Highly Parallel Planar Surface Antibody Array-based Assay to Monitor Protein-protein Interactions

Abstract
Protein-protein interactions have been receiving increasing attention as targets for pharmacological intervention. However, current methods used to detect protein-protein interactions either lack proper throughput, are not quantitative, or rely on highly engineered systems and cumbersome workflows. To address this, we developed a planar surface antibody array-based sandwich assay that enables simultaneous detection and relative quantification of protein-protein interactions from complex solutions. We validated our approach by applying it to receptor tyrosine kinases (RTKs), which are well characterized and are known to form disease relevant, dynamic interactions. The antibody array-based assay revealed co-existing combinatorial RTK interactions that were unique to each cell type tested. The assay allowed the monitoring of ligand-mediated formation of RTK complexes as well as their disruption by small molecule inhibitors. The antibody array sandwich assay described can be applied to a broad range of cases with various topological configurations, thus offering an advantage over technologies that have strict proximity requirement. This protein interaction array-based assay can be used to screen for inhibitors or inducers of protein-protein interactions, thereby aiding in the identification of new chemical modulators with unique properties and novel mechanisms of action.

Summary
- A planar surface antibody array was configured to detect and quantify RTK protein-protein interactions.
- Two sets of antibody pairs were used to distinguish between two conformations of EGFR-c-Met heterodimers.
- The antibody array-based sandwich immunosay was used to monitor the disruption of receptor heterodimers by small molecule inhibitors.

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