Benchmarking Nanopore Optical Interferometry (NPOI) against SPR

Summary

The Silicon Kinetics SKi Pro system advances a new technology for the measurement of biomolecular interactions: Nanopore Optical Interferometry (NPOI). At the core of NPOI is a nanoporous silicon biosurface that provides a heavily porous, three dimensional surface for increased ligand immobilization density and analyte capture compared to much older Surface Plasmon Resonance (SPR) approaches, thereby promising improved sensitivity over conventional biomolecular interaction analysis technologies. Here a variety of archetypical systems will be characterized using both NPOI and SPR in order to benchmark quantitatively determined NPOI results against those of SPR. It is shown that the measurements correlate at 0.86 for k_{on}, 0.9999 for $k_{off}$and 0.99999 for K_D.

Introduction

NPOI differs from SPR in two important ways, as it uses a different substrate and a different read out mechanism. As compared to the hydrogel biosurfaces used in planar SPR substrates, the nanoporous silicon biosurface of NPOI is approximately 10 times as deep and offers at least 100 times the surface area as in a typical SPR hydrogel (Figure 1). This approach puts more analyte within the area of the substrate being optically interrogated.

NPOI replaces the SPR based optical interrogation scheme with white light interferometry[1], so does not require the use of a prism in its optical design. Absent this constraint, the SKi Pro system is able to analyze interactions in flow cells *and* multi-well plate formats. The SPR instrument's optical design absolutely requires a prism in close proximity to the slide substrate, limiting the versatility of this technology. It is this reason, why SPR cannot be used directly as an economical plate reading technology several decades after its invention, whereas NPOI can.

SKi Pro can measure biomolecular interaction analyses in up to 24 individual, self-referenced (i.e. differential) flow cells, and can measure interactions directly in 96 well, SBS format microtiter plates. That is, cost economical configurations of SKi Pro exist that have no fluidics, allowing a wellplate, from e.g. an offline liquid handler, to be placed directly on the system for label-free binding studies. Data provided by SKi Pro includes the on rate (k_{on}), off rate (k_{off}) and affinity (K_D), measured in the flow cell format, and concentration, yes/no, and affinity measured in the well plate format. SKi Pro is a modular instrument system, easily upgradeable from a manual plate reader model to a totally automated 24 flow cell/plate reader dual use system.

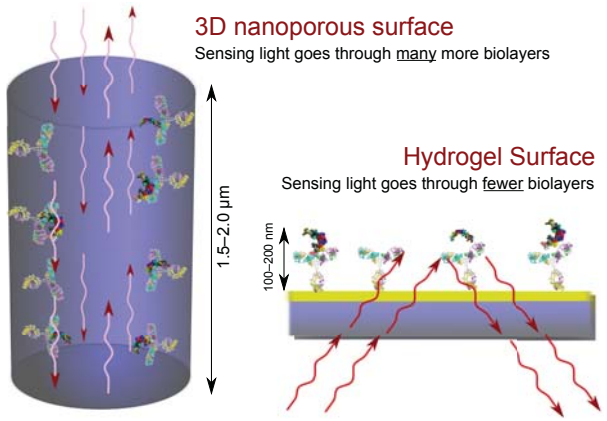

Figure 1: Nanopore optical interferometry analyzes a 1.5–2.0 μm deep biosurface, measuring biomolecular interactions in multiple biolayers. In traditional SPR, the required surface plasmon extends ≈300 nm from the gold surface of the SPR substrate placing constraints on the biosurface area available for analysis.[2]

Table 1: Features comparison of SKi Pro versus commercial SPR platforms. SKi Pro offers greater ligand immobilization, far greater analyte capture capacity, better drift and more versatility than SPR.

Feature	NPOI	SPR
Data	k_{on}, k_{off}, K_D, concentration, & yes/no	k_{on}, k_{off}, K_D
Surface Chemistries	carboxyl, NTA, streptavidin benzaldehyde, Protein A & Protein G	carboxyl, NTA, streptavidin
Biosurface	nanoporous silicon	hydrogel
Depth	1.5–2.0 μm	100–200 nm
Flow cells	1–24	1, 2, 4 or 6
Well Strips	1×8	NA
Drift	0.35 RU/h	18–60 RU/h
Modularity	Yes	No
Capture Capacity	100 ng	0.5 ng

Additionally, SKi Pro offers an unmatched array of chip options compared to other biomolecular interaction analysis platforms (see Table 1). Both flow cells and well strips come with many useful surface chemistries including carboxyl, streptavidin, Protein A, Protein G, NTA and a novel benzaldehyde moiety[3]. How the system performs on some well known assays, for which ample SPR data is available will be shown below.

