Obstacles to translating the promise of nanoparticles into viable amyloid disease therapeutics

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Abstract

Nanoparticles (NPs) constitute a powerful therapeutic platform with exciting prospects as potential inhibitors of amyloid-β (Aβ) aggregation, a process associated with Alzheimer’s disease (AD). Researchers have synthesized and tested a large collection of NPs with disparate sizes, shapes, electrostatic properties and surface ligands that evoke a variety of responses on Aβ aggregation. In spite of a decade of research on the NP-Aβ system and many promising experimental results, NPs have failed to progress to any level of clinical trials for AD. A theoretical framework with which to approach this physical system is presented featuring two simple metrics, (1) the extent to which NPs adsorb Aβ, and (2) the degree to which interaction with a NP alters Aβ conformation relative to aggregation propensity. Most of our current understanding of these two interactions has been gained through experimentation, and many of these studies are reviewed herein. We also provide a potential roadmap for studies that we believe could produce viable NPs as an effective AD therapeutic platform.

1. Background

1.1. Nanoparticles for biomedical applications

For many decades, researchers have investigated nanoparticles (NPs) for their unique properties in a variety of scientific and technological contexts [1, 2]. Materials at this length-scale possess interesting and attractive characteristics that are distinct from the properties exhibited by the surface of the same material in its macroscopic form. This phenomenon of the emergence of properties as a material’s size is reduced to the nanoscale manifests as a result of the increasing influence of surface chemistry and nanoscale morphological features (i.e. nanoscale radii of curvature and greater influence of edges) [3]. These properties and, more significantly, the ability that researchers have to tune them have rendered NPs a widely researched and significant platform for many avenues of science in the 21st century [4].

In the last several decades, an increasing number of efforts have been dedicated to studying the effects of NPs on biological systems [5, 6]. The tunability of these particles’ size, shape and surface functionalization has allowed scientists to engineer materials that can evoke a wide range of biological responses both in vivo and in vitro, thus embodying an attractive characteristic for human disease therapeutics. Many NPs are prospective agents engineered to deliver pharmacologically active concentrations of drugs to a target site, whereas in other cases the NPs themselves serve as the therapeutic agent. This review will focus upon investigation into the latter approach.

When the use of local equilibrium approaches is appropriate, the manner in which the biological environment equilibrates vis-à-vis the NP lends itself to development of NPs as therapeutics. From the perspective of a single NP, also definable as the local level, species in solution are in a constant dynamic process of reorganizing themselves relative to the NP to achieve the lowest free energy state. This notion of equilibration can be defined as that for which the density profiles of solvated species around a NP constitute a minimization of the relevant thermodynamic potential. During this process, solvated species will be attracted or repelled from the NP surface to an extent that is dependent upon the concentration, charge, size and shape of said species as well as the geometry of the local environment. Within experimental and biological environments, these NP-induced changes in solvated species can lead to alterations in local biomol-
ecule concentrations, pH and ionic strength, all factors that strongly influence the aggregation of disease-associated amyloid proteins [7–9]. In fact, NPs have a surprisingly potent and diverse ability to modulate amyloid aggregation.

1.2. Aβ aggregation
Amyloid proteins occur naturally and are characterized by their propensity to aggregate into structures bearing β-sheets oriented perpendicular to the long axis of the aggregate [10]. Such structures are referred to as amyloid fibrils and can both elongate via monomer addition and laterally associate. Amyloid aggregates are associated with a litany of devastating disorders including Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, prion diseases, type II diabetes and others [11]. Though NPs have been studied for their effects on amyloid aggregate formation, a defining characteristic of each of these diseases, this review focuses upon the aggregation of amyloid-β (Aβ), the resulting structures of which are a hallmark of Alzheimer’s disease (AD) [12].

AD is the sixth leading cause of death in the United States and the most common neurodegenerative disease in the world [13]. It is unique among the top ten most lethal diseases in the United States in that it presently has no known cure or even prevention. Significantly, the number of people with AD in the United States is predicted to increase by a factor greater than two by 2050 [14].

AD is characterized by the presence of extracellular aggregates of Aβ and intracellular aggregates of the tau protein [15]. This pathology has inspired researchers to hypothesize that these aggregates are etiological factors for AD [16]. Consequently, many researchers aim to identify and characterize therapeutic agents capable of disrupting the aggregation process of these proteins [17–19]. As a result of evidence suggesting that tau aggregation is a downstream event in the pathology of the disease [20, 21], much anti-aggregation work has focused upon Aβ. Accordingly, this review exclusively addresses NP systems intended to modulate Aβ aggregation.

Aβ is a small intrinsically-disordered protein (IDP) comprised of 37–43 amino acids, the disparate length of which is due to differential cleavage of the amyloid precursor protein. Aβ monomer is subsequently released into the cerebrospinal fluid (CSF) where, in certain circumstances, conditions may become conducive for aggregation [22]. The smallest and first aggregate structures to form are referred to as oligomers. Some of these oligomeric structures are nuclei capable of seeding further growth and the formation of larger aggregate structures, namely the characteristic amyloid fibril structures that deposit as extracellular plaques within the brain [23]. Among the naturally occurring isoforms, Aβ1–40 is the most abundant in vivo and the primary component of plaque deposits [24]. The second most abundant isoform, Aβ1–42, however, aggregates more readily than its shorter counterpart to form stable oligomers, and as a result is the primary component of early-stage diffuse amyloid deposits. While aggregation of these full-length proteins is most relevant to in vivo plaque development, studies of aggregation often employ fragments of the protein that include Aβ’s hydrophobic core, residues 17–21 [25], and readily form characteristic amyloid fibrils.

Recent studies have demonstrated that the severity of AD symptoms better correlates with the presence of Aβ oligomers rather than the deposition of Aβ fibrils [26, 27]. Research aiming to clarify the fundamental pathogenesis of AD has largely shifted toward attempting to delineate the formation and structure of Aβ oligomers and clarify the process of transitioning from these oligomers to fibril structures. The instability and multiplicity of oligomer structures has thus far resulted in their defiance to definitive characterization [28]. Due to the experimental inaccessibility of these structures, many researchers have employed theoretical and simulation methods to elucidate the form and thermodynamics of these elusive species [29, 30].

