CMC – Structure Initiative Protocols
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DOCK180 DHR1 Domain cloning and expression protocol

U-^{15}N-labeled DOCK180 **DHR1** domain (residues 154–256; Swiss-Prot Q99704) was subcloned into the pGEX-6P2 vector (Amersham Biosciences) between the BamHI and EcoRI restriction sites.

Protein is expressed in *E. coli* strain BL-21. 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 protein 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. Expected yields are 10-50 mg/L.

DOCK180 DHR1 Domain for NMR protocol

U-^{15}N-labeled DOCK180 DHR1 domain (residues 154–256; Swiss-Prot Q99704) was subcloned into the pGEX-6P2 vector and produced using the same methods as for the talin F3 domain. U-^{15}N,^{13}C and U-^{15}N,^{2}H doubly labeled talin was produced in the same way, using ^{13}C-glucose or D6-glucose and D_2O.