Following the co-translational folding of a multidomain protein

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Tandem repeat proteins composed of multiple copies of similar domains have a high risk of forming non-native inter-domain contacts during folding that can lead to misfolded states. Therefore, a strong selective pressure may exist to minimize misfolding interaction between adjacent domains during co-translational folding. To explore how this occurs at the molecular level we monitored variations on pulling force generated by the PimA nascent chain as it emerges from the ribosomal exit tunnel during vectorial elongation of a multi-domain protein.

The protein PimA is an essential enzyme of 386 residues responsible for the initial mannosylation of phosphatidylinositol in Mycobacterium smegmatis. The structure of PimA consists of two Rossmann-fold domains with a deep fissure at the interface forming the catalytic center, followed by a long α-helix which connects back the C-terminal domain with the N-terminal domain. By the use of a set of gradually longer fragments of PimA fused to a translational arrest peptide in the *E. coli*-based PURE *in vitro* translation system we generated a series of co-translational force-profiles. The force profile analysis reveals that while the C-terminal domain is able to fold co-translationally as soon as it emerges from the ribosome, the N-terminal domain requires the complementation of the central β-sheet by the very C-terminal portion.