1.3. NPs as therapeutic agents targeting Aβ aggregation
The uncertainty in the molecular structure of the pathogenic target of Aβ-concerned AD therapeutics complicates the task of engineering these agents. Nonetheless, a large number of researchers have made progress in this endeavor as substantiated by encouraging and indisputable metrics. NP systems constitute a promising class of AD therapeutics. NPs of a wide range of surface chemistries, morphologies and sizes have demonstrated potent efficacy as modulators of Aβ aggregation, as we discuss in detail below.

One challenge to developing these agents into effective therapeutics is the propensity for NPs to develop a coating of proteins that adsorb or associate to the NP surface in vivo. This coating is often referred to as a protein corona [31]. The process of protein corona formation and its distribution of constituents is a crucial consideration to researchers engineering therapeutics, as this NP-protein complex is often the operative molecular assembly for pharmacokinetic (PK) and pharmacodynamic (PD) processes. However, the tunability of NP chemistry and morphology offers a means by which this obstacle can be manipulated. Moreover, the prospect of overcoming this challenge is encouraged by the advantages offered by the biodistribution of NPs [32]. It is difficult to achieve high concentrations of therapeutic agents within the brain. The blood-brain barrier (BBB) is a devastatingly effective defense system functioning to exclude exogeneous materials and is thus a barrier that need be reckoned with as therapeutics are almost universally administered outside its borders. In fact, only small lipophilic molecules, representing a small fraction of neurotherapeutics, can cross the BBB unassisted [33].
However, NPs of various sizes and compositions have been observed to cross the BBB via both passive and active transport mechanisms, including passage by ion channels, endocytosis, receptor mediated transcytosis, pinocytosis and tight junction disruption. Furthermore, NPs can be functionalized with ligands that recognize BBB receptors to enhance BBB transport [33].

In spite of the oft-touted promise of NPs as prospective AD therapeutics, the authors of this review are unaware of NP systems in any phase of clinical trials for AD. It is the overarching goal of this review to expound the studies that have demonstrated the remarkable characteristics of NPs as an engineering platform for AD therapeutics and to couch these observations within a theoretical framework in order to steer the field toward a more mechanistic approach to NP engineering in this domain. In section 2, we will outline a general physical framework with which both protein aggregation and NP-protein interactions can be described. In section 3, we will discuss the body of work that exists on NPs as modulators of Aβ aggregation. In section 4, we will formulate a perspective on the present state of NP therapeutics in the context of AD and suggest directions for future efforts.

2. Theoretical perspective for NP-protein interactions

Our objective in this section is to present a theoretical apparatus with which to discuss NP-Aβ interactions and specific experimental observations of this system. We first outline the fundamental equations of self-assembly that are used to describe the distribution of protein aggregate populations at thermodynamic equilibrium and in the absence of modulating NPs. We then discuss the manner in which binary interactions between a single NP and a single protein may be described by a potential of mean force (PMF). We conceptually transition from this isolated interaction into the equilibration of a bath of proteins with a single NP. Finally, we describe the self-assembly of Aβ in the presence of NPs and synthesize two metrics by which NP-Aβ interactions may be characterized with respect to their ability to modulate the Aβ aggregation process.

2.1. Theory of protein aggregation

We begin our theoretical discussion with a general description of self-assembly processes. Specifically, we outline the conditions of thermodynamic equilibrium for systems capable of self-assembly. Our presentation closely follows that of Tanford and Israelachvili [34, 35].

At equilibrium, the chemical potential of every Aβ monomer must be the same at every location inside the system volume, and this concept implies that the chemical potential of each monomer be equal in every thermodynamically possible aggregate structure:

\[
\mu = \mu_i^0 + \frac{k_BT}{i} \log \frac{X_i}{i}
\]  

for all integer values of \(i\), which denotes the number of monomers in an aggregate. Here, \(\mu\) is the chemical potential, which is constant over all space at equilibrium. \(\mu_i^0\) is the mean interaction energy per monomer in aggregates consisting of \(i\) monomers. \(k_B\) is the Boltzmann constant, and \(T\) is the temperature. \(X_i\) is the non-dimensionalized concentration, more rigorously activity, of aggregates of size \(i\) expressed in terms of monomer units. Equation (1) may be rearranged to isolate for the concentration of aggregate \(i\):

\[
X_i = i X_i \exp \left( \frac{\mu_i^0 - \mu_\infty^0}{k_BT} \right)^i
\]

which may then be used to express the total concentration \(C\) of monomers in solution:

\[
C = \sum_{i=1}^{\infty} X_i
\]

We may now propose an expression for aggregates of the simplest morphologies, namely rods, discs and spheres:

\[
\mu_i^0 = \mu_\infty^0 + \frac{\alpha k_BT}{r^d/d}
\]

where \(-\alpha k_BT\) represents the free energy disparity between an isolated monomer and isolated aggregate of size \(i\) relative to that of an isolated aggregate of size \(i + 1\). Rigorously, the value of \(\alpha\) will be dependent on the size and shape of the aggregate to which the monomer is associating as well as the conformation of that monomer. \(\mu_\infty^0\) is the bulk free energy of a monomer in an infinite aggregate, and \(d\) is the dimensionality of the aggregate morphology. With a knowledge of or assumed scheme for the relevant values of \(\alpha\), Equations (2) through (4) uniquely determine the equilibrium distribution of aggregate populations as a function of aggregate size [36].

The above equations are referred to as the fundamental equations of self assembly. These equations provide us with a prescription of what must be true at thermodynamic equilibrium but offer no description of the process by which monomers may come to this state of equilibrium. This process is commonly addressed by molecular dynamics (MD) simulation approaches [37, 38], molecular field theory [39, 40], classical nucleation theory (CNT) or some combination of the three. Due to the limited sample of appropriate modeling techniques, many researchers have developed similar theoretical frameworks specially designed to address the process of protein aggregation [41].

