Investigating the Macromolecular Crowding and Confinement Effects Using High-Performance Computational Simulations: A Case Study with *Escherichia coli* Prolyl-tRNA Synthetase

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Abstract

Proteins are biological machines, whose functions require specific movements of their structural elements, which include domains and loops. These motions are critical for molecular recognition – a central paradigm for designing drug molecules to combat various diseases. The intracellular space is densely packed with metabolites and macromolecules or "crowders" that restrict proteins' motions. Are these crowders able to influence small molecule recognition by an enzyme (protein catalyst), and if so, by what mechanism? One way to investigate the effects of crowding and confinement on an enzyme's dynamics and function is to create a virtual system by constructing molecular models of enzymes encapsulated by solvent and crowder molecules. In the present study, molecular dynamics simulations are being performed to examine the impact of crowding and confinement on the prolyl-tRNA synthetase of *Escherichia coli*. This enzyme plays a vital role in protein production in living cells. With these simulations, the cellular environment can be replicated to probe enzyme dynamics and functions computationally in the presence of synthetic crowders of varying sizes and chemical properties. Furthermore, simulation data can be used to quantify the change in molecular recognition of the target protein. The method of setting up ternary systems comprising solvent molecules, crowders, and the protein of interest and the preliminary simulation results will be presented. Understanding how crowder molecules influence protein dynamics and function could lead to applications in areas of drug discovery and design.

Biological Significance of Macromolecular Crowding

- Within the intracellular space, macromolecules of varying sizes can impact an enzymatic function by influencing substrate binding and product release. This could be due to changes in protein movements in crowded environments.
- The target enzyme of this study is prolyl-tRNA synthetase (Ec ProRS), a member of aminoacyl-tRNA synthetases that functions to covalently attach the amino acid proline to the 3'-end of tRNA.
- The function of this binding is vital to protein synthesis in living cells.

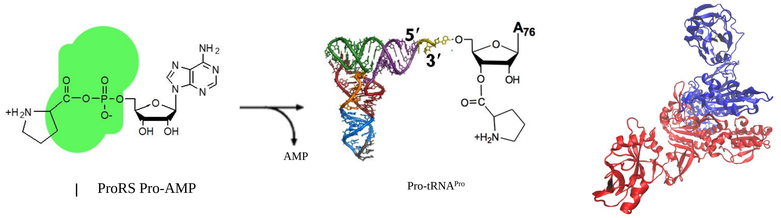


Fig 1. The tRNA synthetase docked with Pro-AMP charges tRNA with proline for protein translation.

Fig 2. Ribbon structure of prolyl-tRNA synthetase of *Escherichia coli*.

Objective

- Development of a series of 3-D models of the target protein in the intracellular-like environment to simulate the target protein in crowded environments and examine the influence of crowder molecules of varying sizes on its dynamics.
- Conduct a binding study using these 3-D models of Ec ProRS in the crowded environment with ProRS Pro-AMP bound.
- Development of a better understanding of how crowders impact enzymatic function.

Design

- Simulate the intracellular-like environment with biologically inert molecules to replicate the intracellular crowder molecules. Water molecules and sodium ions are also added.
- Use high-performance computation to optimize these systems and observe changes in protein dynamics in different crowded environments.

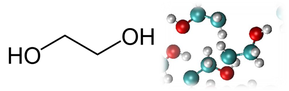


Fig 3. Chemical structure of biologically inert ethylene glycol (EG) alongside 3-D representation.

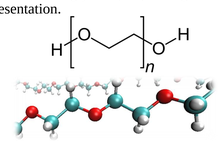


Fig 4. Chemical structure of biologically inert polyethylene glycol (PEG) alongside 3-D representation.

Methods

Development of Protein Systems to Model Intracellular Environments

- Protein structures are retrieved from the Protein Data Bank, the biological molecule databases, as protein data bank files. Polymer chains used to simulate crowder molecules are built using VMD.
- The biological modeling software VMD is used to make 3-D models that include the target protein, solvents, and crowder molecules.

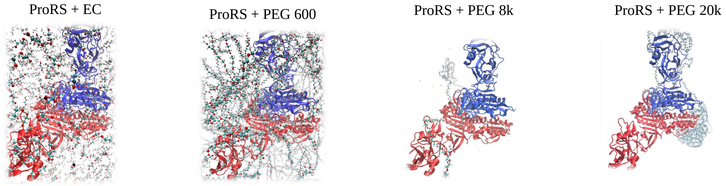


Fig 5. VMD generated 3-D environments of *Escherichia coli.* prolyl-tRNA synthetase in EG, PEG 600, PEG 8k, and PEG 20k.

