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The interaction of peptides and proteins with nanostructures surfaces: a challenge for nanoscience

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Abstract

The impact of nanotechnologies in biomedicine and biotechnology is becoming more and more evident. It imposes practical challenges, for instance, raising specific issues on the biocompatibility of nanostructures. Nanoparticles are characterized by a high surface-to-volume ratio, which makes them reactive to foreign species. Thus, when proteins or peptides approach an inorganic nanoparticle, as well as a flat surface, they are likely to interact with the substrate to some extent. This interaction is crucial for applications in drug delivery, imaging, diagnostics, implants, and other medical devices. Specifically, gold nanoparticles are highly versatile and particularly appealing. It is widely accepted that the surfaces of nanoparticles adsorb proteins either transiently in the soft corona layer or permanently in the hard corona layer. As a consequence, the protein structure and/or function may undergo profound adjustments or remain conserved. Detailing the interaction of different inorganic substrates with proteins and peptides at the atomic level, and designing ways to control the interaction, is the key for biomedical applications of nanoparticles, both from a fundamental viewpoint and for practical implementations. In the last decade, we have addressed protein–nanoparticle interactions, focusing on interfaces of gold surfaces and nanoparticles with amyloidogenic peptides and protein models. We have developed classical force fields, performed advanced molecular dynamics simulations, and compared computational outcomes with data from nuclear magnetic resonance experiments. Protein–gold complexes with differently coated gold nanoparticles have been modeled to explore the effects of charge and size on the protein structure. Our work unravels that a complex interplay between surface properties and characteristics of the biological adsorbate determines whether peptide conformation is influenced and whether protein aggregation is accelerated or inhibited by the presence of the substrate. General guidelines to cope with amyloidogenic proteins could be inferred: these can be essentially summarized with the necessity of balancing the hydrophobic and electrostatic interactions that the amyloidogenic proteins establish with the coating moieties.

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Introduction

Amyloidogenic proteins are implicated in several neurodegenerative diseases and determine the molecular mechanisms that underlie amyloidogenesis. In this overview article, we summarize current knowledge on the behavior of amyloidogenic peptides and proteins in the presence of nanoparticles (NPs). We draw general conclusions from the presented results, which emerge from combined recent experimental and computational studies performed in our group.

NPs are known for their size-related properties and are intensely investigated for diverse uses [1,3–5]. They are ubiquitous in our environment and can be exploited for numerous applications, due to exquisite structuring that manifests extensive sizes, shapes, and related functions [6,7]. There is a fundamental interest in understanding the interaction of amyloidogenic peptides and proteins with NPs as humans are routinely exposed to NPs [8,9]. The effect of protein–NP interactions may control the toxicity of NPs, which is a crucial issue for the applicability in biological systems within emerging research fields, such as nanomedicine and nanodiagnostics [1].

The large surface-to-mass ratio makes NPs attractive for studying surface effects on amyloidogenic peptides and proteins. Surface effects are proposed to play a major role for the assembly of amyloidogenic peptides *in vivo*: in fact, they may explain why these peptides and proteins misfold *in vivo* at concentrations that are insufficient for fibril formation in solution *in vitro*. Surfaces provide an external constraint for the aggregation of amyloidogenic peptides and might, thus, promote the aggregation process. The interaction of AuNPs with proteins is also known to induce co-operative effects, such as self-assembling of NPs. This phenomenon is potentially mediated by protein–protein interaction, which may occur at a high protein concentration on the NP surface. In some cases, protein molecules form bridges between NPs [2]. A better understanding of the microscopic mechanisms occurring at the bionano interface is, thus, crucial for further advances in nanotechnological applications.

In recent years, numerous research groups have investigated the effects of various NPs on amyloidogenic peptides and proteins [10,11]. Depending on the specific chemical composition of the NPs and their size, as well as on the peptidic sequence and the chemicophysical conditions used, disparate effects have been revealed: from a strong acceleration of fibril formation/aggregation to a complete inhibition of fibrillation.

In 2002, Braun realized a pioneering work on protein–NP complexes, carrying out classical atomistic molecular dynamics (MD) simulations of a gold-binding peptide on two different crystal surfaces of gold. There have been scattered attempts to study protein–surface interactions, mostly with atomistic and mesoscopic models [10,12]. More recently, the focus has been again shifted to classical MD simulations, which constitute the single-scale method that presently better compromises among accuracy, size of simulated systems, and explored time scales [3,10,11,13–16].

