

Study of the Hsp90 chaperone with NMR

Eleanor Smith

Abstract:

Hsp90 is a ubiquitous chaperone protein whose function, unlike other chaperone proteins that aid in de novo folding, is to mature and activate already folded proteins. The protein targets of Hsp90 include steroid hormone receptors and protein kinases.

Hsp90 is a homodimer, each monomer consists of three domains (N-terminal, middle and C-terminal). The N-terminal domain shows ATPase. Hsp90 binds to an incredibly diverse range of substrates with the aid of various co-chaperone molecules. These co-chaperones include Hsp70, Hop, Aha1, p23, Cdc37 and PP5 and have functions ranging from control of the ATPase activity to acting as adaptors and recruiters of substrate proteins. Some co-chaperones, particularly those associated with the C terminal TPR binding domain, are still of unknown function. The exact method of substrate activation remains unknown. This project aims to investigate this issue using NMR spectroscopy to monitor structural changes in Hsp90 and its kinase substrates during the ATPase cycle. Previously an NMR assignment of the N-terminal domain has been obtained. We are working to optimise expression and purification of other Hsp90 constructs using a ^{15}N perdeuterated labeling strategy and spectra have so far been obtained for amino acids 271-551 (middle domains) and 1-551 (N-terminal and middle domains). Both constructs show good peak dispersion which should allow assignments of the backbone amides and methyl groups as a further step towards structural studies of the functional protein, which as a 165kDa dimer is a significant challenge for NMR methods.