Biophysical Measurements for the Development of Monoclonal Antibody Therapeutics using the BiOptix 404pi SPR Instrument

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Introduction

The development of monoclonal antibody therapeutics and biosimilars require an understanding of the kinetics and affinity constants (K_D) of a variety of interactions at many stages in the development process. These values have important pharmacodynamic and pharmacokinetic implications. Typically, the affinity constant for antibody/antigen interactions falls within the picomolar to low nanomolar range which makes their accurate determination a difficult process. The BiOptix 404pi is a highly sensitive instrument that is capable of measuring various antibody interactions accurately. Here we present data related to the following:

1. Antibody/Fc receptor Interactions

The Fc domain of an antibody is involved in the immune effector pathway that allows for antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). Understanding the affinity of the Fc region for a Fc receptor can be crucial in therapeutic development. For example, an increased affinity can mean a more effective drug against cancer. The high quality data obtained by the 404pi is demonstrated with a human mAb/CD64 (FcRγ) interaction.

2. Slow Off-rate Antibody Interactions

As mentioned, antibody interactions can have slow off-rates (k_{off}) and thus low (tight) affinity (K_D) constants. The ability to measure these constants accurately is important. An anti-PSA/PSA system is used to demonstrate the ability of the 404pi to measure slow k_{off}.

3. Antibody Screening

During development, it is important to be able to screen antibodies quickly for their kinetics. Eight mAbs are screened against an antigen to yield kinetics for each one using the high throughput afforded by the 404pi.

Methods

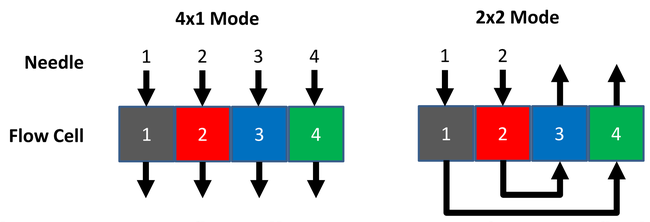


Figure 1. The BiOptix 404pi flow cell structure contains four microfluidic channels that can be controlled independently (4x1) or in referenced series (2x2) - both allowing for testing multiple solutions at one time.

All experiments were performed on a BiOptix CMD200m chip at 20°C. All ligand immobilization was performed with standard EDC/NHS chemistry and ethanolamine for deactivation. Reference surfaces were activated and blocked for kinetic assays. Analyte injections in each kinetic assay were performed randomly and in triplicate with at least 5 "warm-up" injections as well as buffer blank injections every 6th injection, unless otherwise noted.

Table 1. Assay details for the mAb/Fc receptor interaction and anti-PSA/PSA interaction.

Ligand	Analyte	Concentrations (nM)	Association Time (s)	Dissociation Time (s)	Regeneration	Running Buffer
Defucosylated mAb	CD64	16, 8.0, 4.0, 2.6, 1.3, 0.67	180	400	10 mM glycine, pH 1.7	PBST* + 100 µg/mL BSA
Fucosylated mAb	CD64	16, 8.0, 4.0, 2.6, 1.3, 0.67	180	400	10 mM glycine, pH 1.7	PBST* + 100 µg/mL BSA
Anti-PSA	PSA	590, 300, 150, 74, 37, 18, 0	90	90 and 3600	100 mM HCl	PBST* + 200 µg/mL BSA

*PBST = Phosphate Buffered Saline + 0.05% Tween-20

Antibody Screening. Approximately 8,000 RU of anti-mouse Fc was immobilized onto each flow cell of a BiOptix CMD200m sensorchip at 20°C. Each screening cycle was performed by capturing an antibody on flow cells 3 and 4, followed by a blank injection, an injection of analyte, regeneration with 10 mM glycine, pH 1.7, and a final blank injection (see figure 2).[1]

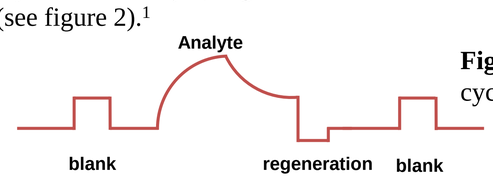


Figure 2. A sensorgram depicting a typical cycle for the antibody screen.

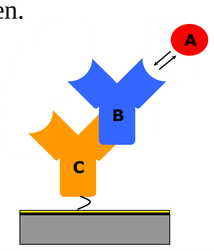


Figure 3. The orientation used for the antibody kinetic screen. "A" represents the antigen, "B" represents the mouse monoclonal antibodies of interest, and "C" represents the anti-mouse Fc antibody covalently immobilized to the sensorchip.

