PNAS COMMENTARY

In the flow, how fluid dynamics shapes 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) (Fig. 1 *A* and *B*). Flow has also been shown to promote the self-assembly of proteins into amyloid fibrils in vitro (13) and in vivo (14). In 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, that 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, sheds 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 (Fig. 1*A*) (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

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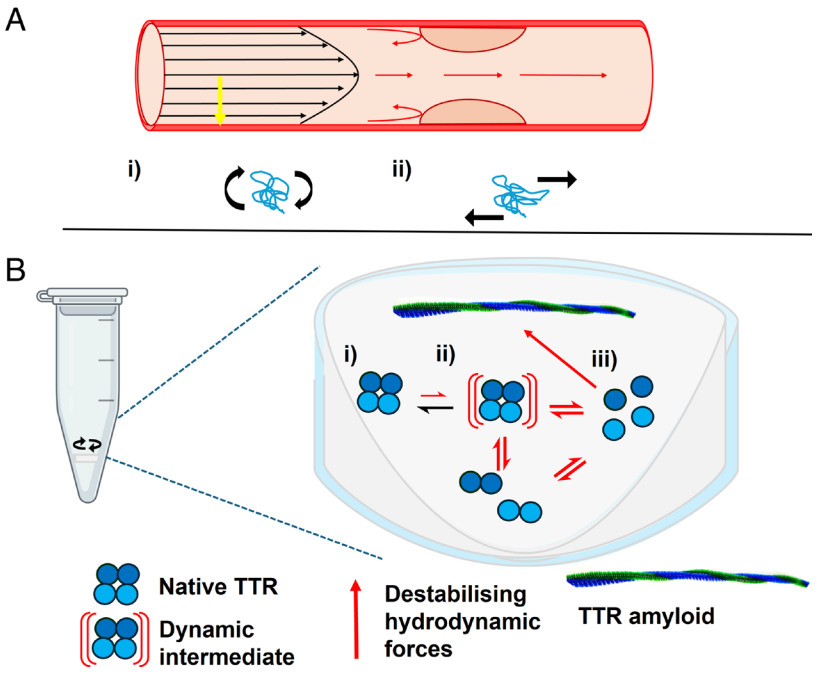

Fig. 1. Modulation of biomolecular systems by hydrodynamic forces. (*A*) Schematic of a blood vessel to demonstrate physiological hydrodynamic forces. i) Shear flow is characterized by a velocity gradient perpendicular to the direction of flow (yellow arrow). Globular proteins are thought to experience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

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 (Fig. 1*A*). 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:solution 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:liquid 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.

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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, posttranslational 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 all being implicated (23). 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 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 and is deposited in 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 (Fig. 1*B*). 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 becomes more fluorescent on binding to amyloid fibrils. Under these conditions, 10 μM 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. This inverse-concentration dependence suggests that the disassembly of the TTR tetramer into monomers is preceded by a rate-limiting conformational change within the tetramer to initiate assembly into amyloid (Fig. 1*B*). 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 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 the 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 Fig. 1*B*).

While there is still much work to do to pin down precisely how flow fields modulate the TTR tetramer, dimers and monomer structures, the work highlights the importance of hydrodynamic flow in triggering TTR to form amyloid at physiologically relevant protein concentrations and pH. 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 mechanosignaling, but is also being increasingly recognized as a potential dangerous perturbant in protein aggregation and amyloid formation.

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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. U.S.A.* **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? Bioprocess online (2024). https://www.bioprocessonline.com/doc/does-flow-play-a-role-in-protein-aggregation-during-bioprocessing-0001. Accessed 6 March 2025.
12. J. Dobson *et al.*, Inducing protein aggregation by extensional flow. *Proc. Natl. Acad. Sci. U.S.A.* **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 Biosens.* **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, Initiation of transthyretin aggregation at neutral pH by fluid agitation. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2425230122 (2025).
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. U.S.A.* **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. U.S.A.* **109**, 9629–9634 (2012).