTABLE SPR



The **P4SPR static** system:

- allows manual sample injection into the flow cell
- analyte and ligand interact through diffusion
- well-suited for **rapid screening** and **quantification**

The **P4PRO kinetic** system:

- utilizes AffiPump to deliver sample into the flow cell
- measurement of **real-time on/off kinetics** for accurate and precise affinity characterizations
- performance of both devices with 5 nM of each other

CAPABILITIES

Device Features	P4SPR	PRO + AffiPump	NEST*
Affinity characterization (K_D)	✓	✓	✓
Kinetics (K_{on} / K_{off})		✓	✓
Qualitative Y/N binding	✓	✓	✓
Concentration analysis	✓	✓	✓
Detection in complex media	✓	✓	
Operation	Manual	Semi automated	Fully automated
Channels	4	2x2	4+
Flow control		✓	✓

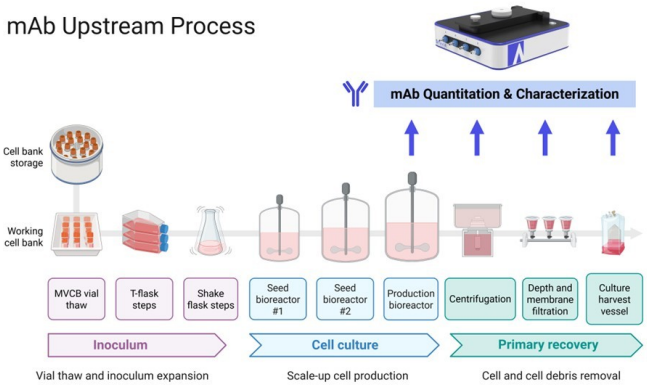
*in development

Available sensors	Carboxyl (Affiocat)	Carboxyl (MHDA)	Streptavidin	Protein A	NTA	Bare gold	Glass
Ligand functional moiety	Amine group, EDC/ NHS coupling		Biotin	FC	His tag	Thiol group	Customized surface
Typical analyte	Antibody, protein, peptide, small molecule					Aptamers	NA

Applicable for cell culture supernatent, crude extract, serum and plasma

CASE STUDIES

1A. Characterization of mAbs During the Biomanufacturing Process



Large-scale production poses challenges in conducting QC characterization of the biologic product. Time-consuming methods like ELISA and lack of access to instrumentation like SPR can hinder timely assessment of bioproduct quality throughout the process.

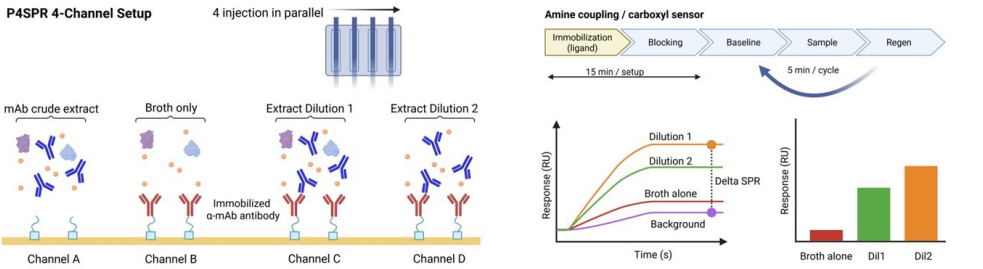
For the production of biologics such as mAbs, protein-based drugs, peptides, ADCs, or large viral-based therapeutics, the P4 series offers a fast, simple, and in-facility alternative.

We demonstrated:

- quantitation of AAV in crude media using a streptavidin sensor immobilized with biotin α-AAV antibodies (2)
- 91-111% correlation to the titers obtained using droplet digital PCR

Using the P4SPR, our customer rapidly analyzed Fabs, nanobodies, membrane proteins, extracellular vesicles, and virus-like particles in crude broth during various stages of Upstream Processing (USP).

1B. Experimental Design and Data



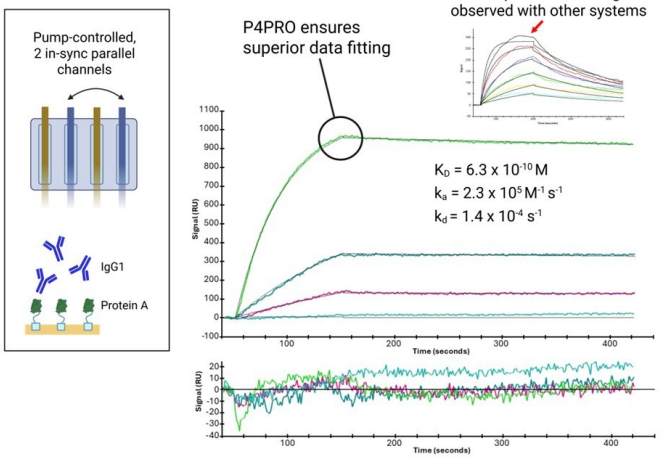
In a **production facility**, our customer set up this experiment (left):

- channels A and B as reference surfaces to measure non-specific binding of the crude extract and the broth
- channels C and D as sample channels to measure binding in two dilutions of the the extract
- all **injections are done simultaneously**

For data processing (right):

- sensorgrams were stacked and corrected for NSB
- the SPR response correlates with the **active concentration** of the expressed target molecule
- each SPR cycles takes 5 minutes
- about **20 conditions were screened in 2 hours at the bench**

2. Quality Fitting of IgG1-Protein A Interaction



Flow rate, analyte concentration, and mass transport affect data quality and can lead to poor data fitting and inaccurate binding affinity. Fitting models are limited in capacity to correct for these effects. The **proprietary design of the P4PRO** reduces these effects by delivering analyte sample evenly across the sensor, minimizing experimental artifacts common in other SPR devices.

Kinetic binding of IgG1 in three steps:

1. immobilize protein A
2. inject IgG1 dilutions into 2 channels simultaneously
3. reference and model data to obtain on/off rates and K_D

The modeled curve shows **excellent fit with the data, exhibiting a K_D in the low nM range, in line with previous reported affinities (4, 5)**. With the P4PRO, accurate and reproducible binding data is obtained with a simple, portable system.

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(1) Technical Note 3 (https://www.affiniteinstruments.com/_files/ugd/645009_22334550097c4d5d81e89023f43e9b3f.pdf)
(2) Application Note 10 (https://www.affiniteinstruments.com/_files/ugd/645009_d4ff2df4433f47058923ad4357018082.pdf)
(3) Technical Note 1 (https://www.affiniteinstruments.com/_files/ugd/645009_ebe1b0bb39ac48c683fa604ec3e4ea8a.pdf)
(4) Braisted AC, Wells JA. Proc Natl Acad Sci. 1996. https://doi.org/10.1073/pnas.93.12.5688
(5) Tsukamoto M, Watanabe H, Ooishi A, et al. J Biol Eng. 2014. https://doi.org/10.1186/1754-1611-8-15