LigandTracer

Application Note

Beyond single binding processes

by Marloes Stokman, researcher at Byondis BV, The Netherlands

The most common use of LigandTracer® is to monitor binding of antibodies (Abs) to target receptors on live cells. In some cases, only one type of ligand-target complex is formed and the binding process is described by a single on- and off-rate. In other cases, multiple types of ligand-target complexes are formed with distinct interaction properties, e.g., due to variation in receptor structure or cellular environment. Furthermore, cellular processes such as internalization will also affect the observed binding curve.

In this example, the binding between a therapeutic Ab, Trastuzumab, and its target receptor HER2 was investigated at various conditions to learn more about the binding behavior in a physiologically relevant environment.

Experimental details

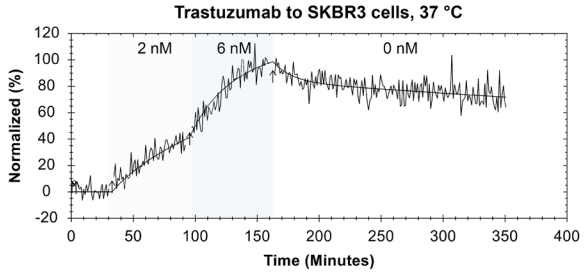
Identifying possible causes for heterogenous interactions

By placing LigandTracer Green into a humidified incubator at 37 °C and 5% CO_2 it is possible to study how Trastuzumab, labeled with AlexaFluor488 (AF488), interacts with HER2 expressed on SKBR3 cells under physiological conditions (Fig. A). The resulting real-time binding trace displayed a slight biphasic dissociation with a small fraction of the Abs releasing quickly. By applying the 1:2 model, the interaction was resolved into two parallel processes (Table 1).

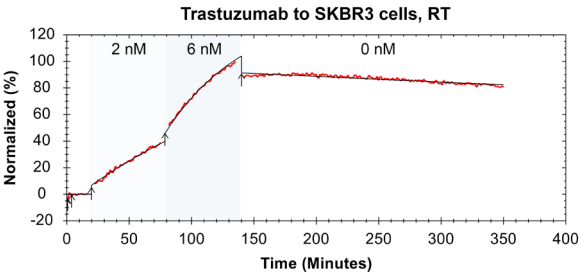
To decrease the influence of cellular processes, the interaction was monitored at room temperature. The 1:1 model fitted well to the resulting real-time binding trace, describing the entire interaction process with one on- and off-rate (Fig. B, Table 1). Typically, the kinetic rate constants become slower at lower temperature, as was observed here.

To learn more specifically if internalization is connected to the biphasic dissociation at 37 °C, experiments with the internalization inhibitors Pitstop 2 and Filipin III were performed. The binding trace showed again a monophasic dissociation that was well-fitted with a 1:1 binding model (Fig. C, Table 1), showing that internalization affects the interaction. Internalization processes often include spatial re-arrangement of receptors on the cell surface, which could be a reason for the observed biphasic dissociation.

A



B



C

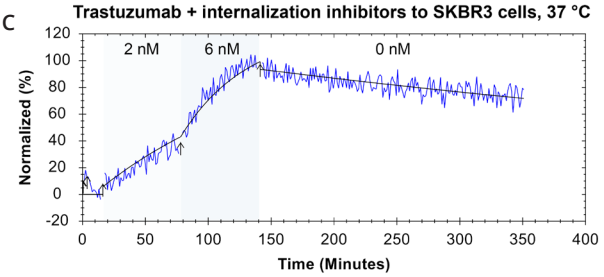


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Table 1. Results from kinetic fitting.

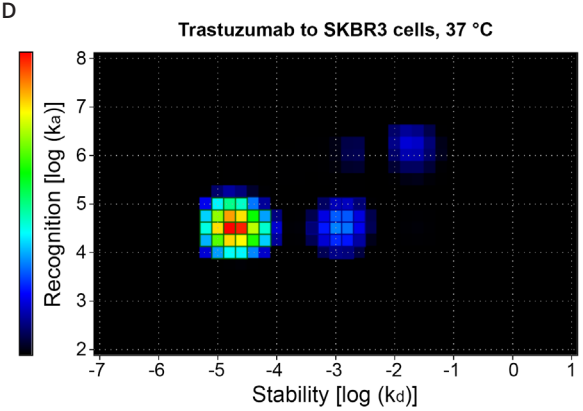
	k_{a1} (1/(M*s))	k_{d1} (1/s)	K_{D1} (M)	k_{a2} (1/(M*s))	k_{d2} (1/s)	K_{D2} (M)
37 degrees	6.0E+04	1.4E-05	2.3E-10	2.3E+04	1.1E-03	4.7E-08
Room temperature	4.1E+04	8.2E-06	2.0E-10	n.a.	n.a.	n.a.
37 degrees + intern. inhib.	6.6E+04	2.3E-05	3.5E-10	n.a.	n.a.	n.a.

InteractionMap as a tool to visualize interaction heterogeneity

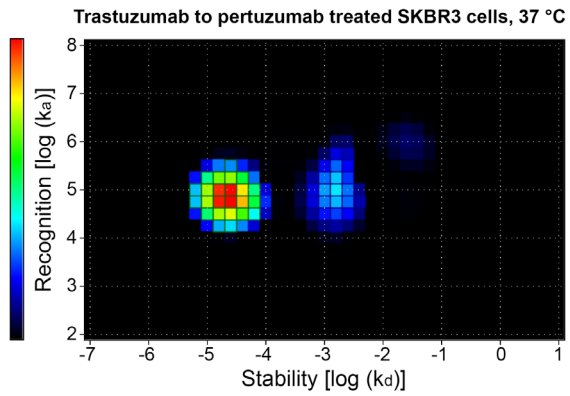
Interactions that are more complex than one binding process, defined by a single on- and off-rate, are also referred to as heterogenous interactions. InteractionMap (IM) is a mathematical tool that searches for distinct 1:1-like interactions contained in a binding trace and presents them in an on- and off-plot. The color represents the relative contribution to the measured curve. Analyzing the Trastuzumab interaction at 37 °C with IM resulted in two major interaction peaks (Fig. D). In addition, there is a third, fainter peak in the upper right corner that represents a very fast on/off binding process caused by unbound fluorescence ligand. The two main interaction peaks have very similar on-rates, likely reflecting recognition of the same molecular target. The main difference lies in the off-rate and thus the binding stability.

The binding of AF488-Trastuzumab at 37 °C was also recorded to SKBR3 cells treated with the dimerization inhibiting antibody Pertuzumab. Interaction Map analysis resulted again in two main interaction peaks on similar positions (Fig. E). However, the less stable of the two peaks (i.e. the one further to the right) was more pronounced and thus contributed more to the overall interaction process. This implies that the proportion of Trastuzumab interacting with HER2 in a less stable manner increases when more HER2 monomers are present. A possible explanation is that an increase in HER2 monomers translates to a larger fraction of Abs being bound monovalently.

D



E



Conclusions

Measuring protein interactions in real-time on live cells makes it possible to determine whether the interaction is dominated by a single binding process or if secondary binding or physiological processes are present. By varying the experimental conditions, it is possible to learn about the nature of these secondary processes.

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