OpenSPR

PUBLISH FASTER WITH BINDING KINETICS & AFFINITY ON YOUR BENCHTOP

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nicoya LIFESCIENCES

"The OpenSPR is being used every day in our department and we love it because it's user-friendly, and we get the data we need from our own bench!"

– **He (Grace) Gu**

Biochemistry, Cellular
& Molecular Biology
Ph.D. Student,
Johns Hopkins University

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ABOUT US & CONTACT INFO

WHO WE ARE

Nicoya Lifesciences is a team of engineers and scientists with extensive experience working at the forefront of nanotechnology, biochemistry, and optical sensors. We come from some of the world's leading research institutions such as the Imperial College of London and the University of Waterloo. We are based in Kitchener-Waterloo, Ontario - known as Canada's Silicon Valley.

OUR MISSION

To provide lifesciences researchers with cutting-edge technology to unleash their limitless potential as they make their next big discovery.

OUR VISION

To extend human life by enabling deeper knowledge of the biological world.

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THE SCRIPPS RESEARCH INSTITUTE
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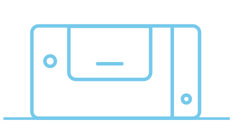
Berkeley UNIVERSITY OF CALIFORNIA

OpenSPR

PUBLISH FASTER WITH BINDING KINETICS & AFFINITY ON YOUR BENCHTOP

Overview

OpenSPR is the world's only benchtop surface plasmon resonance (SPR) instrument. It provides high quality, label-free interaction analysis for a fraction of the cost of existing solutions. Our unique nano-structured sensor surface uses localized SPR (LSPR) to deliver repeatable, highly sensitive kinetic data.

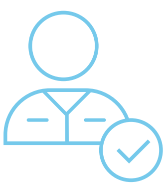


BENCHTOP

Avoid costly & inconvenient core facilities with our affordable benchtop solution

REAL-TIME DATA

Publish faster with label-free binding kinetics & affinity data



USER-FRIENDLY

Train anyone in your lab to become an SPR expert with our user-friendly solution

LOW MAINTENANCE

Forget about expensive service contracts so you can focus on your research

Applications

- Kinetics/affinity characterization
- Competition assays
- Target identification
- Epitope mapping
- Screening
- Yes/No binding
- Concentration

Compatible With

- Proteins
- Lipids
- Carbohydrates
- Antibodies
- Nucleic acids
- Small molecules
- Cells
- Viruses
- Nanoparticles
- & more

Technical Specs

PERFORMANCE

Association Rate (k_{on})	$1x10^3$–$1x10^7$ 1/M*s
Dissociation Rate (k_{off})	0.1–$1x10^{-5}$ 1/s
Affinity Range (K_D)	mM–pM

HARDWARE SPECS

# of Channels	2
Flow Rate	5–200 µL/min
Injection	Semi-Automated
Injection Volume	5–100 µL
Autosampler	Optional upgrade to OpenSPR-XT
Temperature Range	10°C–40°C (max 10°C<ambient)
Temperature Precision	+/- 0.25°C
Buffer Switching	3 ports available, automated switching
Instrument Size	46 x 34 x 21 cm
Weight	42 lbs
File Output	CSV, Tracedrawer

OpenSPR-XT

PUBLISH FASTER WITH AUTOMATED BINDING KINETICS & AFFINITY ON YOUR BENCHTOP

Overview

OpenSPR-XT is built with our powerful yet affordable nanotechnology biosensor platform. It has been seamlessly integrated with our sophisticated autosampler system to allow for fully automated, 24/7 operation.

BENCHTOP
Avoid costly & inconvenient core facilities with our affordable benchtop solution

REAL-TIME DATA
Publish faster with label-free binding kinetics & affinity data

AUTOMATED
Maximize your productivity with premade workflows & user-friendly software

RELIABLE
Get publication-quality data with the highest level of consistency & repeatability

Applications

- Kinetics/affinity characterization
- Competition assays
- Target identification
- Epitope mapping
- Screening
- Yes/No binding
- Concentration

Compatible With

- Proteins
- Antibodies
- Nucleic acids
- Small molecules
- Carbohydrates
- Lipids
- Cells
- Viruses
- Nanoparticles
- & more

Technical Specs

PERFORMANCE	
Association Rate (k_{on})	$1x10^3$–$1x10^7$ 1/M*s
Dissociation Rate (k_{off})	0.1–$1x10^{-5}$ 1/s
Affinity Range (K_D)	mM–pM
HARDWARE SPECS	
# of Channels	2
Flow Rate	5–200 µL/min
Injection	Automated
Injection Volume	5–100 µL
Sample Capacity	2x 96 well plates, 2/10 mL vials
Sample Temperature	Cooled from 20°C-4°C
Unattended Run Time	24 hours
Temperature Range	10°C–40°C (max 10°C<ambient)
Temperature Precision	+/- 0.25°C
Buffer Switching	3 ports available, automated switching
Instrument Size	78 x 55 x 53 cm
File Output	CSV, Tracedrawer

OPENSPR FOR EDUCATION

NOVARTIS, PFIZER, & MERCK RELY ON SPR. KEEP YOUR STUDENTS AHEAD.

