Microfluidics analysis of protein aggregation

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Abstract

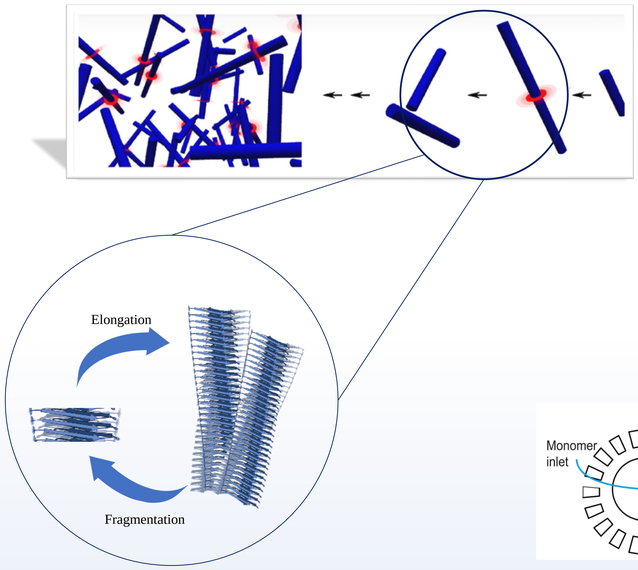
Introduction: Protein aggregation is crucial in many diseases, specifically aging-associated diseases such as Alzheimer's and Parkinson's. Also, studying protein aggregation provides essential information about protein structure and folding/unfolding under different conditions**.**

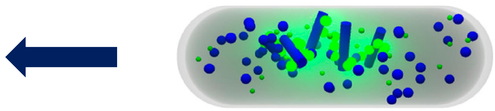
Methods: Microfluidic chips provide high throughput investigation, different conditions, a more cell-like environment by confinement medium, and reduced material consumption [1]. fibrils for industrial, pharmaceutical, medical, and biotechnological purpose. Here we discuss the effects of various factors, such as different flow rates [2], confinement [3], ions, and chemical components in increasing or decreasing aggregation [4].

Results and discussion: The existence of flow accelerates primary and secondary nucleation and decreases the lag time of aggregation by decreasing free energy, which is caused by incrementing collision of molecules. We will see that microfluidics can provide cost-effective information about protein aggregation, its growth rate, different factors affecting it, and how we can control and manipulate aggregation growth, shape, and structure. And this information can be used in many diseases associated with aggregation-prone proteins such as tau protein, α-synuclein, and Amyloid β.

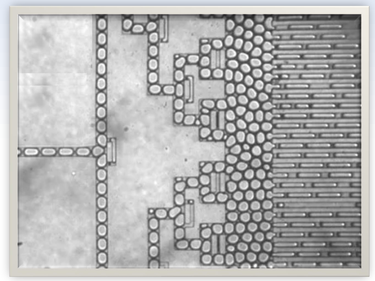
Conclusion: Accordingly, besides providing information about aggregation structure, growth mechanisms, and different factors affecting this process, microfluidics offers information about how aggregation can damage tissues [5] and how we can use aggregation products such as amyloids and s.

- A single primary nucleation event triggers a cascade of spatially correlated subsequent events such as secondary nucleation events.
- The fibrils formed in this manner, therefore, themselves partake in subsequent conversion reactions, indicating that the overall process is a chain reaction.
- The data should be analyzed in the framework of reaction-diffusion phenomena.





The encapsulation of protein molecules (blue spheres) and thioflavin T fluorophores (green spheres) into aqueous droplets stabilized by PFPEPEG block-copolymer surfactants. Misfolded proteins that assemble into cross-β structures can be detected through ThT fluorescence from the droplet after incubation.



The design of the microfluidic device used for studying amyloid fibrillation, fragmentation and elongation.