2.2. Interactions between a single NP and a single protein

The interaction of an isolated protein with an isolated NP in solution may be succinctly described by the PMF acting between the two species. We may consider the PMF acting between a NP and a protein from...
multiple levels of abstraction. To begin, consider the process of bringing a single NP and a single protein into close proximity. At every distance of separation between the centers of mass of these two molecules, the relevant thermodynamic potential may be calculated by an ensemble average of the positions and momenta of the solvent and solvated species as well as the conformations of the NP and protein. In such a circumstance, the grand canonical ensemble should be used with the simple caveat that the centers of mass of the NP and protein are constrained to fixed points in space for each realization of constrained equilibrium [42]. This thermodynamic potential as a function of center of mass separation is the PMF for this binary interaction. This function tells us the force that is exerted between the NP and protein at different separations from the thermodynamic perspective just described. Such a PMF may tell us that, through this statistical mechanical lens, there is a repulsion between the two molecules at all distances or, conversely, that an attraction pervades. Many PMF plots indicate a varying attractive and repulsive character in a separation-dependent manner.

An equivalent conceptualization of the significance of the PMF is to consider the ensemble of trajectories through time of an interacting NP and protein as they propagate through phase space. If we were to ensemble average all of these trajectories, we would recover the same PMF obtained with the previous prescription. It is from this theoretical vantage point that we may more intuitively make the assertion that the conformational ensemble of proteins is always affected when it comes in proximity to a NP. For certain NPs and proteins, the degree of alteration to the conformational ensemble of the protein may be negligible for all engineering purposes. This will likely be the case for proteins possessing and existing in a thermodynamically favored state and that interact with a NP displaying a relatively benign surface. However, for IDPs or NPs possessing influential surfaces, the alteration to the protein’s conformational ensemble may be drastic and, in some cases, critical to the macroscopic physics of the system [43–46].

2.3. Equilibration of NPs and proteins in solution

While calculation of the PMF previously described can be extremely informative, in real-world settings higher order interactions between multiple NPs and proteins are ubiquitous. While NP-NP interactions are certainly a relevant phenomenon to this physical system, we shall assume that they possess a sufficiently repulsive PMF between one another to ensure their stability in solution. Any NPs worth considering in this context would necessarily possess this property. A significant point to appreciate is that the PMF between a protein and a NP-protein complex will differ from the original binary interaction [47]. Likewise, the PMF of a NP and protein-protein complex will differ as well. In general, the PMF between a NP and protein complex will differ depending on the extent to which other proteins are adsorbed to the NP as well as the size and morphology of the protein complex.

The explicit consideration of these types of isolated interactions can be useful for probing the early interactive processes of NP-protein systems. However, the number and increasingly complex geometries that emerge as higher order interactions are considered rapidly precludes an exact and exhaustive treatment of the physics at this level of abstraction. We must resign ourselves to an equilibrium description of a NP exposed to a bath of solvated proteins. As aforementioned, the process of adsorption of proteins to a NP surface is commonly referred to as protein corona formation and has been studied extensively both experimentally and theoretically [48–51]. It is well established that the properties of this protein corona are a function of the NP size, shape and surface chemistry as well as the concentrations of solvated proteins [52–54].

2.4. Theoretical considerations for NP-Aβ interactions

Thus far, we have outlined the thermodynamics of self-assembly processes and developed a general theoretical description of binary and higher order NP-protein interactions. We shall now focus our discussion on NP-Aβ interactions in particular. The nucleation process of Aβ even in the absence of NPs is more complicated than the most idealized cases in CNT. The formation process and morphology of oligomeric structures of Aβ remains relatively poorly understood. It is appreciated in the literature that, under certain conditions, so-called off-pathway Aβ oligomers form [55]. These structures possess some semblance of stability though do not constitute nuclei for the fibril growth process. Some researchers have developed theoretical models of protein aggregation that explicitly account for the occurrence of off-pathway structures [56]. In spite of this complication, Kashchiev and Auer have developed several theoretical models of the nucleation process of Aβ in isolation, which they have refined and extended over the last decade [57–62]. These significant works constitute the state of the art of modeling Aβ nucleation. However, we do not believe that the level of detail inherent to these studies is necessary to broadly classify the mechanisms by which NPs may modulate Aβ aggregation.

The fundamental equations of self-assembly describe conditions at thermodynamic equilibrium but do not provide insight into the dynamics related to the arrival at this state. Readers are referred to the work of Kashchiev and Auer for a more expanded discussion of the functional dependence of the nucleation rate of Aβ on the physical parameters discussed [57–62]. We note only that in excruciatingly simple terms, both greater concentrations of monomer and greater values of α (for species necessary for nucleation) correlate with greater nucleation rates. This functional relationship is significant as the nucleation rate is intimately
related to the lag time of the Aβ aggregation process [63], which is experimentally accessible.

It is at this point that we introduce two metrics with which we will frame our discussion of the existing studies of NP-Aβ interactions. First, we may discuss the extent to which Aβ monomers and aggregates localize on the surface of NPs. Certain NPs are engineered specifically to bind with Aβ, which reduces the effective concentration of solvated Aβ. This effect can be expressed in the fundamental equations of self-assembly by adding a new term to the right hand side of equation (3) representing the concentration of Aβ monomers associated to NPs. Furthermore, the chemical potential of Aβ monomers associated to NPs must be equal to that of monomers in all other aggregate species. A review of these equations makes explicit how the population distribution of Aβ aggregates would be altered by this modification. This metric is illustrated graphically along the y-axis in figure 1.

The second metric concerns the extent to which interaction with a NP alters the conformation of Aβ. This second metric is represented as the x-axis in figure 1. Regardless of whether or not an Aβ monomer adsorbs to a NP surface, the localization of the two structures may alter the conformation of an Aβ monomer and, consequently, its aggregation properties. The effect of the alteration of Aβ’s conformational ensemble would be manifest in a change in the value of α. It is through this mechanism that a NP-induced alteration to Aβ conformation may effect a redistribution of Aβ aggregate populations.

Under most circumstances, both of the phenomena pertinent to the two metrics defined herein are occurring to some extent. However, researchers may be able to point to one metric or the other in some cases and assert its operative dominance. We have thus far discussed these metrics in a purely phenomenological manner. Note that mechanistically, the two metrics will bear some degree of coupling.