Why Teach SPR?

Scientists are a driving force in the global economy. This is why we need to educate our aspiring scientists with the latest research technology and techniques, so that they have enough hands-on experience to innovate and make an impact.

In the past five years, there has been over 35,000 scientific publications involving SPR. SPR is a technique that has been growing in popularity and importance in both research and industry. It is used by thousands of biotechnology companies and every pharmaceutical company in the world. Set your students up for success with OpenSPR - the ideal solution for instructors looking to incorporate SPR into their labs.

BENEFITS FOR STUDENTS

Gain hands-on experience with industry standard technique

Build your résumé and increase employability

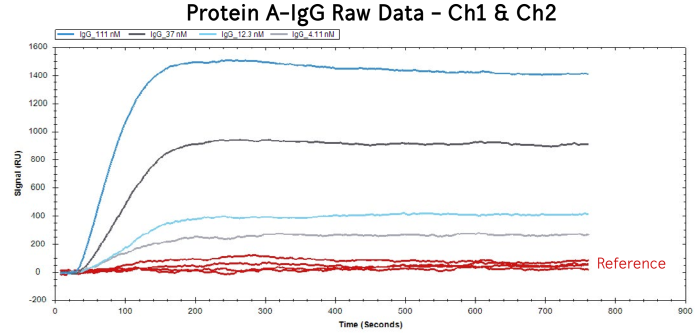
BENEFITS FOR INSTRUCTORS

Very affordable with minimal maintenance costs

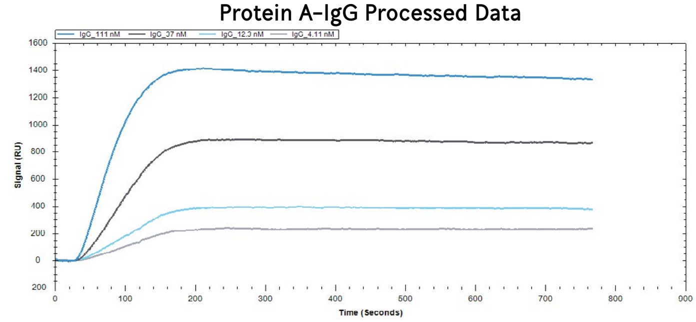
Predesigned labs to minimize your workload

Find the data you're missing.

Protein A-IgG Raw Data - Ch1 & Ch2



Protein A-IgG Processed Data



"OpenSPR will give students a huge learning advantage. With Biacore, if you could afford it, you wouldn't want it being run by 50 undergrads, so a technician would probably run it. That would be a very different experience for the student. With OpenSPR, they can actually do the work themselves in small groups, giving them a far greater impact and learning advantage. They can work with the system themselves; that's really cool."

Dr. Thorsten Dieckmann
Biophysical Chemistry, University of Waterloo

SPR SENSORS

OPTIMIZE EASILY WITH A WIDE RANGE OF AFFORDABLE SENSOR CHIPS

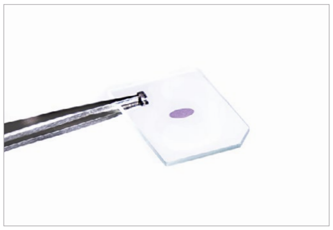
OpenSPR & OpenSPR-XT Sensors

Tired of expensive surface plasmon resonance sensor chips? Our nanotechnology enabled sensors are manufactured to the highest quality to ensure consistent and repeatable measurements, at half of the price of traditional sensors. We achieve less than 2% CV on all critical optical properties. Our sensors are stable in a variety of solvents, buffers and reagents.

Surface Chemistries

OpenSPR sensor surfaces are available as plain gold surfaces for custom surface functionalization or with a variety of functional surface chemistries. These sensor chips reduce the time and effort needed for immobilization while improving repeatability.

Standard Sensor Chip



High Sensitivity Sensor Chip

High Sensitivity Sensors

Our line of High Sensitivity Sensors provide an extra boost of sensitivity for your toughest SPR applications. The increased localized sensitivity is particularly advantageous for small molecule analysis, and can also be used to enhance the signals of other larger biomolecules.