To contribute to the evolution of this field, we have selected two amyloidogenic systems: (i) amyloid-β peptides with 7 and 42 residues and (ii) the beta2-microglobulin (β_2m) and its amyloidogenic variants. The misfolding of these peptides and proteins may have dramatic consequences in pathology and degenerative processes. Furthermore, evidence exists that NPs, in particular, citrate-coated gold NPs (Cit-AuNPs), could interfere with protein dynamics along the pathway leading to fibrillogenesis. Contrary to expectations, our studies revealed no promotion of β_2m aggregation in the presence of Cit-AuNPs. Instead, Cit-AuNPs were able to inhibit *in vitro* fibrillogenesis of highly fibrillogenic protein models, through a mechanism based on fast exchange interactions that are capable of interfering with the early aggregation steps of the protein, thereby inhibiting fibril nucleation events. Systems with differently coated AuNPs or Au surfaces and different amyloidogenic molecules were described by enhanced MD simulations, to explore the effects of NP charge and size on protein conformation. An overview of the effects of gold substrates on fibril-prone peptide systems is presented in Table 1.

Experimental nuclear magnetic resonance (NMR) determinations have been conducted on the β_2m and its amyloidogenic variants. Contrary to previous reports for β_2m at acidic pH [38], the protein solutions with AuNPs were stable for several months in physiological-like conditions, and no evidence of increased aggregation or partial unfolding was observed. Most significantly, however, the presence of AuNPs did not enhance the onset of fibrils, as previously observed [38]. On the contrary, citrate-stabilized AuNPs were shown to inhibit fibrillogenesis of D76N β_2m [4] by interfering with the early aggregation steps of the protein. This inference, showing a decrease of aggregation of the proteins in presence of AuNPs [4,14], was definitely demonstrated by combined NMR and quartz crystal microbalance with dissipation monitoring measurements [35]. In addition

Table 1

Overview of the effects of AuNPs of different size and surface decorations tested in our group on amyloid peptides and proteins.

Substrate and coating	Substrate charge	Peptide/protein	Adsorbate charge	Effect on fibril
AuNP | bare	Neutral	Aβ16-42	0	Inhibition [15]
AuNP		Aβ1-42	−3	Promotion [16]
AuNP | citrate	Negative	$\beta_2m^{(mon,dim)}$	0	Low inhibition [3]
		ΔN6 $\beta_2m^{(mon,dim)}$	−1	Inhibition [14]
		D76N $\beta_2m^{(mon,dim)}$	+3	Inhibition [4,14]
AuNP		Aβ16-42	0	Weak Promotion, *in fieri*
AuNP | PEG-OH	Neutral	Aβ16-42	0	Inhibition, *in fieri*
AuT | $(PEG)_4COO^-$	Negative	$\beta_2m^{(mon)}$	0	Weak promotion, *in fieri*
AuT | phenyl	Negative	$\beta_2m^{(mon)}$	0	Inhibition [13]

Large NP (AuNP) are usually modeled with flat surfaces in simulations (upper five entries). Thiolated NPs (AuT) are usually modeled with nanoclusters (lower two entries).

to citrate coating, other type of stabilizing decorations were tested [34,37] that provided quite general indications for the specific coating properties that must be designed for stabilizing amyloidogenic proteins in NP colloidal suspensions. Computational modeling yields structural information that is in fair agreement with such experimental evidences.

Characterization of the surface effects of nanomaterials on the aggregation of amyloidogenic peptides and proteins may help assess the potential risks of NPs with respect to neurodegenerative diseases and eventually design smart nanomaterials that do not have negative impact on living organisms or even have positive impact. In this review, we address recent advances in the field, focusing on work from our group.

Methodology

Development of force fields for classical MD simulations

When we started this research lines more than a decade ago, while a plethora of force fields (FFs) existed to simulate the Newtonian dynamics of biological molecules and inorganic materials separately, known FF parameterizations for bioinorganic interfaces were elusive. Thus, we undertook a research line devoted to the development of FF parameters to simulate protein–surface and protein–NP interfaces. The development of the GolP FF [18], which explicitly includes protein–Au(111) interaction parameters, has allowed us to access various levels of computational description of protein–surface interactions, from classical MD to Brownian Dynamics (BD). GolP was originally developed in the OPLS/AA format and later made compatible with CHARMM [21]. It includes image charge effects that account for the surface charge induced in the metal by the local charges in amino acids. Its parameterization is based on density functional theory (DFT) of small molecules representative of amino acid side chains adsorbed on the Au(111) surface. The FF parameters are tuned by fitting molecular mechanic total energies and geometries to those obtained by DFT. The (111) face of gold was chosen because it is the most stable among the variety of Au crystal surfaces and therefore widely occurring in NPs [3]. GolP quantitatively reproduced experimental adsorption energies for small molecules on Au and enabled prediction of the binding affinity scale for amino acids on Au coherent with available experiments.