3. Experimental observations of NP-Aβ interactions

A large body of experimental work exists related to the interaction of NPs with Aβ. Due to the aggregation prone nature of Aβ and its involvement in the etiology of AD, aggregation is a factor of either explicit interest or implicit influence in all of these studies. The body of work discussed in this section varies widely with respect to in vitro experimental conditions, including experiments conducted over a range of pH values, ion concentrations and temperatures as well as Aβ and NP concentrations and with different Aβ isoforms. In addition, those experiments including a biological component utilized a variety of different cell types and animal models. Furthermore, the observed influence on Aβ aggregation differs, with some NPs effecting a promotion of Aβ aggregation while others inhibit the process. Interestingly, the degree and character of this influence was often observed to be a function of experimental conditions for the same NPs.

For purposes of organization, we have classified experimental studies into two categories: those with and those without conjugated surface ligands engineered to possess a specific chemical interaction with Aβ. The former category includes NPs presenting peptide fragments and small molecules capable of binding Aβ, whereas the latter features a metallic or polymeric NP surface. This classification scheme is not intended to imply that studies falling into these categories did or did not display interactions between the NPs and Aβ, only whether the NPs bear conjugated surface ligands that were engineered to do so by their very nature.

3.1. NPs with surface ligands engineered for specific chemical interactions

NPs engineered to present surface ligands often pair ligands that possess some ability to either bind or modulate the formation of Aβ aggregates with a second ligand that enhances NP delivery in vivo to the site of the aggregates. We begin our discussion with work that has utilized peptide moieties to achieve either one or both of these objectives and finish with an overview of studies involving NPs with non-peptide surface ligands.

3.1.1. NPs with peptide surface ligands

In a logical approach, several studies have investigated functionalization of NPs with peptide fragments known to modulate aggregation. Shlomo Margel and coworkers designed in 2011 γ-Fe2O3 NPs containing encapsulated fluorescein [64]. These NPs were functionalized using a series of coatings terminating in the attachment of either the full-length Aβ1–40 protein or the peptide LPFFD, which is known to break β-sheet structures [65]. It was observed that the Aβ1–40-coated NPs promoted the fibrillation process, while NPs coated with LPFFD inhibited this process. Thus, this study highlights the importance of incorporating within the design of the conjugated peptide the ability to disrupt aggregation. Using a different inhibitory peptide, Xiong et al. reported in 2015 the effects of peptide-functionalized poly(lactic-co-glycolic acid) (PLGA) NPs on Aβ aggregation [66]. Intriguingly, the authors observed that the peptide engineered for this study, Ac-LVFFARK-NH2, inhibits Aβ fibrillation but possesses intrinsic self-assembling behavior leading to cytotoxic non-fibrillar aggregates. However, when this peptide is localized on the surface of PLGA NPs, the resulting complex modulates aggregation in favor of less toxic Aβ oligomer species. This strategy highlights the complexity of the Aβ aggregation process and the multitude of molecular architectures involved. While it is appreciated that Aβ oligomers as a class of structures are reported to be more pathogenic than fibrillar structures, certain specific molecular interactions may produce a less toxic or less pathogenic subset of oligomers.
In an analogous approach, strategically selected amino acids have been exploited as modulatory elements. In 2012, Margel and coworkers published a study detailing the different modulatory effects of polymeric NPs and reported that NPs composed of a polymer containing phenylalanine units (a constituent of \(\beta\)-sheet breaking) inhibited \(A\beta_1-40\) fibrillation, while those NPs containing alanine units promoted aggregation [67]. In 2010, Xiao et al reported that CdTe NPs functionalized with N-acetyl-L-cysteine were capable of quenching the nucleation and fibril elongation phases of \(A\beta_1-40\) aggregation with remarkable potency [68]. The authors postulate that this effect is attributable to the presence of intermolecular attractive forces. They suggest hydrogen bonding forces between \(A\beta\) fibrils and the NPs result in blocking of fibril elongation sites. Collectively, these studies have demonstrated that NPs, including those with magnetic and fluorescent properties for sequestration and imaging purposes, can be engineered to interact with \(A\beta\) aggregates via functionalization with \(A\beta\) proteins and peptides as well as amino acid groups for rational design of their modulatory effects on the \(A\beta\) aggregation process.

In an alternative approach, peptides analogous to \(A\beta\) have also been exploited for their ability to recognize the full-length \(A\beta\) protein and thus serve as a targeting sequence. Xiaogang Qu and coworkers demonstrated that NPs composed of polyoxometalate/\(A\beta_{15-20}\) were able to inhibit the aggregation of \(A\beta\) in mouse CSF [69]. In this work, polyoxometalate was combined with \(A\beta_{15-20}\) due to research evidencing the former as a \(\beta\)-sheet breaker [70] and the latter as a targeting moiety [71]. In a subsequent study, Xu and coworkers investigated a similar polyoxometalate–peptide therapeutic but, in this instance, integrated with a gold NP scaffold [72]. They report that these nanostructures possess similar efficacy with respect to the inhibition of \(A\beta\) aggregation and expand their experimental scope to confirm the ability of these NPs to cross the BBB of mice.

Additional studies have specifically incorporated moieties to facilitate BBB passage into the design of prospective NP therapeutics. Qizhi Zhang and coworkers developed NPs that designedly possess both an ability to cross the BBB and a binding/inhibiting effect for \(A\beta\) aggregates. This dual functionality was achieved by conjugating a differential distribution of TGNYKALHPHNG (TGN) and QSHYRHISPAQV peptide ligands to the surface of PEGylated poly(lactic acid) NPs. The former peptide serves to facilitate crossing of the BBB [73], while the latter is known to interact with \(A\beta\) [74]. Their work uniquely demonstrated that indeed these NPs effectively target \(A\beta\) aggregates in an AD mouse model [75]. In the same year, Qizhi Zhang, Yong Qian and coworkers demonstrated that NPs with the same two targeting ligands could be further functionalized with the known \(\beta\)-sheet breaker H102 and that the resulting NPs could abrogate spatial learning and memory deficits in an AD mouse model [76]. These studies were bolstered by another in 2017 that more precisely elucidated the biological mechanisms involved in the targeting process [77]. This ability to target \(A\beta\) aggregates in animals via NP systems is critical to the development of an effective therapeutic. Other groups have successfully implemented similar approaches to NP design. In a 2017 study, Jie Liu and coworkers demonstrated that selenium NPs functionalized with TGN, the same peptide used several years prior by Qizhi Zhang and coworkers to achieve BBB passage, and LPFFD, the \(\beta\)-sheet breaker peptide used by Margel and coworkers, could also

Figure 1. Representations of \(A\beta\) molecules interacting with NPs as functions of two discussed metrics: the extent to which NPs adsorb \(A\beta\) and the degree to which interaction with a NP alters \(A\beta\) conformation relative to aggregation propensity.
inhibit Aβ fibril formation [78]. In addition, Morales-Zavala et al reported that gold nanorods functionalized with an Aβ aggregate-targeting peptide and a ligand commonly used to deliver agents to the central nervous system could inhibit Aβ aggregation in vitro and reduce toxicity in a Caenorhabditis elegans AD model [79]. Together, these studies establish that NPs can be designed to both target Aβ and inhibit aggregation and to also reach the challenging physiological environment of the brain.

