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Nanoscale Interfaces to Biology

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Summary

Nanotechnology has held great promise for revolutionizing biology. The biological behavior of nanomaterials depends primarily on how they interface to biomolecules and their surroundings. Unfortunately, interface issues like non-specific adsorption are still the biggest obstacles to the success of nanobiotechnology and nanomedicine, and have held back widespread practical use of nanotechnology in biology. Not only does the biological interface of nanoparticles need to be understood and controlled, but nanoparticles must be treated as biological entities rather than inorganic ones. Furthermore, one can adopt an engineering perspective of the nanoparticle-biological interface, realizing that it has unique, exploitable properties.

Introduction

The combination of nanotechnology and biology has resulted in a rapidly advancing field. Since its inception about two decades ago, innovative approaches for using nanoparticles (NPs) to kill tumors, enhance drug delivery [1], assemble structures, and sense intracellular processes have been envisioned [2–5]. However, these applications are all limited by non-specific adsorption (NSA), where biomolecules stick to NPs non-covalently. Both the NP and the biomolecules it encounters are complex three-dimensional entities, thus their interface is also complex (Figure 1a). NPs are not hard spheres but crystals with facets and edges, and are coated with ligands that enable solubility and stability. Biomolecules have well-defined structures determined by numerous intramolecular and intermolecular interactions. Thus, when a NP interacts with a biomolecule, numerous non-covalent bonds can form between them [$\bullet \cdot 6$], often resulting in denaturation and loss of activity.

NSA affects not only NPs, but any interface between an inorganic surface and biology. Medical devices and implants have faced the same challenges of surface fouling. NSA can cause false positive/negative signals in sensors, compromising sensitivity. These complications dramatically intensify as the inorganic system size shrinks to the nanoscale, because surface to volume ratios increase dramatically, and nanoscale surfaces differ physically and chemically from bulk.

It is clear that the biological behavior and consequences of NPs are largely dictated by how they interface to biology (Figure 1b). Interface issues strongly influence cellular uptake, where

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varying NP size and shape varies uptake behavior [7,8], biodistribution [9], cytotoxicity [10,11], and the unintended consequences of NPs such as adsorption to other species and aggregation. Clearly, NPs cannot be treated as noninteracting species, but rather as biological entities, where their interaction with the environment is mediated by the proteins that adsorb to them.

Unfortunately, the biological interface is the least understood aspect about NPs. Despite the importance of the interface, efforts to characterize it are surprisingly scarce. Biological outcomes of simple experiments such as cellular uptake and cytotoxicity of NPs are determined empirically, and we are far from being able to predict this behavior. The ligand is the most critical aspect of NPs because it is the surface presented to the biological environment. However, even fundamental studies characterizing its properties, such as surface coverage and binding strength, are few [12–15]. When biomolecules are conjugated to NPs, their behavior can be completely opposite to that of its unconjugated form. Frequently, surface issues are simply ignored, where linked proteins are assumed to be fully folded and active. To date, research on NPs in biology has focused predominantly on exploiting the size and material dependent properties of NPs, but not understanding the interface.

Consequently, challenges remain for not only characterizing the biological interface but also controlling it. Surface treatments are highly variable and difficult to reproduce. There is enormous diversity in ligand types (small molecules, branched species, polymers), and in how they bind (covalent or non-covalent, monolayers or multilayers [16–18]). Also, ligand can come on and off the particle, and free ligand can influence biological behavior [19]. Therefore, the biological interface of NPs is a significant challenge that needs to be addressed for their development and application.

Different classes of NSA

It is helpful to categorize NSA and interface effects. NSA can occur by adsorption of 1) the linked biomolecule or 2) other species present in the environment.

Self-adsorption

DNA in NP-DNA conjugates adsorb to NPs *via* the nucleotides (Figure 2a), as reported by Gearheart and Murphy et al. [20] and Zanchet and Alivisatos et al. [21]. DNA self-adsorption inhibits its ability to bind to a target [22], compromising its use for assembly or sensing. Self-adsorption depends on coverage, where lower coverage increases self-adsorption because of larger exposed NP surface areas. Increasing coverage decreases NSA, but can also reduce hybridization ability due to steric hindrance. This varies with NP size, as higher curvature allows for a higher density of oligonucleotides, while limiting steric hindrance. Self-adsorption also depends on DNA sequence [23], since each nucleotide has a different affinity for gold surfaces [24]. Self-adsorption can be alleviated by spacers or chemical modification with thiols for gold NPs, which block DNA adsorption and its ability to hybridize [25].

