Protein-protein interactions measured with LigandTracer®

LigandTracer was originally developed for real-time measurements of biomolecular interactions with cellular systems, but it can be used for the quantification of protein-protein interactions as well. This application note describes binding measurements of labeled ligand interacting with target protein adsorbed to a plastic surface or immobilized to magnetic beads.

Experiment details

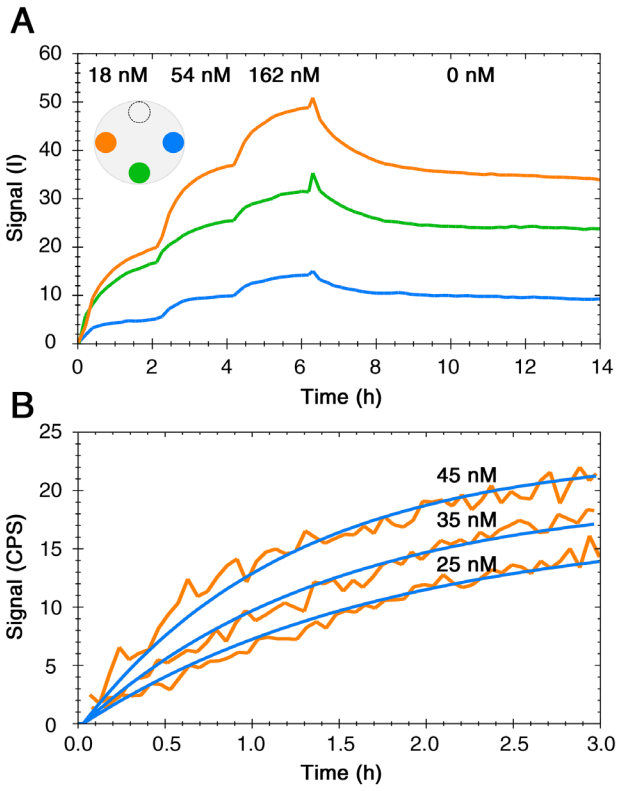
Characterizing the interaction to adsorbed target proteins

FITC-labeled human serum albumin (HSA) interacted with three adsorbed anti-HSA antibodies, evenly distributed in a cell dish together with a target free reference area (empty circle) and measured simultaneously in LigandTracer Green[2,3,4]. Interaction kinetics and affinities varied between the antibodies (Fig. A).

Several other systems have also been measured successfully, such as the interaction between humanized mAb and adsorbed α-kappa[1], and trastuzumab binding to biotin-HER2 captured by adsorbed streptavidin.

Concentration estimation using magnetic beads

The internal magnet of the cell dish holder of LigandTracer enables the use of magnetic beads. The interaction between an antibody and Protein A on beads was characterized in LigandTracer Grey. The result (Fig. B, blue) was used for the estimation of concentration and specific activity of additional antibody samples (Fig. B, orange) with the Concentration Through Kinetics method (% CV < 20 %, n=3 for each concentration).[1]



Conclusions

LigandTracer can be used for real-time quantification of protein-protein interactions, through protein adsorption or magnetic beads. The specificity and kinetics of protein-protein binding pairs can be evaluated reproducibly using this general method.

Reference and protocols

1. Wang E, et. al. *Automated functional characterization of radiolabeled antibodies: a time-resolved approach.* Nucl Med Commun. 2014. 35(7):767-776.
2. Protocol: Adsorb protein to a plastic dish
3. Protocol: A typical LigandTracer measurement
4. Protocol: Protein labeling with FITC

Protocols can be downloaded at www.ridgeview.eu/download/