3.1.2. NPs with non-peptide surface ligands

The vast field of AD research has also identified an array of synthetic and naturally occurring small molecules capable of binding Aβ and/or modulating aggregation. Like peptides, these molecules have been adapted for the functionalization of NPs to design inhibitors of Aβ aggregation. In an approach utilizing small molecule functionalization as a targeting moiety, Shlomo Margel and coworkers observed that magnetic γ − Fe₂O₃ NPs conjugated with the amyloid-binding fluorescent dye Congo red selectively bind to Aβ₁−₄₀ fibrils [80]. Furthermore, these NP-bound fibrils can be selectively removed from solution through the application of magnetic field. Lipids and lipid-derived small molecules known to interact with Aβ have also shown promise as NP functional groups for the targeting of Aβ. In 2010, Gobbi et al demonstrated that liposomes and solid lipid NPs possess high binding affinity for Aβ₁−₄₀ fibrils when they were functionalized with the lipid head group phosphatidic acid and the mitochondrial-derived lipid cardiolipin [81]. Four years later, Song et al observed that an apolipoprotein E3-reconstituted high density lipoprotein nanocomposite material could bind to Aβ monomer and oligomer structures [82]. Moreover, these NPs facilitated the clearance of Aβ and reversed memory deficits in an AD mouse model.

In an approach that leverages the anti-aggregation properties of small molecules, Jie Liu and coworkers investigated selenium NPs functionalized with the well-studied Aβ aggregation inhibitor epigallocatechin-3-gallate (EGCG) [83] and subsequently coated with the Tet-1 peptide [84], an agent that can bind and thus target neurons [85, 86]. These NPs were capable of inhibiting the formation of Aβ aggregates as well as dissociating pre-formed aggregates. Similarly, the reported aggregation inhibitor curcumin [87] has been conjugated to NPs. In 2014, Palmal et al demonstrated that gold NPs functionalized with curcumin could inhibit fibril formation and dissociate pre-formed aggregates [88]. In the following year, Cheng et al reported that superparamagnetic iron oxide NPs (SPIONs) coated with curcumin colocalized with Aβ aggregates in the brains of Tg2576 mice [89], a widely studied AD mouse model. Yoo et al explored the inhibitory capabilities of CdTe NPs stabilized with thioglycolic acid [90]. While not an identified inhibitor of Aβ aggregation, this functionalization was chosen due to its similarity in terms of physical characteristics to proteins. Indeed, Yoo et al observed that these NPs are able to inhibit Aβ₁−₄₀ aggregation and that the mechanism and efficiency of inhibition is similar to protein inhibitors.

3.2. NPs without surface ligands engineered for specific chemical interactions

A substantial volume of research has been conducted concerning the interactions of Aβ and NPs displaying what we have termed passive surface chemistries, or surfaces not functionalized specifically for the recognition of Aβ. Some of these studies have tested NPs with a metallic core, these studies almost universally employing gold, while others feature a completely polymeric construction.

3.2.1. Metallic NPs

Metallic NPs were among the first NP structures to be investigated for their ability to modulate Aβ aggregation. As early as 2008, Wu and coworkers evaluated an array of different metallic NPs (TiO₂, SiO₂, ZrO₂, CeO₂, C₆₀, C₇₀) for their ability to modulate the fibrillation of Aβ₁−₄₂ [91]. This work found that TiO₂ NPs in particular promote the fibrillation of Aβ₁−₄₂ in a dose-dependent manner. Yokoyama et al also pioneered early efforts via a study what has become the most widely investigated NP material, gold. In contrast to Wu and coworkers, this group focused their study upon this single NP material to facilitate elucidation of the influence of NP physical properties and solution conditions upon NP-Aβ interactions [92, 93]. In two separate papers, the effects of pH, temperature and NP size were examined with respect to their influence on the association of Aβ to the gold NP surface as well as the stability of the resulting NP-Aβ complexes. Although the deionized aqueous conditions under which these experiments were conducted are not representative of a physiological environment, their work did demonstrate early on that these physical parameters can significantly influence NP-Aβ interactions. NP physical properties subsequently became an important aspect of experimental investigations.

Several years later, the role of NP surface charge was studied for its influence upon Aβ aggregation. Liao et al reported that both bare gold NPs and gold NPs functionalized with negative surface groups could inhibit Aβ fibrillation and additionally effect fragmentation of preformed fibrils [94]. However, the effect was not observed for the same NPs functionalized with positive surface groups. That same year, Chan et al reported that gold NPs of various negative surface charges could modulate the elongation phase of the Aβ fibrillation process [95]. They observed that this process is highly sensitive to surface charge density such that slight alterations can produce opposite effects on Aβ fibril elongation. In the following year, Dawson, Lynch and coworkers investigated the
interaction between Aβ and SPIONs [96]. Several configurations of these NPs were synthesized: those with no modifications and those with one or two dextran layers incorporating either carboxyl or amino conjugates. It was observed that all of these NP configurations inhibited Aβ fibrillation at lower concentrations but promoted fibrillation at higher NP concentrations. Furthermore, this promotion was more potently effected by the positively charged NPs than negatively charged NPs with an equivalent charge density. In 2016, Mirsadeghi et al. explored the influence of PEGylated SPIONs on the Aβ fibrillation process when exposed to an external magnetic field [97]. The authors found that higher concentrations of NPs promoted aggregation while lower concentrations inhibited the aggregation process. The authors note that the coating charge of the SPIONs also influenced the effect on fibrillation. Namely, at lower NP concentrations negatively charged NPs displayed greater inhibitory capabilities than positively charged NPs. Both the effects of SPION concentration and surface charge are consistent between the work of Dawson, Lynch and coworkers and that of Mirsadeghi et al. in spite of the fact that a magnetic field was applied in the latter study. Ethayaraja Mani and coworkers reported in 2017 that NPs with charge opposite to that of the residues comprising the β-sheet structures in amyloid fibrils can inhibit the formation of these structures regardless of the material constituents of the NPs [98]. This study tested the efficacy of gold and silica NPs as inhibitors of Aβ aggregation and observed that negatively charged gold and silica NPs inhibit the formation of Aβ1-40 fibrils while positively charged NPs had no effect.

