ELSEVIER

feature

High-throughput epitope binning of therapeutic monoclonal antibodies: why you need to bin the fridge

Q1 **Benjamin D. Brooks**[1,*], bbrooks@microfl.com, ben@brooks.nu, **Adam Miles**[1] **and Yasmina Abdiche**[2]

Analytical tools are evolving to meet the need for the higher-throughput characterization of therapeutic monoclonal antibodies. An antibody's epitope is arguably its most important property because it underpins its functional activity but, because epitope selection is innate, it remains an empirical process. Here, we focus on the emergence of label-free biosensors with throughput capabilities orders of magnitude higher than the previous state-of-the-art, which can facilitate large assays such as epitope binning so that they can be incorporated alongside functional activity screens, enabling the rapid identification of leads that exhibit unique and functional epitopes. In addition to streamlining the drug development process by saving time and cost, the information from epitope binning assays could provide the basis for intellectual property protection.

Introduction

The pharmaceutical industry is experiencing a 'biologics boom' with the increasing emergence of therapeutic monoclonal antibodies in the clinic. Although small molecules still rule the current pharmaceutical market in terms of profit, it is anticipated that biologics revenue will eventually outpace the small molecule market (http://www.firstwordpharma.com/node/1030586?tsid=17#axzz2zAieR100). By 2016, biologics are projected to have captured ~17% of total global spending for pharmaceutics with an overall market value reaching US$210 billion [1]. Furthermore, it is projected that seven of the top ten medicines, as defined by spending, will be biologics within five years [1].

The production of large numbers of monoclonal antibodies against therapeutic targets has become highly commoditized, yet analytical tools are lagging behind in terms of meeting the demand for higher-throughput characterization. Although numerous biophysical characteristics must be optimized in discovering and developing a therapeutic antibody, an antibody's epitope is arguably the most important, because it determines its functional activity. Unlike an antibody's affinity, which is routinely optimized by standard molecular biology methods, an antibody's epitope is an innate property that cannot be engineered. Although epitope selection remains an empirical process, it is too often overlooked early in the drug discovery process because current analytical tools lack the throughput required to characterize large panels of antibodies. Label-free biosensors continue to evolve to meet the needs for drug discovery, but emerging platforms are often only reaching specialized laboratories, mainly owing to their high cost, need for skilled operators and niche applications. To capture a wider commercial market, the vendors of biosensor technologies compete with one another on factors such as low cost, ease of use, robustness, sensitivity and stability [2,3]. In addition, vendors are developing differentiated applications for biosensors. The epitope binning of monoclonal antibodies is an application that is particularly relevant to the discovery of therapeutic antibodies and, because a binning campaign scales geometrically with the size of the antibody panel, it demands tools with significantly higher throughput than current state-of-the-art platforms.

High-throughput epitope binning assays enable a large panel of antibodies to be organized into epitope families

Epitope binning is a competitive immunoassay used to characterize and organize a panel of monoclonal antibodies that bind a specific antigen into epitopic families or bins. By testing

1359-6446/06/$ - see front matter © 2014 Published by Elsevier Ltd. http://dx.doi.org/10.1016/j.drudis.2014.05.011

Please cite this article in press as: Brooks, B.D. et al., High-throughput epitope binning of therapeutic monoclonal antibodies: why you need to bin the fridge, Drug Discov Today (2014), http://dx.doi.org/10.1016/j.drudis.2014.05.011

