In the Flow, How Fluid Dynamics Shapes Amyloid Formation

Or

Flowing Towards Fibrils Reveals a Crucial Link in Amyloid Formation

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Physicochemical factors, such as pH, temperature and chaotropes, have long been known to modulate the structure, stability and dynamics of proteins (1, 2). The role that fluid flow plays in manipulating protein structure, however, is comparatively understudied and often overlooked in our quest to understand protein behavior. Yet, conformational changes under flow are known to be pivotal to many biological assembly processes such as blood clotting (3, 4), spider silk formation (5, 6) and the aberrant unfolding and aggregation of proteins (7–12) (Figure 1a,b). Flow has also been shown to promote the self-assembly of proteins into amyloid fibrils *in vitro* (13) and *in vivo* (14). In the current issue of PNAS, Ritsch *et al.* examine the role that fluid flow plays in the aggregation of transthyretin (TTR) into amyloid under physiological pH conditions (15). The inspiration for the work was the finding, based on decades of research, which has shown the low pH is required for TTR amyloid formation *in vitro* (16). This left unanswered the question of what triggers aggregation at neutral pH in a physiological setting. The work by Ritsch *et al.* addresses this question, with fluid flow being brought to the fore and, combined with powerful NMR experiments, shed new light on a molecular mechanism which has, until now, remained elusive (15).

Determining the effects of fluid flow on biomolecular conformation is challenging due to the variety of hydrodynamic forces that can be generated, combined with the synergy of these forces with gas:solution and solid:solution interfaces that are ubiquitous in Nature and unavoidable in a laboratory setting. Shear flows are generated when fluid flows over a surface, whether this be a blood vessel (Figure 1a) (3), a microfluidic chip (5), or the rotation of a stirrer (15, 17). Hence shear is a feature in many experiments in the laboratory, where samples are pumped, stirred, flowed or shaken. This includes the use of peristaltic pumps, columns and other protein purification devices, with potential risks to the large-scale production of proteins e.g., as biopharmaceuticals (11). In a biological setting, shear stress is imparted on the walls of

blood vessels, which is sensed by endothelial cells, and has implications in the progression of diseases, such as atherosclerosis (18). Constrictions in a pipe, such as a stenosed blood vessel, generates a different kind of flow force, known as extensional flow (Figure 1a). The acceleration of the fluid caused by such a constriction can lead to breakdown of laminar flow (characterized by Reynold's number (19)), generating turbulent flow. All these types of flow can modulate protein behavior and impact biology. It is also important to remember that these bulk hydrodynamic phenomena take place in the presence of an interface that also plays a critical role (10, 20). This could be a solid-liquid interface (e.g. test tube surface to solution *in vitro*, capillary wall to blood, or a spider silk duct in a biological setting) and, in some cases, an air-water interface (10, 20, 21). Both phenomena have been shown to induce changes in protein conformation, dynamics and stability, including protein unfolding and self-assembly (7–9, 12). While the surface area of these deleterious interfaces can be limited, flow forces act to amplify their effect by continually removing adsorbed potentially remodeled proteins into the bulk, and providing a fresh interface for further protein interactions (11). In essence, flow and surfaces can act synergistically to influence macromolecular behavior, with potentially devastating effects on biology and in the use of proteins as biopharmaceuticals.

One of the most important challenges facing human health today are amyloid diseases, including Alzheimer's and Parkinson's, type 2 diabetes, and others, in which normally well-behaved soluble proteins (which can be globular or intrinsically disordered) misfold and self-assemble into amyloid fibrils, which have the strength of steel (22). What triggers this catastrophic misbehavior of proteins is mysterious, with mutation, post-translational modifications, mislocalization or errors in protein transport (e.g. from nucleus to cytoplasm or endoplasmic reticulum to the cell surface); imbalances in protein production and degradation; and/or overloading of chaperones being implicated (23). Transthyretin (TTR) belongs to the family of amyloidogenic proteins. In its native, tetrameric form, TTR is involved in the transport of retinol in the blood and cerebrospinal fluid. However, stimulated by mutation, proteolysis, or an individual's age, the protein can misbehave, self-assembling into amyloid fibrils that accumulate in the heart or brain with devastating consequences (24). TTR remains the only amyloid disease in which a small-molecule therapy has reached the clinic (24).

