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Extending the throughput of Biacore 4000 biosensor to accelerate kinetic analysis of antibody-antigen interaction

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ABSTRACT

The surface plasmon resonance (SPR) biosensors are being routinely used in different stages of drug discovery and development. However, the lack of high throughput SPR biosensors continues to be a primary bottleneck for the rapid kinetic screening of large panels of monoclonal antibodies (mAbs). To further increase the throughput of the Biacore 4000 biosensor, we have developed three kinetic screening assays to characterize mAb-antigen interactions – (i) 16-mAb capture kinetic, (ii) single cycle kinetic (SCK), and (iii) parallel kinetic (PK). The performance of all three kinetic assays was evaluated by characterizing the binding of kinetically diverse human mAbs to four antigens with molecular weights of 14kD, 29kD, 38kD, and 48kD and binding affinities ranging from 130pM to 200 nM. The binding rate constants measured using all three kinetic assays were reproducible across multiple experiments and correlated with the values generated using the conventional 8-mAb capture kinetic assay on the Biacore 4000 ($R^2 > 0.94$). Moreover, the 16-mAb capture assay decreased experiment time and analyte consumption by 35% and 50%, respectively. This work illustrates the significance of the 16-mAb capture kinetic, SCK, and PK assays to increase the throughput of Biacore 4000 and to support rapid kinetic screening of mAbs.

Introduction

The research and development of therapeutic monoclonal antibodies (mAbs) has become a key focus for the treatment of different diseases. Diverse biophysical, biochemical, and functional assays are being developed to meet the current throughput needs of the biopharmaceutical industry. Kinetic screening of mAbs has become an integral screening technique of today's mAb discovery workflow [1]. Among the different biosensor technologies capable of measuring mAb binding affinities, the surface plasmon resonance (SPR) and the surface plasmon resonance imaging (SPRi) biosensors are the preferred platforms to measure the kinetics of a mAb binding to its target antigen [2,3]. However, the lack of high throughput SPR/SPRi biosensors capable of reliably characterizing mAb-antigen interactions continues to be a critical bottleneck in the discovery and development of biotherapeutics. The traditional serial flow SPR biosensors, such as Biacore 3000 and Biacore T200, have limited throughput and can only screen approximately 100 mAbs per day from crude conditioned media (CM) [4], whereas the parallel flow biosensors such as Biacore 4000 (formerly Biacore A100) and MASS-1 assist in the kinetic screening of approximately 400 mAbs per day [5,6]. Array based SPRi biosensors such as IBIS MX96, Horiba XelPleX, Horiba OpenPlex, Plexera PlexArray enable the design of multiplex assays and allow simultaneous characterization of several hundred molecular interactions, thus enabling rapid kinetic screening of mAbs [7–9]. Newer technology platforms such as Biacore 8k and MASS-2 are currently emerging to meet the increasing demand for higher throughput SPR/SPRi biosensors.

Apart from the development of new biosensor technologies, researchers are also developing new methods to enhance the throughput of existing biosensors for kinetic screening of mAbs [10–12]. Taking advantage of the crisscrossing flow path design of the Biorad's ProteOn XPR36 biosensor, Bravman et al. developed the “one-shot” kinetic assay which enables the measurement of six different ligands simultaneously binding to six analyte

Abbreviations: RT-LF, real time label free; SPR, surface plasmon resonance; SPRi, surface plasmon resonance imaging; mAbs, monoclonal antibodies; SCK, single cycle kinetic; PK, parallel kinetic; CM, conditioned media; k_a, association rate; k_d, dissociation rate; K_D, equilibrium dissociation constant; SICK, single injection cycle kinetic; HA, hydrodynamic addressing; CHO, Chinese Hamster Ovary.

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concentrations [10]. The 36-ligand array kinetic assay developed by Abdiche et al. allows simultaneous characterization of up to 36 analyte-antigen interactions; further expanding the throughput of ProteOn XPR36 [11]. The use of ELISA detection reagents resulted in 1000-fold increase in the sensitivity of bio-layer interferometry (BLI) biosensor and the BLI-ELISA assay developed by combining ELISA detection and BLI biosensor allowed high throughput and accurate measurement of solution based binding affinity of mAb [12]. In this study, we focused on increasing the throughput of the Biacore 4000 biosensor and the three new kinetic assays that were developed in this study were used to characterize of mAb-antigen interactions.

The Biacore 4000 is an array-based biosensor with four independent flow cells (labeled as Flow Cell-1 through Flow Cell-4). Each flow cell contains five detections spots (labeled Spot-1 through Spot-5) that can simultaneously measure binding events [6]. The hydrodynamic addressing (HA) technology of the Biacore 4000 allows sample injection over either the outside spots (Spot-1 or Spot-5) or both outside spot and its neighboring inside spot (Spots-1 and Spot-2 or Spots-4 and Spot-5), but fails to deliver samples over just the inside spots (Spot-2 or Spot-4). Therefore, even though the Biacore 4000 has 16 detection spots, it only allows the design of kinetic assays by simultaneously capturing up to eight mAbs per cycle (two mAbs per flow cell) [5,6]. Moreover, the Biacore 4000 restricts users to design newer and more advanced assays single cycle kinetic (SCK) and parallel kinetic (PK) assays that were developed to eliminate the need to regenerate the sensor surface. To address all the aforementioned challenges, we have developed three kinetic assays using different combinations of pre-existing injection commands to accelerate the kinetic analysis of mAb-antigen interactions on the Biacore 4000 biosensor: i) 16-mAb capture kinetic, (ii) SCK, and (iii) PK assays.

