

# Antibody Screening and Characterization by Nanopore Optical Interferometry

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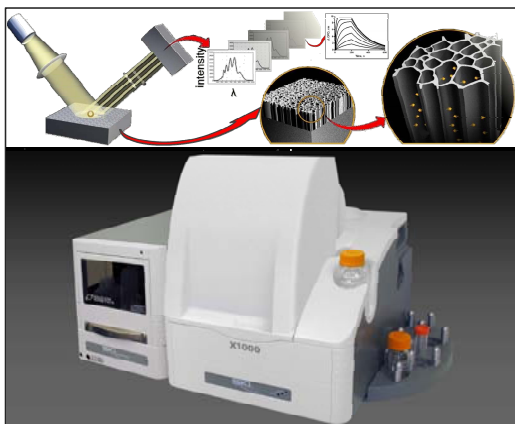
## Introduction



A leading application of biomolecular interaction analysis (BIA) technology is the characterization of antibody-antigen interactions. The desired capabilities of BIA instruments include both fast screening of primary clones and in-depth characterization of analytical and therapeutic antibodies including ability to measure ultra-high affinity interactions.

Described is a new technology for measuring biomolecular interactions, nanopore optical interferometry (NPOI). The unique characteristics of NPOI are that it is capable of using both multi-well plate and flow cell formats on a single platform and accurately characterize ultra-high affinity antibodies.

## Nanopore optical interferometry (NPOI)



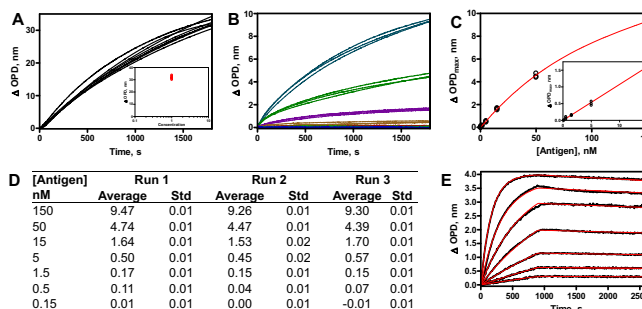
Nanopore optical interferometry utilizes a unique 3D nanoporous silicon biosurface with 80-100X the available surface area as that of the hydrogel biosurface utilized in SPR platforms. Reflected light from the porous silicon/fluid interface and reflected light from the bulk silicon/porous silicon interface are focused onto a diffraction grating from where emitted light is measured by a photo detector. Shifts in spectral properties as a result of protein binding are computed and translated into an optical path difference (OPD) shift, which is proportional to the mass of surface bound biomolecule. The instrument drift afforded by this design is three logs less than that of traditional SPR designs, enabling direct measurement of very slow off rate interactions. Unlike SPR, NPOI technology does not require a prism in physical contact with the chip. That allows SKI Pro instruments to acquire data in both flow cell and multi-well formats.

## Results and Discussion

### Screening Applications

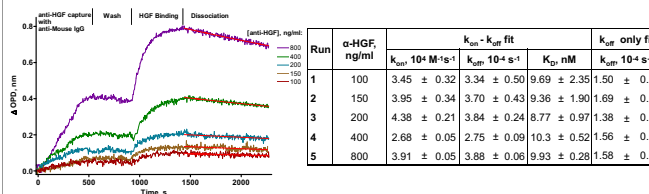
Multi-well plate reader capability of Silicon Kinetics instruments allows the use of multiple sample analysis in initial stages of antibody development. Typical applications include IgG quantitation and hybridoma clone selection by either full kinetic characterization or  $k_{off}$  ranking. The following experiments were carried out on a dual mode SKI Pro X1000 instrument in a plate reader configuration.

### Immunoglobulin capture and quantitation



**A:** An anti-mouse IgG antibody is immobilized on 8-well strips of SKI Sensor carboxyl biochips using amino-coupling (EDC/sulfo-NHS) chemistry. **B:** Half-log serial dilutions of the antigen at 0.15-150 nM concentrations were prepared in microplate wells. The binding-regeneration cycle was conducted 3 times in a single automated run. **C:** Maximum  $\Delta$ OPD values are used to generate a calibration curve. **D:** Statistical analysis of maximum  $\Delta$ OPD values shows lower limit of detection (LLOD) at 0.5 nM. **E:** Kinetic characterization of interaction between capture antibody and mouse IgG. Global fit with a two-state model was done using SKI Report software analysis function.  $k_{on} = 1.20 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{off} = 3.08 \times 10^{-5} \text{ s}^{-1}$  and  $K_D = 2.57 \times 10^{-9} \text{ M}$ .

