CryoEM of a Variant of the SDD1 Arrest Peptide Reveals the Mechanism of Enhanced Arrest

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When a nascent polypeptide begins to fold into its three-dimensional structure while still being synthesized by the ribosome, this process is referred to as cotranslational protein folding. Traditional methods that are used to study protein folding like nuclear magnetic resonance (NMR) and X-ray crystallography fail to adequately capture intermediates of this dynamic process. The lack of effective tools to study cotranslational folding *in vivo* led to the development of Force Profile Analysis (FPA), which utilizes short tracts of amino residues that stall translation, called arrest peptides (APs). APs have been found and optimized in both *E. coli* (SecM) and human cells (XBP1u), and work as sensors which are sensitive to the amount of force exerted on the nascent chain due to protein folding or membrane insertion.

One of the most robust, versatile, and well characterized eukaryotic organisms is *Saccharomyces cerevisiae.* However, it was not until recently that *S. cerevisiae* was found to have an uncharacterized protein called SDD1 with an arrest peptide that induces ribosome quality control (RQC). RQC rescues stalled or collided ribosomes in eukaryotes when triggered.

This presents an opportunity to characterize the region of SDD1 that promotes arrest and create a dynamic range of variants as a tool to study cotranslational protein folding. We thus present single amino acid mutations which led to the discovery of an arrest-enhanced variant. We then characterize the relationship between the structure and the function of this variant to propose that additional π-stacking between the variant and the ribosome tunnel wall prevent induction of the peptidyl-transferase center, leading to enhanced stalling and growth defects.