Interaction forces between filamentous actin and ezrin as a function of its activation measured by means of force spectroscopy

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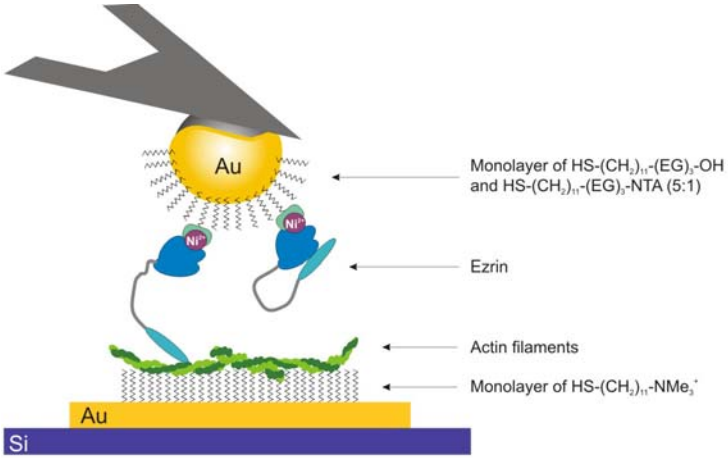

Schematics showing a colloidal sphere coated with ezrin for investigating interactions with F-actin.

Ezrin, a member of the ERM (ezrin/radixin/moesin) protein family, provides a regulated linkage between the plasma membrane and the actin cytoskeleton. As a membrane-associated protein, it contributes to the organization of structurally and functionally distinct cortical domains participating in adhesion, motility and other fundamental plasma membrane processes.

Ezrin is negatively regulated by an intramolecular interaction of the terminal domains that masks, at least to some extent, membrane and F-actin binding sites. A known pathway for activation, i. e. dissociation of amino and carboxy termini, involves the interaction of ezrin with phosphatidylinositol 4,5 bisphosphate (PIP_2) in the membrane, followed by phosphorylation of the threonine 567 residue in the carboxy terminal domain. The mutant protein ezrin T567D mimics the phosphorylation induced activated state, whereas ezrin T567A is constitutively inactive. In preliminary experiments, the activation mechanism of ezrin wild type from the dormant to the active, F-actin binding state by recognition of PIP_2 has been studied. The positive influence of PIP_2 binding on the F-actin association capability of ezrin has been demonstrated using colloidal spheres equipped with firmly attached actin filaments to measure the maximal adhesion forces and the work of adhesion of the ezrin-actin interface. However, depending on our polymerization conditions long actin filaments were formed on flat substrates. Investigation by means of confocal laser scanning microscopy (CLSM) revealed a persistence length of 5 to 20 µm. Since F-actin is known to be a rather stiff macromolecule and the spheres used in the described colloidal probe experiments were about 15 µm in diameter, the setup will be modified. F-actin immobilized on a planar substrate will be probed with an ezrin coated bead using the atomic force microscope (AFM). This model system will allow a thorough characterization of the acting forces at the ezrin-actin interface. In particular, we expect that the systematic study of the interaction forces between F-actin and the mutants will yield valuable information concerning the activation mechanism of ezrin.