

**P002** Talin FERM domain interactions with PIP kinase type 1 $\gamma$ : NMR and fluorescence study.

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Talin is an essential component of focal adhesions (FA) that couples  $\beta$ -integrin cytodomains to F-actin and provides a scaffold for signaling proteins. It is a large (270kDa, 2541 residues), flexible rod-shaped molecule. Talin globular head domain (residues 86-410) is homologous to the N-terminal FERM domain of the band 4.1, ezrin, radixin, moesin family of cytoskeletal proteins and contains binding sites for the integrin  $\beta 3$  cytodomain and PIP kinase type 1 $\gamma$ . The information on the location and structural characteristics of these binding sites is required for the understanding of the mechanism of FA assembly regulation. Here we used NMR to map the binding site of PIP kinase, and fluorescence to determine kinetic properties of the interaction. Our data demonstrate that PIP kinase peptide containing the minimal talin-binding site forms a 1:1 complex with F2F3 talin fragment and the binding site overlaps that of the integrin. Mutation of R358 in the binding site increases  $K_d$  for the interaction from 6  $\mu$ M to 35  $\mu$ M, evidencing the role of the arginine side chain in stabilisation of interaction with both integrin and PIP kinase. The results suggest that ternary complex formation with a single talin FERM domain is unlikely, although both integrins and PIP kinase may bind simultaneously to the talin anti-parallel dimer.