Amyloid diseases are a broad class that includes familiar ailments such as type 2 diabetes and Alzheimer’s disease as well as more exotic conditions such as Creutzfeldt-Jakob disease and its animal variant, bovine spongiform encephalopathy or “mad cow” disease. Amyloid diseases are thought to be caused by the formation and deposition in the body’s tissues of highly-ordered, thread-like protein aggregates called amyloid fibrils or plaques. Common to all amyloid diseases is the improper folding of a specific protein or peptide, called an amyloid protein, resulting in its transformation from a soluble form into an insoluble fibrous form through a process known as protein fibrillation. Only around 30 of the estimated 100,000 proteins in the human body are linked to formation of protein fibrils that cause disease. Moreover, the body has evolved a whole host of mechanisms to inhibit unwanted protein fibrillation. It is only when these mechanisms fail that protein fibrillation results in disease. This backgrounder covers the causes and effects of amyloid diseases and the role nanoparticles might have in causing, diagnosing or treating them. 

Nanoparticles and amyloid diseases

Nanoparticles and Amyloid Diseases

Nanoparticles are being explored for their role in diagnosing, preventing, treating or even causing amyloid diseases.

Uses of nanoparticles in diagnosing amyloid diseases

A variety of papers has been published on the use of nanoparticles to diagnose or better understand amyloid disease. In most cases, this detection occurs ex vivo, i.e., outside the body, by testing a sample of blood or cerebral spinal fluid (CSF). Therefore the patient is not exposed to the nanoparticles. The basic concept is that a nanoparticle binds to a particular biomolecule, producing a characteristic signal that is absent or weaker without the biomolecule-nanoparticle combination. Early preclinical work includes the use of gold nanoparticles to detect a marker for Alzheimer’s disease in the blood and the use of quantum dots to provide better image contrast to improve understanding of Alzheimer’s amyloid fibril structure. Representative papers

Uses of nanoparticles in preventing or treating amyloid diseases

Much work is being done to investigate the potential for nanoparticles to inhibit the formation of amyloid protein fibrils associated with Alzheimer’s disease (prevention) or to slow down the progress of the disease (treatment). Of particular interest is work demonstrating that two different types of nanometer-scale capsules can inhibit the aggregation of proteins associated with Alzheimer's disease thereby preventing amyloid fibers from forming. In the treatment arena, nanoparticles are being investigated as novel agents for penetrating the blood-brain barrier to deliver drugs to diseased brains. Most of this work targets Alzheimer’s disease. At this point, none of the treatments have been
approved for use in humans but the choice of using biocompatible materials is motivated by that ultimate goal. Representative papers

Nanoparticles implicated in causing amyloid disease

Artistic rendering of amyloid protein fibrillation in the presence of nanoparticles. Insulin fibrils on mica were the basis for this imagery. [A] Depicted here are large NPs (blue) and an amyloid protein (green) in its monomeric and folded state. [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.

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Recent work suggests that nanoparticles may provide a novel mechanism for the onset of amyloid diseases. One paper raises the prospect of nanoparticles accelerating the onset of protein-misfolding diseases by providing a surface upon which protein fibrillation can begin. The Linse et al. study observes that several types of nanoparticles (NP) can significantly enhance the rate of a protein aggregation process, called fibrillation, which results in smaller proteins assembling into fibrous strands (fibrils). They argue that the potential to induce protein fibrillation is linked to the attachment of proteins to nanoparticle surfaces. Bound proteins generally experience structural and functional perturbations; when those particular proteins belong to the ‘amyloid’ family, as was studied in this work, these alterations can promote their aggregation into oligomers which are known precursors to forming the larger and longer fibrils. The fibrils themselves are correlated with a number of “amyloid” diseases ranging from Alzheimers to Type II diabetes. The authors call for further research into the potential for NP to accelerate protein fibrillation while acknowledging that these protein aggregates may have advantageous or even therapeutic roles.

When proteins go bad: Unwanted effects of protein fibrillation

Aggregation of insoluble protein fibrils causes disease as a result of toxicity to or interference with the normal functioning of cells, disruption of cell membranes, interference with other molecules critical to some physiological process, or overloading of the waste clearance processes found in healthy tissue. Processes that accelerate protein fibrillation may accelerate the onset of amyloid diseases. The formation of protein fibrils is not always associated with disease; rather, some protein fibrils have been found to exhibit useful functional properties, such as the proteins involved in melanin production. For those protein fibrils that are linked to disease, both hereditary and environmental factors have been identified as contributing to the development of the disease.

One type of amyloid protein, called beta-2-microglobulin (β2m), is linked to the disease dialysis-related amyloidosis (DRA), which results in deposits of plaque in bone, joints and tendons. β2m builds up in the bloodstream of patients on dialysis for kidney malfunction, undergoes protein fibrillation and deposits in the tissues of the body causing
joint stiffness, pain, fluid buildup and occasionally bone fractures. Carpal tunnel syndrome is one unwanted outcome of DRA. For more information on DRA and other amyloid diseases, see the links section below.

Misfolding of the protein β2m (left) results in formation of fibrils (center, scale bar = 100 nm) that can deposit in the joints causing carpal tunnel syndrome and other unwanted outcomes (right).


