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Research paper

Measuring affinity constants of 1450 monoclonal antibodies to peptide targets with a microarray-based label-free assay platform

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ABSTRACT

Monoclonal antibodies (mAbs) are major reagents for research and clinical diagnosis. For their inherently high specificities to intended antigen targets and thus low toxicity in general, they are pursued as one of the major classes of new drugs. Yet binding properties of most monoclonal antibodies are not well characterized in terms of affinity constants and how they vary with presentations and/or conformational isomers of antigens, buffer compositions, and temperature. We here report a microarray-based label-free assay platform for high-throughput measurements of monoclonal antibody affinity constants to antigens immobilized on solid surfaces. Using this platform we measured affinity constants of over 1410 rabbit monoclonal antibodies and 46 mouse monoclonal antibodies to peptide targets that are immobilized through a terminal cysteine residue to a glass surface. The experimentally measured affinity constants vary from 10 pM to 200 pM with the median value at 66 pM. We compare the results obtained from the microarray-based platform with those from a benchmarking surface-plasmon-resonance-based (SPR) sensor (Biacore 3000).

1. Introduction

Highly adaptive structures in paratope regions of antibodies afford their specific recognition capabilities and thus enable them the primary defense against foreign pathogens in a living organism. This remarkable molecular attribute also makes antibodies the leading choice of reagents for diagnosis and extraction of biomarkers from samples in clinical laboratories and in laboratories of life/medical sciences. In recent years, monoclonal antibodies are actively and in some cases successfully explored as one of the major forms of biologic drugs, for inherently high target-specificity and in turn low required dosage to achieve same therapeutic efficacy (Beck et al., 2010; Nguyen et al., 2007; Weiner et al., 2010). Recent Ebola outbreaks in Africa and other parts of the world and the remarkable promise of combinations of monoclonal antibodies as an effective cure of infected patients highlight the importance of and urgent need for antibody-based drugs and antibody research in general (Qiu et al., 2014; Weingartl et al., 2012).

Despite the aforementioned, most monoclonal antibodies from commercial vendors and in academic laboratories are not well characterized, in terms of quantitative binding properties against specific and non-specific targets. It is a common and often costly experience that one finds monoclonal antibodies against same antigen target but from different vendors or from the same vendor but of different lots to yield significantly different outcomes in "identically" executed assays. There are extensive studies revealing that on average 50% of commercial antibodies do not produce expected binding results as advertised and the success rate varies from 0% to 100% for different vendors (Perkel, 2013). Even from the same lot, qualitative outcomes of antibody-antigen binding assays may vary from one type of assay to

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another; and from one laboratory to another. Some variations originate from changes in the paratope of the antibody that are often inadequately characterized. Others have to do with assay conditions, protocol details, and conformational presentations (denatured vs. natural form, free form vs. constrained form as a conjugate to a large carrier or as an integral part of a large protein) of antigen targets that can be understood and anticipated only if kinetic and thermodynamic information on antibody-antigen binding reactions are known even in limited circumstances, instead of merely IHC and Western Blot data or even less. The main reason that most antibodies are so insufficiently characterized and validated is the cost, in terms of materials, instrumentation, and skilled labor. There clearly is a need for cost-effective assay platforms that yield high-quality kinetic and thermodynamic parameters on antibody-antigen binding reactions and on how outcomes of the reactions may vary from assay to assay as protocols and conformational constraints on antigens change.

We report a microarray-based label-free assay platform that affords high-throughput cost-effective measurement of binding curves of antibodies to antigen targets (Fei et al., 2013, 2008; Landry et al., 2012; Zhu et al., 2007). We applied this platform to determine binding constants of 1410 rabbit monoclonal antibodies and 46 mouse monoclonal antibodies to synthetic peptide targets that are immobilized through a terminal cysteine residue on a functionalized glass slide surface. The results compare well with measurements using a benchmark (but low throughput) SPR-based label-free sensor (Biacore 3000). Furthermore we find that the measured binding constants do not change when the target density changes by more than a factor of 4 (comparable to the target density in the SPR measurement) so that the average target separation is twice the dimension of a captured antibody, indicating that the measured binding constants are affinity constants instead of avidity constants that would involve both paratopes of bivalent antibody molecules.