SENSOR CHIP	COATED FOR IMMOBILIZATION OF:
Carboxyl	any amine group via EDC/NHS
NTA	his-tagged targets
Streptavidin	biotin tagged targets
Biotin	streptavidin coupled targets
GST	GST-tagged biomolecules/proteins
Protein A	IgG based antibodies
Amine	any carboxyl group via EDC/NHS
Liposome Binding (LIP)	liposomes/membrane proteins
Hydrophobic (MEM)	lipid monolayer
Thiol	maleimide-taged ligands
Standard Gold	non-functionalized and perfect for custom surface chemistry development and ligands with thiol groups

SPR ANALYSIS SOFTWARE

OUR CUSTOM OPENSPR SOFTWARE IS USER-FRIENDLY, INTUITIVE, AND POWERFUL.

OpenSPR Software Suite

Our OpenSPR software is for exclusive use with the OpenSPR instrument. It allows for control of the instrument and real time data acquisition. The OpenSPR software is included with the purchase of an OpenSPR Starter Kit.

Features

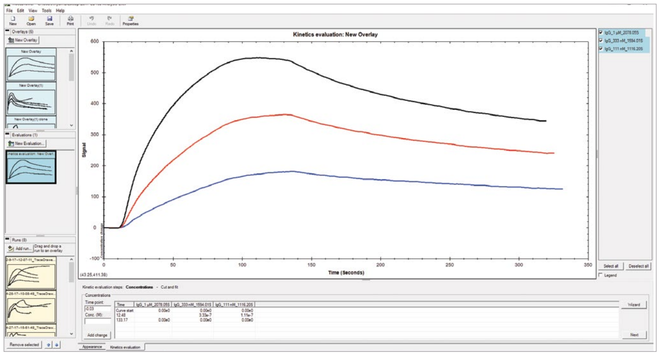
- Intuitive and easy to use
- Guided setup procedure
- Injection markers
- CSV export for flexible data processing options
- TraceDrawer export for quick and easy analysis

Minimum PC Requirements (Mac On Virtualization)
Communication: USB 2.0
Platform: Windows 10. Microsoft .NET Framework 4.0
Memory: 4 GB RAM
CPU: i3

TraceDrawer Software

TraceDrawer is the ultimate solution for real-time interaction data handling and works seamlessly with data produced from the OpenSPR instrument. It can also be used with data generated from any real-time analysis tool once the data is in the appropriate format (.txt or .csv file formats).

TraceDrawer can be used to post process data (subtract references, cut, move, combine data from several experiments), evaluate kinetic constants (k_a and k_d), evaluate affinity constants and EC50, create reports and produce publishable figures. For kinetic analysis, several different models are built in for analysis. TraceDrawer is a great tool for organizing, processing and presenting your data.



Kinetic Analysis Models

- 1:1, with diffusion correction and two-state analysis options
- 1:2
- Bivalent interactions
- Kinetics competition

"Nicoya has a very helpful and insightful training session! After one session, I was able to run my experiments immediately using the LIP chips and look at protein binding to different lipid mixtures. The data is beautiful and the instrument is by far one of the most easiest and user-friendly that I have ever used!"

– **DR. TONYA N. ZECZYCKI**

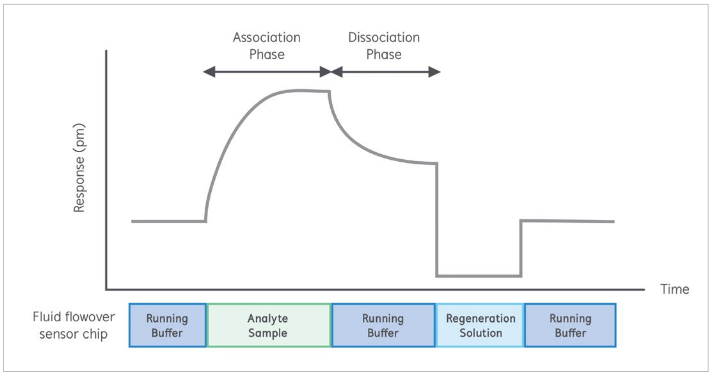
Assistant Professor of
Biochemistry & Molecular
Biology, East Carolina University

SURFACE PLASMON RESONANCE

What is Surface Plasmon Resonance? (SPR)

SPR is an analytical technique for studying molecular interactions.

