Exclusive interview with Michael Metterlein – ChromoTek GmbH

A part of Proteintech Group released in addition to the AppNote - **Optimizing Kinetics Assays to Avoid Avidity Effects.**

Tell us a little about yourself and your work.

I have been working for ChromoTek as a Scientist for almost nine years. Since 2020, ChromoTek has been a part of Proteintech. Together we develop nanobodies and conventional antibodies as new tools for research. My main responsibilities are protein analytics using bio-layer interferometry (BLI), nanoDSF and dynamic light scattering. Further, I am strongly involved in developing immunoassays, such as immunofluorescence (IF), immunoprecipitation (IP) or western blotting for antibody validation and in-project management workflows.

What is the difference between (antibody) affinity and avidity?

Affinity is the strength of a single interaction (1:1 binding), such as the interaction between the epitope on an antigen and the antibody at a single binding site. The affinity of an antibody to its antigen is measured by the dissociation constant (K_D), which is the rate constant of dissociation at equilibrium. The K_D is defined as a ratio of k_{off} / k_{on}, where k_{off} describes the rate of dissociation of the antibody from the antigen, and k_{on} is the rate of association of the antibody to the antigen. As such, the smaller the K_D value the greater the binding affinity.

Affinity is influenced by hydrogen bonds, electrostatic bonds, Van der Waals forces, and hydrophobic forces. Avidity, also known as the functional or apparent affinity, describes the cumulative strength of multiple affinities between interacting biomolecules, which arises from two or more interaction sites. It is influenced by binding affinity, valency, and structural arrangements. Avidity can, for example, define the strength of a bivalent antibody to its antigen.

For example, IgG and IgE have two antigen-binding sites, as opposed to a dimerized IgA, which has four binding sites, and IgM with 10 binding sites. In case of

an IgM, it is unlikely that all 10 antigens will disengage from the IgM pentamer simultaneously. Therefore, the avidity of IgM can be relatively high, while the binding affinity of a single binding-site may be low.

What would be the method of choice to measure affinity and avidity: ELISA or a label-free real-time approach?

Analysis of antibody and antigen complexes has traditionally been done using enzyme-linked immunosorbent assays (ELISAs). However, a major shortcoming of this end-point assay is the lack of kinetic, thermodynamic, or stoichiometric information. ELISA cannot accurately describe the affinity or avidity of an antibody. Thus, we use BLI early on in our antibody discovery process to get important information on affinity and rate constants.

ChromoTek provides nanobody-based reagents. Can you explain the role of avidity in this work as avidity is typically associated with multivalent analytes like IgG or IgM?

Most of our nanobody-based products are single-domain antibodies that were derived from Camelid immune libraries and screened for high affinity, which is crucial for example for efficient IP and IF experiments (ChromoTek Nano-Traps, Boosters and Labels). These reagents show efficient 1:1 binding in case of monomeric antigens. Nevertheless, in some cases we exploit the avidity effect by using bivalent nanobody formats to increase the apparent affinity.

In your experimental setup, how would you recognize interactions that contain a bivalent or multivalent component?

In most cases we know our test samples quite well. By comparing monovalent and bivalent formats we can observe significant differences in k_{off}. Bivalent proteins usually show much slower k_{off} values than monovalent proteins – but note that this is only true for bivalent proteins used as analytes (free in solution and not when immobilized).

Can you tell us what your recommendations are to resolve issues arising from avidity effects in your assay setup?

The easiest way to prevent avidity effects is to immobilize the bivalent protein sample. Another approach is titrating down a target protein, but this requires a lot of optimizations.

Can you tell me a bit about your collaboration with Sartorius?

Since we purchased our first BLI instrument in 2015 we have been in close contact with the Sartorius Field Application Scientists and the support center. They helped us a lot to establish a set of assays for screening and characterization of our samples. In 2018, we supplied nanobody-based samples for an industry kinetics workshop that we also participated in. Out of this workshop the idea for a collaboration was born as we recognized that avidity is an underappreciated concept.

How does the Octet® BLI platform help you to meet your scientific goals?

We use the BLI platform in early development during clone screening after ELISA. A ranking of clones regarding their k_{off} is of high importance for many of our projects. It accelerates our projects by reducing the number of clones of interest and thus saves us money and time. In addition, we use BLI to characterize final candidates regarding their affinity and kinetics, as well as for epitope binning studies. Quantification of hybridoma supernatants or binding specificity assays are further important applications of the BLI platform in our workflows.

Any other final thoughts on everything we discussed today?

A robust assay design helps to limit assay artifacts and helps you measure the desired binding kinetics and affinity data. Having accurate affinity data early on can accelerate your research tremendously!

Thank you very much for the interview!