mAb avidity/affinity measurement using oblique-incidence reflectivity difference method

A. Introduction

Oblique-incidence reflectivity difference (OIRD) as a recently developed biosensor compatible with microarray technology can enable monitoring the kinetics of biomolecular interactions in a label-free and high-throughput manner [1-6]. In principle, OIRD method measures the fractional difference between the reflectivity of the p- and s-polarized lights from a surface at oblique incidence, termed as "$\Delta_p - \Delta_s$," and can acquire two signals consisting of an imaginary part $Im\{\Delta_p - \Delta_s\}$ and a real part $Re\{\Delta_p - \Delta_s\}$, which provide us with phase and amplitude information of the "$\Delta p - \Delta s$". Based on the common functional slide, the $Im\{\Delta p - \Delta s\}$ signal is applied for detection in our experiment because of its higher sensitivity than $Re\{\Delta p - \Delta s\}$. A small change in thickness or mass density of the molecular layer leads to changes of reflectivity of the p- and s-polarized lights which enables OIRD method a high sensitivity optical biosensor [7].

Here, we will introduce the specific binding of antigen-monoclonal antibody (mAb) and their affinity measurement applied by OIRD method.

B. Materials

1. Antigen microarray

Antigen proteins (0.5 mg/mL) were first arrayed in 384-well titer dish and printed onto an epoxy-grafted glass slide (ArrayIt, Inc.) to form three identical sub-arrays using a microarrayer. The format of the sub-arrays fits the design of a flow-cell containing multiple chambers that allows for parallel kinetics measurements in multiple reactions.

2. Monoclonal antibodies

CDI mAbs were diluted in 1xPBS to different concentrations and stored at 4 C until use.

3. Binding Buffer

1xPBS (phosphate-buffered saline)
1xPBST (1‰TWEEN20 in 1xPBS)
1% BSA in 1XPBS
These buffers are stored at room temperature with the exception of the 1% BSA which is stored at 4℃.

4. Microarrayer

We used VersArray ChipWriter™ Pro systems (Bio-Rad, USA) for antigen array fabrication.

5. Flow-cell

Home-made flow-cells were designed for the OIRD system that allows simultaneous measurements of four binding reactions in different chambers. Each chamber is in a 8x10x1 mm dimension and manipulated by a peristaltic pump, which controls the flow rate and direction in the chamber.

C. Methods:

1. Antigen array fabrication

The antigen proteins and negative control BSA are diluted to a final concentration of 0.5 mg/mL in 1x PBS with 30% glycerol and 0.03% Triton in a 384-well titer dish and then printed by a contact-printing machine on epoxy or aldehyde glass slide (**See note 1**). After printing, the antigen microarray is incubated at 4 C overnight to ensure firm attachment to the glass surface.

2. OIRD measurement:

2.1. Assemble the flow-cell onto the antigen array and scan with OIRD system. The OIRD signals were collected and compiled with LabVIEW (NI, USA) to create a first 2-D image of OIRD intensity after printing (**See note 2**).

2.2. Block the antigen slide by incubating with 1 mg/mL BSA in 1xPBS for 1 hr at RT.

2.3. Sequentially pump 1xPBS and 1xPBST buffers into the flow-cell to wash the antigen slide for 10 min at a flow rate of 1 mL/min, followed by acquiring a second 2-D image OIRD image.

2.4. Scanned across the centerlines of the printing spots on the antigen array using the 632.8 nm laser beam to confirm the coordinate of the starting point and to give the real-time scanning program a 5 min baseline scan (**See note 3**).

2.5. Pump CDI mAbs at different concentrations into their respective reaction chambers in the flow cell, while the scanning program records binding event to generate on-curves (**See note 4**).

2.6. After the OIRD signals (i.e., on-curves) reach saturation, pump 1xPBS into the flow-cell at a flow rate of 200 μL/min (**See note 5**), while collecting the OIRD signals that generated the off-curves. To obtain a better off-curve, a 2 times of on-curve scanning time is taken.

2.7. Obtain a final OIRD 2D image by the third 2D scanning program after the off-curve measurement.

3. Analysis of OIRD sensorgrams

3.1. Subtract the second 2D image from the third 2D image after the off-curve scanning to generate the net 2D image using the LabVIEW program. The net 2D image can be used to deduce the OIRD signal change on the antigen sample. Given that mAbs are usually of high affinity, the off-curves are minimal.

3.2. Extract the on- and off-curves from LabVIEW into OriginPro 9.0 (OriginLab,

USA).

3.3. Fit the simulated curves with the observed on- and off-curves using the OriginPro program to calculate the k_{on} and k_{off} values, respectively. See note 7 for additional details regarding fitting models (**See note 6**).

3.4. Deduce the K_D value by taking the k_{off} / k_{on} ratio. The average K_D and relevant standards are calculated from the dilutions of monoclonal antibody used in the flow cell chambers.

D. Notes

1. To maximize the OIRD signals, the printing concentration should be saturated at the printing area. For proteins and DNA (~10 – 50 kD), 0.1-0.5 mg/mL is sufficient. Sub-arrays are printed to be compatible with the flow-cell construction.
2. The scanning program related with a stepping motor code in LABVIEW is easy to learn. The 2D image after printing will point out the start scanning coordinate for the real-time procedure.
3. The baseline and the next on-curve data are defined by the subtraction of average pixels (10-20) of the unprinted region adjacent the sample from average pixels (10-20) of the sample.
4. This scanning process of the printed spots is repeated every ten seconds. The reflective light intensity proportionately corresponding to Im $\{\Delta_p$-$\Delta_s\}$ signals is directly collected to obtain the on-curves of the binding events.
5. 200 μL/min is the empirical result based on the flow-cell and the peristaltic pump. Experimentally, we determined that the flow rate should be a double the rate beyond which the association-dissociation curves are affected for the flow-cell used in the present experiment.
6. One 1:1 Langmuir model is applied in the fitting method. This model displays the simplest situation of an interaction between Ligand (L) and immobilized Receptor (R). The 1:1 Langmuir model was developed by Irving Langmuir in 1916. The fitting method is used and developed in SPR and other real-time detection biosensors. [9, 10]

References

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