The average lag time $\langle\tau\rangle$ of the amyloid growth depends on the system size and is defined by two distinct contributions:

- The time required for a primary nucleus to form, $\langle\tau_n\rangle = c_n V^{-1}$
- The time required for the chain reaction associated with secondary nucleation, τ_g

For small system volumes $\tau_g \ll c_n V^{-1}$

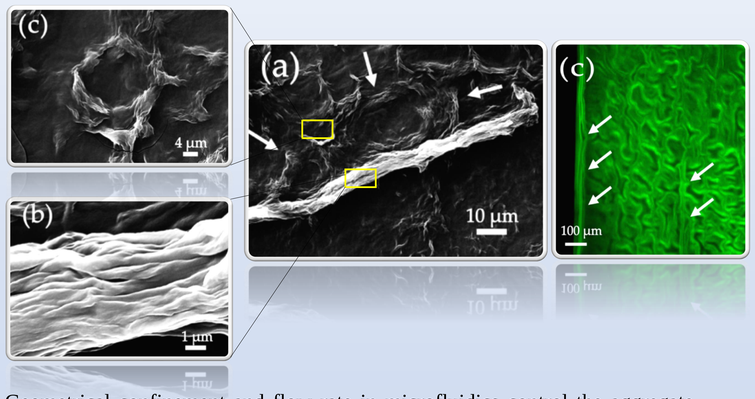
- the lag time is dominated by the primary nucleation time.

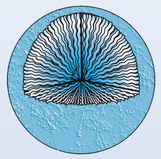
For large system volumes, $\tau_g \gg c_n V^{-1}$.

- The occurrence of primary nucleation events in the system becomes frequent;
- the lag time is determined by the propagation time of the chain reaction.

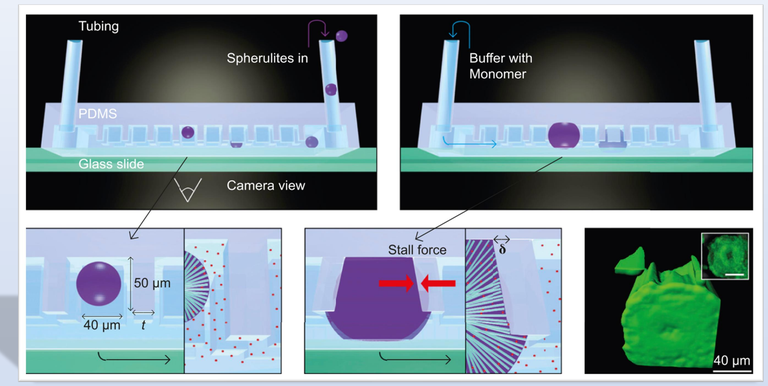
The observed lag time is the sum of the two times, and sample size dependence of the lag time:

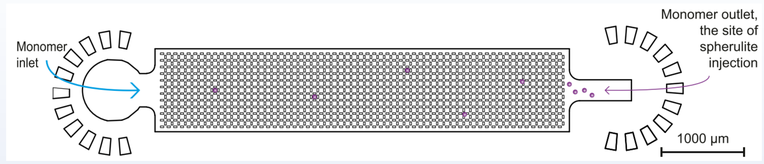
$$\langle\tau\rangle_V = c_n V^{-1} + \tau_g$$





If fibrils arrange in a radial arrangement, they will form spherulites.





- To evaluate the force generated as a result of amyloid growth, force against microcantilevers in a microfluidic device has been used.
- Amyloid growth generates sufficient forces to deform soft interfaces with elastic moduli comparable to that of the cell membrane and tissues with low moduli, such as those of the brain
- The forces generated by amyloid growth can reach the same order of magnitude as those resulting from the polymerization of cytoskeletal proteins

- Geometrical confinement and flow rate in microfluidics control the aggregate morphology type.
- Increasing the flow rate in a confined geometry leads to the formation of clusters of very thick fibers.
- The flow acts on a higher hierarchical level:
  - large-scale (3D) packing is strongly modulated by the flow rate,
  - the building blocks of the aggregates are still characterized by a high content of β-sheets that their arrangement stay constant.

References

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