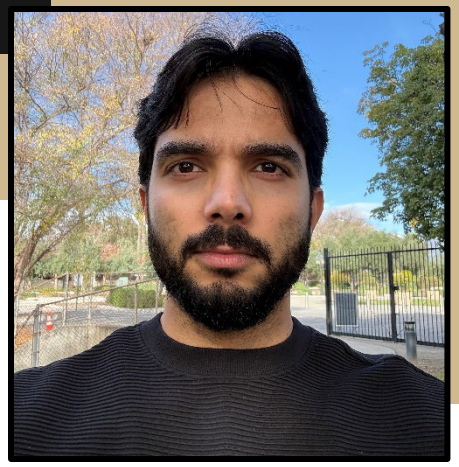
BIOCHEMISTRY SEMINAR



Rohan Bhardwaj

Graduate Student | Schlebach Group | Purdue University

"Surveying the Sequence Constraints of Folding on the Ribosome by Deep Mutational Scanning"

Though protein folding begins during the early stages of protein biosynthesis on the ribosome, relatively little is known about how ribosomes may alter these initial folding pathways. Several recent investigations of cotranslational folding have largely relied upon the use of arrest peptides that interact within the ribosomal exit tunnel in a manner that requires mechanical folding forces to prevent translational stalling. Applications of arrest peptides as cotranslational force sensors are beginning to reveal when proteins begin to form structure. While this approach identifies the points during elongation in which structures form, the nature of these conformational transitions often remains unclear. To identify regions of nascent polypeptide chain involved in these transitions, we developed a deep mutational scanning approach to explore the sequence constraints of Escherichia coli dihydrofolate reductase (DHFR) folding on the ribosome. To this end, we developed an arrest peptide-based fluorescence reporter for cotranslational DHFR folding in live-cells. To probe the profile of the amino acids that play key roles in the cotranslational folding of DHFR, we generated a diverse codon-saturated library of ~9,500 DHFR variants in the context of this biosensor. Using fluorescence activated cell sorting and deep sequencing, we have mapped the mutational landscape for co-translational folding of DHFR, providing a comprehensive view of sequence constraints associated with the late-stage co-translational folding transition. Moreover, we profiled the same library in the context of a variant of the sensor bearing a defective arrest peptide, we are currently determining how these mutations differentially disrupt the cotranslational folding reaction. Ongoing efforts integrate sequence, structural, and nucleotide features, revealing determinants of DHFR folding on the ribosome and enabling prediction of folding outcomes across the mutational landscape. This generalizable approach could potentially be used to map the cotranslational folding intermediates of a wide variety of other proteins.

PURDUE UNIVERSITY | James Tarpo Jr. and Margaret Tarpo Department of Chemistry

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