The first extension of GolP to describe NPs was performed for Cit-AuNPs: the FF parameters for classical MD simulations of proteins on Cit-AuNPs have been tested and released [3,14]. FF parameters for differently functionalized NPs are currently being derived, according to the same strategy used to develop GolP but taking explicitly into account the functional groups with different chemical nature and size. The parameterization of thiol-protected AuNP has been performed in line with GolP [13] but also to be compatible with the AMBER FF [19]. Various other bare surfaces have been parameterized using the GolP paradigm [20,21], but here, we focus on Au(111) and functionalized NPs.

The FF for citrate anions (as well as other surfactants) decorating gold NPs can be developed, starting from structural optimization and electronic structure calculations of the unbound citrates at the *ab initio* (e.g. DFT) level of theory; we have used the Gaussian09 computer package. The missing bond and angle parameters can be generated from a Hessian matrix determined at the *ab initio* level of theory. Charges on individual atoms can be derived by fitting the *ab initio* electrostatic potential grid points following the Merz-Kollman scheme. From these, a set of restrained electrostatic potential atomic charges are derived. The interactions of the AuNP with the surfactants include dispersive forces, which are described with a Lennard-Jones (LJ) potential. To model these interactions, the atom–atom 12-6 LJ parameters can be obtained using mixing rules, which were shown to reproduce the experimental interaction energies for a test set of molecules [18]. In our atomistic model, a positive gold core has been covered with a reasonable citrate surface concentration of 2.8×10^{-10} mol/cm^2, resulting in a surface charge density of -0.3 e/nm^2, consistent with the experimental zeta potential [3,17].

General strategies for simulating and predicting the acceleration/inhibition of the aggregation of amyloidogenic peptides or proteins in the presence of NPs is concisely reviewed in the next two subsections. Such strategies are based on the combination of explicit solvent, enhanced sampling, implicit solvent simulations, and postprocessing of trajectories using entropy calculation.

Computational strategies for peptides on substrates

Computational attempts to define the conformational ensemble of intrinsically disordered peptides in solution have been carried out [22]. Recently, two different computational strategies have been established by our group to investigate peptides interacting with bare or coated Au(111) substrates, both based in the use of advanced sampling techniques and all-atom MD simulation in explicit solvent.

The first strategy is based on the combination of temperature replica exchange (T-REMD) and metadynamics (MetaD) techniques. The use of T-REMD aims at facilitating the sampling of the conformational space of the peptide. In particular, with the use of high temperatures, the crossing of the energy barriers can be accelerated. Thus, statistically significant sampling of

complex systems can be attained. On the other hand, MetaD can be used to force the system to explore specific coordinates [15,23]. A useful reaction coordinate is, for example, the 'vertical' component of the distance between the peptide and the gold surface, which is effective to describe the adsorption and desorption. Other useful reaction coordinates are the torsional angles that embed the inherent conformations of the peptide adsorbates [23]. MetaD coupled with T-REMD can be performed with GROMACS [24] in conjunction with the PLUMED plugin [25]. The systems can be modeled with the GolP FF in explicit solvent, for example, using the SPC water model. Methods have been used concurrently by other groups [26,27].

A second strategy consists of a series of three MD simulations, the first of which is aimed at sampling the conformational ensemble of the peptide in water (without surface or NP), often found to be crucial to obtain a reliable picture of the relevant phases of the peptide adsorption process [16]. The first step is achieved through an initial T-REMD simulation (over a proper temperature range), covering a number of replicas that depend on the target system. The second step is a free MD simulation at 300 K of multiple different systems extracted from the T-REMD in water to disclose the early stage of adsorption of peptides on Au(111). The third step is a Hamiltonian-temperature replica exchange (HT-REMD) simulation over a cumulative period of some microseconds, which should be initialized from the final structures of the adsorption trajectories in step two [16]. By scaling the specific gold-protein potential in the modified Hamiltonian replicas, it is possible to decrease the high-energy barriers due to the interactions between the surface and the peptide, whereas with the use of high temperatures, it is possible to accelerate the crossing of the energy barriers of the whole system. The HT-REMD protocol is available with the PLUMED plugin [25]. It is a robust method to sample heterogeneous systems where the interactions among the various species can be very different in intensity and where the complexity and size of the peptide do not allow a profitable use of specific conformational sampling techniques.

