

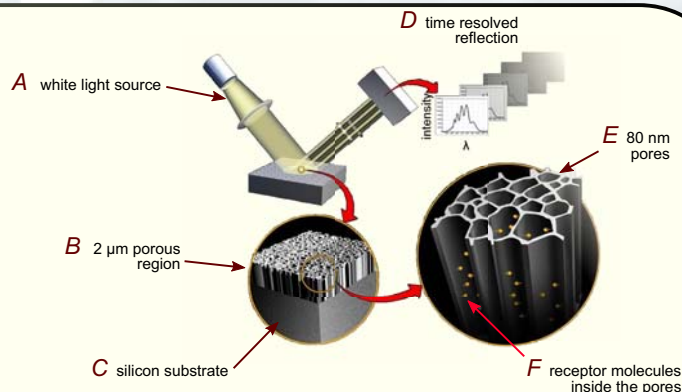
Nanopore Optical Interferometry (NPOI) Used to Characterize Antibody-Antigen Interactions



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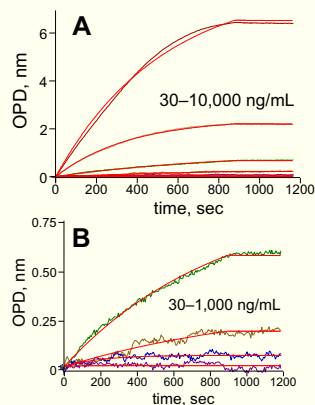
Summary

A new technique which allows protein interactions to be studied without labels, nanopore optical interferometry (NPOI), is introduced. In NPOI biochips are formed by etching 80 nm wide, 1,500 nm deep parallel pores into the face of a silicon wafer. For measurement, a white light probe passes through these light transmitting pores and interacts with the length of a protein monolayer attached to the pore walls. This creates an interference pattern, detected by reflection, which tracks the biomolecular binding signal in real time. By using a porous structure, the sensitivity of the technique is enhanced as compared to planar approaches, while the use of white light interferometry as the probe allows for wide instrument configurability. To date the technique has been used to study a wide range of protein/protein, protein/peptide, and protein/DNA interactions in order to quantify the affinity and kinetics of these. Here the technique will be introduced, and a variety of antibody/antigen interactions studied by the sensorgram and kinetic titration techniques will be described.

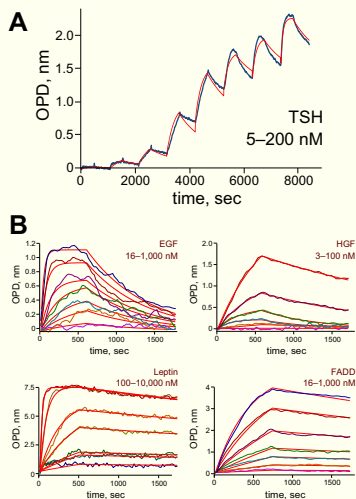


Results

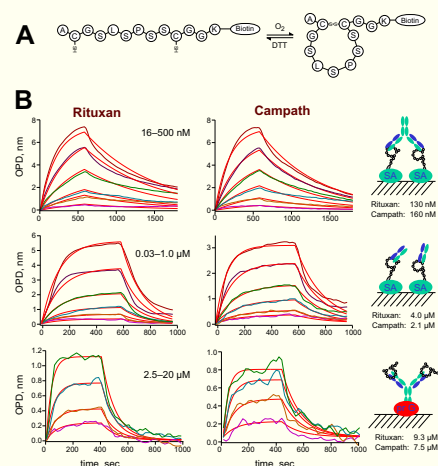
Antibody Capture from Hybridoma Media



Kinetic Characterization



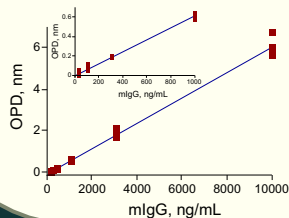
Antibody/Peptide Interactions



Antibody Capture from Hybridoma Media

NPOI is generally quite insensitive to non-specific binding due to the nature of the porous silicon surface. Here it is demonstrated that IgG can be effectively recruited from hybridoma media at concentrations lower than 100 ng/mL.

SKi Sensor™ carboxyl chips are used to prepare Fc specific anti-mouse IgG surfaces at 30 nm of coverage (1.5 ng/mm²). A concentration series from 30–10,000 ng/mL of mouse IgG in Hybri-Max Medium (Sigma-Aldrich, St. Louis, MO) supplemented with Pen-Strep and 0.1% BSA was introduced from low to high twice on two different sensor chips for a total of four runs at each concentration. The total data set is shown in **A** while the lower concentrations runs are shown in **B**. The a solid line, representing a single exponential rise, is also shown to guide the eye. The calibration curve is shown below.



Kinetic Characterization

	$k_{on}, M^{-1} sec^{-1}$	k_{off}, sec^{-1}	K_D, nM
a-TSH	9.0×10^4	1.1×10^{-3}	12
a-HGF	9.5×10^3	4.5×10^{-4}	48
a-EGF	2.6×10^4	1.3×10^{-3}	51
a-Leptin	3.9×10^3	1.3×10^{-4}	33
a-FADD	2.1×10^3	1.7×10^{-4}	80

For characterizing the affinity and kinetic constants for an antibody/antigen interaction NPOI flow cells may be used with either the kinetic titration (**A**) or sensorgram (**B**) methods. In the case of TSH† Rabbit anti-mouse antibody is used to capture anti-TSH and varying concentrations of TSH are introduced *without* complete dissociation. In the case of anti-Leptin the antibody is covalently immobilized using amino coupling to carboxyl chips. For the other three antibodies protein G chips are used for antibody capture, followed by BS3 Ab/protein G cross linking.

For the four antibodies in **B** concentration series are introduced and dissociation is allowed to go to completion. For EGF, a 6,000 Da antigen, a detection limit of 6 ng/mL was determined (z -factor = 0.45). This is a concentration 50× lower than the affinity.

†We would like to acknowledge and thank Lakshman K. Bindu at SAIC Frederick for performing this work.

Antibody/Peptide Interactions

Two, ca. 900 Da, peptides were selected by phage display to bind either Rituxan or Campath antibodies.† These peptides exist in equilibrium between a closed oxidized form (**A**) and an open reduced form. Only the oxidized form actively recognizes the peptides. The goal of the work was to both characterize the affinity of the interactions, as well as show whether the peptide/Ab interaction was through the Fab region.

The whole Ab interacting with the peptides has a much higher affinity than in the other two cases due to an avidity affect (**B**), namely that the Ab can bind to two separate sites on the surface.

	$k_{on}, M^{-1} sec^{-1}$	k_{off}, sec^{-1}	$K_D, \mu M$
Rituxan			
peptide/Ab	7.5×10^3	9.8×10^{-4}	0.13
peptide/Fab	1.6×10^3	6.5×10^{-3}	4.0
Ab/peptide	7.7×10^2	7.1×10^{-3}	9.3
Campath			
peptide/Ab	8.1×10^3	1.3×10^{-3}	0.16
peptide/Fab	2.5×10^3	5.1×10^{-3}	2.1
Ab/peptide	1.0×10^3	7.6×10^{-3}	7.5

†The selection of the peptides, creation of antibody Fabs, and supply of the whole antibodies was work done by Bradley Messmer and Ana Sanchez at the Moores Cancer Center at UCSD.

Discussion