

Nanoparticles as catalysts for protein fibrillation

Vicki L. Colvin* and Kristen M. Kulinowski

Department of Chemistry, Center for Biological and Environmental Nanotechnology, MS 60, Rice University, Houston, TX 77025

The study by Linse *et al.* (1) published in this issue of PNAS observes that nanoparticles (NPs) can significantly enhance the rate of protein fibrillation, or the formation of fibrils, potentially leading to novel mechanisms for amyloid diseases as well as therapeutic opportunities for their treatment. NPs are materials with dimensions between 1 and 100 nm whose small sizes confer properties distinct from those of bulk systems (2–5). Their potential to induce protein fibrillation is a function of both the NP surface charge, which promotes adherence of the protein, and its large surface area. In this case, NP–protein binding induces significant structural and functional perturbations to the protein, a fact that could be important for a more general understanding of the biological interactions of engineered NPs. The observation of fibrillation, which is a specific kind of aggregation phenomenon relevant for amyloid proteins, raises the possibility that NPs could play a role in increased risk of amyloidosis and other protein-misfolding diseases (Fig. 1). The authors call for further research into the potential for NPs to accelerate protein fibrillation and acknowledge that the same variables associated with new protein assemblies of this sort may have beneficial or even therapeutic roles.

One of the most important messages of this work for chemists is that when NPs enter the biological world they become very different materials (1). The small sizes of NPs convey the potential to access many biological compartments, where they are met with a smorgasbord of possible binding partners from the complex and concentrated soup of biomolecules. As a result, NPs develop in a biological system that these authors aptly term a “corona” (6). The nature of these natural surface coatings will define everything important about the NP in an organism: its surface charge, its stability against aggregation, and even its hydrodynamic size. These nonspecific associations are by no means fixed in time, and indeed this paper shows that over several hours one particular amyloid protein can experience multiple adsorption and desorption events.

Because this corona will define the interface between NPs and organisms, it begs the question of whether the biological interactions of NPs depend in any way on their composition. Certainly the nature of the surface will control which of the many biomolecules will interact with the NPs. Experiments using hydrophobic NPs,

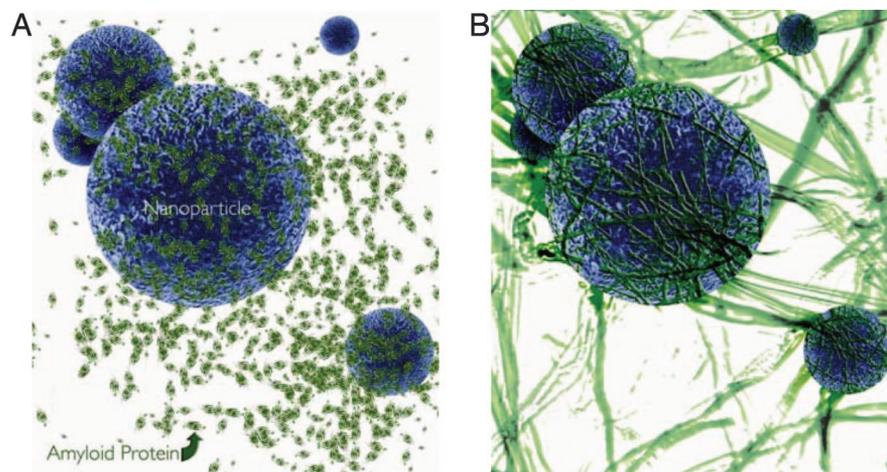


Fig. 1. Artistic rendering of amyloid protein fibrillation in the presence of nanoparticles. The formation and deposition in the body's tissues of highly ordered, thread-like amyloid protein aggregates have been linked to a family of diseases. For example, fibrillation of insulin has been linked to diabetes. Linse *et al.* (1) studied 70-nm polymer particles and β_2m protein, which in its normal state is an ≈ 3 -nm globular protein; however, the dimensions and morphology for insulin fibrils on mica were the basis for this imagery (28). (A) Depicted here are large NPs (blue) and an amyloid protein (green) in its monomeric and folded state (1). (B) This artistic rendering shows the association of the amyloid protein with the NP surfaces, perhaps with the generation of small oligomers, which are the precursors to fibrils. In solution, larger protein fibrils appear as their growth is enhanced by the surface association of proteins.

such as single-walled carbon nanotubes (SWNTs), have indicated that these systems will bind with proteins through the neutral amino acids (7, 8). Particle shape also will likely play a role; anisotropic particles like SWNTs are well suited to “wrapping” with anisotropic biomolecules such as DNA (9).

Interestingly, particle size may be less important than composition or surface character in defining how biomolecules bind to NP surfaces. One study of the denaturation of proteins onto gold NPs of various sizes found little difference between the NP-tethered proteins and bulk solutions until NP diameters were well under 15 nm (10). Such observations are echoed by Linse *et al.* (1), who found that the NP-induced fibrillation depended more on the hydrophobic character of the surface than on particle size. Someday, researchers may develop computational models that use basic physiochemical features of a particle, coupled with information about a particular biological setting, to predict the size and structure of its corona and, consequently, the nature of the biological interaction.

Biologists may take away an equally important but different message from the publication by Linse *et al.* (1). The fibrillation induced by NPs illustrates that many classes of biomolecules will exhibit distinctive behaviors when they encounter NPs.