Multistep simulation protocol for proteins on substrates

In the case of larger proteins, an established protocol for the computational description of protein–NP interactions consists of a multiscale computational pipeline that includes an initial extensive sampling of the adsorption orientations of the adsorbate on the substrate and the corresponding driving forces. This first step is performed in an efficient way by BD and generates reasonable configurations of encounter complexes of one protein with the substrate by following the diffusional progression of the protein toward the substrate. The docked configurations are then clustered to extract a small number of representative structures to be used for atomistic T-REMD refinement. Considering a proper temperature range, it is possible to sample the conformational space and energy profile [3,28]. The protocol is based on different software packages implemented in a consistent and compatible way at the different scales, that is, the SDA7.2 software [29] for docking using the ProMetCS continuum solvent model (derived from GolP) for protein–gold association [28] and the GROMACS package [24] for T-REMD simulations using the GolP FF [18].

The same protocol can be broadened to investigate the early stages of aggregation of proteins on surfaces or NPs, allowing for the possibility of dimeric protein aggregates existing in solution. To this aim, we devised and assessed the following chain of steps: (1) BD in solution of two proteins to extract a number of viable dimeric configurations; (2) T-REMD of such selected dimers for structural refinement of the dimers; (3) BD of each dimer with the surface or NP in the presence of the solvent; and (4) T-REMD to refine a small number of most representative encounter complexes, each constituted of a protein dimer attached to the substrate. The results can then be analyzed against those obtained for monomeric adsorption. This protocol has been applied to the case of the native β_2m and its amyloidogenic variants D76N and ΔN6 [4,14], providing results in agreement with NMR experiments.

Implicit solvent models have been recently developed [30] within methods to compute the configurational entropy due to internal and external degrees of freedom, on the basis of the nearest neighbor method [31,32]. The combination of explicit solvent, enhanced sampling, and implicit solvent simulations with post-processing of trajectories using improved implicit solvent methods and entropy calculations will eventually provide a thermodynamic picture where all contributions are known.

Results

$A\beta_{16-22}$ and $A\beta_{1-42}$ peptides at the interface between gold and water

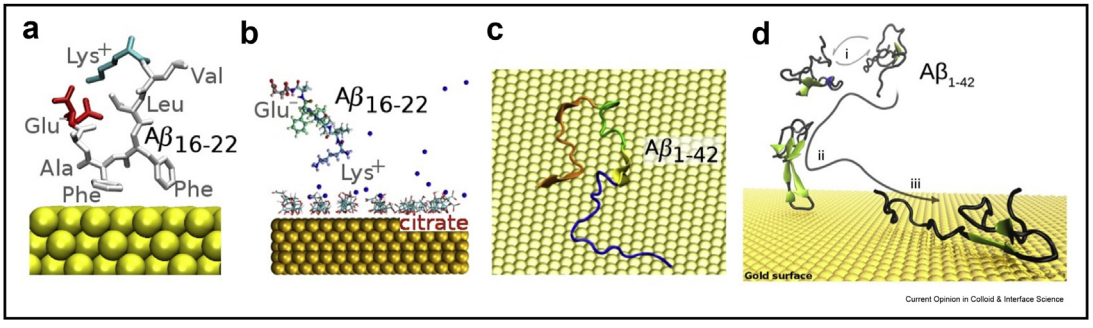
The alanine dipeptide (i.e. Ac-Ala-NHMe) is the simplest peptidic chain. Its adsorption on a gold surface was investigated [23] as a case study to evaluate the first methodological strategy reported previously for peptides on substrates. By computing the free-energy landscape of the adsorption process of the peptide onto a gold surface through MetaD simulations, we found that the substrate modified the structure and dynamical behavior of the alanine dipeptide [23]. This evidence stimulated us to progress toward more complex peptides interacting with gold surface or NP.

