

Analysis of profilin dynamics at the cell membrane by image pair correlation and number and brightness analysis

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Profilin is known to bind and sequester G-actin but also to interact with phosphatidylinositol polyphosphate at the cell membrane. Many *in vitro* studies have investigated the role of profilin in actin filament assembly and disassembly. However, the dynamics and role of profilin at the membrane in live cell is still unknown. We used image pair correlation and Number & Brightness analysis of MDA cells expressing profilin-GFP to investigate profilin's barrier to diffusion and aggregation. When Cytochalasin D disrupts actin polymerisation, we see a decrease in the barriers for profilin diffusion, which is independent of profilin's actin-binding properties. In addition, we also see a decrease in the barriers to diffusion when the profilin phosphatidylinositol polyphosphate binding site is perturbed, suggesting two possible modes of restricting profilin diffusion at the membrane. Finally, the disruption of actin filament leads to an increased rate of profilin cluster assembly/disassembly possibly highlighting a cell mechanism to generate actin filaments.