Like surface charge, alterations in NP size can lead to the modulation of Aβ aggregation toward both inhibition and promotion. Chan et al. also studied gold NPs of various sizes (2–15 nm) and showed that, like charge, NP size could modulate fibril elongation in a highly sensitive manner and with opposing effects [95]. In addition, Gao et al. studied the influence of size of L-glutathione stabilized gold NPs on the aggregation of Aβ1-40 [99]. Larger NPs (18 and 36 nm) were observed to promote aggregation whereas smaller NPs (6 nm) inhibited the process, emphasizing the significance of size on NP-Aβ interactions. In certain regimes and across certain ranges of experimental design space, the size of the NPs may not assert a distinguishable effect on Aβ aggregation. However, as demonstrated by these studies, varying size has the potential to reverse the modulatory effect of NPs.

In 2017, Moore et al. investigated the influence of both the surface chemistry and diameter of gold NPs on the aggregation of Aβ1-40 [100]. This study demonstrated that 18 nm NPs with different electrostatically-associated surface layers including citrate, polyacrylic acid and polyallylamine HCl were all capable of inhibiting Aβ fibril formation. Notably, however, those NPs featuring the negatively charged surface layer of polyacrylic acid were most effective, completely abrogating Aβ fibril formation at ratios of NP to Aβ of 1:2 000 000. To explain such low ratios, the authors suggest that local interactions with these NPs, but not necessarily binding events, are capable of sufficiently altering Aβ conformation such that aggregation is inhibited. In parallel, the authors demonstrate that the inhibition of Aβ aggregation is a function of NP size, with large 40 nm NPs being incapable of eliciting the inhibition observed for 8 nm and 18 nm NPs, thus emphasizing the important interplay between NP size and surface chemistry.

In accordance with this complexity of NP-Aβ interactions, some studies extend their analysis beyond simple detection of the presence of aggregates. Among the first of these studies, Ma et al. reported in 2013 the influence of bare gold NPs on the aggregation of Aβ25-35 [101]. Under their experimental conditions, these NPs promote fibril formation, and this study evaluated the mechanism of this effect and the resulting morphological and electrical characteristics of the aggregates. Similarly, the 2017 study by Mani and coworkers, which observed the importance of negative surface charge on the efficacy of gold and silica NPs as inhibitors of Aβ aggregation, combined experimental observations with MD simulation in order to bolster molecular detail of the NP-Aβ binding mechanism [98]. In a subsequent study, these researchers presented a kinetic model of NPs and aggregating Aβ molecules [46]. This kinetic model was unique in its incorporation of mechanisms by which NPs may adsorb, deactivate and desorb Aβ monomers. In this context, deactivation refers to the modulation of a monomer’s conformation such that it is no longer capable of aggregation. This kinetic model was parameterized to fit experimentally measured aggregation behavior of Aβ in the presence of citrate-stabilized gold NPs.

Leveraging the affinity that gold NPs have for Aβ, several research groups have developed techniques to use gold NPs as indicators of Aβ fibril presence rather than as disruptors of the aggregation process. Geng et al. published a screening method based on this principle [102] in 2010. They demonstrated that gold NPs may be used to visually detect the inhibitory efficacy of small molecules on the aggregation of Aβ12–28. In a similar effort, Elbassal et al. exploited the same affinity to develop a method for fibril detection [103]. Their methodology is predicated on the principle that the surface plasmon resonance frequency of the gold NPs used is sensitive to Aβ1-40 content in solution.

3.2.2. Polymeric NPs

Polymeric NPs offer a broad diversity of materials with potential to interact with Aβ and modulate aggregation, facilitating further insight into mechanisms. The tunability of these materials has been demonstrated by studies that have explored copolymeric NPs. Dawson, Lynch and coworkers studied the influence of the monomeric ratio of NiPAM:BAM NPs on the aggregation of Aβ [104].
While these NPs have an insignificant effect on the elongation and fibrillation processes, they function to extend the lag phase of aggregation in a manner that is dependent upon the NP surface properties, tuned by the monomeric ratio. They posit that this behavior is the result of an adsorption process, a hypothesis that is supported by a subsequent study that utilized polymeric NPs presenting conjugated amino groups. Here, the researchers discovered that the ratio of Aβ to NP surface area could dictate either promotion or inhibition of aggregation [105]. At high Aβ to NP surface area ratios, aggregation was promoted, while at low ratios, aggregation was inhibited. This same correlation was later replicated using SPIONs as aforementioned [96]. The authors explain this interesting behavior by proposing that Aβ adsorbs to the surface of the NPs and that adsorbed Aβ nucleates and/or induces fibril elongation at an increased rate provided the Aβ is not adsorbed too sparsely to the surface. Additional support for this proposed mechanism was facilitated through the use of capillary electrophoresis with laser-induced fluorescence detection. Karine Andrieux and coworkers monitored the interaction between polyethylene glycol (PEG)-bonded or non-PEGylated poly(alkyl cyanoacrylate) nanostructures and aggregating Aβ to observe that the PEGylated form of these NPs was able to adsorb Aβ and function as a catalytic center for the aggregation process, promoting fibril growth [106]. Interestingly, the non-PEGylated form of these NPs had no significant effect on Aβ aggregation kinetics. Andrieux and coworkers also published studies in 2011 and 2012 that applied a similar methodology to NPs composed of other polymers [107] and studied the effect of these polymeric NPs on Aβ aggregation both in solution and in human serum [45]. In further support of the aforementioned hypothesis, this latter study included a MD simulation component that focused upon the conformational change that Aβ undergoes when interacting with PEG.

