Rapid, label-free selection of antibodies binding to different epitopes using Biacore A100

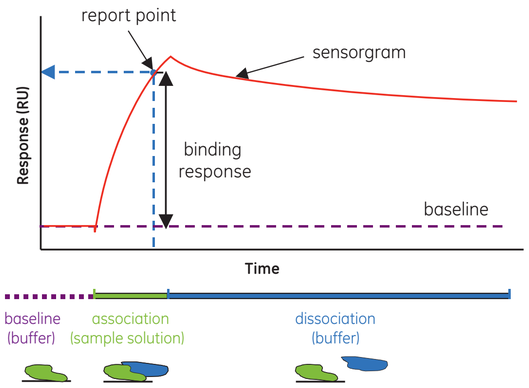
Markku Hämäläinen[1], Trevor Wattam[2], Anna Sylvan[1], Pär Säfsten[1]
[1]GE Healthcare, Uppsala, Sweden. [2]GlaxoSmithKline, Stevenage, UK.

Introduction

Development of biotherapeutics is a rapidly expanding area and increased productivity is a challenge. Generation of large numbers of antibodies and their derivatives has previously exceeded the capacity for thorough characterization of their activity. The binding epitope and stability of binding (off-rate) of a therapeutic antibody determines its *in-vivo* effect. For biomarker assay development, the identification of two antibodies binding with high stability to different epitopes is crucial. We have developed new and effective assays finding and classifying antibody pairs that binds to different epitopes on a target molecule. These methods are based on Biacore™ A100, a high productivity instrument for label-free analysis of protein interactions. The workflow consists of primary and secondary screens, identifying multiple antibodies with good kinetic properties – high affinity and slow off-rates. These antibodies are then epitope mapped using a new method that produces a high quality picture of the binding epitopes and pair-wise binding stabilities.

Detecting protein interactions with Biacore

Biacore systems monitor protein interactions in real-time using a label free detection method. One of the interacting molecules is immobilized onto a sensor surface, while the other is injected in solution and flows over the sensor surface. As molecules from the injected sample bind to the immobilized molecules, this results in an alteration in refractive index that is proportional to the change in mass concentration at the surface. Using the phenomenon of surface plasmon resonance (SPR), these changes are detected in real time and data is presented as a *sensorgram* (SPR response plotted against time). These plots display the formation and dissociation of complexes over the entire course of an interaction, with the kinetics (association and dissociation rates) revealed by the shape of the binding curve.



The sensorgram provides real-time information about the entire interaction, with binding responses measured in *resonance units* (RU). Binding responses at specific times during the interaction can also be selected as *report points*. After the analysis, any remaining bound sample molecules are removed in a *regeneration* step that prepares the surface for the next sample injection.

More information can be found at **www.biacore.com**

Workflow

- Screen a large set of hybridoma supernatants to find antibodies with good kinetics properties
- Perform a large-scale pair-wise epitope mapping to find the best antibody pairs

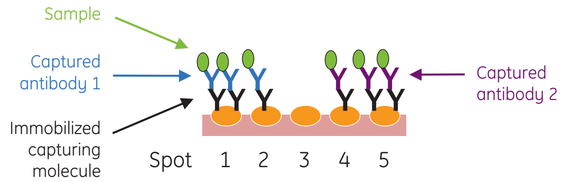
Screening

Off-rate ranking

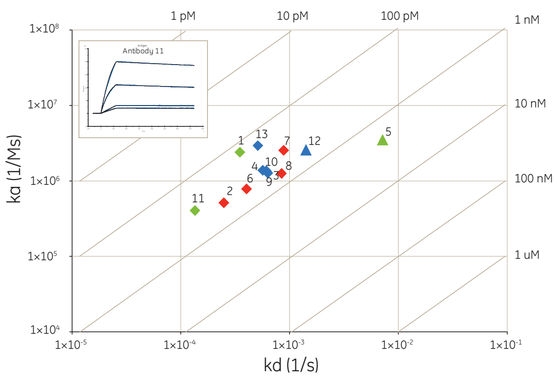
Biacore A100 can be used to screen hundreds of unpurified hybridoma supernatants, capturing 8 different antibodies per cycle (2 per flow cell) and injecting antigen over all of them to rank according to the off-rate. The most interesting antibodies can then be selected for more detailed kinetic analysis.

Kinetics

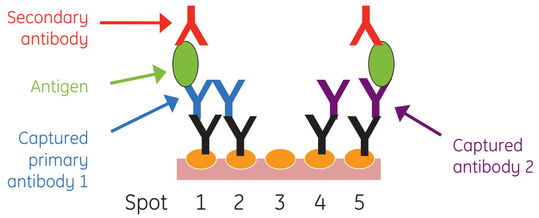
To enable kinetic determinations with high precision, injection of multiple concentrations of antigen is the most commonly used method. In many cases the throughput of such assays can be improved dramatically by utilization of two levels of capturing antibody together with two antigen concentrations ("2over2" kinetics). The Biacore A100 Antibody Extension Package that we have developed here enables this type of analysis at higher throughput and with a 50% reduction in the amount of antigen needed.



