Title
Spatio-temporal fluorescence fluctuation analysis of paxillin-EGFP in cellular adhesions using scanning FCS, ICS and PCH

Journal
BIOPHYSICAL JOURNAL, 88(1)

ISSN
0006-3495

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Publication Date
2005

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49th Annual Meeting of the Biophysical Society, Long Beach, California, 2005.
Biophys J. 2005; Suppl, 1821-Plat.

Abstract

Fluorescence methodologies based on two-photon laser excitation and confocal imaging provide detailed spatio-temporal maps of protein dynamics during cellular processes. Single point fluorescence correlation spectroscopy (FCS) provides information about protein concentration and dynamics within a very small region of the cell without providing any spatial information. We developed novel extensions of FCS to probe spatial correlations and previously inaccessible temporal windows. Scanning FCS (sFCS) and image correlation spectroscopy (ICS) of more dynamic proteins have been proposed as alternative methods to gain information bridging the temporal scales of FCS (ms-ms) and ICS (ms-s). sFCS rapidly measures many focal points, providing the same concentration and dynamic information as FCS as well as information on the spatial correlation between points along the scanning path and reveals slower dynamics. This can be done by either scanning the beam in a circular path or by using confocal imaging techniques (line scanning), and thereafter calculating correlation functions along both the fast (scan axis) and slow (interline) axis. We show that by exploiting the hidden time structure of the scanning method in which adjacent pixels are few microseconds apart we can recover information about dynamic processes such as molecular diffusion in the microseconds to second time scales. Results show multiple diffusion constants for paxillin at and near adhesions suggesting that the dynamics of the associations in these regions are quite complex. We have also extended the Photon Counting Histogram (PCH) analysis to include sFCS and ICS images to measure the brightness (aggregation state) of protein complexes across ... [truncated at 250 words]