LigandTracer

Application Note

Real-time proximity assay

Proximity of cell-bound ligands can be evaluated with LigandTracer® Green by using fluorescently labeled ligands together with ligands labeled with a corresponding quencher. Measuring proximity of bound ligands allows to draw conclusions about the recognized epitopes as well as the co-localization of targets on the cell surface. The biological activity of many receptors depends on their arrangement into dimers, higher-order oligomers or clusters. Thus, knowledge about target proximity in connection with binding kinetics allows for a more detailed characterization of your ligand-receptor system.

Experimental details

Co-localization of antibodies targeting the same receptor

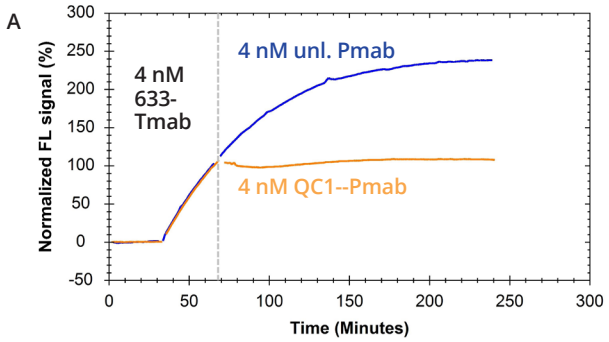
The HER2 targeting antibody Trastuzumab (Tmab) was labeled with the fluorophore Atto633 and its binding to SKOV3 cells was measured with the RedNIR detector. Addition of a second HER2 targeting antibody (Pertuzumab; Pmab) labeled with the quencher IRDye QC-1 diminished the fluorescent binding signal, while addition of unlabeled Pmab did not affect the binding trajectory of Tmab (Fig. A). The lack of competition between Tmab and Pmab implies that the antibodies target different epitopes on HER2 and that the signal disruption is indeed due to quenching which translates to the antibodies binding in close proximity.[1]

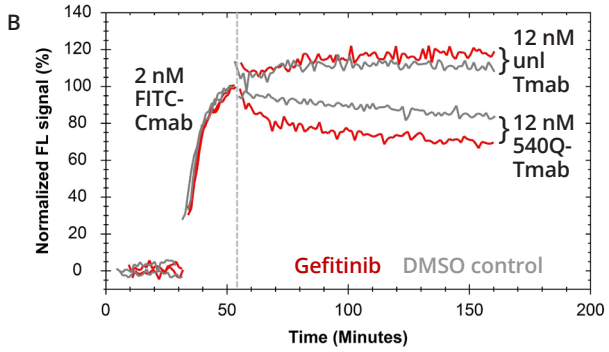
Relative quantification of receptor dimers

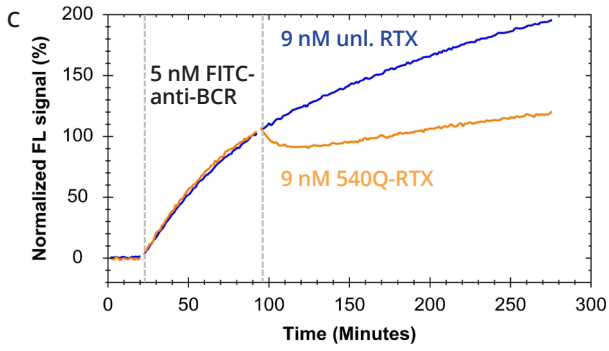
Binding of FITC-labeled Cetuximab (Cmab) targeting the epidermal growth factor receptor (EGFR) was detected on SKOV3 cells. Addition of quenching anti-HER2 Ab (540Q-Tmab) resulted in a signal decrease relative to the curve with unlabeled Tmab (Fig. B). Quenching implies that their target antigens; EGFR and HER2, are co-localized, presumably as heterodimers. Treating the cells with the tyrosine kinase inhibitor Gefitinib increased the quenching effect by 50 % without affecting the interaction properties of the antibodies, indicating a relative increase in EGFR-HER2 heterodimer level of 50 % upon drug treatment.[1]

Co-localization of receptors

Binding of a FITC-labeled antibody towards the B-cell receptor (BCR) on Daudi cells was measured with the Blue-Green detector. Rituximab (RTX), which targets CD20, was added as either an unlabeled variant or labeled with quencher Atto 540Q (540Q-RTX). The quenching RTX variant reduced the fluorescent binding signal (Fig. C), indicating that the BCR and CD20 are co-localized on the cell surface.[1]



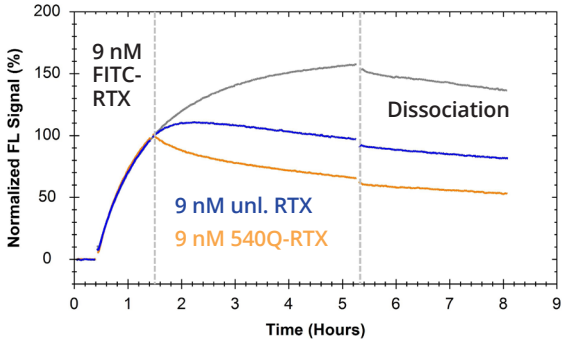




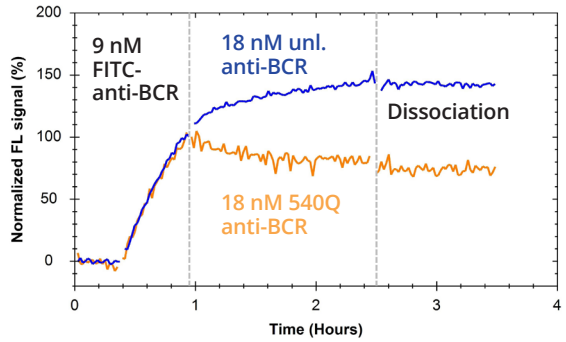
Clustering of receptors

In the examples above, ligands targeting different epitopes have been used, but it can also be evaluated if the same target receptors are in proximity to each other. When using the same ligand as both the fluorescent and quenching variant, self-competition is expected, which requires that the quenching effect is compared with the signal decrease caused by unlabeled ligand. In the example on the right, the signal decrease caused by addition of RTX labeled with a quencher is larger than the decrease caused by addition of unlabeled ligand, showing that not only competition but also quenching occurs (Fig. D). A similar pattern is observed for an antibody targeting the BCR (Fig. E). In both examples, a likely cause of antibody proximity is receptor clustering. Since the data above demonstrated co-localization of CD20 and the BCR, the receptors are presumably present in the same clusters.[2]

D



E



Conclusions

As quenching efficiency is very sensitive to the distance between fluorophores and quenchers, it can be used in LigandTracer Green measurements to assess co-localization of bound ligands and the proximity of targeted receptors on the cell surface. The real-time proximity assay can thus be used for epitope mapping, to study co-localization of receptors and relative changes in their co-localization, such as differences in dimer levels.

References

1. Bondza, S., Björkelund, H., Nestor, M., Andersson, K. & Buijs, J. Novel Real-Time Proximity Assay for Characterizing Multiple Receptor Interactions on Living Cells. ***Anal. Chem.*** **89**, 13212–13218 (2017).

2. Bondza, S., ten Broeke, T., Nestor, M., Leusen, J. H. W. & Buijs, J. Bivalent binding on cells varies between anti-CD20 antibodies and is dose-dependent. ***mAbs*** **12**, 1792673 (2020).