Coarse-Grained Simulations of Globular Proteins in Crowded Conditions

Lucas Zimmer

Mentors: Dr. Sanchita Hati and Dr. Sudeep Bhattacharyay, Department of Chemistry and Biochemistry, UW-Eau Claire, WI

University of Wisconsin Eau Claire

Introduction

Background

Within cells, proteins coexist with lipids, carbohydrates, nucleic acids, ions, and metabolites. These biomolecules interact through noncovalent interactions, which define protein structure and, consequently, function [1]. Under certain cellular conditions, these interactions can drive the reversible assembly of molecules into condensates, altering local molecular concentrations and modulating protein function. In some cases, changes in cellular conditions can also lead to the irreversible formation of protein aggregates.

Atomistic molecular dynamics simulations, together with experimental studies using polymers to simulate crowding, have provided valuable insights into the mechanisms underlying condensation and aggregation of intrinsically disordered proteins/regions which lack well-defined structures. However, less is known about how proteins with reduced flexibility, such as globular proteins, behave in crowded environments and whether they preferentially form aggregates or condensates.

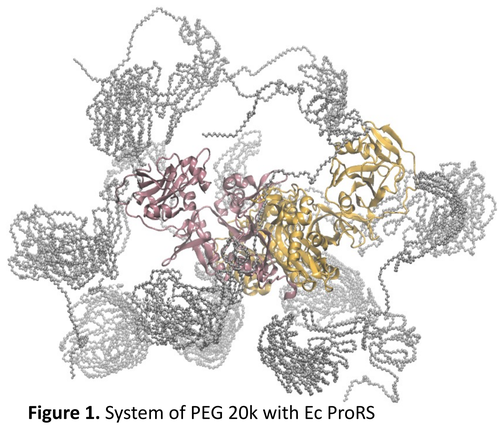
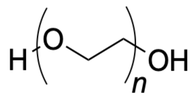


Figure 1. System of PEG 20k with Ec ProRS

Our current system of study is *Escherichia coli* (*E. coli*) Prolyl t-RNA Synthetase (Ec ProRS) in the presence of polyethylene glycol (PEG 8k and PEG 20k) and Dextran (DEX 40k)

a.



b.

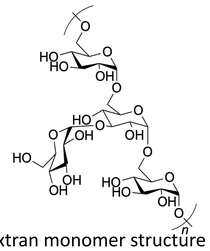


Figure 2. a) PEG monomer structure b) Dextran monomer structure

Aminoacyl t-RNA synthetases (AARS) such as ProRS are common targets for drug development as they are crucial to cell survival yet vary greatly in structure between different species [2].

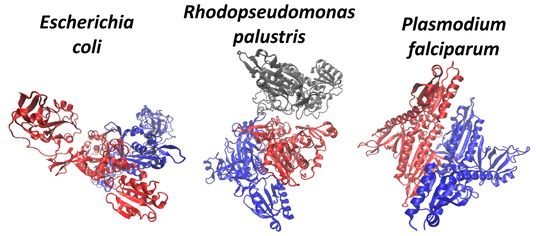


Figure 3. Structures of ProRS within different species

Objectives

We are using coarse-grained molecular dynamics simulations to investigate the behavior of globular proteins under crowded conditions and to determine the reversibility of their phase separation. Coarse-grained simulations enable efficient modeling of large systems by reducing the number of particles, providing a practical approach to studying phase separation processes. This method allows us to distinguish between condensation and aggregation and explore the underlying molecular mechanisms. To ensure accuracy, interaction parameters in the coarse-grained models will be derived from both experimental data and atomistic simulations.

Methods

- **MARTINI** – Coarse-grained Ec ProRS and produced interaction parameters for the coarse-grained model.
- **POLYPLY** – Used to generate coarse-grained polymers from a random walk within the system.
- **GROMACS** – Molecular dynamics software used to minimize the system, run molecular dynamics simulations, and analyze data.
- **Visual Molecular Dynamics (VMD)** – Employed to visualize and analyze systems.

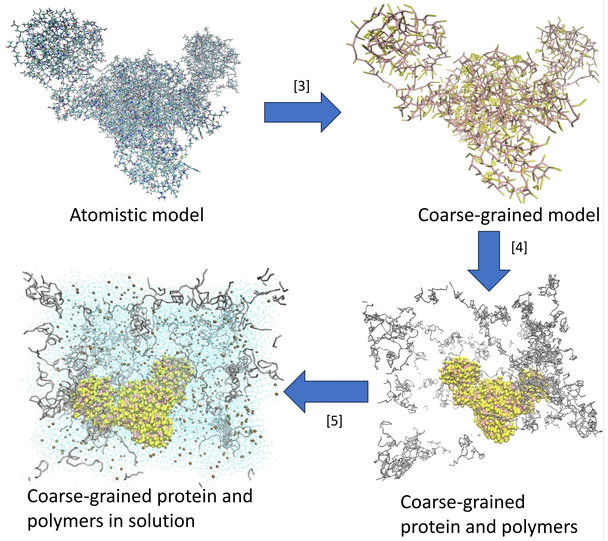


Figure 4. Workflow of producing a coarse-grained system

$$V_{LJ} = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right]$$

Equation 1. The Lennard Jones (LJ) Potential, where ε is the potential well depth, σ is the collision diameter, and r is the distance between the centers of two particles

