The Driving Force for Co-translational Protein Folding is Weaker Near the Ribosome Surface due to Greater Water Ordering

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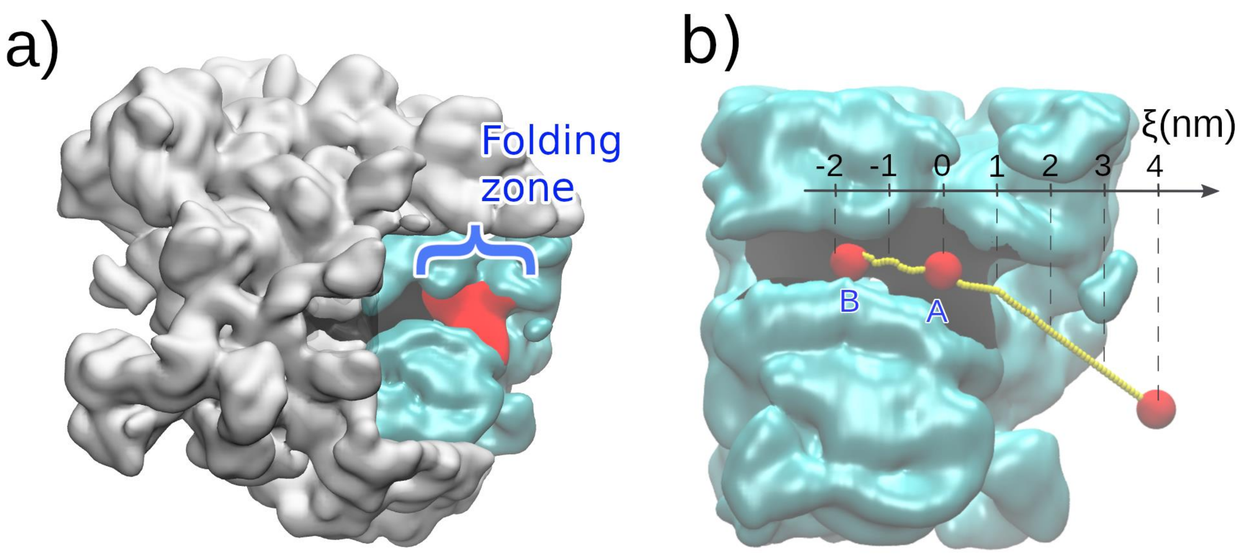
Introduction and Motivation

The association of hydrophobic side chains is the primary driving force for protein folding[1,2]. The first location that tertiary protein folding can occur is in the ribosome, when the nascent polypeptide chain passes through a 10 nm 'exit' tunnel, lined with ribosomal proteins and RNA, and out into the cellular milieu. Single molecule Laser Optical Tweezer[7–9] experiments have observed that the folding process for some proteins becomes slower the closer the folding domain is to the ribosome surface, and NMR[10] and pulse proteolysis[11] experiments have found individual domains are less stable.