Amyloidogenic peptides are the subject of intensive studies because of their involvement in neurodegenerative disorders. For example, Alzheimer's disease [5] is related to the self-assembly of the intrinsically disordered peptide amyloid-β (Aβ). The self-assembly of Aβ into insoluble fibrils is mainly influenced by the physicochemical features of the segment 16–22 (A$\beta_{16\text{-}22}$). As the full length peptides A$\beta_{1\text{-}40}$ (Aβ40) or A$\beta_{1\text{-}42}$ (Aβ42), A$\beta_{16\text{-}22}$ in solution does not attain a stable structure and, under certain conditions (e.g. increasing concentration), can aggregate to form bionano structured fibrils. It has been shown that the presence of NPs in the environment can profitably accelerate or inhibit the fibrillation process [33]. Before such experimental evidence, we studied by computer simulations the interaction between A$\beta_{16\text{-}22}$ and the Au(111) surface: the results of this pioneering work identified atomistic mechanisms behind the amyloidogenesis driven or mediated by the presence of a substrate [15]. By applying a combination of T-REMD and MetaD, we found that the A$\beta_{16\text{-}22}$ conformations were strongly perturbed by the presence of the substrate. We observed [15] that in solution, the peptide assumed fiber-like conformations in agreement with its propensity to fibrillate, but onto the surface, fiber-like conformations were not populated. In other words, the interaction of the peptide with the surface suppressed the fiber-like conformations. In particular, we observed a depletion of the fiber-like conformations *via* a mechanism that was intermediate between induced fitting and conformational selection, which relied on the high affinity of the surface toward the two contiguous Phe$_{18}$ and Phe$_{19}$ residues of A$\beta_{16\text{-}22}$ (Figure 1a). Considering that fiber-like conformations have been correlated with fibrillation propensity, we concluded that the effect of the surface was to inhibit the fibrillation propensity of A$\beta_{16\text{-}22}$. However, one should not exclude a possible opposite effect due to the increased local concentration on the NP, which could act as a catalyst for peptide nucleation.

The results obtained for A$\beta_{16\text{-}22}$ on Au(111) [15] opened the way to rationalize, at the atomic level, the effects of metal substrates on the structure and related function of peptides. Currently, we are investigating the A$\beta_{16\text{-}22}$ peptide on functionalized gold substrates, specifically on the Cit-AuNP, represented by a citrate-coated Au(111) surface, and on the gold surface decorated with hydroxy-terminated polyethylene glycol (PEG), with PEG attached to the gold via a thiolate functional group. Preliminary analysis indicates that A$\beta_{16\text{-}22}$ adsorbs preferentially onto the PEG-functionalized surface, whereas the Cit-AuNP mildly attracts the charged terminals of the peptide, mainly in an elongated conformation (Figure 1b). As a consequence, Cit-Au(111) surface would enhance fibril-like conformations, eventually triggered by the citrates, while PEG-functionalized surfaces would act as inhibitors.

The investigation of the mechanism ruling the interaction of the Aβ42 peptide with the Au(111) surface is more challenging because of the higher complexity of the system [16]. By means of the three-step computational protocol described previously (T-REMD, MD, HT-REMD), the adsorption mechanism of Aβ42 on a gold surface in aqueous solution was disclosed. The protocol included as the first step one of the most extensive T-REMD simulations of the Aβ42 peptide realized so far. The obtained conformations for the Aβ42 peptide adsorbed on the Au(111) surface in the presence of explicit solvent unequivocally prove the ability of the gold surface to induce elongated, fibril-prone states of the peptide [16] at the interface (Figure 1c). Overall, we provided an exhaustive description of the conformational ensemble of Aβ in solution, describing how the peptide approaches the gold surface and the

Figure 1



Summary of the amyloidogenic peptide simulations on gold. **(a)** Atomic representation of a stable conformation of A$\beta_{16\text{-}22}$ on bare Au(111), with a 'U-shape' peptide conformation. **(b)** Atomic representation of a stable conformation of A$\beta_{16\text{-}22}$ on citrate-coated Au(111), with an 'elongated' peptide conformation. **(c)** Atomic representation of a stable conformation of Aβ42 on bare Au(111), with an 'elongated/fibril-like' peptide conformation. (d) The main phases of the adsorption of Aβ42 onto the gold surface in water. **(i)** The peptide in solution is mainly disordered. **(ii)** The early stage of the adsorption mainly occurs in a random way; eventually, portion with high affinity toward gold surface is preferentially bounded. **(iii)** After binding, Aβ42 can form amorphous protein-corona structures or relax toward extended conformations, eventually forming a fibril-template.

early stage of the adsorption and the effect of Au(111)/AuNP on the amyloidogenesis of Aβ42 (Figure 1d). This finding has also reinforced the notion that the behavior of short peptide segments extracted from Aβ42 cannot be safely extrapolated to the full-length chain. In fact, the behavior of A$\beta_{16\text{-}22}$ described in the previous paragraphs is opposite to that of Aβ42, thanks to the preponderant role of the diphenylalanine core of such a short peptide, which is easily captured by the Au surface [15] (Figure 1a). Instead, the role of the diphenylalanine dimer is not so central in the full-length Aβ42 peptide.

