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MONITORING *IN VIVO* PROTEIN-PROTEIN INTERACTIONS BY COUPLING BIMOLECULAR FLUORESCENCE COMPLEMENTATION (BIFC) AND FLOW CYTOMETRY

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We present a high-throughput approach to study weak protein-protein interactions by coupling bimolecular fluorescent complementation (BiFC) to flow cytometry (FC). Bimolecular Fluorescence Complementation (BIFC) is a method to detect protein-protein interactions based on the formation of a fluorescent complex by fragments of the yellow fluorescent protein (NYFP and CYFP) brought together by the association of two interaction partners fused to the fragments[1]. For weak protein-protein interactions, the detected fluorescence is proportional to the interaction strength, thereby allowing *in vivo* discrimination between closely related binders with different affinity for the bait protein[2,3]. FC provides a method for high-speed multiparametric data acquisition and analysis; the assay is simple, thousands of cells can be analyzed in seconds and, if required, selected using fluorescence-activated cell sorting (FACS). The combination of both methods (BiFC-FC) provides a technically straightforward, fast and highly sensitive method to validate weak protein interactions.

On the other hand, the combination of both technologies has also been applied to detect the interference of protein interactions[4]. In a test case, we demonstrate that the inhibition of protein interactions results in a concomitant decrease in fluorescence emission. It has to be taken into account that the identification of molecules able to modulate protein contacts is of significant interest for drug discovery and chemical biology. Therefore, the combination of BIFC with flow cytometry might provide an effective means to detect interaction modulators among large chemical or peptidic libraries by directly reading out changes in the reporter signal.

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