SPR is an optical effect that can be utilized to measure the binding kinetics and affinity of molecular interactions in real-time without the use of labels. SPR is unique because it is one of the few techniques that allows determination of binding kinetics and not just binding affinity, as you would get from traditional techniques like ELISA. The binding kinetics, or the on and off rates, can only be determined with a biosensing technique that gives real-time binding data of both the association and dissociation phases of the interaction. This data gives detailed insight into the binding strength and stability of the interaction, which is critical for many industries and research areas. It helps researchers determine which molecules interact, why they interact, and how strongly they interact.



Why SPR?

SPR is becoming a standard technique available in every single biochemistry lab.

1. **REAL-TIME MONITORING**
   Provides binding kinetics (on/off rates, yes/no binding & affinity values)

2. **LABEL-FREE DETECTION**
   Avoid spending time & money on expensive labeling reagents and protocols

3. **CONSERVE PRECIOUS SAMPLES**
   Saves money & reduces time needed for expression and purification

4. **REUSABLE SENSOR CHIPS**
   Regeneration buffers are used to disrupt the interaction between the analyte and ligand

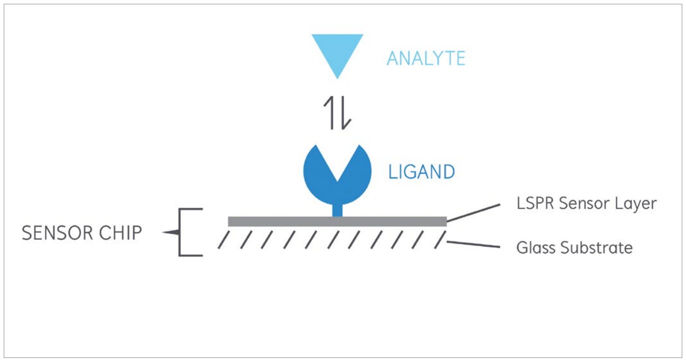
5. **COMPLEX SAMPLE TESTING**
   Test crude samples to reduce time & cost associated with purification

6. **RELIABILITY & ACCURACY**
   Obtain accurate results from repeatable measurements

How Does SPR work?

The SPR instrument consists of an optical measurement system, a fluid handling system, and a sensor chip.

An SPR sensor consists of a very thin gold layer coated onto a glass substrate. When a light source illuminates the gold layer, a plasmonic wave is generated with an electric field extending above the surface of the sensor. This electric field of the sensor is very sensitive to changes in the dielectric constant and can detect changes in mass or refractive index on the sensor surface, such as a molecular binding event. An optical detection system is used to measure the changes of the sensor properties, creating a response signal to measure molecular interactions in real-time.



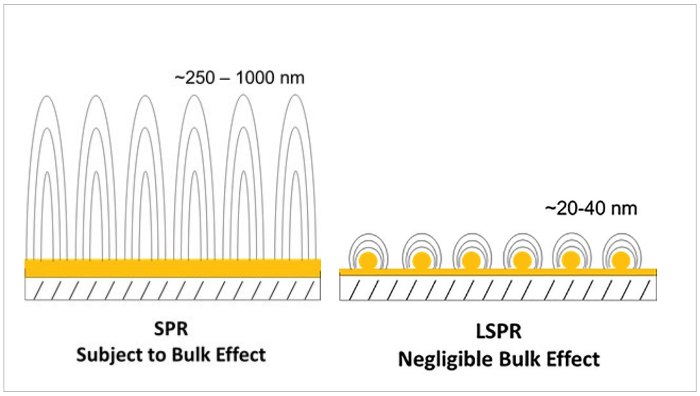
How is LSPR Different from SPR?

Localized surface plasmon resonance is generated by gold nanoparticles as compared to a continuous gold film as used in traditional SPR.

OpenSPR uses localized surface plasmon resonance (LSPR). LSPR produces a strong resonance absorbance peak in the visible range of light, with it's position being highly sensitive to the local refractive index surrounding the particle. Therefore, OpenSPR measures small changes in the wavelength of the absorbance position, rather than a reflected angle as in traditional SPR.