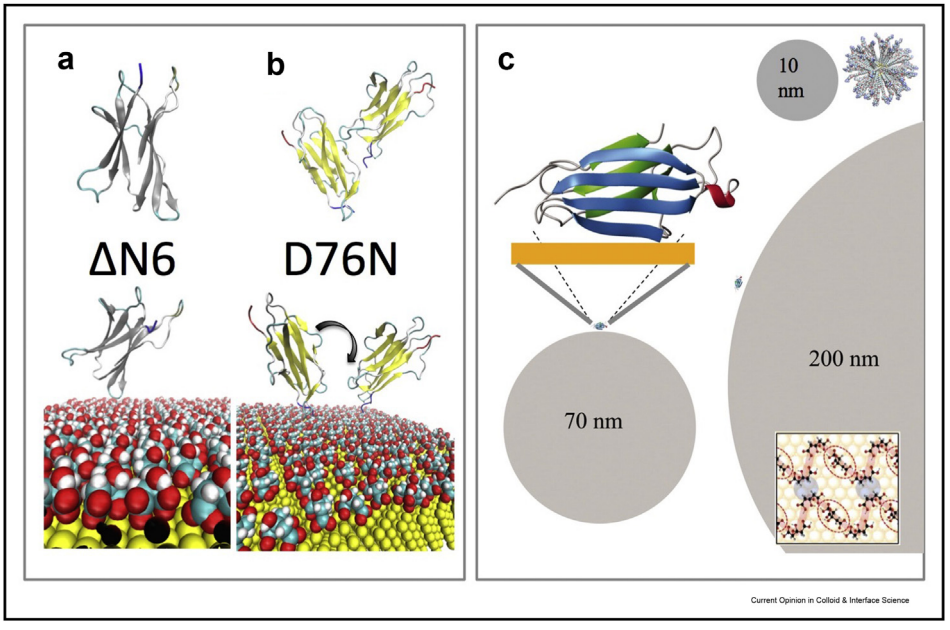
The role of gold substrates on β_2m aggregation

A systematic investigation of the interaction of the amyloidogenic protein β_2m (and its naturally occurring variants ΔN6 and D76N) with Cit-AuNPs [3,4,13,14,34,35] was carried out in our group (Figure 2a). The interaction between β_2m and Cit-AuNPs was modeled at multiple levels, and the results were in excellent agreement with the NMR experiments. The Cit-AuNPs were modeled by either a coated Au(111) surface or a Au_{25} cluster decorated with thiol-attached ligands. In most reviewed work, AuNPs that expose an Au(111) face significantly larger than the protein diameter (3–4 nm) are approximated as a flat crystalline surface: this treatment is motivated by the fact that the space available on the surface of a single large NP is roughly hundreds of times that occupied by a single peptide/protein. Instead, for the interaction of peptides and proteins with NPs smaller than 3 nm, our models include full atomistic details of the NP core and organic ligands. *Via* the combination of experiments and simulations, we could explain in terms of electrostatic interactions the origin of the observed protein signal perturbations mostly localized at the amino-terminal region [3,4,13,14,35].

β_2m and its amyloidogenic variants represent a prototypical amyloidogenic system. Our motivation to study this class of proteins stems from the crucial relevance of protein misfolding in pathology and degenerative processes. Furthermore, there are experimental hints that AuNPs could have a concerted action, with the protein along the pathway leading to fibrillogenesis. β_2m naturally occurs in class I major histocompatibility complexes. Owing to renal failure and consequent high concentration from impaired clearance or because of a mutation, a pathologic fibrillar aggregation of β_2m takes place, leading to amyloid deposition [4].

Colloidal gold NPs are unstable in solution and thus are usually stabilized with carboxylic acids, alcohols, or polymers. The stabilized NP surface is then covered with a biomolecule layer, the corona, on contact with a biological medium. This corona determines the NP surface properties. When a protein approaches this complex object, the competition between the protein

Figure 2



Summary of the protein–NP simulations. **(a)** Representative conformations of the □N6 monomer in solution (top) and adsorbed on a surface representing a Cit-AuNP (bottom): the computational outcome is compatible with NMR intensity reduction. (b) Representative conformations of the D76N dimer in solution (top) and adsorbed on Cit-AuNP; adsorption leads to complete dissociation of the dimeric adducts present in solution. **(c)** Conceptual scheme of the studied systems. NP, nanoparticle; Cit-AuNP, citrate-coated gold NP; NMR, nuclear magnetic resonance.

