Molecular Dynamics Simulations to Investigate Macromolecular Condensate Formation in the Aqueous Solution of Polyethylene Glycol of Variable Sizes

Amber Lock

Mentors: Dr. Sanchita Hati and Dr. Sudeep Bhattacharyay

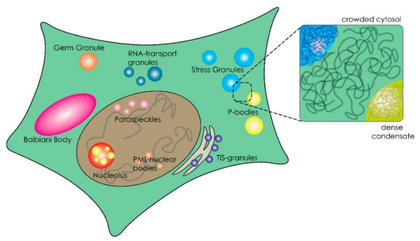
Department of Chemistry and Biochemistry, UW-Eau Claire, Wisconsin - 54701

Abstract

Biomolecular condensates are membraneless organelles formed by the reversible assembly of biomolecules such as proteins, nucleic acids, and lipids. The formation of these biomolecular condensates increases the local concentration of the respective biomolecules and facilitates various biological processes such as gene expression, DNA damage response, and signal transduction. For understanding the molecular mechanism of biomolecular condensate formation, different commercially available synthetic polymers, such as polyethylene glycol (PEG) and Ficoll 70, a synthetic polysaccharide, are often used for in vitro studies. These hydrophilic polymers can induce phase separation and condensate formation by altering the solvation properties of biomolecules. Also, weak interactions between synthetic polymers and biomolecules are believed to play a crucial role in inducing condensate formation. However, the exact molecular mechanism of the condensate formation by synthetic polymers in water is poorly understood. We are employing atomistic molecular dynamics simulations to investigate the molecular mechanisms of macromolecular condensate formation. Our initial focus is on PEG of variable sizes, ranging from PEG 100 Da to PEG 20,000 Da. The high-performance computing facilities at UWEC are being used to perform long-timescale molecular dynamics simulations and explore intricate dynamics in macromolecular condensate formation.

Background

- Biomolecular condensates (BMCs) are naturally occurring membraneless organelles in eukaryotic cells that play crucial roles in various cellular processes, including signal transduction, gene expression, and stress response.[1]
- Often described as a gel-like substance, BMCs result from protein crowding that undergoes liquid-liquid phase separation (LLPS). BMCs contain a variety of biomolecules and form reversible assemblies.



- Growing evidence suggests that biomolecular condensates are connected to neurodegenerative diseases characterized by protein aggregation, including Alzheimer's disease and amyotrophic lateral sclerosis.[2]

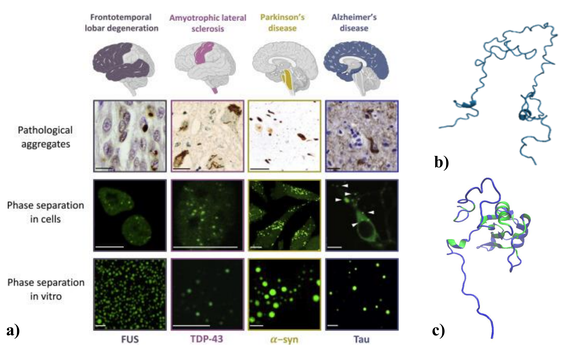


Figure 2. a) Biological effects of condensates[3] b) Tau Protein c) FUS

- The thermodynamics of condensate formation involves a complex interplay between entropy and enthalpy. The loss in entropy due to ordered assembly formation inside the liquid-like condensate is compensated by the increase in intermolecular interaction enthalpy. The main factors that promote LLPS include changes in biomolecule concentration and intermolecular interactions.[4]

Objective

- A thorough understanding of the molecular mechanism of condensate formation by biomolecules and the impact of phase separation on their functions.
- Understanding the mechanisms and environment that facilitate the formation of BMCs is crucial to addressing issues related to cellular physiology. It could lead to new drug discovery, especially in the realm of Alzheimer's disease. It can aid in drug delivery and development methods, such as the disruption of pathological condensates. Also, BMCs are crucial for viral replication and are promising targets for novel antiviral therapies.[5]
- Synthetic crowders like polyethylene glycols (PEG) of variable sizes (PEG 600, PEG 8k, and PEG 20k; molar mass of 8,000 and 20,000 g/mol, respectively) are being used to understand the molecular mechanism of phase separation and condensate formation.
- The LLPS process is sensitive to ionic strength, temperature, and pH. Variable salt concentrations is being employed to understand the effects of multivalent interactions in BMC formation.

Methods

- **System setup:** Each PEG system (600, 8k, and 20k) was prepared with the Visual Molecular Dynamics program (VMD). Using its onboard tools, systems were hydrated with water and were balanced with varying concentrations of NaCl. All data presented here come from 0.15 M NaCl systems.

Table 1. Size and make-up of the PEG systems.

PEG System (Number of chains)	Number of atoms in simulation (no water)	Number of atoms in simulation (with water)
PEG 600 (24)	23,030	671,795
PEG 8k (18)	23,112	589,515
PEG 20k (8)	25,384	914,728

- **Atomistic simulations:** Using the NAMD and CHARMM programs,[6] simulations were run on the BOSE computing cluster provided by the Blugold Center for High-Performance Computing at UW-Eau Claire.