Method

For benchmarking studies two protein/protein, two small molecule/protein and three antibody/antigen systems were studied. The details of these methods will be discussed in turn.

protein/protein

For p27 KID/CDK2 studies carboxyl chips were used with EDC/sNHS activation (400 mM/100 mM, in pH 6.0 MES buffer).[a] Immobilization of p27 KID was performed with 500 μg/mL of protein in pH 4.5 acetate, followed by ethanolamine blocking (pH 8.0, 1M). CDK2 analyte was introduced at several concentrations between 250–2,000 nM in 20 mM HEPES, pH 7.5/ 300 mM NaCl/ 5 mM DTT for 10 minute association. Following 10 minutes of free dissociation the chip was regenerated for 10 minutes using PBS with 1M NaCl followed by an injection of 50 mM DTT.[b]

Details of the Bcl-2/p53 plate reader and flow cell measurements are identical to those reported earlier.[4]

protein/small molecule

Carbonic anhydrase II (CAII) from bovine erythrocytes was purchased and used as is from Sigma (St.Louis, MO). Carboxyl chips were activated with EDC/sNHS (200 mM/50 mM in water) and CAII was immobilized at 200 μg/mL in pH 6.0 acetate buffer. Furosemide and sulpiride were used as is from Sigma and were introduced in PBS with 0.0005% TWEEN running buffer at concentrations from 0.03–5,000 μM.

antibody/antigen

Biotinylated anti-GFP antibody (aGFP) was used as is from Santa Cruz Biotechnology (Santa Cruz, CA). This was immobilized on streptavidin chips in pH 4.5 acetate at 1 mg/mL, giving 20 nm (1 ng/mm^2) of aGFP surface coverage. Green fluorescent protein (GFP) acquired from Sigma, was reacted with the antibody surface from 0.15–150 nM in PBS. Between injections the surface was regenerated using 60 seconds of 1M, pH 10.5 ethanolamine solution.

The aTSH/TSH system was studied using carboxyl chips.[c] Rabbit anti-mouse capture (RAMC) protein was immobilized on an activated chip at 100 μg/mL followed by ethanolamine blocking. Mouse anti-TSH antibody (aTSH) was interacted with RAMC at 50 μg/mL in PBS,

[a]These proteins were kindly provided by Dr. Richard Kriwacki of the St. Jude Children's Research Hospital, Memphis, TN.
[b]This work was performed in the laboratories of Professor Ian de Belle at the Centre de Recherche du CHUL, Québec, Québec Canada.
[c]This work was performed in the laboratories of SAIC-Frederick by Lakshman Bindu.

giving a stable 6.5 nm surface. Thyroid Stimulating Hormone (TSH) was introduced at concentrations from 5–200 nm without allowing complete dissociation and without regeneration. Data were analyzed using the kinetic titration approach.

Conditions for hIgG/ahIgG measurements have been previously published.[5]

The results for the measurements and analyses are summarized in Table 2. As the molecules studied here all generally well known, essentially similar results are expected with NPOI and SPR.

Table 2: Binding affinity and kinetic characterization data obtained with SKi Pro compared to referenced commercial SPR platform obtained data. SKi Pro delivers at equivalent data on archetypical systems, and exhibits less matrix effect artifact in rat plasma (RP). SKi Pro measurements are from the flow cell configuration with the exception of one set of Bcl-2/p53 measurements which are from the plate reader configuration (PR). Units of k_{on} are everywhere $sec^{-1}M^{-1}$, units of k_{off} are sec^{-1} while K_D is presented in nM.