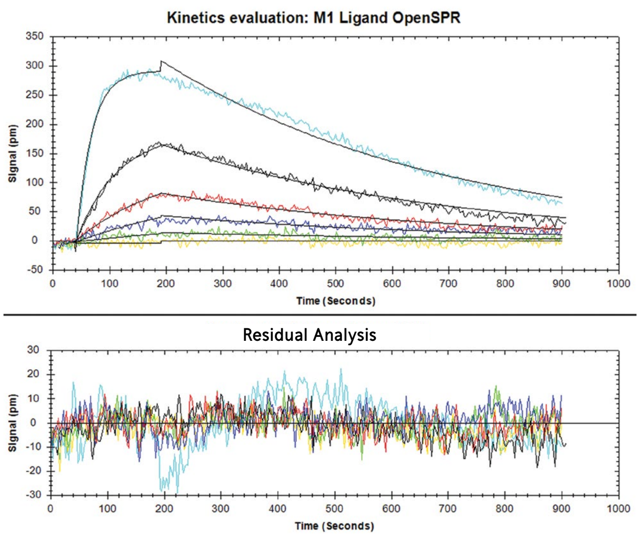
Advantages of Using LSPR Instead of SPR

- The optical hardware for LSPR is less complex, so the instrument is smaller and more affordable
- Since the angle is not important, the instrument is more robust against vibration and mechanical noise
- LSPR is not as sensitive to bulk sample effects, which causes errors in experimental data, because it has a much shorter electromagnetic field decay length
- No strict temperature control is needed
- The sensor chips can be manufactured at a much more affordable price
- Easier to use and maintain



APPLICATIONS

Protein-Protein

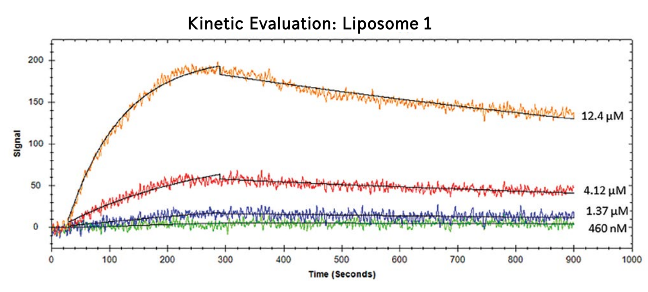


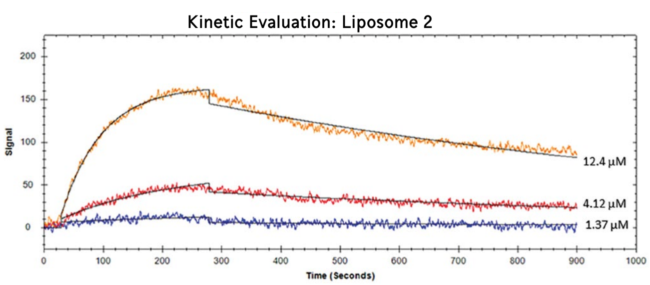
Ligand: Biotinylated protein on streptavidin sensor chip

Analyte: Mutant protein

k_{on} = 0.35x10^{6} M^{-1}s^{-1}
k_{off} = 2.0x10^{-3} s^{-1}
K_D = 5.7 nM

Protein-Lipid





Ligand: Liposomes formed with 2 different phospholipids, immobilized onto Liposome (LIP-1) Sensor Chips

Analyte: Proprietary protein

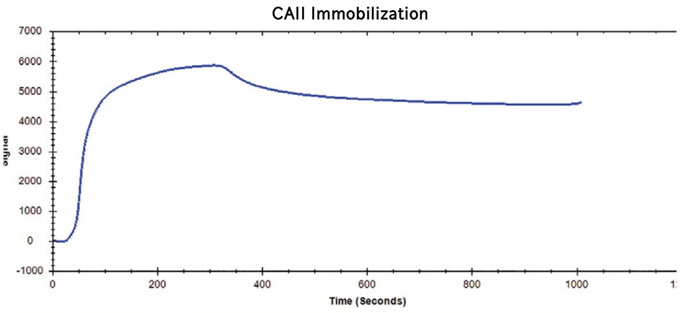
Liposome 1

k_{on} = 810 M^{-1}s^{-1}
k_{off} = 5.53x10^{-4} s^{-1}
K_D = 682 nM

Liposome 2

k_{on} = 1100 M^{-1}s^{-1}
k_{off} = 9.24x10^{-4} s^{-1}
K_D = 841 nM

Protein–Small Molecule



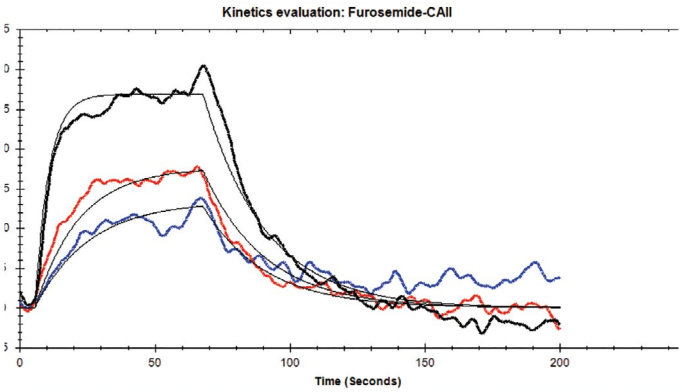
Ligand: CAII protein immobilized onto a High Sensitivity Carboxyl Sensor Chip