Based on the results of the off-rate ranking 13 antibodies were selected for kinetics determination and epitope mapping. The kinetics were determined using 2over2 kinetics for each antibody. The kinetic parameters were plotted as an on/off rate plot with isoaffinity diagonals. The subsequently determined epitope specificities are marked with blue, red and green in the chart. The sensorgrams for the antibody with the slowest off-rate are included as an example.



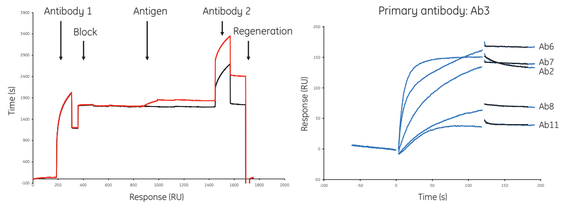
Epitope Mapping



The assay format utilizes a capturing antibody covalently attached to the sensor surface on spot 1, 2, 4 and 5. The antibody is used to capture the 2 different primary antibodies (spots 1-2 and 4-5). Antigen is then injected on spots 1 and 5, and all spots are blocked by injecting of a mixture of non-specific antibodies. Finally two secondary antibodies are injected on spots 1-2 and 4-5. This is performed in parallel for 4 flow cells, thereby testing 8 antibody pairs per cycle.

Spot 2 is used as a reference for spot 1 and spot 4 for spot 5.

The format gives a perfect reference in terms of non-specific binding and drift since the active and the reference spot are subject to exactly the same conditions except for the antigen injection.



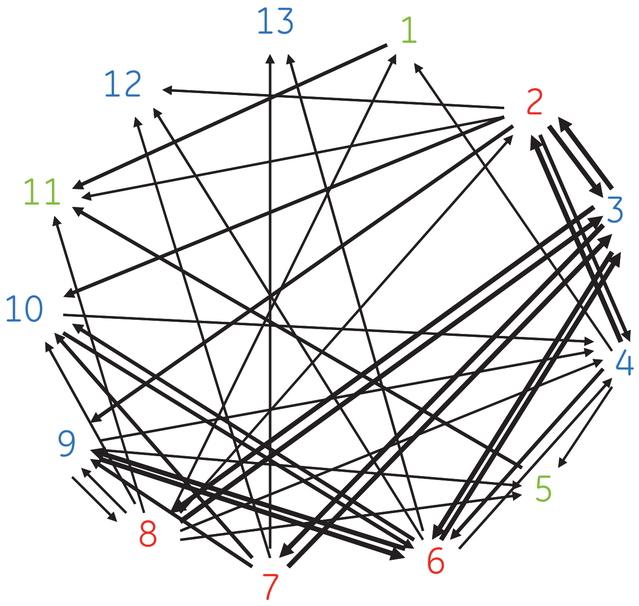
The software includes a dedicated module for epitope mapping. The evaluation is performed in a series of steps where a threshold is set in each step to remove non-binders. In the third step the threshold between positive and negative pairs is set. For positive pairs, the off-rate is calculated (see figure). In this case one antibody has a much faster off-rate compared to the others.

The results are presented in a matrix with primary antibody as rows and the secondary antibodies as columns. Positive pairs are marked with blue, negative with white and the ones where a value is missing with dots.

	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab11	Ab12	Ab13
Ab1											$4*10^{-2}$		...
Ab2			$2*10^{-4}$	$6*10^{-2}$					$3*10^{-4}$	$4*10^{-4}$	$2*10^{-4}$	$5*10^{-2}$	...
Ab3		$3*10^{-2}$				$1*10^{-6}$	$1*10^{-6}$	$6*10^{-4}$					...
Ab4		$4*10^{-2}$											...
Ab5											$1*10^{-3}$		...
Ab6			$1*10^{-6}$						$1*10^{-6}$	$1*10^{-5}$		$4*10^{-2}$	...
Ab7			$1*10^{-6}$						$1*10^{-5}$	$1*10^{-4}$		$3*10^{-2}$	...
Ab8			$1*10^{-6}$						$1*10^{-6}$	$1*10^{-6}$		$6*10^{-2}$	...
Ab9				$1*10^{-6}$	$1*10^{-6}$	$2*10^{-4}$					...	...	...
Ab10				$1*10^{-5}$		$7*10^{-4}$					...	...	...
Ab11			$5*10^{-2}$								...	...	...
Ab12	...	...	...	...	...	...	...	...	...	...	...	...	...
Ab13	...	...	...	...	...	...	...	...	...	...	...	...	...

The 13 tested antibodies. Each interaction was tested 2-3 times in both directions. For each positive pair an arrow was drawn from the primary to the secondary antibody, the thicker line the more replicates showed this behavior.

Three different types of antibodies with respect to pairing capacity are marked with red, blue and green. Red and blue often give positive pairs between each other and never within the group. Green have few pairs overall which indicate that they bind somewhere between the blue/red epitope and block binding.



Conclusions

- **Rapid label-free method for pair-wise epitope mapping**
- **Streamlined analysis in dedicated software module**
- **Essential tool for development of new antibody reagents**

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imagination at work

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