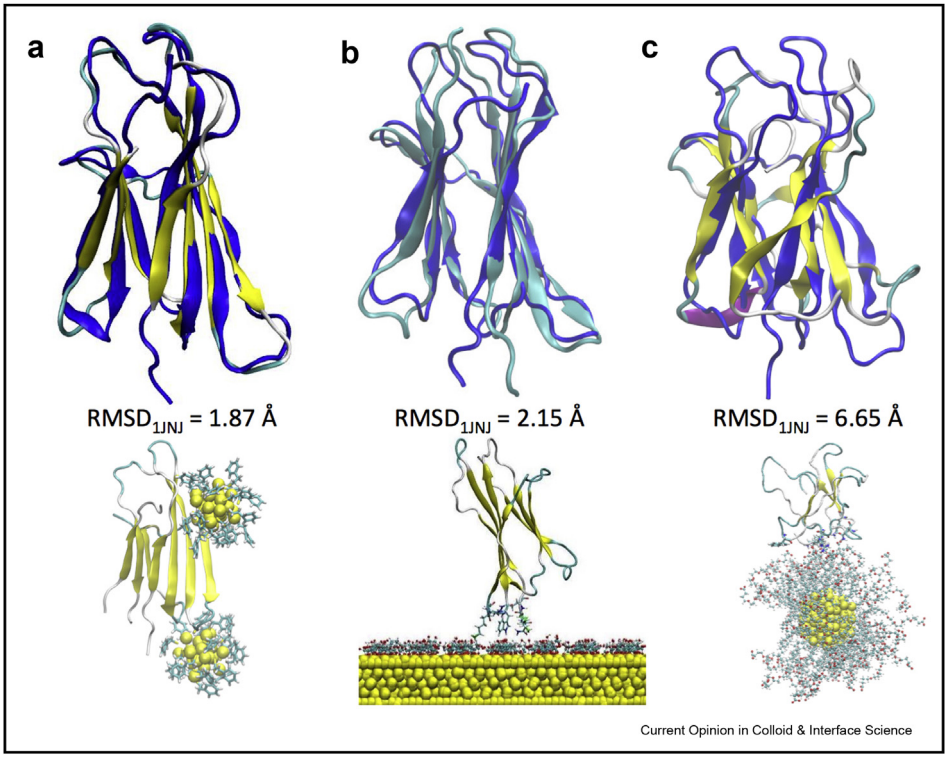
intrinsic propensity for aggregation and the protein–NP attraction governs the behavior of the amyloidogenic protein in solution [10,11].

However, a clear picture detailed at the atomic level of the effects of AuNPs and their coatings on protein interactions is still missing. Experimentally, the atomic-level structure resolution of NP–protein systems is tackled by NMR [36]. Atomistic MD simulations are an effective computational complement to NMR measurements [28].

We investigated three different variants of β_2m in the early stages of aggregation [3,13], at the dimer level. Specifically, we included in our study (i) wild-type β_2m; (ii) the naturally occurring amyloidogenic single mutant D76N β_2m [4] (Figure 2b); and (iii) the amyloidogenic variant ΔN6 β_2m (Figure 2a). ΔN6 β_2m is a truncated form of wild-type β_2m, devoid of the first six residues; it can trigger the fibrillar conversion of wild-type β_2m *in vitro* in physiological conditions [14]. Our computational results allowed us to propose a comparative mechanism for how different protein modifications can affect protein dimerization and fibril formation. Noteworthily, the role and the strength of intermolecular salt bridges at the protein–protein interface, at a physiologically relevant pH, has been correlated with the experimentally established degree of amyloidogenicity of each individual species (D76N >ΔN6 > wild-type).

Computational and experimental studies were conducted by us for the three selected β_2m forms on Cit-AuNPs of variable size at physiological-like pH. We found that the protein solutions with AuNPs at physiological-like pH were stable for several months: no evidence of increasing aggregation or partial unfolding was observed [4]. Most significantly, however, in our study, the presence of AuNPs did not enhance the onset of fibrils, as instead was previously observed. We found that Cit-AuNPs inhibit fibrillogenesis of D76N β_2m [4] by interfering with the early aggregation steps of the protein (Figure 2b). This inference, derived from both MD simulations and NMR measurements that show a decrease of protein aggregation in the presence of AuNPs [4,14], was eventually confirmed by combined NMR and quartz crystal microbalance with dissipation monitoring measurements [35].