Efforts by Brezesinski and coworkers demonstrated that these conformational changes can both promote and inhibit aggregation. In 2008, this group reported that interactions between Aβ and nanoscale (4 nm) polyampholyte complexes modulated Aβ fibril formation via inhibition or promotion depending on whether these complexes bore associated fluorinated or hydrogenated dodecanoic acid, respectively [108]. Circular dichroism was used to show that this differential modulation correlates with a disparate abundance of α-helix versus β-sheet presence in the Aβ conformations. These results mirror the implications of the kinetic model proposed by Mani and coworkers using gold and silica NPs, presented ten years later. This initial study was proceeded by two others by Brezesinski and coworkers, one of which demonstrated that differential conformational disruption could be effected by the same polyampholyte complexes for Aβ fibrilization [106] thus altering the propensity of oligomer formation by this isoform [43]. Finally, Brezesinski and coworkers found that polystyrene microgel and latex NPs were able to both adsorb Aβ and disrupt the conformational properties of the protein, effectively inhibiting oligomer and fibril formation [44]. They attribute the efficacy of these particles to a specific balance between hydrophilic and hydrophobic regions on the NP surface.

In addition to surface hydrophobicity, NP surface charge has been explored for its influence upon polymeric NP interaction with Aβ. In 2018, Yan Sun and coworkers released a study in which they altered the relative concentrations of chitosan and hyaluronic acid composing the NPs to achieve disparate zeta potentials ranging from 35.8 to −35.3 mV [109]. They report that higher magnitudes of surface charge densities produced the greatest inhibitory effects on Aβ aggregation for both positive and negative surface charges, a result that echoes observations with metallic NPs illustrating the importance of charge density.

The intercalating effects of surface hydrophobicity and surface charge have also been investigated. In 2016, Yan Sun and coworkers released a study in which they explored the inhibitory capabilities, with respect to Aβ fibrillation, for a series of polymeric NPs that bore the same surface composition with regard to hydrophobic groups but different negative surface charge densities [110]. In an intriguing result, the authors find that there exists a charge density that constitutes a local optimum with respect to inhibitory efficacy. The authors explain this behavior by proposing that the hydrophobic surface would readily adsorb Aβ in the absence of any electrostatic repulsion between the two species. In parallel with the previously mentioned hypothesis involving adsorption and protein conformational change, they suggest that the adsorbed Aβ would not present nucleation sites for fibrillation due to presumed conformational changes of the Aβ monomers sufficient to reduce aggregation propensity. Thus, the remaining solvated Aβ has a lower effective concentration due to the sequestration and inactivation of monomers on the NP surface. As the negative surface charge density is increased, the electrostatic repulsion increases between the NP and the Aβ, which is negatively charged at the experimental pH. At sufficiently high negative charge densities, the authors explain that this electrostatic repulsion is sufficient to reduce the amount of Aβ that can attain the proximity necessary to hydrophilically adsorb. Hence, the mutual countering of these two effects presents an optimum.

The effect of polymeric NP size on modulation of Aβ aggregation has also been investigated, although to a much lesser extent than for metallic NPs. In the previously discussed study reported by Yan Sun and coworkers [110], which explored NPs comprised of chitosan and hyaluronic acid, the diameters of these particles varied widely. The authors tested and deemed insignificant the effect of NP diameter with respect to influencing Aβ aggregation. However, if the hypoth-
esized mechanism of action involves adsorption, the observation that two otherwise equivalent NPs with radii of curvature that vary by more than a factor of 3 and surface areas that vary by a factor of nearly 10 modulate Aβ aggregation to indiscriminate extents has significant implications. Namely, it would imply that variations of the curvature of the NP surface, its total area and diffusivity in this range do not substantially alter the physics of the interaction. This raises the question: would an equivalent planar surface display these properties as well?

Experimentation with polymeric NPs has also expanded the exploration of the effect of solution conditions on the ability of NPs to modulate Aβ aggregation. In 2013, Ghavami et al reported that the interaction of polystyrene and silica NPs with aggregating Aβ can depend quite sensitively on temperature within the physiological range [111]. The authors demonstrate that slight temperature changes can affect exposure of hydrophobic versus hydrophilic NPs, leading to selective inhibition by hydrophobic NPs at higher temperatures. While this sensitivity may not be present for all NP-Aβ systems, this important work substantiates the cautionary point that the behavior of a NP with respect to Aβ aggregation should not be assumed to be insensitive or even proportional to temperature. Indeed, Ghavami et al present a non-trivial case for which a NP that inhibits Aβ aggregation may promote the same process at 5 °C lower temperature.

3.3. Summary of experimental studies

The numerous studies outlined herein have revealed a substantial amount of detail within the NP-Aβ interaction landscape. Collectively, these studies evidence the significance of NP diameter and surface chemistry as well as the bulk pH, temperature and concentrations of the NPs and Aβ. Many researchers have reported the successful inhibition of Aβ aggregation under certain in vitro conditions and others have reported encouraging results for in vivo settings. Though we know far more than ever before about this promising therapeutic platform, many of these studies are limited to isolated instances of physical phenomena that cannot be directly compared nor mutually reconciled. Unfortunately, the crystallization of a cohesive picture of the physics of this interaction remains a distant aspiration.

4. Outlook for the platform

4.1. What has been systematically missed?

The existing body of work on NPs as potential therapeutics for AD with the specific intent of abrogating the aggregation of Aβ is vast and remarkably varied. It is abundantly apparent that there exists exciting potential for this platform. The diversity of the mechanisms through which these NP complexes modulate the aggregation process is an attractive property as it indicates that there are multiple molecular mechanisms that can be exploited to achieve the desired inhibitory behavior. This robustness will be a critical attribute for these types of NP systems should they progress from in vitro and animal studies to clinical trials.

However, in spite of the promise that has been demonstrated by NPs in this domain, there exist no NPs in any stage of clinical trials for AD. This surprising fact was raised by Saraiva et al in their excellent review on the delivery of NPs to brains for neurodegenerative diseases [112]. It should be noted that the regulatory process needed to approve such trials poses unique challenges for NP systems relative to many other small molecules [113]. Nonetheless, as of 2016, there were 51 FDA-approved NP medicines and 77 NP products in clinical trials for a wide range of applications other than AD [114].