How proteins go bad: Formation of amyloid fibrils

Component 1: The amyloid protein

Because protein fibrillation is implicated in serious diseases, much work has been done to understand the mechanisms of fibril formation. Some proteins need to partially unfold, or denature, before they can form amyloid fibrils. Unfolding is promoted by certain experimental conditions such as low pH or high temperature. Therefore, experiments that seek to explore the role of protein misfolding on fibril formation often employ conditions not typically found in the body to accelerate the rate of protein fibrillation, akin to the extremely large doses of toxins given to lab animals to assess toxicity. Measuring the early stages of fibrillation requires that researchers employ acidic and salty conditions that are not particularly physiologically relevant but well suited to investigating the processes that lead to protein fibrillation.
Component 2: Soluble oligomers of amyloid proteins

The predominant mechanism by which protein fibrillation is believed to occur is referred to as “nucleated growth.” Once protein unfolding begins, there is a lag phase during which no protein fibrils are observed followed by rapid production of protein fibrils. During the lag phase many non-fibrous short peptide sequences known as oligomers form and act as seeds for fibril formation. The figure to the left shows an atomic force microscopy image of these oligomers; they are larger than single proteins and typically round. In this example, the aggregates are of insulin that has been heated to 70°C to promote fibril growth. As soon as there is a sufficient population of these oligomers, growth of the fibrils occurs rapidly by addition of proteins to the seeds. Seeding the solution with these soluble oligomers can reduce the lag time thereby accelerating the rate of protein fibrillation. Recent evidence suggests that it is the protein oligomers, as opposed to the larger and more fibrous aggregates, that are the causative agents of neurodegenerative amyloid diseases such as Alzheimer’s.
Component 3: The fibril formation

The figure to the left shows amyloid fibrils formed from insulin at 70° C. The thread-like nature and small dimensions are apparent. The fibrils seen in this image consist of multiple filaments that are intertwined to create the twisted fiber characteristic of mature amyloids. Biologists do not know exactly how the proteins are arranged in these insulin fibrils; while ordered, these systems do not have a single crystal arrangement necessary to derive atomically precise structures. However, other techniques such as nuclear magnetic resonance and fiber x-ray diffraction have indicated that specific secondary architectures are common in fibrils. In particular, beta sheets are a common motif in the broader class of amyloid proteins; with some amount of unfolding, beta sheets from multiple proteins will stack on top of one another. Much like the steps of a spiral staircase, they form a twisted columnar architecture that is perpendicular to the plane of the sheets. A schematic of the single and multiple filament structure is shown in the figure below.

Mature insulin fibrils.

Resources on Amyloid Diseases

Peer-reviewed Studies

Background on amyloid diseases


Uses of nanoparticles in diagnosing amyloid diseases

These papers investigate the use of nanoparticles to diagnose amyloid disease. In most cases, this detection occurs *ex vivo*, i.e., outside the body by testing a sample of blood or cerebral spinal fluid (CSF). Therefore the patient is not exposed to the nanoparticles.

   A review of mostly *ex vivo* applications of nanoparticles to amyloid disease detection.

   Core-shell (CdSe)ZnS quantum dots provide better image contrast to improve understanding of amyloid fibril structure.

   This paper investigates the use of gold NPs as part of a bio-barcode assay to
detect a biomarker for Alzheimer’s disease in cerebral spinal fluid.


Uses of nanoparticles in preventing or treating amyloid diseases
These studies investigate the potential for nanoparticles to inhibit formation of amyloid protein fibrils associated with Alzheimer’s disease. At this point, they have not been approved for use in humans but the choice of using biocompatible materials is motivated by that ultimate goal.

5. Ikeda, K., et al., Inhibition of the formation of amyloid beta-protein fibrils using biocompatible nanogels as artificial chaperones. Febs Letters, 2006. 580(28-29): p. 6587-6595. This ex vivo study showed that biocompatible nanogels 20-30 nm in diameter can prevent aggregation of proteins associated with Alzheimer's disease and inhibit amyloid fibers from forming. Chaperones are proteins that help other proteins properly fold.


7. Liu, G., et al., Nanoparticle iron chelators: A new therapeutic approach in Alzheimer disease and other neurologic disorders associated with trace metal imbalance. Neuroscience Letters, 2006. 406(3): p. 189-193. This paper demonstrates the ability of nanoparticles to assist in chelators getting across a simulated blood–brain barrier, binding to metals and then carrying the metal ions back out through the BBB. Chelation therapy is a promising avenue for restoring the proper balance of metal ions in the brain. An imbalance of metal ions has been implicated in degeneration of brain tissue associated with Alzheimer’s disease. This study was done in cell culture.


**Nanoparticles implicated in causing amyloid disease**


**Other information sources**

- **Imaging the Brain 2007**: Good images and descriptions of brain diseases caused by protein aggregates
- **Amyloidosis and Kidney Disease**: Basic description of dialysis-related amyloidosis from the National Kidney and Urologic Diseases Information Clearinghouse
- **National Institute of Neurological Disorders and Stroke**: Information on Creutzfeldt-Jakob disease (CJD) with links to resources on CJD and Alzheimer’s disease
- **Wikipedia entry on Amyloid**: Very technical but has excellent links to web resources on amyloid diseases