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Engineering Zap70 Biosensor Through Directed Evolution for Applications in Single-Cell Imaging and Immunotherapy

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Abstract: Genetically-encoded biosensors based on Fluorescence Resonance Energy Transfer (FRET biosensors) have been widely used to dynamically track the activity of Protein Tyrosine Kinases (PTKs) in living cells because of their sensitive ratiometric fluorescence readout, high spatiotemporal resolution. However, the limitation in sensitivity, specificity, and dynamic range of these biosensors have hindered their broader applications, and there was a lack of efficient ways to optimize FRET biosensors. Here we established a rapid, systematic and universal approach for FRET biosensor optimization through directed evolution which involves generating genetic diversity and screening for protein variants with desired properties at the same time and achieves simultaneously screen a large number of biosensor variants for the response to kinase activity. ZAP70 kinase plays a central role in early T cell and NK-lymphocytes activation and 65536 FRET biosensor variants for ZAP70 kinase were generated and introduced into mammalian cells at the same time. Then the cells were sorted using FACS based on FRET ratio and sent for next-generation sequencing. The sequence information of selected biosensor variants was revealed by high-throughput sequencing which allows us to explore the protein sequence space of selected biosensors compared with the input library. Based on the information of protein sequence space, functional substrate peptide sequences were successfully predicted and verified in signal live cells by using live-cell imaging. The successfully identified Zap70 biosensors could be further used for unraveling the spatiotemporal dynamics of Zap70 kinase activities in single live cells, advance our understandings in T cell regulation and pave the way for appropriate therapeutic designs in the future.

Keywords: FRET biosensor; directed evolution; ZAP70; immuno-engineering

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