This observation is not surprising because it has long been recognized that the bioinorganic interface is rich with complexity; protein denaturation, crystallization, and even fibrillation have been reported as a result of protein interactions with bulk surfaces (11–14). When the surfaces are of nanoscale dimensions, however, these familiar processes can change in character and magnitude (8, 10). In the study by Linse *et al.*, fibril formation was put into overdrive compared with the protein's unperturbed behavior, an observation that was remarkably general across a range of NP sizes, surface coatings, and particle compositions.

The mechanism proposed in studies of NP–fibril formation suggests that nanoscale surfaces can act as platforms for protein association. For proteins bound directly to NPs, this association can induce significant changes in protein structure, and, as with low pH or high temperature, proteins may unfold and produce structures more likely to form fibrils (15). However, Linse *et al.* (1) saw no evidence

Author contributions: V.L.C. and K.M.K. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 8691.

*To whom correspondence should be addressed. E-mail: colvin@rice.edu.

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that the final fibrils were associated with NPs, as might be expected if they originated at surface-bound proteins.

A more subtle possibility is that NPs act much like conventional catalysts in promoting fibrillation. In this model, NPs would reduce the energetic barriers to fibril formation by enhancing the population of prefibril aggregates. Protein association at NP surfaces could lead to easier production of the soluble protein aggregates known as oligomers that may act as fibril building blocks. Much research has been directed at understanding the role of oligomers in accelerating fibril formation; recently, oligomers have themselves been implicated as being the causative agents of neurodegenerative amyloid diseases such as Alzheimer's (13). The high surface areas of NPs, coupled with the dynamic exchange of proteins between bound and free forms, may increase local protein concentrations and promote oligomer formation. Under the highly acidic conditions studied by Linse *et al.* (1), such an increase would lead to more rapid fibril formation, in agreement with their data. Interestingly, NP surfaces have been shown to nucleate the formation of inorganic structures, but in those cases the NP becomes incorporated as a core into the final product (16). For this biological process, NP surfaces are not permanently affixed to the fibrils, and, in principle, NP surfaces function multiple times to enhance fibrillation, much like a conventional catalyst.

Whether these observations will hold for other amyloid proteins or for β_2 -microglobulin (β_2m) under physiological conditions remains an outstanding question. Linse *et al.* (1) set out to test the hypothesis that a 3D NP surface would promote fibrillation of a model protein that fibrillates readily and is associated with dialysis-related amyloidosis (17). To observe fibrillation in reasonable time frames, they studied the process outside of a biological system under conditions in which fibril growth is thought to occur by means of a nucleation-dependent model. At physiologically relevant pH

and ionic strength, amyloid fibril formation proceeds through a different, but related, process (18). Whether the NP surfaces would be as active in catalyzing fibril formation under more realistic biological settings remains to be seen. Perhaps even more significant is that in a real biological setting, amyloid proteins will compete with a multitude of other biomolecules for access to the NP surface. Amyloid association is likely to be vastly reduced under these circumstances, and, if so, the catalytic properties of NPs for fibril formation may also be reduced.

Although this study found that NPs can promote fibrillation, there are ongoing efforts to use NPs to detect, prevent, and treat protein-misfolding diseases such as Alzheimer's (19–22). Therefore, interpreting the results of this paper as unequivocally bad news for NPs is unwarranted. Of particular relevance are recent reports of the use of organic NPs to prevent fibrillation (23). In one *ex vivo* study, biocompatible phospholipid nanomicelles with diameters of ≈ 14 nm inhibit the aggregation of a protein associated with Alzheimer's disease (24). These NPs are coated with a biocompatible polymer and thus would show different but not inconsistent behavior from that found by Linse *et al.* (1), whose NPs were uncoated. Indeed, designing coatings that limit or prevent protein adherence may prove to be of critical importance to the safe application of NPs to medicine.

Given the incredible variety of nanoparticle sizes, shapes, surface coatings, and compositions, it would be remarkable to find any biological response that is universal; however, this is a possible conclusion from Linse *et al.* (1), and this generality has implications for science policy. The current practices for evaluating nanobiological interactions rely on a case-by-case framework that assesses the effects of particular nanostructures in the context of specific exposure scenarios. Although this is the best response to regulatory issues at this time, the case-by-case approach is time-consuming and ultimately impractical given the many ways chemists can alter

NP surface properties, size, and function. If more fundamental and general trends can be identified and validated, then specific and simple tests for screening new NPs will be enabled; such work will highlight size or composition thresholds, for which deeper scrutiny is warranted. Ultimately, such fundamental science would lead to predictive models that would aid government agencies in their oversight functions, create faster commercialization pathways for emerging nanomedicines, and guide researchers to produce safe NP systems at the very earliest stages of design. Governments worldwide are just starting to develop research strategies that have such goals in mind (25–27).

It is tempting to overinterpret the fascinating science of Linse *et al.* (1) given the dearth of information about NP biological interactions and the growing desire of consumers and policymakers to have better information about NP risks. In such a climate and through no fault of the researchers themselves, single studies can become focal points for public scrutiny and be given far more significance than is warranted. The observation that NPs can catalyze fibrillation is important and should make all of us think harder about how best to use these new materials. However, the actual experiments occurred in small tubes under pH and ionic strength conditions that would kill most cells and used a model protein that is well known to fibrillate. The experiments were perfectly designed to test the authors' hypotheses about the consequences of NP-protein association yet poorly suited to inform the public broadly about the risks of NP exposures. This last issue will require the concerted effort of the scientific community and is not something that any single publication should be expected to address. In the meantime, all of us should welcome landmark publications such as the one by Linse *et al.*, which teach us about yet another fascinating way that engineered NPs can interact with biological systems.

This work was supported by National Science Foundation Grant EEC-0647452.

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