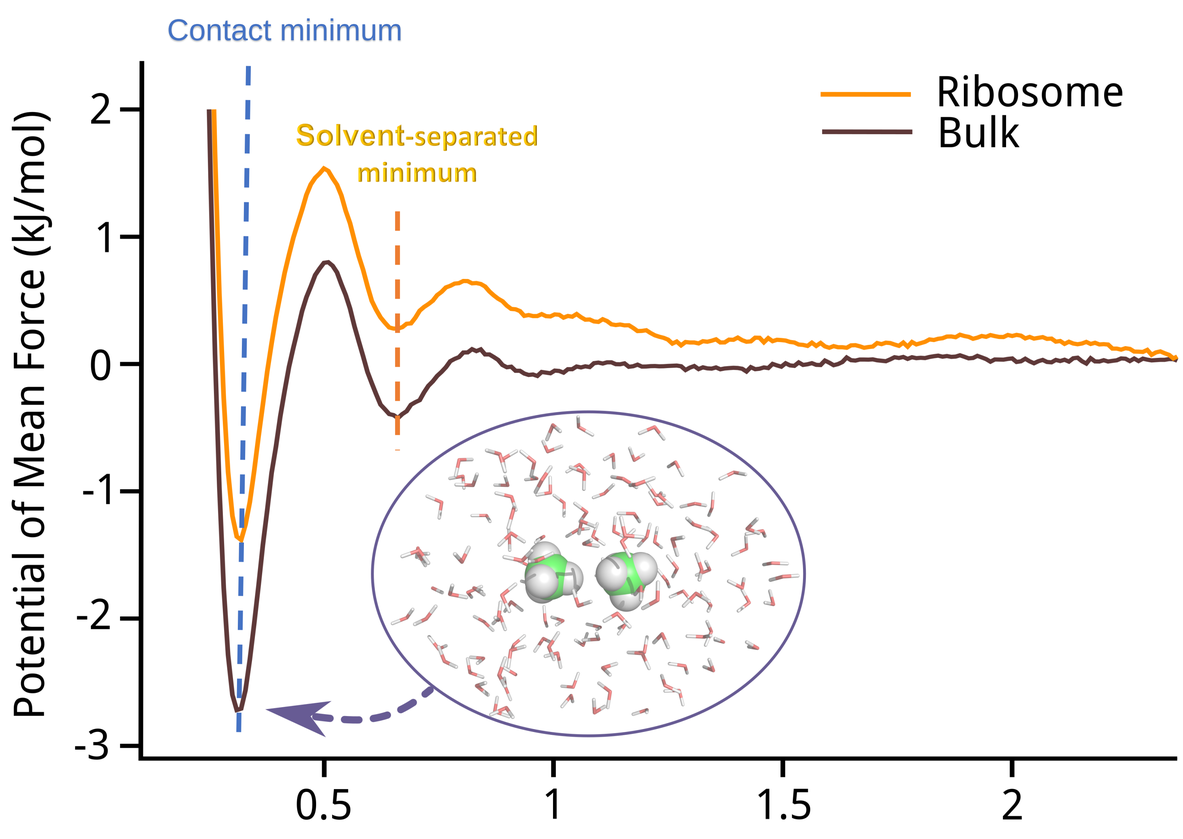


Here, We test the novel hypothesis that the environment around the ribosome weakens the hydrophobic effect, thereby contributing to decreased protein stability and slowing folding.

To do this we:

- Use the conventional physical chemistry approach we calculate the potential of mean force between two hydrophobic molecules in the ribosome exit tunnel and in bulk solution.
- Compare thermodynamic and water structure properties.

Potential of mean force between two hydrophobic molecules in the ribosome exit tunnel and in bulk solution



Results:

- The hydrophobic effect is weakened in the presence of the ribosome: Near the ribosome the contact minimum between two methane molecules is half as stable as compared to in bulk solution.
- The hydrophobic effect contributes 60% to the free energy difference between the folded and unfolded states, we estimate that the free energy of protein stability is decreased by 30%.

