Imaging protein activity in living cells: Src kinases at the leading edge

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Signaling networks that control cell behavior are tightly regulated in space and time. Fluorescent biosensors for living cells can provided a valuable window on the dynamics of these networks, providing quantitative information on the kinetics and localization of protein activity in vivo. However, biosensors for living cells currently require considerable optimization for each target and are also limited by the availability of naturally occurring ligands/binding elements with appropriate target specificity. In this talk, I will describe a new approach of generating biosensors based on an engineered fibronectin monobody scaffold that can be tailored to bind different targets via high throughput screening. Using the artificial monobody scaffold and extremely bright reporter fluorescent dyes, we generated a biosensor that can report the activation of endogenous, unmodified Src family kinases (SFK) in living cells. The new SFK biosensor in conjunction with automated analysis of cell edge dynamics offer revealing insights into the role of Src kinases in cell migration. We are now generating biosensors for individual Src family kinases that are important in cancer and other diseases and also developing a new class of biosensors based on small molecules that are specific for active conformations of signaling proteins. I will also discuss new strategies for probe development for various aspects of cell physiology using high throughput, high content imaging.