Decades of research on TTR amyloid formation have used acidification (pH 4.4) to trigger amyloid formation, with the mechanism requiring disassembly of the native tetramer (comprising a dimer of dimers) into the aggregation-prone monomer (24). Under physiological

pH, by contrast, TTR does not readily form amyloid, leading Ritsch *et al.* (15) to question how this protein aggregates into the hearts of ~ a quarter of elderly adults. As the heart generates hydrodynamic forces in the cardiovascular system, Ritsch *et al.* decided to explore how fluid flow might influence TTR aggregation *in vitro* by generating turbulent flows inside microcentrifuge tubes using a controlled stirrer apparatus (Figure 1b). Both wild-type TTR and an F87E variant were subjected to agitation at pH 7 at 37˚C, for up to one month, with aggregation quantified using turbidity and fluorescence of the dye, thioflavin T, which binds to amyloid fibrils. Under these conditions, 10μM TTR was found to form amyloid at a similar rate to protein incubated quiescently at pH 4.4 and 25˚C. By varying the initial protein concentration, the authors showed that the rate of amyloid formation actually slows down as the concentration of protein is increased, with such an inverse-concentration dependence suggesting that the disassembly of the TTR tetramer into monomers, preceded by a rate-limiting conformational change within the tetramer, initiates assembly into amyloid (Figure 1b). Note that while the air:liquid interface was removed from their experiment, the solid:liquid interface between the container and protein solution was still present, hence this interface may also play a key mechanistic role.

The authors then used NMR to determine how their flow field influences TTR structure and assembly. These experiments revealed the presence of a "dynamically perturbed tetramer," which may be in rapid exchange with the aggregation-competent monomer, formed in the presence of the fluid flow. More specifically and based on previous work, the authors note that residues found in the AB and GH loops on the weak dimer interface become more dynamic under flow and thus promote the dissociation of the tetramer into dimers and subsequently monomers. Intrinsic tryptophan fluorescence experiments provided further evidence that long-lived conformational changes occur in TTR near W79, which neighbors the key loops above. Taking all this evidence together, the authors suggest that agitation promotes formation of a damaged 'activated' tetramer, that dissociates more readily into dimers and monomers, with the flow field potentially also activating monomers to misfold into amyloid-competent conformations (summarized in Figure 1b).

While there is still much work to do to pin down precisely how flow fields modulate the TTR tetramer and monomer structures, the work highlights the importance of hydrodynamic flow in

triggering TTR to form amyloid at physiologically-relevant protein concentrations, pH and hydrodynamic environment. It is also one of few studies to capture a protein conformation induced by hydrodynamic forces at the molecular level. More broadly, the work highlights the ability of flow to induce small, but important, conformational changes in protein structure. This ability has been exploited by Nature for functional purposes, such as mechano-signaling (21), but is also being increasingly recognized as a potential dangerous perturbant in protein aggregation and amyloid formation.

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References

1. R. Lumry, H. Eyring, Conformation changes of proteins. *J Phys Chem* **58**, 110–120 (1954).
2. T. R. Jahn, S. E. Radford, Folding versus aggregation: Polypeptide conformations on competing pathways. *Arch Biochem Biophys* **469**, 100–117 (2008).
3. T. A. Springer, von Willebrand factor, Jedi knight of the bloodstream. *Blood* **124**, 1412–1425 (2014).
4. D. Butera, P. J. Hogg, Fibrinogen function achieved through multiple covalent states. *Nat Commun* **11**, 5468 (2020).
5. S. Rammensee, U. Slotta, T. Scheibel, A. R. Bausch, Assembly mechanism of recombinant spider silk proteins. *Proc Natl Acad Sci USA* **105**, 6590–6595 (2008).
6. M. Andersson, *et al.*, Biomimetic spinning of artificial spider silk from a chimeric minispidroin. *Nat Chem Biol* **13**, 262–264 (2017).
7. L. Ashton, J. Dusting, E. Imomoh, S. Balabani, E. W. Blanch, Susceptibility of different proteins to flow-induced conformational changes monitored with Raman spectroscopy. *Biophys J* **98**, 707–714 (2010).
8. D. E. Dunstan, P. Hamilton-Brown, P. Asimakis, W. Ducker, J. Bertolini, Shear flow promotes amyloid-β fibrilization. *Prot Eng Des Sel* **22**, 741–746 (2009).
9. T. A. Hakala, *et al.*, Accelerating reaction rates of biomolecules by using shear stress in artificial capillary systems. *J Am Chem Soc* **143**, 16401–16410 (2021).

10. C. Moino, F. Artusio, R. Pisano, Shear stress as a driver of degradation for protein-based therapeutics: More accomplice than culprit. *Int J Pharm* **650**, 123679 (2024).

11. L. F. Willis, S. E. Radford, N. Kapur, D. J. Brockwell, Does flow play a role in protein aggregation during bioprocessing? (2024). Available at: https://www.bioprocessonline.com/doc/does-flow-play-a-role-in-protein-aggregation-during-bioprocessing-0001.

