# The Folding Pathways of Knotted Proteins

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When protein structures which had topological knots in their protein backbones were first discovered, they were a surprising find. Up to that point, it had been thought impossible to efficiently knot and fold a polypeptide chain. Using several different algorithms, it is now estimated that there are some 300 or so knotted proteins in the protein structure database. How these proteins knot and fold is an important question, and one which cannot be addressed by using existing models of protein folding which have been developed from the extensive study of small, monomeric protein folding systems. Experimental folding studies on these unusual and complex structures began relatively recently, and our group was the first to show that these types of proteins could be reversibly unfolded and refolded *in vitro* without the need for proteins such as molecular chaperones which play an important role in aiding the folding process of some proteins *in vitro* and *in vivo* [1]. A comprehensive kinetic analysis of the folding pathway of the dimeric knotted methyltransferase YibK has established that this protein folds along two parallel pathways (due to the presence of a *cis* proline in the native structure) with several intermediate states. As has been observed for many dimeric proteins, the rate-limiting step is the dimerisation of two highly structured monomeric intermediates [2]. Despite the presence of the knot, YibK folds with kinetics that are not dissimilar to other dimeric proteins of the same size. A kinetic analysis of the folding pathway of another dimeric knotted methyltransferase, YbeA, showed a less complex mechanism attributable to the fact that this protein has no *cis* proline residues and the fact that there are fewer populated intermediate states during the folding of YbeA maybe a result of its lower stability [3]. Both YibK and YbeA are dimers and this makes a full thermodynamic and kinetic analysis laborious. A monomeric variant of YibK has been made which has simplified kinetics. However, this monomeric form has lost some structure in comparison to the monomer subunit in the native wild-type protein, and it is incapable of binding the co-factor necessary for function [4]. In recent studies, novel knotted fusions were created in which YibK was fused at either or both N- and C-termini to the super-stable protein ThiS. This enabled a series of kinetic experiments in which the YibK but not the ThiS part of the fusion was specifically unfolded and then refolded. The refolding of the YibK thus took place in the presence of a significant folded domain of ThiS which has to pass through the knotting loop during the folding process. The results from these experiments suggest that it is highly likely that the knotting process takes place very early on during folding, and that the protein may even be knotted in the denatured state [5].

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