Figure 3



Deviation of each β_2m refined structure (excluding the N-terminal and C-terminal tails) from the starting NMR structure (PDB:1JNJ). **(a)** Representative conformation of β_2m associated to two hydrophobic phenyl-terminated NPs, $(Au_{25}[S(CH_2)_2Ph]_{18})^-$ (bottom), and superposition of the adsorbed structure to the NMR structure (top); (b) Representative conformation of β_2m associated to a Cit-AuNP (bottom) and superposition of the adsorbed structure to the NMR structure (top). (c) Representative conformation of β_2m in solution (top) associated to carboxyl terminated NPs, $Au_{144}[S(CH_2)_9(OC_2H_4)_4COO]^{60-}$ (bottom), and superposition of the adsorbed structure to the NMR structure (top). Modest internal rearrangements are observed in panels (a) and (b) on adsorption; a more pronounced change emerges in (c). NMR, nuclear magnetic resonance; NP, nanoparticle; Cit-AuNP, citrate-coated gold NP.

In addition to citrate coating, other types of stabilizing decorations are currently tested in our group [14,34,37]. We are focusing now our computational efforts on promising candidate that are likely to have an effect on amyloidogenic proteins in NP colloidal suspensions (Figure 3); thus, we focus our computational efforts on promising candidates. Preliminary simulations indicate that hydrophilic carboxylic coatings (Figure 3c) establish important and structure-destabilizing interactions with the protein: this is the opposite effect to that found for surfaces with anionic citrates (Figure 3b). We also found that the interaction of the β_2m native monomer with a small AuNP (2-nm diameter) terminated with hydrophobic phenyl groups [13] stabilizes the protein structure (Figure 3a) against unfolding induced by large hydrophobic surfaces (e.g. graphite). These preliminary findings on different functionalizations are in line with recent experimental findings [37].

Overall, our simulations suggest that the balance among hydrophobicity, ligand chain length, and NP size is further modulated by the charge effects. These results stress the complexity of the bionano interface and the need for fine tuning the NP properties to master protein–NP interactions.

Discussion and conclusion

The results obtained for the Aβ42 peptide on Au(111) enable a generalization to the fate of the full amyloidogenic peptide on AuNPs, at least in cases where the NPs expose rather wide surfaces. We have proposed that NP-induced acceleration of the fibrillation process of Aβ42 is closely linked to the ability of the NP facets to bind the amyloidogenic species at the surface/solvent interface. The flat interface geometry favors the formation of critical nuclei, which in turn can facilitate the fibrillation process [12,16,39].

The mechanism beyond the inhibition is less intuitive. A mechanism is based on the assumption that NPs recruit peptides from the solution decreasing the concentration, thus lowering the probability of peptides to clump together [12,39,40]. An alternative mechanism of inhibition, which we hypothesize and for which we have preliminary evidence, relies on the ability of NPs to prevent the propensity of the amyloidogenic species to assume fiber-like conformations [15,39]. In fact, NPs affect the dynamical behavior of the amyloidogenic species through a conformational selection/induced-fit mechanism [4,15]. This property hampers the formation of fiber-like conformations and thus inhibits the development of ordered supramolecular structures. Our simulations [15,16] reveal a shallow selectivity of the gold surface for individual amino acids, which implies that many of the 42 amino acids significantly interact with the surface, which is possible only by realizing elongated, linear-like structures. We propose that higher binding selectivity can be achieved by using small NPs, with a diameter of about 5 nm or less, of the same order of magnitude of the interatomic distances of Aβ42 in water [16]. Such small NPs will only recruit a fraction of highly reactive amino acids in the peptidic chain, selectively binding a small portion of the peptide species and thus preventing the formation of fiber-prone conformations. Thus, the size of AuNPs and their affinity toward specific segments of Aβ42 may hence be used as a control knob for modulating the fibrillation process [15,16]. This operation is likely extendable to other amyloidogenic species, such as β_2m [13]. Remarkably, a recent experimental work [33] revealed that large L-glutathione-coated AuNPs enhance the fibrillation process, whereas small AuNPs with the same coating (diameter of about 6 nm) significantly reduce fibrillation. In the same work, the authors observed that coated AuNPs with diameter of the order of 2 nm can completely inhibit the fibrillation process, further corroborating our prediction.

In summary, experimental findings complemented by computational modeling have demonstrated that the protein–NP interaction can be controlled by tuning the size, the spatial orientation, and the electrical properties of the NP. The binding propensity of various chemical functionalizations of AuNPs depends not only on the exposed surface area but also on the surface charge density that is imposed by the functional groups. These fundamental insights into the interaction between proteins and functionalized NPs offer opportunities for developing novel core/shell receptors with highly specific protein–substrate recognition ability, which would possess promising properties for applications in protein stabilization, unfolding, and delivery.

Conflict of interest statement

Nothing declared.

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