System	NPOI	SPR
p27 KID/CDK2[6]	$k_{on} = 2.3(4) \times 10^3$	$k_{on} = 2.6(2) \times 10^3$
	$k_{off} = 4.1(8) \times 10^{-4}$	$k_{off} = 3.8(9) \times 10^{-4}$
	$K_D = 190$	$K_D = 150$
Bcl-2/p53[7]	$K_D = 490$	$K_D = 540$
Bcl-2/p53 (PR)	$K_D = 680$	$K_D = 540$
CAII/furosemide[8]	$k_{on} = 1.8 \times 10^4$	$k_{on} = 9.6 \times 10^4$
	$k_{off} = 1.0 \times 10^{-2}$	$k_{off} = 5.0 \times 10^{-2}$
	$K_D = 550$	$K_D = 510$
CAII/sulpiride[8]	$k_{on} = 3.9 \times 10^2$	$k_{on} = 3.4 \times 10^3$
	$k_{off} = 0.14$	$k_{off} = 0.64$
	$K_D = 360,000$	$K_D = 190,000$
aGFP/GFP*	$k_{on} = 1.0 \times 10^5$	$k_{on} = 2.2 \times 10^6$
	$k_{off} = 1.1 \times 10^{-3}$	$k_{off} = 0.012$
	$K_D = 11$	$K_D = 5.4$
aTSH/TSH†	$k_{on} = 9.0 \times 10^4$	$k_{on} = 6.5 \times 10^5$
	$k_{off} = 1.1 \times 10^{-3}$	$k_{off} = 2.6 \times 10^{-3}$
	$K_D = 11(2)$	$K_D = 4(2)$
hIgG/ahIgG‡ (buf)	$k_{on} = 2.3 \times 10^3$	$k_{on} = 3.8 \times 10^3$
hIgG/ahIgG (RP)	$k_{on} = 2.6 \times 10^3$	$k_{on} = 2.6 \times 10^3$
	$K_D = 600$	$K_D = 1,000$

*SPR data for this pair was taken by David Myszka at the center for biomolecular interaction analysis Salt Lake City, UT.

†This SPR value for this pair comes from unpublished results at SAIC, Frederick, MD.

‡This pair was measured with identical samples using SKi Pro and a BiaCore 3000 at UCSD.

Discussion

Two systematic differences in the data can generally be seen. First, the k_{on} measured by NPOI tends to be slower for all systems studied. SPR surfaces are often designed to attract molecules using electrostatic interactions (the so called pre-concentration effect). This is used to advantage when immobilizing molecules, however is highly pH dependent. NPOI surfaces are not designed to be as heavily charged as those for SPR so do not have this preconcentration effect.

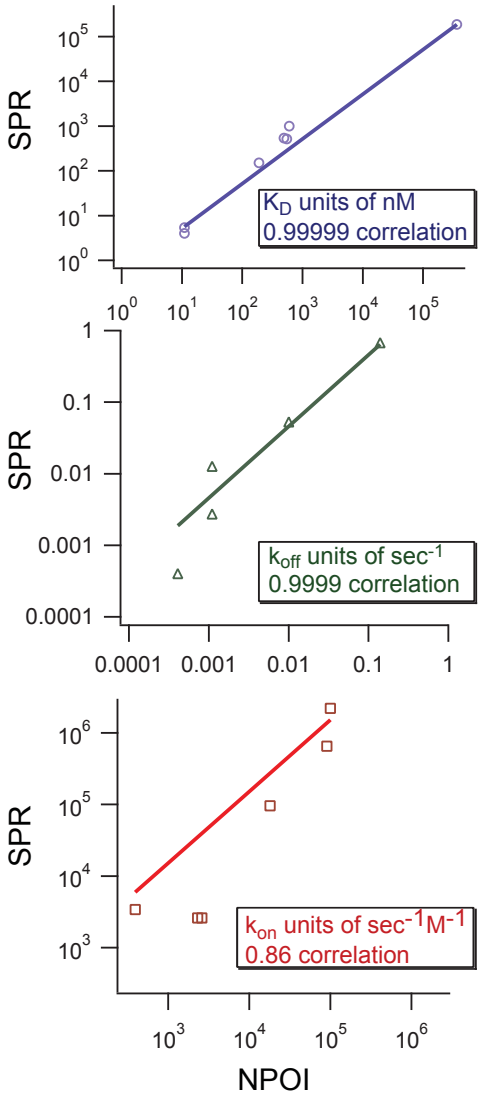

Figure 2: Comparison of NPOI and SPR data.

The second effect is the apparent immunity to spurious matrix affects seen in the hIgG/ahIgG data. Here the k_{on} values measured with and without rat plasma are quite similar for NPOI but differ for SPR. In fact the k_{on} for SPR in rat plasma approaches the k_{on} for NPOI without rat plasma. This can be explained by

the SPR chips having a variety of non-specific binding sites. In the presence of rat plasma these sites fill allowing the true rate to be seen.

But the key feature of this data set is the high correlation between the values measured by the two approaches. The data for the parameters measured were plotted against each other and a linear fit, constrained to pass through zero, was performed and the correlations between the sets of values were calculated. As shown in Figure 2, these correlations are everywhere quite high.

Given the wide range of systems studied and the high correlations seen, NPOI is shown to be a more than viable alternative to SPR for the accurate quantification of biomolecular interactions.

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