2. Methods and materials

The essence of the present assay platform is as follows. Antigen targets are immobilized on a functionalized glass slide in form of a microarray in such a way that epitopes on the targets are available to subsequent solution-phase antibodies. The antigen microarray is incubated in solutions of specific antibodies raised against the targets at a series of concentrations. Afterward the microarray is kept in a constant flow of the buffer to allow antibody-antigen complexes formed during incubation to dissociate. Surface mass densities of antibody-antigen complexes on the microarray during incubation and subsequent dissociation are recorded in real time with a scanning ellipsometry sensor (Landry et al., 2012). The sensor measures the phase change of an illuminating optical beam as a result of antibody-antigen complex formation. The phase change has been shown proportional to the surface mass density of antibody-antigen complexes. The optical data yield binding curves that are subsequently used to extract binding kinetic constants (Landry et al.,2012).

2.1. Peptide antigen microarray

1456 antigens are synthetic peptides (15-aa with average molecular weight of 2 kDa) supplied by Epitomics, Inc (Burlingame, CA). They originate from a large collection of source proteins (See Supplemental Information) that are mostly targeted in drug discovery. At either N- or C-terminus, a cysteine residue is added intentionally. The peptides are lyophilized as received. To make printing solutions, each peptide is dissolved in 2 μL DMSO and diluted further in 38 μL 1 × PBS to a final concentration of 0.25 mg/mL (~125 μM). The solutions are deposited in a 384-well plate for microarray fabrication. Peptide microarrays are printed on epoxy-functionalized glass slides (ArrayIt, Sunnyvale, CA) using an OmniGrid 100 contact-printing robot (Digilab, Holliston, MA) with 100 μm diameter stainless steel pins (Majer Precision Engineering, Tempe, AZ). Primary amine residues and thiol residues on the peptides react covalently with epoxide groups on the glass surface and anchor the peptides. A significant fraction of the peptides is immobilized through the terminal cysteine, making functional regions of these immobilized peptides intact and available in subsequent binding assays.

As shown in Fig. 1, on one functionalized glass slide, we print 6 identical peptide microarrays so that after assembled in a fluidic cartridge each microarray is housed in a separate reaction chamber (12 mm L × 6 mm W × 0.4 mm D). In the present study, each peptide microarray (Fig. 2(a)) consists of 4 identical subarrays as replicates, each having 100 distinct peptides and 20 control features in form of 8 rows × 15 columns. The left-most and the right-most columns consist of bovine serum albumin (BSA) and one blank. 100 peptides, 3 BSA and 1 blank form the remaining 13 columns (Fig. 2(b)). The diameters of printed peptide spots vary from 80 μm to 160 μm due to variations in wetting property of the peptide solutions. For the present study, 1456 peptides are printed on 16 slides. We use 4 out of 6 microarrays on a glass slide to acquire a set of binding curves. The remaining two microarrays are for back-up. Before binding curve measurements, the peptide microarray is washed and blocked with a solution of BSA at 2 mg/mL in 1 × PBS for 30 minutes and then washed and maintained in 1 × PBS (Fei et al., 2008).

2.2. Monoclonal antibodies (MAbs)

To produce monoclonal antibodies, these peptides are conjugated to carrier protein KLH through the terminal cysteine residue using the standard maleimide conjugation scheme and Sulfo-SMCC cross-linkers (for example, see http://www.piercenet.com/product/maleimide-activated-klh-kit.) The conjugates are then introduced into rabbit or mouse for production of monoclonal antibodies. Harvested monoclonal antibodies are screened using direct peptide coating ELISA and western blot at Epitomics, Inc (Burlingame, CA) for specific binding. For direct peptide coating ELISA, we coated the ELISA plates using same peptides with terminal cysteine residues, either directly or having the peptides first conjugated to BSA and then using BSA-conjugated peptides for coating. We then added rabbit hybridoma supernatants to the plates and the binding of rabbit antibodies to the peptides were detected by HRP conjugated goat anti-rabbit secondary antibody (Jackson Immunoresearch). 1410 rabbit mAbs and 46 mouse mAbs were