CMC – Structure Initiative Protocols
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TALIN F3: CLONING AND EXPRESSION PROTOCOL: DOMINANT NEGATIVE MUTANTS

Labeled and unlabeled Talin F3 domain for NMR studies

Murine talin F3 domain (residues 309-405), C336S mutant, is subcloned into a pGEX-6P-2 vector (Amersham Biosciences) between the BamHI and EcoRI restriction sites. The C336S substitution in talin F3 has no effect on integrin -tail binding.

Protein is expressed in *E. coli* strain BL-21. U-^{15}N-Labeled protein is expressed in M9 minimal media. Cells are harvested by centrifugation and resuspended in phosphate-buffered saline containing lysozyme (1 mg/ml), $MgSO_4$ (10 mM), and DNase I from Sigma (20 µg/ml). Cells are lysed by freeze/thaw cycles before adding Triton X-100 (Roche Applied Science) to a final concentration of 0.1% (v/v) and centrifuging. The supernatant is loaded onto glutathione-Sepharose 4B (Amersham Biosciences) and purified according to the product manual. Glutathione *S*-transferase fusion protease $3C^{pro}$ is added to cleave the fusion protein overnight at 4 °C, and the talin F3 domain is purified from the glutathione S-transferase and protease by using glutathione-Sepharose 4B. The identity and purity of the final protein are confirmed by electrospray mass spectrometry and SDS-PAGE.

U-^{15}N,^{13}C and U-^{15}N,^{2}H doubly labeled talin is produced in the same way, using ^{13}C-glucose or D6-glucose and D_2O.

Talin F3 mutants were generated using the QuikChange mutagenesis kit (Stratagene). Mutants were confirmed by DNA sequencing. The following mutants are available:

F3 mutants L325R, S365D, S379R, Q381V: Mutants fold correctly, as judged by their dispersed NMR spectra, and chemical shifts induced by chimeric peptide indicate that the interaction with membrane-distal region are unchanged. However, they bind to the membrane-proximal region weakly or not at all. Transfection of cells with cDNA encoding the F2 + F3 subdomains (F23) of talin (residues 206–405) activates several integrins, but each of the four mutations diminished the ability of F23 to activate integrin αIIbβ3.