Preliminary Results

Our preliminary results show that the coarse-grained nonbonding crowder-crowder and crowder-protein interactions are not optimized. This is in reference to the atomistic simulations of the same systems. RMSD of the particles relative to their initial position as well as editing to catalytic domain distances are not consistent with atomistic values.

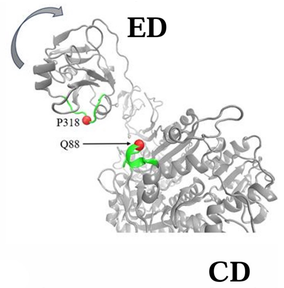


Figure 5. Locations of catalytic and editing domain interfaces

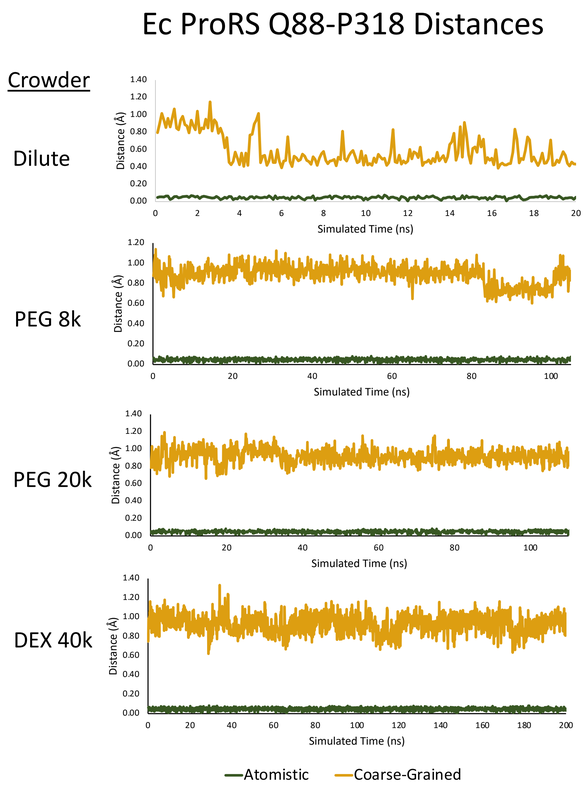


Figure 6. Distance between the interface of editing (ED) and catalytic (CD) domains in the presence of different crowders over the simulated time

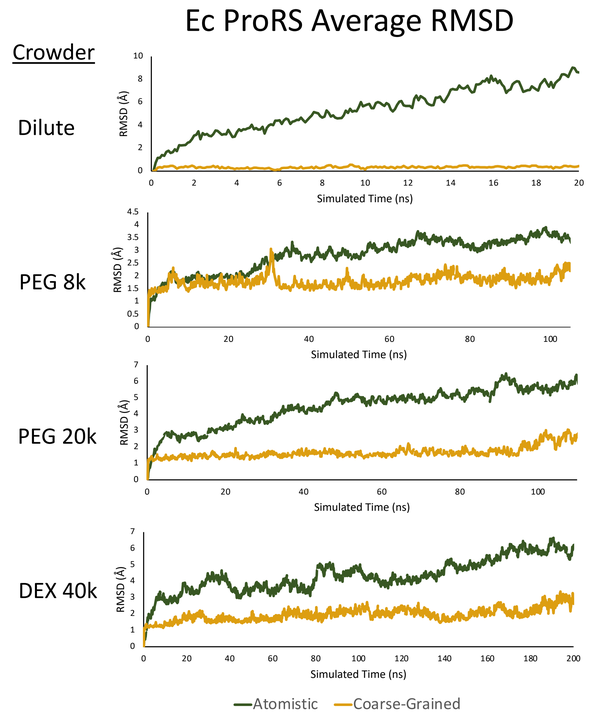


Figure 6. Average RMSD of Ec ProRS from starting coordinates in the presence of different crowders over the simulated time

Conclusions

- The results of these coarse-grained MD simulations have shown a deviation from standard atomistic simulations and reveal a need to refine parameters and system conditions in order to accurately model our system
- In the future we plan to reparametrize our coarse-grained model and once accurate, apply it to observe LLPS mechanisms

Acknowledgements

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