Proteins also adsorb onto NPs and denature, damaging protein function (Figure 2b). This is further complicated by the fact that the NP sterically hinders substrates from accessing the active site, so activity loss can be due to both effects. Because proteins are more complex than DNA, probing protein-NP interfaces is challenging. However, there has been progress in determining “design rules” for self-adsorption onto NPs [26–30]. Cytochrome *c* unfolds on gold NPs with charged ligands but not for neutral ligands. However, for CoFe₂O₄ NPs, the dominant interaction is between the COO⁻ on residues and Co or Fe surface atoms. In this case, PEG was not effective as molecules containing COO⁻ for reducing NSA. Experiments changing the labeling site on cytochrome *c* [29] have elucidated that denaturation is partial and

varies with labeling position, as different protein motifs have different stabilities and roles in folding.

Adsorption of other species

Another type of NSA is where other species adsorb to the NP (Figure 2c). This type of NSA is impossible to avoid because biological environments are innately crowded [••6]. Biological fluids such as blood are highly concentrated [31,32], and intracellular protein concentration is >300 mg/mL, significantly higher than the dilute solutions used for NP conjugation and biophysical characterization. Consequently, when NPs are introduced to these environments, proteins adsorb to the NP, shrouding it in a “protein corona,” which can follow the migrating particle [••33]. Unfortunately, adsorption of other species is complex and difficult to prevent. [34] It is also challenging to predict, where NPs coated with ligands thought to be inert, such as PEG, still encounter NSA even in dilute solutions. [30,35] Furthermore, NSA in cells or biological fluids results in NP aggregation or precipitation and deleterious side effects. [36] There have been advances in surface chemistry to render NPs inert to adsorption [32,37,38], such as cloaking particles with polymers [39]. Allen and Bawendi et al. [40] have coated quantum dots with polymeric imidazole ligands which resist adsorption, and thus have yielded unprecedented images of tumor vasculature. However, this level of control over surface chemistry is relatively new, and is still an exception rather than the standard.

Both types of NSA are prevalent in nanobiotechnology and nanomedicine, and can completely obscure the biological purpose of the NP and cause undesirable side effects. To further complicate things, both types of NSA vary with surface chemistry, NP material, size [27,41, 42] and shape, and surface chemistry can be highly variable not only between labs but also day to day, making it challenging to ascertain its mechanism. Thus, NSA is typically viewed as an impediment.

Characterizing NSA

If NPs are going to be employed for practical biological applications, it is imperative to characterize and understand their biological interface, so that ultimately it can be controllable and predictable. NSA is challenging to characterize because it is due to formation of non-covalent bonds between the biomolecule and NP surface or ligand. Because these interactions are numerous, non-covalent, and dynamic, they are difficult to directly probe. However, there has been substantial progress in measuring their effect on the biomolecular structure and activity, yielding information on how adsorption occurs and the interactions involved [•43].

R_H measurements can infer the effects of NSA on biomolecular structure [21,25,44]. If covalently linked DNA adsorbs to the surface, or if other species adsorb to the NP, R_H will change (Figure 3a) [32]. Methods to determine R_H such as Ferguson analysis, dynamic light scattering, size exclusion chromatography, surface plasmon resonance (SPR), and ultracentrifugation [35] have been successful in quantifying NSA of proteins and DNA to NPs. Centrifugation assays have also been effective in identifying the adsorbed proteins, a critical issue [••33].

Effect of NSA on protein structure can be measured directly for proteins with well-defined structures, elucidating how much the protein is denatured (Figure 3b). Circular Dichroism (CD) spectroscopy [45], NMR, and FRET can [28,30] quantify the degree of denaturation when proteins are interfaced to NPs. These approaches typically measure averages in an ensemble, so single molecule experiments have worked well to complement them [46].

While measurements of protein structure yield information on the interface, they must be coupled with activity measurements (Figure 3c), because if function is compromised, then

conjugation is pointless. For DNA, this is simply its ability to bind to complement. For proteins, this may be ligand binding, which can be quantified by spectroscopy [22,47], isothermal titration calorimetry (ITC), and SPR [48]. For enzymatic proteins, activity assays are necessary [49,50], and changes in activity could be due to either NP-induced denaturation or sterics.

