

Visualizing single EGFR-YFP molecules on the surface of living cell

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Abstract : Single-molecule imaging is now an ideal technology across the biology, chemistry and physics science. It allows one to study the molecular mechanisms of biological reaction in vivo, as well as measure intermediates and follow the time dependent pathways of chemical reaction. Here we describe our study of the behavior of individual EGFR (epidermal growth factor receptor)-YFP protein molecules within living CHO cells based on an objective-type total internal reflection fluorescence microscope. We have used cultured CHO cell that were stably expressed. At first, EGFR-YFP was not detectable as fluorescent spots in the culture medium because of a little high expression level and background autofluorescence of cells. After half-hour modest laser illumination, the background fluorescence was greatly reduced and most of EGFR-YFP molecules were photobleaching. Single fused protein can then be visualized sudden entering the evanescent field as individual diffraction-limited spots and disappear in one step, indicating a dynamic course that EGFR-YFP molecules arrived at and departed from the plasma membrane. To find out whether the disappearance is caused by internalization or quenching, internalization inhibitor is added and some result is presented.