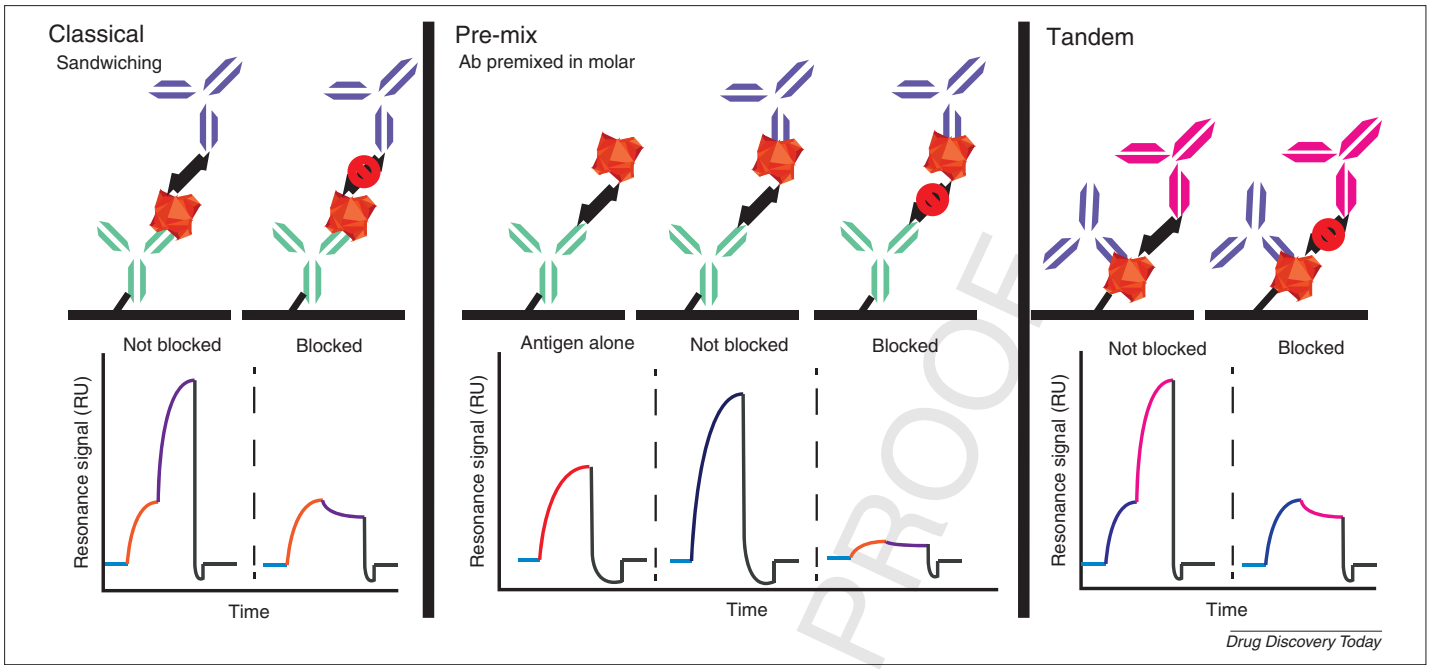

FIGURE 1

Graphic illustrating three alternate formats for epitope binning with label-free biosensor technologies. Sample sensorgrams are provided for practical application. *Abbreviation*: Ab, antibody.

whether antibodies block one another's binding to their antigen in a pairwise and combinatorial fashion a competitive blocking profile is generated for each antibody relative to the others in the test panel. Antibodies that exhibit a closely related binning profile indicate that they target a similar or overlapping epitope and, therefore, are assigned to the same bin [6]. Antibodies that belong to the same epitope bin are likely to share similar functional characteristics. The need for epitope binning assays that are reliable and high-throughput is borne from the ability to generate large numbers of antibodies easily that are specific for a protein target of interest (i.e. the antigen) early in the drug discovery process.

Epitope binning is referenced in the literature under different names, such as epitope mapping, epitope characterization and epitope competition assays. Epitope binning assays are often used in the pharmaceutical industry during the later stages of the screening process, because the limited throughput of existing methods enables the study of only small panels of antibodies. Surface plasmon resonance (SPR) imaging array biosensor platforms enable epitope binning assays to be performed on larger panels of antibodies and consume very little sample, making them accessible to the early stages of a screening process [4].

When it comes to therapeutic antibody selection it is 'all about the epitope'

A successful therapeutic monoclonal antibody must bind the native target with high selectivity and an appropriate affinity. Although affinity-based selection has been a traditional criterion for choosing a few antibodies from a large panel for further characterization, a multitude of problems arise from this approach. First, an antibody's epitope and function are correlated, so only particular epitopes influence the function of the target protein. Second, an antibody's epitope is an innate property that cannot be engineered by rational design, and so it is crucial to identify antibodies that influence the functional domain on the target. An antibody that binds an inert epitope – one that fails to influence the functional domain of the target – is destined to fail *in vivo*, regardless of how high its binding affinity is for the target. Furthermore, most other biophysical properties of an antibody can be engineered to optimize an antibody's pharmacokinetic and pharmacodynamic profile, stability and ease of formulation. The bottom line is that epitope selection remains an empirical process and tools are needed to match the antibody capacity.

An epitope binning assay can be oriented in various ways on a biosensor

Historically, cost and throughput considerations have steered the scientific community toward using ELISAs for conducting epitope binning assays. Recently, however, the approach has been adapted to Luminex®, magnetic bead ELISA and label-free biosensor technologies [6,7]. Abdiche *et al.* outlined different assay formats in which an epitope binning assay can be performed using label-free biosensors [8] (Fig. 1). In a classical sandwich assay, an immobilized antibody is used to capture an antigen and then a second antibody is tested for binding to the preformed antibody–antigen complex. In a premix assay, an immobilized antibody is tested for binding to a solution of the antigen that has been premixed with a saturating concentration of another antibody. In an in-tandem assay, two antibodies are bound, one after another, to an immobilized antigen to test whether the first antibody blocks binding of the second antibody. These assay formats are complementary to one another because each has their unique advantages and disadvantages and all provide unique information [10]. They are used to identify antibody pairs that block or do not block one another's binding to the target antigen. Two antibodies that compete for overlapping epitopes on the target antigen are said to block one another. Conversely, two antibodies that bind nonoverlapping epitopes on the target antigen can bind the antigen at the same time and thus form a 'sandwich' complex; they are said to 'not compete with' or 'not block' one another. The blocking information from these experiments determines the family or bin into which the antibodies are placed. Allosteric antibodies pose unique challenges and warrant further studies to enable proper interpretation of binning results. Array-based SPR technology can be used to epitope bin large panels of antibodies

Please cite this article in press as: Brooks, B.D. et al., High-throughput epitope binning of therapeutic monoclonal antibodies: why you need to bin the fridge, Drug Discov Today (2014), http://dx.doi.org/10.1016/j.drudis.2014.05.011