The 16-mAb capture kinetic assay enables simultaneous characterization of up to 16 unique mAb-antigen interactions (four mAbs per flow cell). Fig. 1 provides a detailed schematic of different steps required to design the 16-mAb capture kinetic assay on the Biacore 4000. Depending on the mAb under investigation, the entire sensor surface can be immobilized with either an anti-Fc or an anti-Fab capture molecule (Fig. 1; Step-1). First, mAb is captured on Spot-1 and Spot-2 and later on Spot-4 and Spot-5 (Fig. 1; Step-2 and Step-3), followed by the regeneration of mAb captured on the outside spots; Spot-1 and Spot-5 (Fig. 1; Step-4 and Step-5). Finally, mAbs are captured on Spot-1 and Spot-5 (Fig. 1; Step-6 and Step-7) to achieve a total of 16 mAb capture surface. In this assay, Spot-3 is used as an internal reference to correct for changes in bulk refractive index.

To test the throughput and performance of the 16-mAb capture, SCK, and PK assays, we selected a panel of 16 mAbs that bound to 14kD, 29kD, 38kD, and 48kD antigens (four mAbs per antigen) with varying binding kinetic profiles. All three assays were designed as capture kinetic assay, where the mAb was first captured using an anti-human Fc immobilized sensor surface followed by the injection of antigen titration series. The reliability and reproducibility of the association rate (k_a), dissociation rate (k_d), and equilibrium dissociation constant (K_D) generated using the 16-mAb capture, SCK, and PK assays were assessed. All three kinetic assays developed in this study have the potential to significantly increase the throughput of the Biacore 4000 biosensor for the characterization of mAb-antigen interactions, and in turn will be able to meet the throughput requirement for rapid selection of lead therapeutic mAbs.

Materials & methods

Reagents

Coupling reagents, N-(3-dimethylaminopropyl)-N-ethyl-carbodiimide (EDC) and N-hydroxysuccinimide (NHS), ethanolamine—HCl (Catalog# BR100633), anti-human Fc specific capture mAb (Catalog# BR-1008-39) and CM5 sensor chips (Catalog# BR-1005-30) were purchased from GE Healthcare. Sodium acetate, pH 5.0 was purchased from Ricca Chemical (Catalog# 61-1). HEPES (Teknova, Catalog# H1035), NaCl (Gibco, Catalog# 24740-011), EDTA (BDH, Catalog# BDH8730-1) and Tween-20® (Bio-Rad, Catalog# 161-0781) were used to prepare running buffer.

All recombinant proteins were expressed using stable Chinese Hamster Ovary (CHO) cell lines and purified in-house. The mAbs used in this study were fully human mAbs expressed with either human IgG1 or IgG4. The antigens used in the study are monomeric ecto-domain of the single transmembrane receptor expressed with a C-terminal myc-myc-6xHis tag. The antigens from CM were purified using the HisTalon superflow cartridge (Clontech) following the manufacture's protocol. Briefly, the CM containing antigen was passed through the HisTalon superflow cartridge to allow antigen binding to the resin. After washing the cartridge with 20 mM sodium phosphate buffer containing 5 mM Imidazole, 0.5 M NaCl, pH7.2, the column bound antigen was eluted using 20 mM sodium phosphate buffer containing 200 mM Imidazole, 0.5 M NaCl, pH7.2. The mAbs were purified using the Protein A Fast Flow HiTrap cartridge (GE Healthcare) following the manufacture's protocol. The resin bound mAb was later eluted using Pierce IgG elution buffer, pH2.8 and the elute was immediately neutralized to pH7.0 using 1 M Tris-HCl pH8.0. The purified mAbs and antigens were later dialyzed into PBS containing 5% Glycerol, pH7.2 and further purified using size exclusion chromatography in PBS containing 5% Glycerol, pH7.2 buffer. Finally, all the purified mAbs and antigens were aliquoted and stored at −80 °C until used for kinetic assay.

Immobilization of anti-human Fc mAb on Biacore 4000

The CM5 sensor surface was first immobilized with an anti-human Fc mAb using a standard protocol, reported previously [3]. Briefly, the entire sensor surface was first activated by injecting a 1:1 (v/v) mixture of 400 mM EDC and 100 mM NHS at a flow rate of 10 μL/min for 10 min. Monoclonal mouse anti-human Fc (10 μg/mL) prepared in 10 mM sodium acetate, pH5.0 was then injected at a flow rate of 10 μL/min for 10 min followed by the injection of 1 M ethanolamine, pH8.5 for 7 min at a flow rate of 10 μL/min. During the entire immobilization procedure, 10 mM sodium acetate, pH5.0 was used as the running buffer. Finally, the uncoupled residual protein was removed by treating the sensor surface with 10—12 s injection of 20 mM phosphoric acid, pH2.0, 8—10 times before performing kinetic assays.

Binding studies performed on the Biacore 4000

All of the binding studies were performed in freshly prepared, filtered and degassed running buffer containing 10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.05% Tween-20® (HBS-ET) at 25 °C. Before performing the binding studies, the CM5 sensor surface immobilized with anti-human Fc mAb was equilibrated with HBS-ET by priming the instrument at least three to four times. For the 8-mAb capture kinetic, PK and SCK assays, mAbs were first captured on both outside spot and its neighboring inside spot (Spot-1, Spot-