Studying how proteins behave within cells by replicating cellular conditions through advanced supercomputer simulation

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Introduction

Abstract
The interior of a living cell is densely crowded, and macromolecules such as proteins, nucleic acids, carbohydrates, and lipids function in that crowded environment. A protein's behavior will be different in crowded intracellular environments than in dilute conditions used commonly in biochemical studies; the high level of molecular crowding is expected to impact a protein's structure and dynamic, thus its function. To gain a molecular-level understanding of molecular crowding effects on protein structure-dynamics-function relationships, we have chosen an important family of enzymes called prolyl-tRNA synthetases, which are involved in protein synthesis in all living organisms. We constructed protein systems in crowded environments for computational simulations using Visual Molecular Dynamics, a molecular modeling and visualization computer program, and performed molecular simulations on the Blugold supercomputer. We initiated our computational study with prolyl-tRNA synthetase from *E. coli* and ficoll 70, a synthetic polysaccharide (carbohydrate), as crowders. We conducted 100 ns molecular dynamics simulations and analyzed intermolecular interactions and protein backbone fluctuations to investigate the effects of crowding. The preliminary results of our study will be presented. The findings of this study may help provide a molecular-level understanding of protein behavior in crowded cellular environments, which we know is crucial for the design of drugs targeting prolyl-tRNA synthetases of pathogenic organisms.

Background

- The intracellular environment is often described as complex and diverse, made up of many proteins, nucleic acids, carbohydrates, lipids, and other biomolecules that are packed tightly together, with some estimates suggesting that up to 40% of the cellular volume is occupied by macromolecules (*1*).
- In an intracellular environment, the behavior of proteins and other macromolecules can be extremely different from what we observe in vitro. The high concentration of molecules and the resulting steric hindrance, electrostatic interactions, and hydrodynamic effects all can impact how a protein structure can change and function (*3*, *4*).

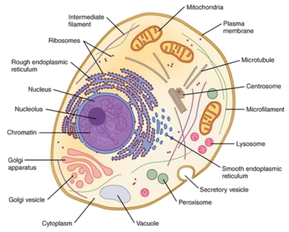
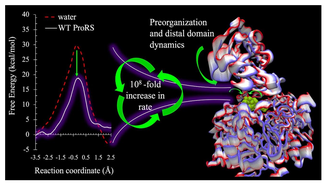


Figure 1. Inside of a cell (*2*).

- Prolyl-tRNA Synthetases (ProRSs) are a specific example of aminoacyl-tRNA synthetases (AARSs), which help translate genetic information into proteins. ProRSs are multidomain and each domain has a specific function. For example, the catalytic domain (CD) catalyzes the attachment of proline to its tRNA, and an editing domain (ED) ensures accuracy by removing incorrectly attached amino acids**.**
- The dynamic coupling between distant domains of ProRSs is crucial for effective catalysis (*4*). Hybrid quantum mechanical/molecular mechanical (QM/MM) simulations revealed that the global dynamics of ED preorganize the active site and the local fluctuations reorganize the electrostatics of the catalytic pocket, impacting the height and width of the Gibbs free energy profile (4). Overall, the ED dynamics contribute to ~1/2 of the catalytic power of the Ec ProRS.



- Crowded environments are expected to impact the editing domain dynamics and therefore the catalytic function of the multidomain ProRS.

Figure 2. The role of pre-organizing dynamics of the editing domain of *E. coli* ProRS observed previously (*4*).

- AARSs, including ProRSs, are common targets for antibiotic drug discovery. This is because inhibiting these enzymes can disrupt protein synthesis, which is essential for bacterial growth and survival. The variations in domain architecture of AARSs from the different species (Fig. 3) allow researchers to design antibiotics that specifically target bacterial AARSs without affecting the human versions.

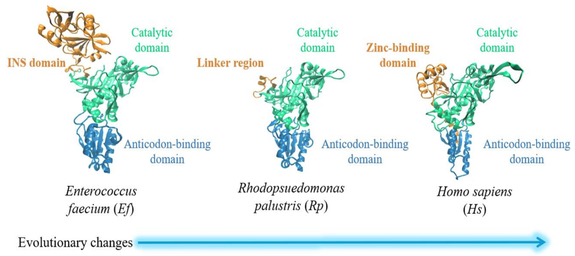


Figure 3. The structurally diverse ProRSs from different species.

Objective
This study aimed to see the effects of molecular crowding on the structure and dynamics of Prolyl-tRNA Synthetase (ProRS) using ficoll 70 as the crowding agent. Ficoll 70 is a synthetic polysaccharide (Fig. 4) commonly used to mimic crowded cellular environments, and by using it we hoped to see how the enzyme's function and structure changed.

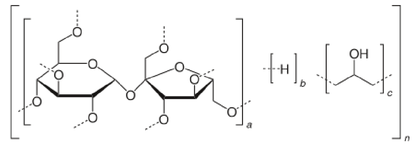


Figure 4. The two-dimensional structure of ficoll 70.

Methods

- Molecular dynamics simulations use classical mechanics to model atoms in a molecule as point masses with charges, ignoring electrons and treating bonds as permanent. Force field interactions are mathematical equations and constants that describe energy fluctuations, due to changes in molecular geometry changes, encompassing bonding and nonbonding interactions (*5*).

