NON-EQUILIBRIUM ENTROPY CALCULATIONS OF MEMBRANE PROTEINS DURING THE AGGREGATION PROCESS

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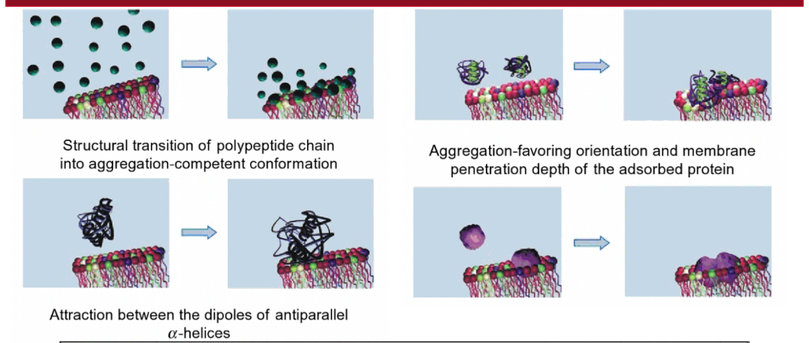
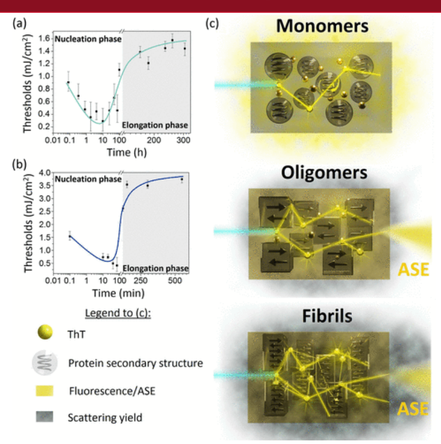
Abstract

Aggregation of membrane proteins is a complex biological phenomena with potentially detrimental consequences. It is recognized as the hallmark of neurodegenerative diseases, suffered by millions of people each year. Abnormal deposits of amyloid fibrils accumulate causing irreparable damage that results in the deterioration of brain tissue and the death of vital neuron cells. This leads to severe impairment in cognitive functioning that progresses at an exponential rate. Membrane protein aggregation is known to be a highly dynamic, irreversible process which is the source of its difficulty to understand and develop new technologies for therapeutic intervention and early detection of neurodegenerative diseases. The design of our study is to interpret the mechanics of membrane proteins that misfold and self-assemble into highly structured fibrils. The aim is to gain a deeper understanding of protein-membrane interactions and the misfolding mechanisms that attribute to the aggregation process. The complexity this biophysical process cannot be accurately modeled using statistical physics and statistical thermodynamics of equilibrium processes. Which suggests, according to numerous studies, that membrane protein aggregation is a non-equilibrium process. Based on non-equilibrium physics, one of the best ways to understand aggregation is through the Langevin equations and the Fokker-Planck equations. Langevin equations describe the stochastic dynamics of non-equilibrium processes and the Fokker Planck equation is used to calculate the probability distribution that reveals the trend in entropy of a model independent protein aggregation process