Unfortunately, the aforementioned experiments cannot yield molecular information about the NP-biomolecule interface. For example, CD yields only secondary structure. Therefore, molecular dynamics (MD) simulations can naturally complement these techniques, since it can elucidate interactions between the biomolecule, NP, and ligand on a molecular level [51]. Recent experiments combining MD with CD and electrophoresis [29] have been able to elucidate rules for how protein structure is affected by NP labeling.

Exploiting interface effects

Due to the major challenges in characterizing and predicting its behavior, NSA is typically viewed as an impediment for nanobiotechnology [39,52]. However, by shifting to an engineering perspective, one can regard NSA as having unique, exploitable properties. The fact that NP surface chemistry can strongly influence biological response [9] can potentially be a means for manipulating biology in ways not previously possible. By realizing that these interface problems are actually an opportunity, one can potentially engineer the NP-biomolecular interface to achieve new capabilities [53]. Listed below are examples of some new approaches that biologically exploit NP interface effects.

Tunable release from NPs

The fact that the non-covalent interactions between NPs and adsorbed molecules change with environment can be used to release a payload from the NPs. Han and Rotello et al. exploited the fact that intracellular concentrations of glutathione are high, and can release NPs bound to DNA [54] to make it available for transcription [55] (Figure 4a). Thus, the NP acts as a smart delivery vehicle.

Enhancing biological reactions with NP chaperones

Another way that interface effects can be exploited is to use NPs as chaperones for enhancing biological reactions. NPs are approximately the same size as proteins, so adsorbed species are brought within nanometer proximity (Figure 4b). Furthermore, if the reaction involves specific nucleic acids (such as mRNA) the NP can be decorated with DNA that binds specifically to it. Along these lines, NP adsorption has been used to double *in vitro* protein synthesis selectively by Park and Hamad-Schifferli [56]. The very same aspects that make NSA problematic is actually useful for enhancement – since involves weak binding, it permits species to come on and off for turnover, which would not be possible if binding was strong.

Perturbing protein structure via protein corona

When NPs are introduced to a biological fluid, proteins adsorb to their surface resulting in a dynamic “protein corona” [48]. It is clear that identifying the proteins in the corona and understanding how adsorption occurs and evolves is critical for useful application of NPs. Still, one can imagine ways in which the corona could potentially be used to induce a desired biological function [53](Figure 4c). Proteins in the corona may be denatured due to interaction with the NP surface, and if this can be controlled, can be used to induce a response. Because the amount of denaturation in the protein can be tuned by changing the surface properties of the NP [28,57], corona properties may be tuned by modifying NP surface. Evidently, this will require “design rules” for how the corona behaves.

Conclusions

The interface of NPs to biomolecules and biological systems presents a formidable challenge for practical application. While it has been challenging understanding NSA, there have been promising advances in its qualitative characterization. Furthermore, there has been a shift in perspective about how to exploit the unique properties of interface effects.

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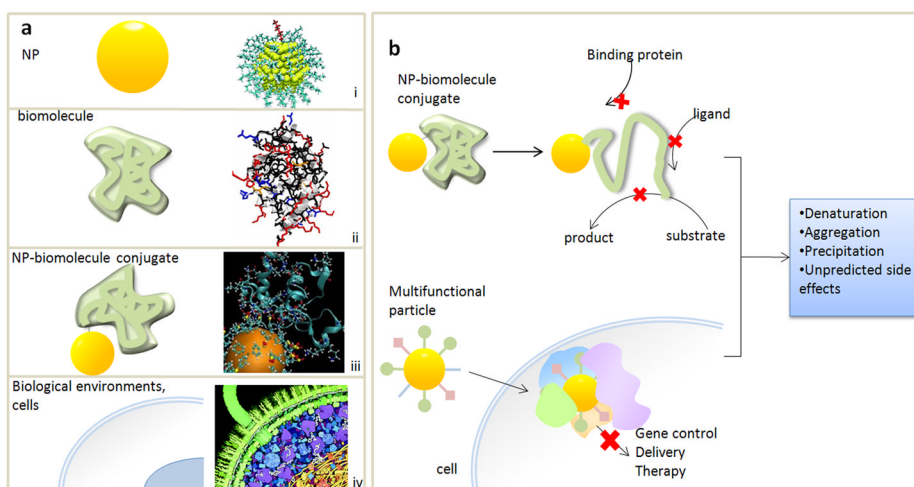