Thermodynamic decomposition of Free Energy into Entropy and Enthalpy

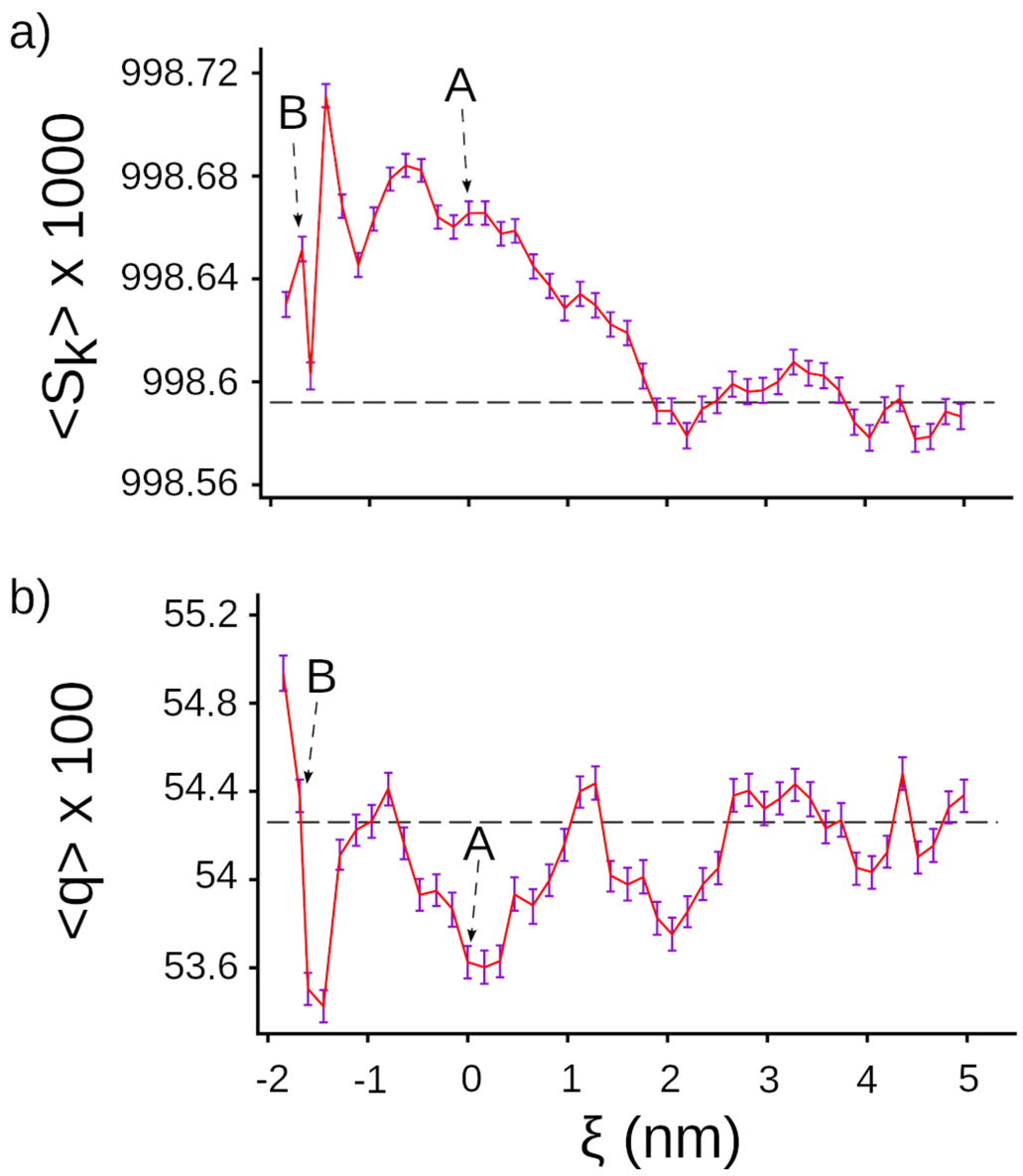
To understand, in terms of thermodynamics, why the hydrophobic effect is weakened by the ribosome we calculated the entropy and enthalpy of association at 310 K using data from multiple temperatures:

Results: The ribosome-induced weakening of hydrophobic association arises from a smaller gain in entropy upon going from the solvent separated configuration of the methanes to the associated state.

System (one methane is fixed at point A)	**Contact Minimum minus Solvent-Separated Minimum (kJ / mol) [mean and 95% confident interval]**		
	ΔΔG	ΔΔH	TΔΔS
Bulk	-2.31 (-2.37, -2.26)	3.81 (2.88, 4.77)	6.12 (5.23, 7.07)
Ribosome	-1.74 (-1.78, -1.70)	2.54 (1.09, 3.81)	4.28 (2.83, 5.55)
P-value (threshold: 0.05)	$<1\times10^{-6}$ (significant)	0.08 (insignificant) X	0.02 (significant)

Water structure properties along the reaction coordinate

To understand why the gain in association entropy in the presence of Ribosome is less than in bulk, we tested whether we could detect signatures of greater water ordering in the exit tunnel by using the tetrahedral orientational (q) and translational (S_k) order parameter[11].
[NOTE: S_k (q) higher means waters are more ordered and vice versa]



Results:

- S_k is higher in the exit tunnel than in bulk, indicating that the water molecules adopt a more tetrahedral structure in terms of their distances.
- q fluctuates above and below the bulk value, indicating the ribosome distorts the water cluster angular configuration to be more or less tetrahedral at different points along the tunnel.
- The angular degrees-of-freedom of the tetrahedron are softer than the distance degrees-of-freedom, meaning that it takes more energy to change the distances than the angles.

Conclusions

- Near the ribosome the contact minimum between two methane molecules is half as stable as compared to in bulk solution, demonstrating that the hydrophobic effect is weakened in the presence of the ribosome.
- Thermodynamic decomposition and structural analyses reveal that the weakening of the hydrophobic effect is due the increased ordering of water molecules in the presence of the ribosome.

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