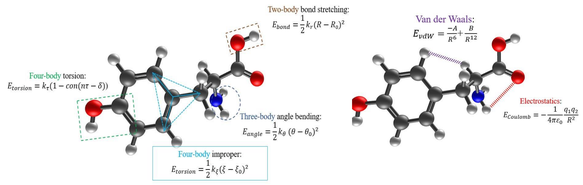


Figure 5. Force fields and interactions.

- To create the molecular dynamics simulation, we developed the 3D model structure of *E. coli* ProRS using *E. feacium* ProRS structure (PDB code: 2J3L) as a template. The crowder molecules, ficoll 70, were built using Visual Molecular Dynamics (VMD) (6).
- We generated the protein-crowder system by combining the protein with the desired number of crowders using VMD. To manipulate them into the position we used VMD commands, and then saving the coordinates of each crowder when the desired 3D confirmation was reached.
- We then solvated the system by creating a solvation box, adding the psf and pdb files of the molecule dimer, and adjusting settings such as box padding and solvent options. We then proceeded to ionize the system.

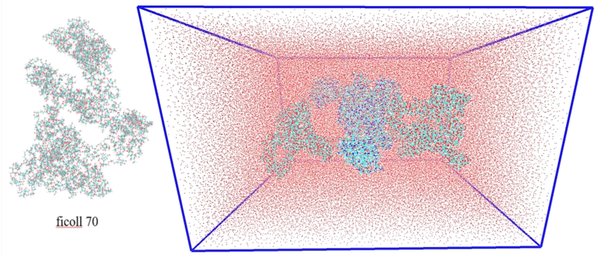


Figure 6. The *E. coli* ProRS in a solvent box containing ficoll.

Results

The results suggest that the crowder may have a confinement effect on the protein. Where the crowder wraps over the protein surface like a cage. The initial distance between the SUBB and SUBA was around 7.08 and 7.06 Å, indicating a compact conformation. When the crowding agent was introduced, the distance increased, with SUBA and SUBB showing a growth of 29.58 Å and 16.48 Å, respectively. With the observation of the crowder never directly touching the protein but wrapping around it, coupled with the data, this suggests that the crowding agent has a confining effect.

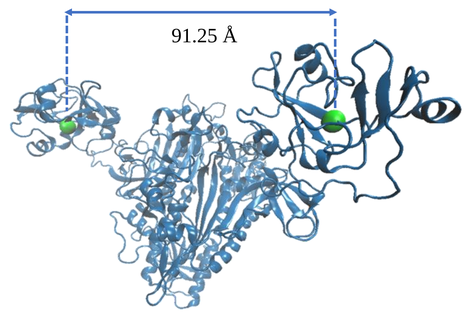


Figure 7. The intra-editing domain distance. The center of mass of two subunits is shown as green spheres. The distance between the two Eds were 110 Å in absence of PEG 600.

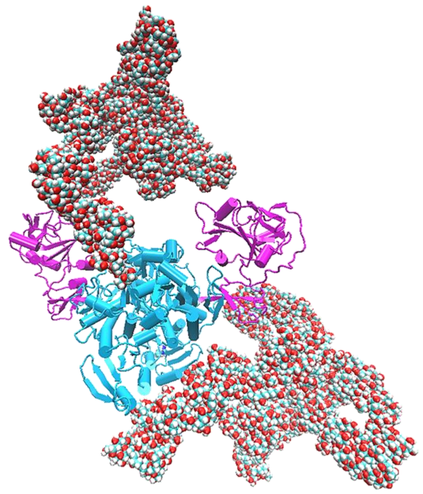


Figure 8. Two ficoll 70 polymers surround the dimeric ProRS.

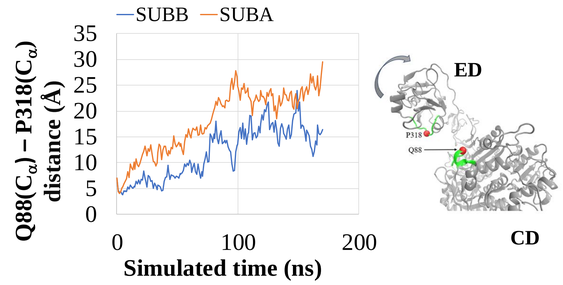


Figure 9. Dynamic changes in the active site cleft of Ec ProRS at the interface of editing (ED) and catalytic (CD) domains in the presence of PEG 600 for SUBA and SUBB over the simulated time.

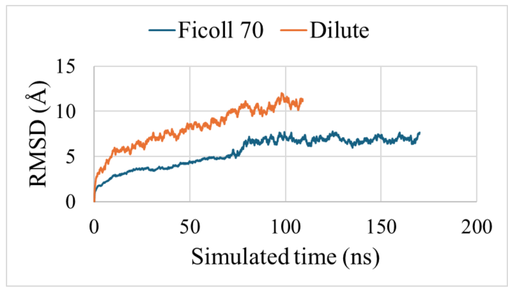


Figure 10. The RMSD of the backbone C_α atoms of *E. coli* ProRS in dilute and crowded conditions over the simulated time.

Conclusions

- The results of these MD simulations revealed that the crowder had a confinement effect on the protein. They wrapped around the protein surface and impacted protein flexibility and compactness. This may be the reason for the significant impact on protein function as observed earlier (*3*).
- Future research can branch off to examine if the type of crowding agent, or the concentration of crowing agent affects the protein's behavior. Or maybe explore the specific mechanism behind the confinement effect we observed here.

Acknowledgment

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