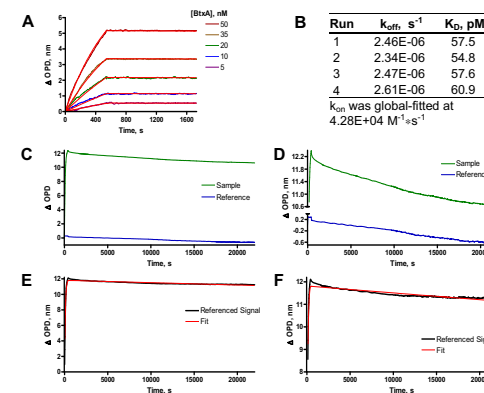
### Kinetic characterization of a monoclonal antibody captured from hybridoma growth medium



A monoclonal anti-Hepatocyte Growth Factor (HGF) antibody was diluted to indicated concentrations in the individual wells of a 96-well plate and captured on anti-mouse IgG pre-coated chips of an 8-well strip. Strip was transferred to wells with HGF at a fixed concentration and then to wells with buffer only to measure binding and dissociation kinetics respectively. The resulting sensograms were fit with a two-state model using SKI Report software analysis function. Kinetics of antigen binding were determined with high repeatability in a wide range of initial antibody contents.

## Characterization of High Affinity Antibodies

SKI Pro technology uses a silanized and PEGylated nanoporous silicon biosurface that is more stable than gold-carbohydrate surfaces used in SPR and allows for the industry's lowest signal drift of <0.1 nm/hr equivalent to <5 RU/hr). In addition, SKI Pro instruments are equipped with two continuous-flow pumps that enables smooth data acquisition without artifacts caused by syringe pump switchover and restroke.



An anti-botulinum neurotoxin A antibody was immobilized on a sample channel of a carboxyl SKI Sensor™ flow cartridge. An isotype-matched control antibody was immobilized on a reference channel. The results of a multi-concentration run with short dissociations (A) were used to global fit on-rate. No measurable dissociation was observed in 15 minutes. Experiments with long 6 hour dissociations were performed in order to determine off-rates (C-F). The results of four separate experiments (B) demonstrate that Silicon Kinetics instruments can be used to accurately and reproducibly measure dissociation rates in low  $10^{-6} \text{ s}^{-1}$  range.

Several high affinity binding pairs were characterized in similar experiments. Some representative results are listed in the table below:

Antigen	Antibody Type	Immobilized Partner	$k_{on} \cdot \text{M}^{-1} \text{ s}^{-1}$	$k_{off} \cdot \text{s}^{-1}$	$K_D \cdot \text{pM}$
*Botulinum neurotoxin A	Modified human IgA	Antibody	4.28E+04	2.47E-06	57.7
Anthrax protective antigen	Modified human IgA	Antibody	1.93E+04	1.63E-05	845
Staphylococcal enterotoxin	Single chain Fv	Antigen	5.61E+04	5.90E-06	105
Human Angiotensin-2	Humanized IgG	Antibody	8.64E+04	1.10E-05	128
Human Cytokine	Humanized IgG	Antigen	1.58E+05	1.70E-06	10.8
Human Cytokine in 50% Serum		Antigen	1.58E+05	3.88E-06	24.5
Protein	DNA Aptamer	Aptamer	9.93E+04	5.09E-06	53.6

\*Kinetic parameters shown are average of 4 runs

## Summary

- Nanopore optical interferometry is a versatile and powerful label-free method to measure protein-protein interactions.
- Dual mode NPOI-based instruments can be used for fast screening of multiple samples in initial stages of antibody development in a multi-well plate reader configuration and for in-depth lead characterization in a flow cell mode.
- The results of experiments presented here show lower limit of immunoglobulin detection (LLOD) at 0.5 nM. This sensitivity is sufficient for antibody capture and analysis for even low producing hybridoma clones.
- NPOI enables experiments with long dissociation times for characterization of high affinity antibodies. NPOI-based Silicon Kinetic instruments are successfully used to accurately measure  $k_{off}$  rates in  $10^{-6} \text{ s}^{-1}$  range.