Minimization

- Program NAMD with CHARMM parameters is used to optimize simulated cellular environments.

Dynamics

- Using Newtonian mechanics, each atom of the simulated cellular environment is tracked as point charges with some mass.
- The simulation is carried out with a set number of steps showing molecular interactions over 100 ns.
- Using VMD, visual representations of dynamic simulations can be generated allowing the observance of molecular changes.

Dynamic Effects

- Changes in restriction of protein motion by crowder molecules.
- Effects of crowder molecules on enzyme active site and substrate binding.

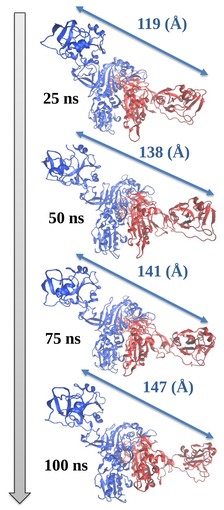


Fig. 6: Ec ProRS editing domains flex outwards during 100 ns dynamic simulations in the absence of crowders.

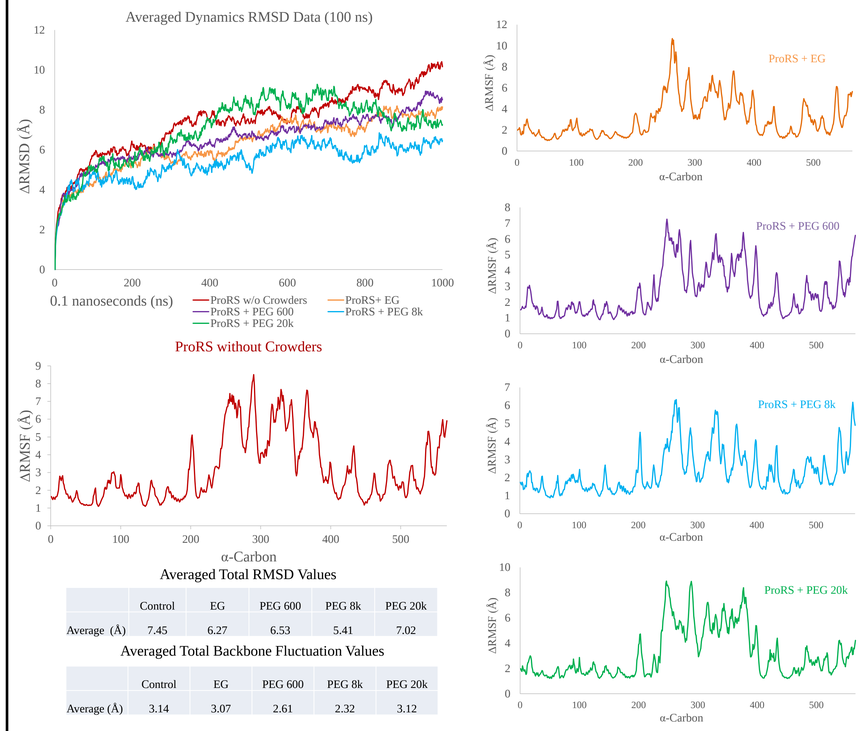
Methods (Continued)

Analysis

- Using the program CARMA, principal component analysis was performed to visualize how each molecular system evolved over time.
- The deviation of the backbone C_α atoms' fluctuation from the original structure demonstrates the influence different crowders have on specific regions and overall enzyme motion.

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N} r_i^2}$$

Preliminary Results



Averaged Total RMSD Values

	Control	EG	PEG 600	PEG 8k	PEG 20k
Average (Å)	7.45	6.27	6.53	5.41	7.02

Averaged Total Backbone Fluctuation Values

	Control	EG	PEG 600	PEG 8k	PEG 20k
Average (Å)	3.14	3.07	2.61	2.32	3.12

Conclusions and Future Directions

- Principal component analysis shows restricted protein movement with increasing crowder size.
- Complete a binding study with inhibitor molecule PLP bound to Ec ProRS in the presence of crowder molecules such as EG, PEG 600, and PEG 20k.

References-

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Commitment to equity, diversity, and inclusion

- We acknowledge that the University of Wisconsin-Eau Claire occupies the sacred and ancestral lands of Indigenous Peoples. We honor the traditional land of the Ojibwe and Dakota Nations.
- We commit to a teaching and learning environment that ensures a culture of equity, diversity, and inclusion.

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