Analyte: Furosemide. (330 Da)

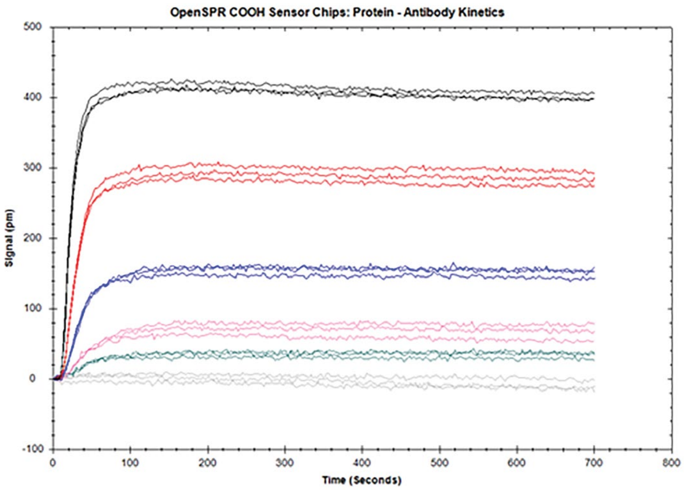
k_{on} = $1.42x10^{4}$ M^{-1} s^{-1}

k_{off} = $6.6x10^{-2}$ s^{-1}

K_D = 0.430 mM



Antibody–Antigen



Ligand: Anti-PSA immobilized onto Gold Sensor Chip with custom chemistry

Analyte: PSA

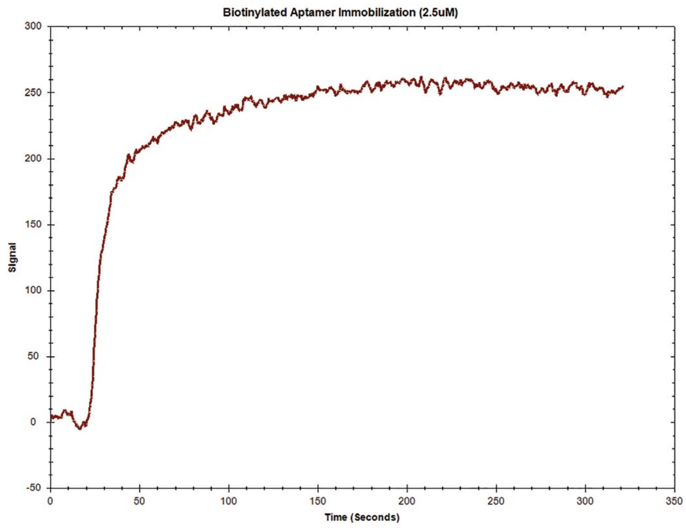
k_{on} = $4.0x10^{4}$ $M^{-1}s^{-1}$

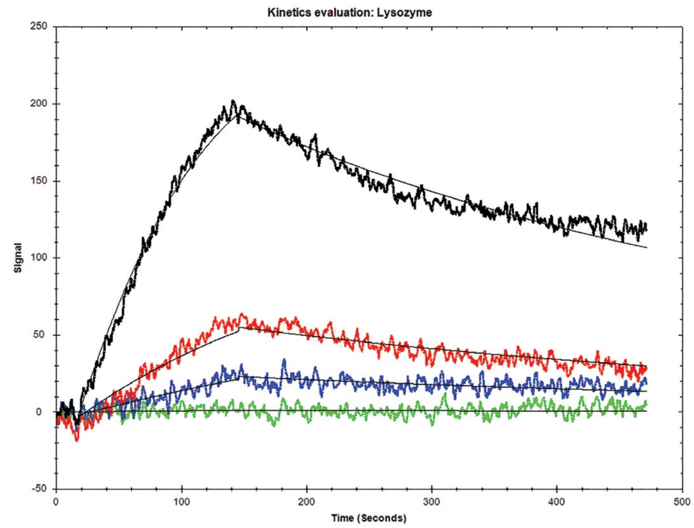
k_{off} = $1.8x10^{-6}$ s^{-1}

K_D = 4.5 nM

CV=3.2%

Protein–Aptamer



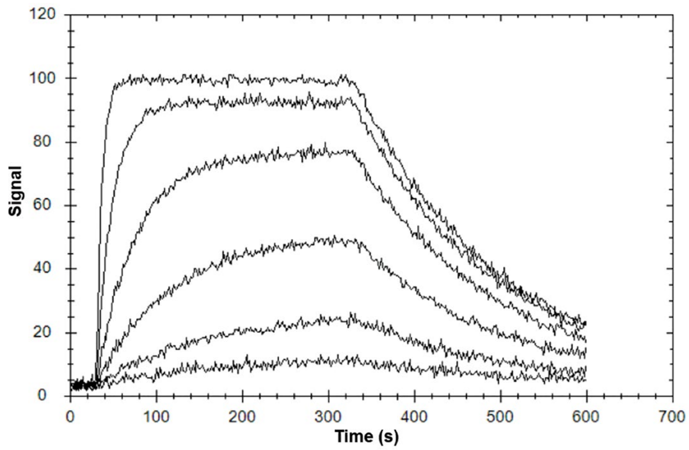


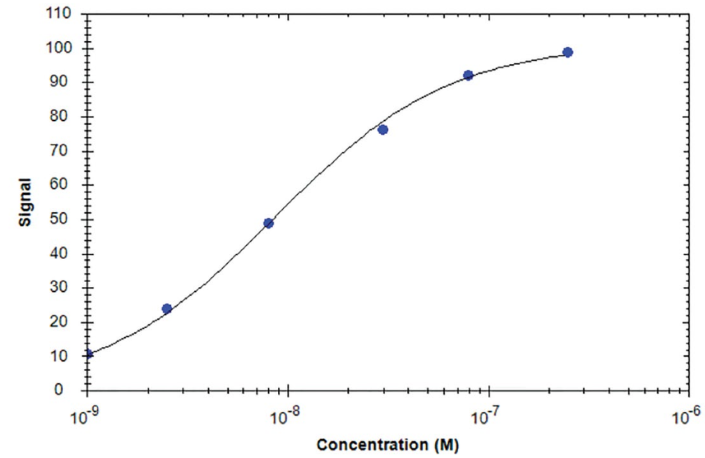
Ligand: Aptamer (biotinylated) immobilized onto Streptavidin Sensor Chip

Analyte: Lysozyme

k_{on} $= 1.8 \times 10^{5}\ M^{-1}s^{-1}$
k_{off} $= 2.6 \times 10^{-3}\ s^{-1}$
K_D $= 14.6$ nM

Concentration Determination





Generation of a standard curve equation can be used to determine concentration of an unknown sample.

Y $= R_{max}{*}c/(c+K_D)$ (1)
K_D : 8.72 nM
R_{max} : 101
Y = 101*c/(c+8.72 nM)

OPENSPR VS BIACORE

PROTEIN-PROTEIN BINDING KINETICS COMPARISON STUDY

SUMMARY

A protein-protein interaction was analyzed on the OpenSPR™ and Biacore™ T200™

The K_D was found to be 1.53 nM with OpenSPR™ and 0.686 nM with the Biacore™, confirming the accuracy of the OpenSPR instrument

Overview

OpenSPR™ is a powerful instrument providing in-depth label-free binding kinetics for a variety of different molecular interactions. In order to demonstrate the powerful capabilities and accuracy of the OpenSPR™, a side by side experiment was conducted against a Biacore T200™. The Biacore T200™ costs hundreds of thousands of dollars, but is considered a standard in the