Single Molecule Imaging of Actin-Tropomyosin Complexes

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The actin cytoskeleton is fundamental for cellular function and highly organized in space and time. The numerous functions of actin filaments ranging from determining cell shape to cell motility are regulated by a variety of actin-binding proteins. Tropomyosins are highly conserved critical constituents of actin filaments and differentially regulate filament stability and function. Tropomyosins are dimeric coiled-coil proteins that form head-to-tail polymers along the major groove of the actin filament. However, the mechanism of tropomyosin polymerisation and how it regulates actin functional diversity in combination with other actin-binding proteins is still uncertain. To understand these assembly mechanisms we have developed an imaging approach combining a microfluidic device and total internal reflection fluorescence (TIRF) microscopy to investigate the architecture of actin-tropomyosin filaments at different stages of the assembly process.

Actin filaments were assembled in vitro and visualised utilising tropomyosin labelled with a fluorescent dye. Biochemical analysis confirmed that the fluorescent tropomyosins bound cooperatively to the actin filaments. Actin-tropomyosin filaments were then tethered to the bottom glass surface of a microfluidic device. The fluid flow inside the microfluidic channels stretched out and aligned the captured actin-tropomyosin filaments for observation with TIRF microscopy (Jegou et al Plos Biol 2011, 9(9), e1001161). Our preliminary results indicate that clusters consisting of approximately ten tropomyosins form at different locations along the actin filament during the early stages of the assembly process at concentrations below EC50. Binding of two tropomyosin isoforms labelled with different fluorophores shows that both isoforms bind to the same filament but appear to segregate into separate clusters. These observations indicate that the formation of distinct actin-tropomyosin filaments (homopolymers) that are observed inside cells require additional sorting factors. Ultimately our approach will allow us to investigate the interaction of different tropomyosin isoforms with different populations of actin filaments at the single molecule level.