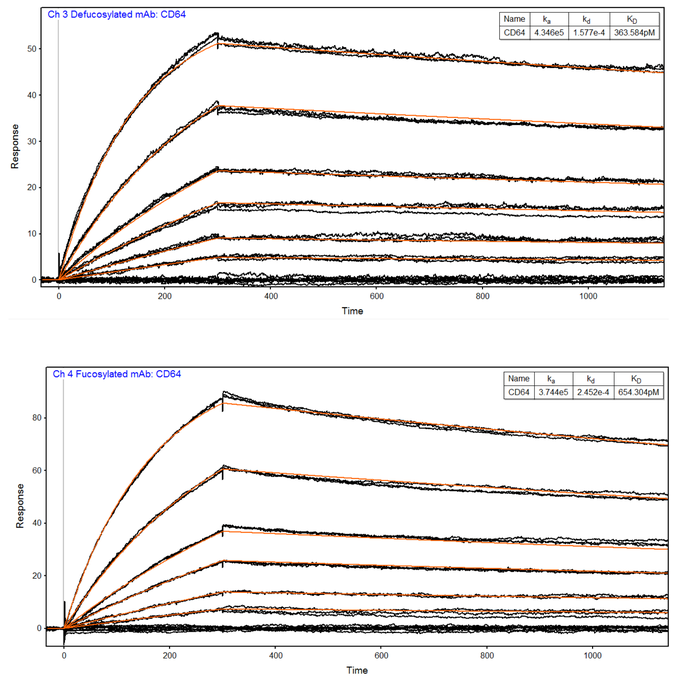
References

1. Canziani et al. (2004) Analytical Biochem, 325: 301-307.
2. Katsamba et al. (2006) Analytical Biochem, 352: 208-221.

1. Antibody/Fc Receptor

Figure 4. The effect of fucosylation on mAb binding to CD64 (FcRγ). Shown are sensorgrams of defucosylated human mAb (top) and fucosylated human mAb (bottom) binding to CD64 in a six point titration series. The randomized triplicate injections within each concentration (black sensorgrams) lie precisely on top of each other and the model (orange lines) provides an excellent fit to a 1:1 interaction model.

The BiOptix 404pi allows the user to determine minor differences between two similar antibodies. In this figure, the affinity values between the two antibodies vary by approximately a factor of 2. From a thermodynamic standpoint, the data suggests fucosylation adversely affects binding to the FcRγ receptor. However, the real-time nature of the biosensor allows the user to further distinguish the differences by evaluating their respective kinetic properties. This information can be invaluable when it comes to biosimilar development and future PK/PD analysis. (Note that this analysis is based on a single titration series and additional replicates are necessary to verify the differences).



2. Slow Off-Rate Interaction

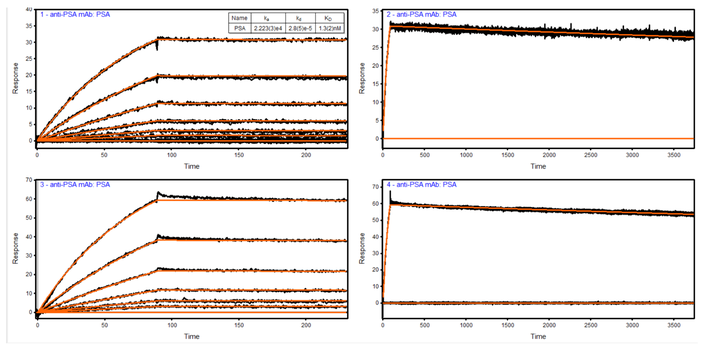
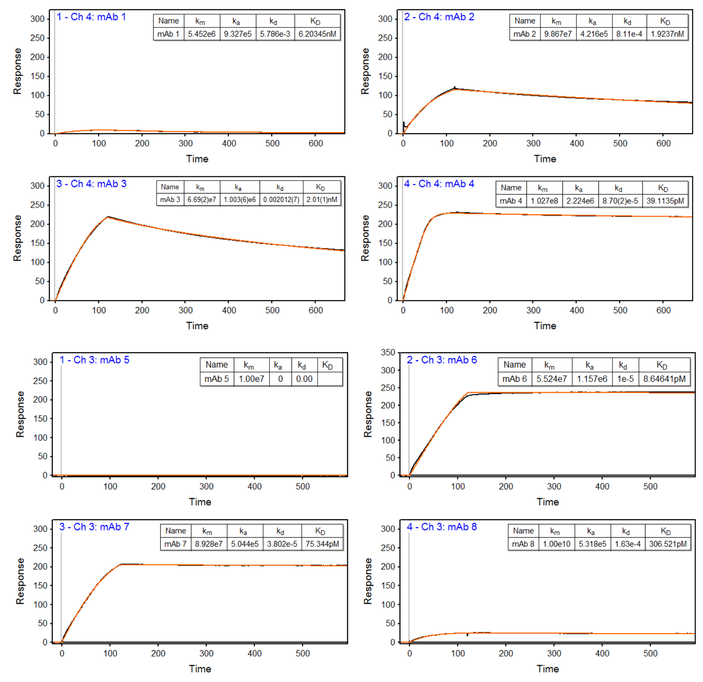


Figure 5. Anti-PSA/PSA interaction. (Left) Prostate-specific antigen (PSA) binding to anti-PSA monoclonal antibody (mAb) in a six point titration series. The data were fit globally from two different flow cells with different surface capacities of the anti-PSA mAb to a 1:1 interaction model. (Right) Triplicate injections of 590 nM PSA following dissociation for 3600 seconds allow for a more accurate estimate of the slow k_{off} observed for this interaction.

The data resulted in an off-rate (k_{off}) of 2.8 x 10^{-5} s^{-1} and an affinity (K_D) of 1.3 nM which were in good agreement with previously reported kinetic data.[2]

3. Antibody Screening

Figure 6. Screening data of eight different monoclonal antibodies (mAbs) against a particular antigen. This screen was performed using the 2x2 kinetic mode which allows for an estimation of the on-rate, off-rate, and affinity of each mAb. This screen results in an estimation because only a single antigen concentration is flowed over each mAb. Therefore, a semi-quantitative ranking of each mAb results. A more accurate on-rate, off-rate, and affinity can be attained, of course, with a full kinetic assay for the mAb of interest.



	Protein
Association/ Dissociation time (seconds)	90s/120s (Plus 120s capture and 2x 30s regeneration injections)
Wash after each injection	Buffer
Blanks	Every injection prior to analyte injection in the same cycle
Samples per day	96
Samples per 5 day work week	480

Table 2. Throughput of the BiOptix 404pi enhanced SPR instrument using a typical format for screening proteins such as antibodies. The values provided in the table demonstrate throughput using the 2x2 kinetic mode.