pharmaceutical industry. In order to show that OpenSPR is able to generate comparable results to the Biacore T200™ but at a fraction of the cost, a protein-protein interaction was analyzed using both instruments under similar conditions. The Biacore T200™ was run by a trained technician in a testing lab while the OpenSPR™ was run by a Nicoya scientist.

Materials and Equipment

- OpenSPR™ Instrument
- Nicoya Lifesciences Carboxyl Sensor Chip & Amine Coupling Kit
- TraceDrawer Kinetic Analysis Software
- Ligand Protein (MW 30 kDa)
- Analyte Protein (MW 15 kDa)
- Running Buffer: PBS + 150 mM NaCl + 0.05% Tween 20, pH 7.4
- Regeneration Buffer: 10 mM Glycine HCl pH 2.5
- Biacore T200™
- CM4 Sensor Chip
- Immobilization buffer: Sodium Acetate pH 5.25

Procedure

OpenSPR™ Experiment

1. Following the start-up procedure found in the OpenSPR™ manual, setup the OpenSPR™ instrument and load a Carboxyl Sensor Chip.
2. Following the instructions included in the Amine Coupling kit, activate the carboxyl surface with EDC and NHS.
3. Dilute ligand at concentration of 82.5 μg/ml into immobilization buffer, and inject 100 μL at 20 μL/min for a 5 minute interaction time.
4. Block the surface with an injection of 100 μL of blocking buffer.
5. Increase the flow rate to 30 μL/min, and inject analyte at the following concentrations: 6.25, 12.6, 25, and 50 nM. Use an association time of 150 seconds and a disassociation time of 400 seconds.
6. Regenerate the ligand with an injection of regeneration buffer at a flow rate of 150 μl/min in between each analyte injection.
7. Data was single referenced with blank buffer injections.

