Informal Seminar on Mathematics and Biochemistry-Biophysics

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Image-driven Analysis of Molecular Transport and Activation in Single Live Cells

Abstract:

Genetically encoded biosensors based on fluorescence resonance energy transfer (FRET) have been widely applied to visualize the molecular activity in live cells with high spatiotemporal resolution. However, the rapid diffusion of biosensor proteins hinders a precise reconstruction of the actual molecular activation map. Based on fluorescence recovery after photobleaching (FRAP) experiments, we have developed a finite element (FE) method to analyze, simulate, and subtract the diffusion effect of mobile biosensors. This method has been applied to analyze the mobility of Src FRET biosensors engineered to reside at different sub-compartments in live cells. The results indicate that the Src biosensor located in the cytoplasm moves 4-8 folds faster than those anchored on different compartments in plasma membrane. The mobility of biosensor at lipid rafts is slower than that outside of lipid rafts and is dominated by two-dimensional diffusion. When this diffusion effect was subtracted from the FRET ratio images, high Src activity at lipid rafts was observed at clustered regions proximal to the cell periphery, which remained relatively stationary upon epidermal growth factor (EGF) stimulation. This result suggests that EGF induced a Src activation at lipid rafts with well-coordinated spatiotemporal patterns. Our FE-based method also provides an integrated platform of image analysis for studying molecular motility and reconstructing the spatiotemporal activation maps of signaling molecules in live cells. Furthermore, we developed a correlative FRET imaging microscopy (CFIM) approach to quantitatively analyze the subcellular coordination between the enzymatic activity and the structural focal adhesion (FA) dynamics. By CFIM, we found that different FA subpopulations have distinctive regulation mechanisms controlled by local kinase activity. Therefore, our work highlights the importance of dynamic single live-cell imaging and its integration in-depth mathematical analysis.

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