# A nanoparticle platform for heterogeneous nucleation events in amyloid formation and protein aggregation

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Heterogeneous nucleation processes and surface-induced aggregation in protein solution are observed in a variety of fundamental and practical problems in biological and biomedical sciences. Here we develop a platform based on polymeric nanoparticles which provides a highly controlled surface-mediated driving force for aggregation under both stagnant and shaking conditions. The high surface-to-volume ratio of the nanoparticles and the flexibility of polymer chemistry allow to accurately control both the total area and the chemistry of the surface exposed to the proteins. This high-throughput assay represents a convenient system to perform kinetic assays and investigate the fundamental physics underlying surface-induced protein aggregation. In particular, in this work we demonstrate the potential of this strategy by unraveling the combined effect of surfaces and hydrodynamic flow on protein aggregation. We show that under physiological conditions hydrophobic surfaces remarkably promote the formation of amyloid fibrils from soluble human insulin. We further show that this effect is due specifically to a dramatic increase in primary heterogeneous nucleation events. In contrast, mechanical forces accelerate the formation of amyloid fibrils by favoring mass transport and further amplify the number of fibrils by promoting fragmentation events. Thus, surfaces and agitation have a combined effect on the kinetics of protein aggregation observed at the macroscopic level but, individually, they each affect distinct microscopic reaction steps: the presence of interfaces generates primary nucleation events of fibril formation, which is then amplified by mechanical forces. These results suggest that the inhibition of surface-induced heterogeneous nucleation should be considered a primary target to suppress aggregation and explain why in many systems the simultaneous presence of surfaces and hydrodynamic flow enhances protein aggregation.

[1] F. Grigolato, C. Colombo, R. Ferrari, L. Rezabkova, P. Arosio, *ACS Nano* **2017**, 11, 11358−11367