Biacore T200^{TM} Experiment

1. Follow the Biacore™ startup procedure, loading the CM4 sensor chip in the Instrument.

2 Activate the COOH surface with EDC & NHS.

3. Dilute ligand at concentration of 100 μg/ml into acetate buffer at pH 5.25, and inject 100 μL.

4. Block the surface with ethylenediamine.

5. Using the Single Cycle Kinetics mode, increase the flow rate to 45 μL/min, and inject analyte at the following concentrations: 6.25, 12.6, 25, and 50 nM. Use an association time of 100 seconds and a dissociation time of 100 seconds. No regeneration is needed with this mode.

6. Data was double referenced with blank injections and a deactivated reference channel.

Results and Discussion

Results from the protein-protein interaction measured on the OpenSPR™ instrument can be found in *Figure 1* and the Biacore™ instrument in *Figure 2*. Data was fit in each with a 1:1 binding model. Both data sets show excellent fits to the theoretical models with very low errors and Chi² values. The OpenSPR™ produced larger analyte signals due to the 4x higher amount of ligand density and higher sensitivity. The kinetic constants determined from the fit are shown in *Table 1*. The calculated on-rates are identical, while the off-rates differ by approximately 50%, which causes the K_D to differ by the same amount. OpenSPR™ determined a K_D of 1.53 nM while the Biacore™ determined a K_D of 0.686 nM. These are extremely close considering the Biacore™ experiment was done with a single cycle without regeneration and used a significantly shorter off rate period compared to the OpenSPR™.

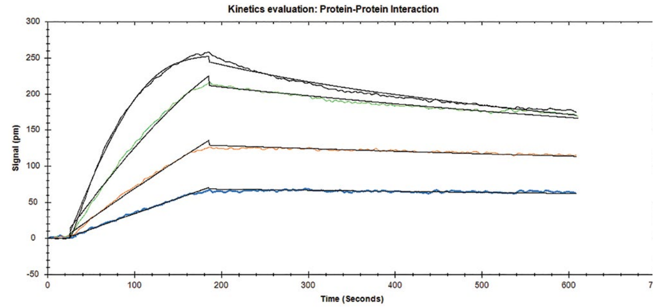


Figure 1. Protein-protein interaction analyzed using OpenSPR™ with analyte protein concentrations of 6.25, 12.6, 25, 50 nM

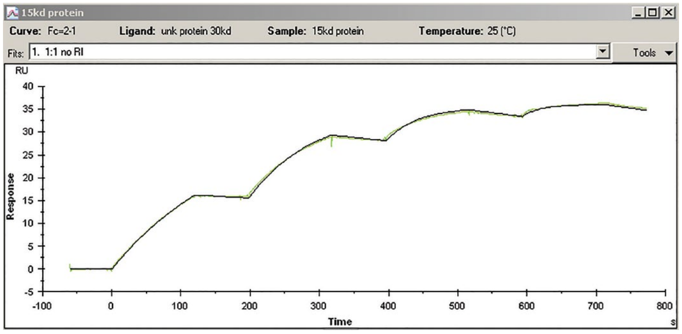


Figure 2. Protein-protein interaction analyzed using Biacore™ with analyte protein concentrations of 6.25, 12.6, 25, 50 nM

***Table 1.* Kinetic and affinity constants of protein-protein interaction measured on OpenSPR™ and Biacore™**

	OPENSPR™	BIACORE™
k_{on} [1/M*s]	8.18×10^5	8.18×10^5
k_{off} [1/s]	1.25×10^{-3}	5.61×10^{-4}
K_D [nM]	1.53	0.686

Conclusions and Summary

This study demonstrates that OpenSPR™ can generate kinetic and affinity data that is comparable to data that can be obtained from a Biacore™ T200 instrument

“We spent many hours trying to run our protein-protein interaction on the Biacore with no success. After running the same experiment on the OpenSPR, we immediately got the data we needed.”

– **DR. NING**
CEO, Kyinno Biotechnology