We propose that the failure of NPs engineered to disrupt Aβ aggregation to progress through the developmental pipeline for therapeutics is fivefold. The first significant obstacle is the extremely limited comparability across the existing literature. It is universally appreciated by those that study the Aβ aggregation process in vitro, both in isolation and in the presence of modulating molecules, that this process depends sensitively on the bulk pH, temperature, ion content, and the isoform and concentration of Aβ. When bulk solution conditions and the properties of Aβ are varied, it can be nearly impossible to extrapolate the effect that one NP type would have on Aβ aggregation under the new conditions. The introduction of NPs into an in vitro system brings about a host of experimental parameters that will possess a functional relationship with regard to modulatory effects and, as is often the case, do so in the experimentally relevant range. Such NP properties are the size, morphology, electrostatic character and surface functionalization (rendered graphically in figure 2). These characteristics give rise to the emergent physical concepts of zeta potential, hydrophobicity and diffusivity.

Furthermore, it should be appreciated that in many cases, an operative mechanism through which NPs influence Aβ aggregation is the modulation of local solution conditions. From a design perspective, therapeutically viable concentrations of NPs should only negligibly alter bulk solution conditions. Therefore, in the absence of direct physical interaction with the atoms of the NP, it is the alterations of local solution conditions that mitigate the effects on aggregation. Of course, many NPs are explicitly engineered to exploit direct physical interactions between the NPs and Aβ. However, even these NPs undoubtedly effect deviations from bulk solution conditions proximal to their surface. Such local solution conditions as pH, ion concentrations and temperature are illustrated in figure 2.

Moreover, complications to comparability discussed thus far only encompass those inherent to in
vitro studies. The topic becomes further muddled when the discussion is expanded to in vivo work. Nonetheless, the spirit of the critique is still relevant even when finer molecular control of experimental conditions is relinquished as the transition is made to a more realistic biological setting.

A second reason why NPs may be stalling in their progress to therapeutic viability is an underdeveloped understanding of the mechanisms of action of many of these NP systems. The difficulty in making these determinations is compounded by the poor comparability that exists in the field, making existing work difficult to leverage for new studies with different conditions. Unfortunately, many of the studies outlined herein constitute isolated observations of specific NPs interacting with Aβ. We contend that time and resources would be better spent taking advantage of one of the characteristics that makes the NP platform so powerful: namely the trait that, to an excellent approximation, the physical properties of the NPs are continuously tunable. Carefully designed experimentation that leverages this trait could allow researchers to uncover the relationships between NP size, morphology and surface functionalization and their corresponding modulatory effects on Aβ aggregation. Such studies would make testing and validating hypothetical mechanisms of action much more facile.

The third obstacle to translating NP therapeutics into realized therapies is one of applicatory myopia. Of the more than 50 studies published specifically on the subject of NPs as modulators of Aβ aggregation, few studies make direct attempts to address the issue of the inevitable protein corona that forms to some degree around NPs when administered to humans. To our knowledge, the recent works of Mirsadeghi et al and Lotfabadi et al are the only two exceptions to this observation [115, 116]. The reality of this effect as an engineering problem is incontrovertible. While the in vivo studies that exist in the literature do implicitly capture this effect, these studies lack an explicit attempt to characterize the corona that forms thus making hypothesis testing of molecular-scale mechanisms of action difficult if not impossible. A complete embrace of the fact that were these systems to progress to human trials, they would invariably be exposed to a host of other proteins with disparate adsorption affinities both before and during any exposure to Aβ is critical to the future success of the platform.

The fourth obstacle in this regard is a delay in integrating new pathophysiological conclusions into experimental designs. The studies discussed above have investigated the ability of NPs to modulate the formation of amyloid fibrils from monomeric protein or to alter the size or conformation of pre-formed amyloid fibrils. However, the effect of NPs on the earliest stages of aggregation, including the formation of oligomers, has not been widely explored. In light of evidence that identifies oligomers as the most pathological aggregate structures, the implications of this

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**Figure 2.** Physical factors influencing the Aβ aggregation process in NP-Aβ systems. The top row features graphical illustrations of NP size, functionalization and shape. The bottom row illustrates local solution conditions effected by the presence of NPs.
concept for the engineering of therapeutics are substantial [117]. Importantly, this pathophysiological finding introduces the paradigm that dissociating larger fibril aggregates may be detrimental. Because the exact etiology of AD is not definitively established, it is important to explore the effect of NPs at all stages of the aggregation process, including early oligomer formation, to ensure a comprehensive approach to therapeutic development.

The fifth obstacle is a deficiency of coupling experimental results with the capabilities of theoretical analysis. If properly designed and implemented, theoretical and computational studies have enormous potential to aid experimentalists in bridging the gaps between isolated observations to form a coherent understanding of the physical phenomena at play. In addition, theoretical and computational approaches provide useful apparatus with which to better understand the protein corona that forms around NPs as well as the specifics of the structure and formation pathways of the Aβ oligomer population. As has been emphasized, NPs constitute a nearly continuously tunable platform capable of evoking a range of effects on the Aβ aggregation process. However, experimental conditions are almost always discretely evaluated. This limitation is not manifest in many theoretical frameworks that are applicable to NP-Aβ systems. Ultimately, theoretical predictions need to be confirmed experimentally but the extensibility that is afforded offers an unparalleled opportunity to support mechanistic hypotheses, predict NPs with optimized characteristics and generally connect the disjointed web of experimental observations that currently exists. In short, an integration of theorists and computational scientists into efforts to develop the NP-Aβ therapeutic platform could be hugely productive.

4.2. Closing remarks

In summary, NPs constitute a powerful platform that has and continues to fundamentally change nanotechnology as it is applied to biomedical applications. The capacity of these systems to modulate the aggregation of the AD-associated protein Aβ has been discussed in this review. Over a decade of research conducted by groups around the globe has demonstrated that NPs can be engineered to potently inhibit the formation of toxic Aβ aggregates, displaying their promise as prospective AD therapeutics. Researchers have tested the influence of NPs bearing a variety of core materials and surface functionalizations both in vitro and in vivo. In spite of this body of work, there remain several obstacles to the translation of NPs into viable AD therapeutics. The acknowledgment and addressing of these challenges in the field could allow researchers to unlock the potential of this attractive platform and develop a therapeutic for this as of yet incurable and untreated disease.

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