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A FRAP-based approach to study spatial regulation of actin turnover

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Under spatial and temporal control of numerous actin-binding proteins, actin filaments are organised into dense meshworks and bundles in cells. Proteins which change filament turnover and promote or inhibit actin-associated cellular processes such as spreading and motility have been studied individually *in vitro* and *in vivo*. However quantitative assessment of how actin-binding proteins work in concert under physiological conditions is still a challenging task. Here, we studied the reaction kinetics of actin cytoskeleton components using laser-assisted confocal microscopy-based Fluorescence Recovery After Photobleaching (FRAP) in two experimental configurations. In both cases we employed analysis approach that we had previously developed to quantify fluorescence recoveries from time-lapse confocal images, to fit them with mathematical models and to statistically evaluate the estimated parameters (http://actinsim.uni.lu). The image analysis was further extended to measure fluorescence redistribution associated with the turnover of filaments. First, actin polymerisation dynamics was analysed in a cell-free biomimetic motility assay. By changing concentrations of regulatory proteins we examined how capping and severing of actin filaments working in concert influence actin exchange in bulk filament meshworks nucleated by ARP2/3 complex. Second, similar approach served to investigate cytoskeleton dynamics in cell-matrix adhesions of living cells. Taking as an example focal adhesion protein zyxin and its binding partner Tes we studied how their interaction regulates actin cytoskeleton dynamics. Taken together our results extend the understanding of the interaction network of actin-binding proteins with actin.

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