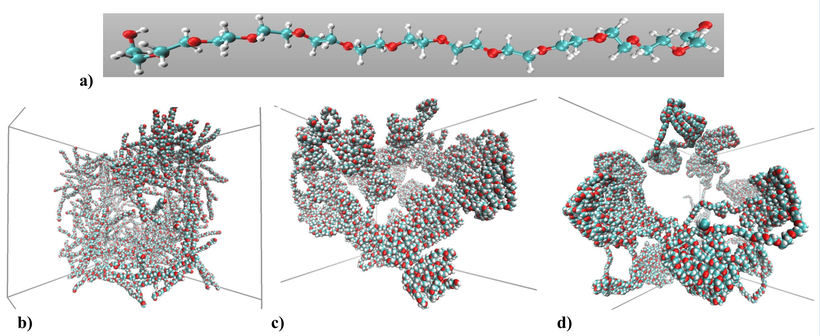


Figure 3. Three systems were developed containing PEG chains of varying lengths, and a varying number of those lengths. a) Example PEG chain, H [-O-CH_2CH_2]$_n$-OH b) PEG 600 c) PEG 8k d) PEG 20k

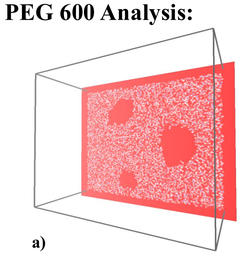
- **Data analysis:** Radial Distribution Functions (RDF), along with volumetric maps, were calculated for each system using VMD's on board tools for analyzing data generated by the atomistic simulations.

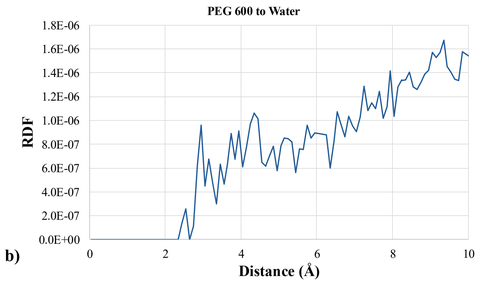
Preliminary Results

Table 2. Progression of each PEG system in nanoseconds. PEG 20k will not be presented.

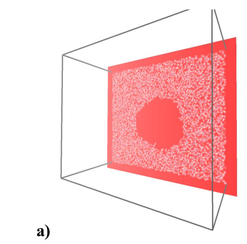
PEG System	Simulation Length (ns)	Simulation Status
PEG 600	132	Completed
PEG 8k	94	Running
PEG 20k	0	Running

PEG 600 Analysis:





PEG 8k Analysis:



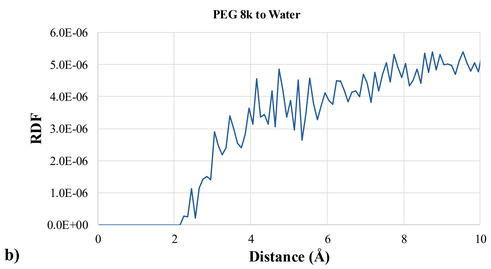


Figure 4. Results for the PEG 600 and PEG 8k systems, respectively. a) Volumetric data describing the system. White dots indicate water and ions, while red indicates empty space/PEG presence. b) RDF data of the system.

Conclusions and Future Directions

- The preliminary results suggest each system forms a condensate and clusters together in ways consistent with previous experimental and computational findings from our research group. As seen above, PEG 600 forms multiple smaller condensates, while the 8k system tends to form a single larger condensate.
- Each system will be run until at least 100 ns has been reached for each to allow the systems to develop.
- The effect of temperature and varying levels of salt concentrations will be studied.

Acknowledgments

- This research was funded by the National Science Foundation Research for Undergraduates grant OAC-2447779. Computational resources for this study were provided by the Blugold Center for High Performance Computing under NSF grant CNS-1920220.
- Departments of Chemistry and Biochemistry for providing access to their equipment and facilities.
- We recognize that UW-Eau Claire occupies the sacred and ancestral lands of Indigenous Peoples. We honor the land of the Ojibwe and Dakota Nations.

References

1. Gorsheneva NA, Sopova JV, Azarov VV, Grizel AV, Rubel AA. Biomolecular Condensates: Structure, Functions, Methods of Research. Biochemistry (Mosc). 2024 Jan;89(Suppl 1):S205-S223. doi: 10.1134/S0006297924140116. PMID: 38621751.
2. Visser BS, Lipiński WP, Spruijt E The role of biomolecular condensates in protein aggregation. Nat Rev Chem. 2024 8(9):686-700. doi: 10.1038/s41570-024-00635-w. Epub 2024 Aug 12.
3. Zbinden A, Pérez-Berlanga M, De Rossi P, Polymenidou M. Phase Separation and Neurodegenerative Diseases: A Disturbance in the Force, Developmental Cell, Volume 55, Issue 1, October 2020. doi: 10.1016/j.devcel.2020.09.014.
4. Poudyal et al. Intermolecular Interactions Underlie Protein/Peptide Phase Separation Irrespective of Sequence and Structure at Crowded Milieu. Nat. Commun. 2023, 14, 6199.
5. Erik W. Martin, Christiane Iserman, Balaji Olety, Diana M. Mitrea, Isaac A. Klein, Biomolecular Condensates as Novel Antiviral Targets, Journal of Molecular Biology, Volume 436, Issue 4, 2024, 168380.
6. Phillips, et al.Scalable Molecular Dynamics with NAMD. J. Comput. Chem. 2005, 26, 1781– 1802.