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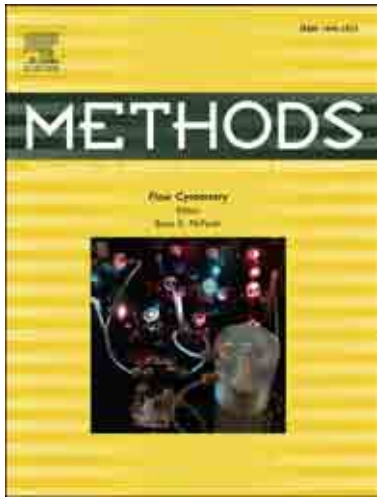

Dissecting single–cell molecular spatiotemporal mobility and clustering at Focal Adhesions in polarised cells by fluorescence fluctuation spectroscopy methods

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Dissecting single–cell molecular spatiotemporal mobility and clustering at Focal Adhesions in polarised cells by fluorescence fluctuation spectroscopy methods

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Abstract

Quantitative fluorescence fluctuation spectroscopy from optical microscopy datasets is a very powerful tool to resolve multiple spatiotemporal cellular and subcellular processes at the molecular level. In particular, raster image correlation spectroscopy (RICS) and number and brightness analyses (N&B) yield molecular mobility and clustering dynamic information extracted from real-time cellular processes. This quantitative information can be inferred in a highly flexible and detailed manner, i.e. 1) at the localisation level: from full-frame datasets and multiple regions of interest within; and 2) at the temporal level: not only from full-frame and multiple regions, but also intermediate temporal events. Here we build on previous research in deciphering the molecular dynamics of paxillin, a main component of focal adhesions. Cells use focal adhesions to attach to the extracellular matrix and interact with their local environment. Through focal adhesions and other adhesion structures, cells sense their local environment and respond accordingly; due to this continuous communication, these structures can be highly dynamic depending on the extracellular characteristics. By using a previously well-characterised model like paxillin, we examine the powerful sensitivity and some limitations of RICS and N&B analyses. We show that cells upon contact to different surfaces show differential self-assembly dynamics in terms of molecular diffusion and oligomerisation. In addition, single-cell studies show that these dynamics change gradually following an antero-posterior gradient.

Keywords: Fluorescent Fluctuation Spectroscopy; Molecular Brightness; Protein dynamics; Protein clustering; Focal adhesions; Cell Biophysics.