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Influence of the ribosome on protein ejection and folding

Abstract

Proteins are synthesized by macromolecular machines called ribosomes, which are found in cells across all species, from bacteria to humans. They perform various tasks necessary to support life. To carry out their functions, many proteins must first self-assemble into a specific configuration known as the native state. The process of a protein attaining its native state is termed protein folding. The folding of proteins in isolation has been extensively studied for over a half-century. However, within cells, proteins are translated by the ribosome based on information contained in an mRNA sequence and emerge through the exit tunnel to the cytosol after synthesis. Proteins can acquire tertiary structure at any stage: during their biosynthesis, as they are ejected through the ribosome's exit tunnel, or posttranslationally – after their release from the ribosome. Indeed, several computational and experimental studies have shown that proteins can start to fold while they are still being synthesized by the ribosome. This phenomenon, known as cotranslational folding, is mediated by the spatial constraints of the ribosomal exit tunnel as well as the interactions between the nascent chain and the ribosome surface. These factors can potentially impact the kinetics and pathways of protein folding. Therefore, gaining a comprehensive understanding of protein behavior during their early stages of existence is of utmost importance and remains a significant focus of ongoing research.

This thesis contains three computational studies related to protein ejection and folding on the ribosome. The introduction to the ribosome and protein folding on the ribosome is summarized in Chapter 1. Chapter 2 describes the computational methods with a focus on the computational modeling and analyses used in the research presented in this dissertation.

In Chapter 3, the ejection process of nascent protein out of the ribosome exit tunnel is described. This process has not been studied before as it is believed to be fast, show little variation between proteins, and have no biological significance. Using a combination of multiscale modeling, and ribosome profiling experimental data, we find a greater than 1000-fold variation in ejection times. Nascent proteins enriched in negatively charged residues near their C-terminus eject the fastest, while nascent proteins enriched in positively charged residues tend to eject much more slowly. More work is required to pull slowly ejecting proteins out of the exit tunnel than quickly ejecting proteins, according to all-atom steered molecular dynamics simulations. An energetic decomposition reveals that the slow ejection is due to the strong attractive electrostatic interactions between the nascent chain and the negatively charged ribosomal-RNA lining the exit tunnel, while the quick ejection of proteins is due to their repulsive electrostatic interactions with the exit tunnel. Ribosome profiling data from *Escherichia coli* reveals that the presence of slowly ejecting sequences correlates with ribosomes spending more time at stop codons. This indicates that the ejection process might delay ribosome recycling and could influence the cotranslational behavior of proteins.

Chapter 4 presents the results of the all-atom simulations of hydrophobic interactions in the presence and absence of the ribosome. Interactions between the ribosome and nascent protein can destabilize folded domains in the ribosome exit tunnel's vestibule, the last 3 nm of the exit tunnel where tertiary folding can occur. Here, we test if the contribution to this destabilization is the weakening of the hydrophobic association, which is the driving force for protein folding. The potential-of-mean force between two methane molecules along the center line of the ribosome exit tunnel and in bulk solution was calculated. The results indicate that the associated methanes are half as stable in the ribosome's vestibule as compared to bulk solution, demonstrating that the hydrophobic effect is weakened by the presence of the ribosome. We demonstrate that the weakening of the hydrophobic effect is due to the increased ordering of water molecules in the presence of the ribosome. These findings mean that nascent proteins pass through a ribosome vestibule environment that can destabilize folded structures. This, in turn, can potentially impact cotranslational protein folding pathways, as well as their energetics and kinetics.

In Chapter 5, the influence of protein synthesis and posttranslational folding on protein folding efficiency is described and compared to the folding from denatured states in bulk solution. To make this comparison, coarse-grained molecular dynamics simulations were performed for dihydrofolate reductase (DHFR), type III chloramphenicol acetyltransferase (CAT-III), and D-alanine–D-alanine ligase B (DDLB) proteins. The results indicate that the influence of ribosomes on folding efficiency depends on the protein size and complexity. For small, simple folds (DHFR), the ribosome facilitates efficient folding by preventing misfolding. However, for larger, more complex proteins (CAT-III and DDLB), the ribosome may not promote folding and may contribute to intermediate misfolds during translation. Additionally, it was found that the folding efficiency correlates with the presence of tertiary structural elements known as entanglements in the native structure.

Finally, Chapter 6 summarizes the conclusions that can be drawn from this work and directions for future research.

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