12. J. Dobson, *et al.*, Inducing protein aggregation by extensional flow. *Proc Natl Acad Sci USA* **114**, 4673–4678 (2017).

13. Y. Goto, *et al.*, Peristaltic pump-triggered amyloid formation suggests shear stresses are in vivo risks for amyloid nucleation. *NPJ Biosensing* **2**, 4 (2025).

14. C. N. Trumbore, Shear-induced amyloid formation in the brain: II. An experimental system for monitoring amyloid shear processes and investigating potential spinal tap problems. *J Alzheimer's Dis* **59**, 543–557 (2017).

15. I. Ritsch, H. J. Dyson, P. E. Wright, Aggregation of transthyretin by fluid agitation. *Proc Natl Acad Sci U S A* (2025) https://doi.org/10.1101/2024.11.08.622726.

16. Z. Lai, W. Colón, J. W. Kelly, The acid-mediated denaturation pathway of transthyretin yields a conformational intermediate that can self-assemble into amyloid. *Biochemistry* **35**, 6470–6482 (1996).

17. A. S. Sediq, R. B. Van Duijvenvoorde, W. Jiskoot, M. R. Nejadnik, No touching! Abrasion of adsorbed protein is the root cause of subvisible particle formation during stirring. *J Pharm Sci* **105**, 519–529 (2016).

18. C. Souilhol, *et al.*, JAG1-NOTCH4 mechanosensing drives atherosclerosis. *Sci Adv* **8**, 1–14 (2022).

19. R. P. King, *Introduction to Practical Fluid Flow* (Butterworth-Heinemann, 2002).

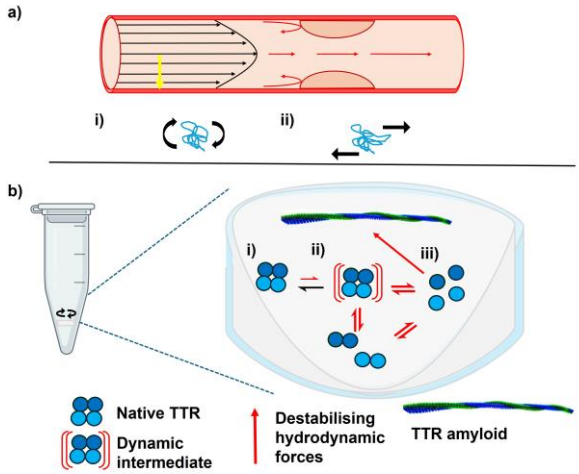
20. Z. Toprakcioglu, *et al.*, Adsorption free energy predicts amyloid protein nucleation rates. *Proc Natl Acad Sci USA* **119**, e2109718119 (2022).

21. M. R. G. Kopp, *et al.*, Surface-induced protein aggregation and particle formation in biologics: Current understanding of mechanisms, detection and mitigation strategies. *J Pharm Sci* **112**, 377–385 (2023).

22. M. G. Iadanza, M. P. Jackson, E. W. Hewitt, N. A. Ranson, S. E. Radford, A new era for understanding amyloid structures and disease. *Nat Rev Mol Cell Biol* **19**, 755–773 (2018).

23. N. Louros, J. Schymkowitz, F. Rousseau, Mechanisms and pathology of protein misfolding and aggregation. *Nat Rev Mol Cell Biol* **24**, 912–933 (2023).

24. C. E. Bulawa, *et al.*, Tafamidis, a potent and selective transthyretin kinetic stabilizer that inhibits the amyloid cascade. *Proc Natl Acad Sci USA* **109**, 9629–9634 (2012).



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173 **Figure 1. Modulation of biomolecular systems by hydrodynamic forces.** a) Schematic of a
174 blood vessel to demonstrate physiological hydrodynamic forces. i) Shear flow is characterised
175 by a velocity gradient perpendicular to the direction of flow (yellow arrow). Globular proteins
176 are thought to experience rotation in these flow fields (lower left image). ii) Constriction of a
177 vessel, causes fluid to accelerate through the constriction, resulting in an extensional flow that
178 may perturb protein structure (lower right image). Elongated proteins may stretch and tumble
179 or align under shear flow which can promote further conformational changes and/or self-
180 assembly. b) Triggering TTR aggregation at neutral pH by fluid agitation. i) Native TTR exists
181 as a tetramer, comprising a dimer of dimers ii) Hydrodynamic forces promote the formation of
182 an activated tetrameric intermediate, which can dissociate into dimers and monomers. iii) A
183 further, gross conformational change is necessary for TTR to form the cross-β structure of
184 amyloid. Fluid agitation is thought to promote this step, although how this occurs remains
185 unresolved.

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