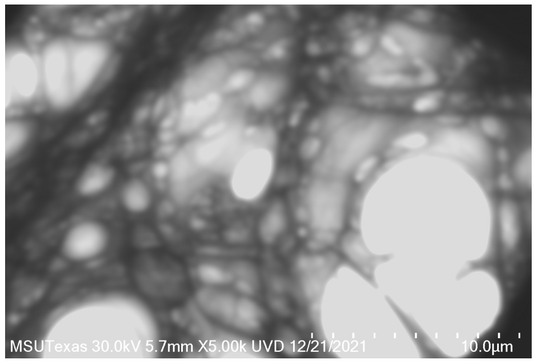
Protein-Membrane Interactions



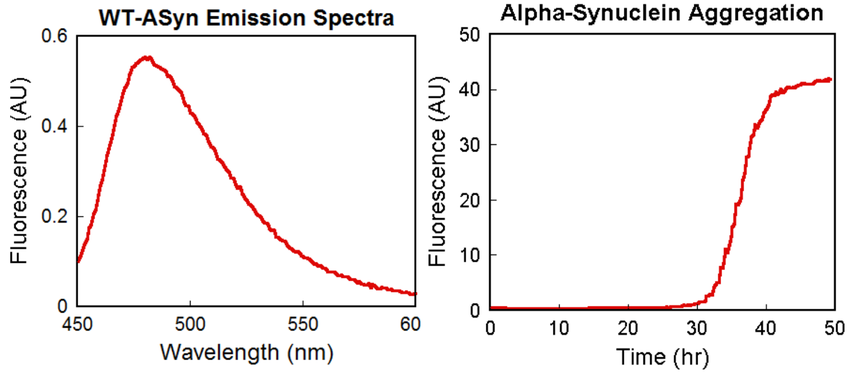
Model Background



STEM imaging of Alpha-Synuclein Fibrils



Laboratory Results



Langevin and Fokker Planck Model Equations

Using the Langevin equation we can write this as

$$P = \langle \sum f_i(\mathbf{x})[f_i(\mathbf{x}) + r_i(t)] \rangle$$

and so S_g can be written as

$$S_g = \frac{\tau}{T_0} \langle \sum ([f_i(\mathbf{x})]^2 + D_i f_{ii}(\mathbf{x})) \rangle$$

where $f_{ii} = \frac{\partial f_i}{\partial x_i}$ Considering the mean value, we can finally write this as

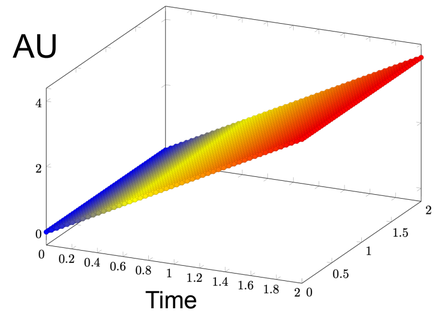
$$S_g = \frac{\tau}{T_0} \int \sum ([f_i(\mathbf{x})]^2 + D_i f_{ii}(\mathbf{x})) P_\gamma(\mathbf{x}, t) d\mathbf{x}$$

and hence

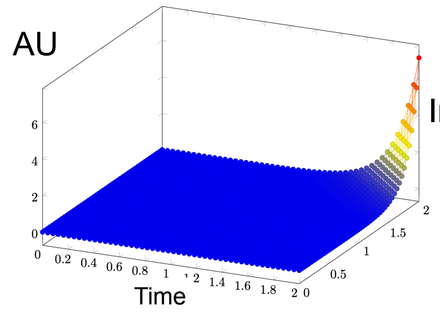
$$S_g = \frac{\tau}{T_0} \int \sum f_i(\mathbf{x}) J_i(\mathbf{x}, t) \; d\mathbf{x}$$

where the last term is related with the Fokker-Planck equation.

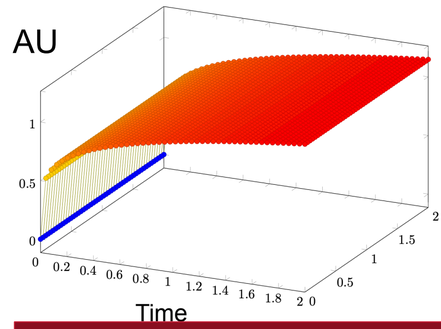
Entropy Calculation Results



Early Stage
(nucleation)



Intermediate Stage
(elongation)



Final Stage
(fibril formation)

Conclusion

Our calculations are based on a model independent process that is compared to an in vitro study on Alpha-Synuclein in the Biochemistry research laboratory at MSU Texas. Our work shows that the mathematical model follows a similar sigmoidal growth curve as the protein aggregation using ThT fluorescence in real time. The model is consistent with very slow growth in the early stage to rapid, exponential growth at the intermediate stage, and ending with a plateau at the final state of fibril formation. Alpha-synuclein fibril formations were confirmed via STEM imaging.

References & Acknowledgments

1.Lella, M., & Mahalakshmi, R. (2018). Direct structural annotation of membrane protein aggregation loci using peptide-based reverse mapping. *The journal of physical chemistry letters*, *9*(11), 2967-2971.
2.Hanczyc, P., & Fita, P. (2021). Laser Emission of Thioflavin T Uncovers Protein Aggregation in Amyloid Nucleation Phase. *ACS photonics*, *8*(9), 2598-2609
3. Reynolds, Nicholas P., et al. "Mechanism of membrane interaction and disruption by α-synuclein." *Journal of the American Chemical Society* 133.48 (2011): 19366-19375.
4. Parres-Gold, Jacob, et al. "Real-time characterization of cell membrane disruption by α-synuclein oligomers in live SH-SY5Y neuroblastoma cells." *ACS chemical neuroscience* 11.17 (2020): 2528-2534.

We would like to give special thanks to Sheila Tucker, Dr. Hansen, Dr. Liang, Dr. Hallford, Dr. Sharma Garret Baughman, Hunter Baker, Kendra Pierce, Anna Roland, and the Welch Foundation.