Figure 1. NPs and biomolecular interfaces. a) NPs, biomolecules, NP-biomolecule conjugates, and biological environments are much more complex (right) than typically depicted (left). (i) from [13]; (ii) PDB ID 1YCC from [58], (iv) Illustration by David S. Goodsell, the Scripps Research Institute. b) Interface effects can diminish the biological function of NP-biomolecule conjugates (upper) and NPs used for therapy in cells and biological fluid, leading to undesirable and unpredictable side effects.

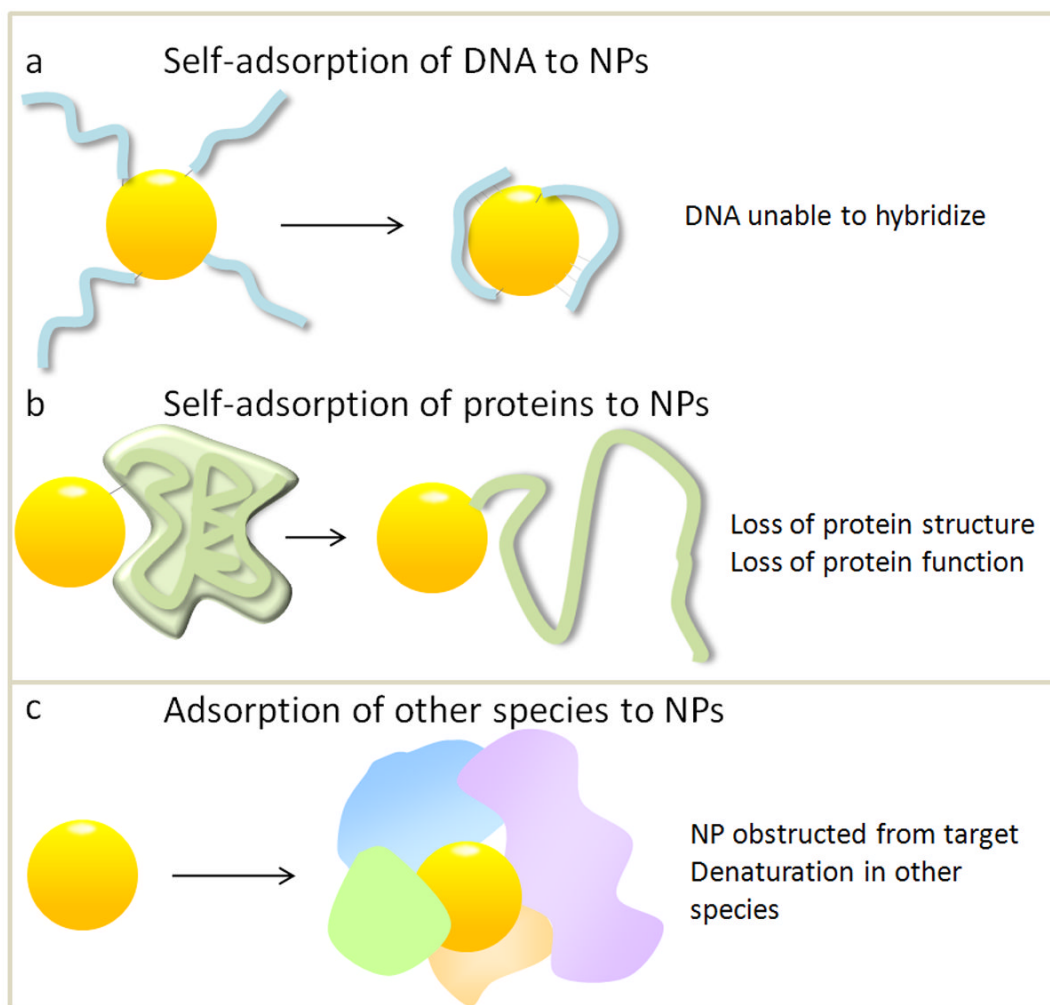


Figure 2. Different classes of NSA. Self adsorption of a covalently linked a) DNA molecule or b) protein; c) adsorption of other species to the NP.

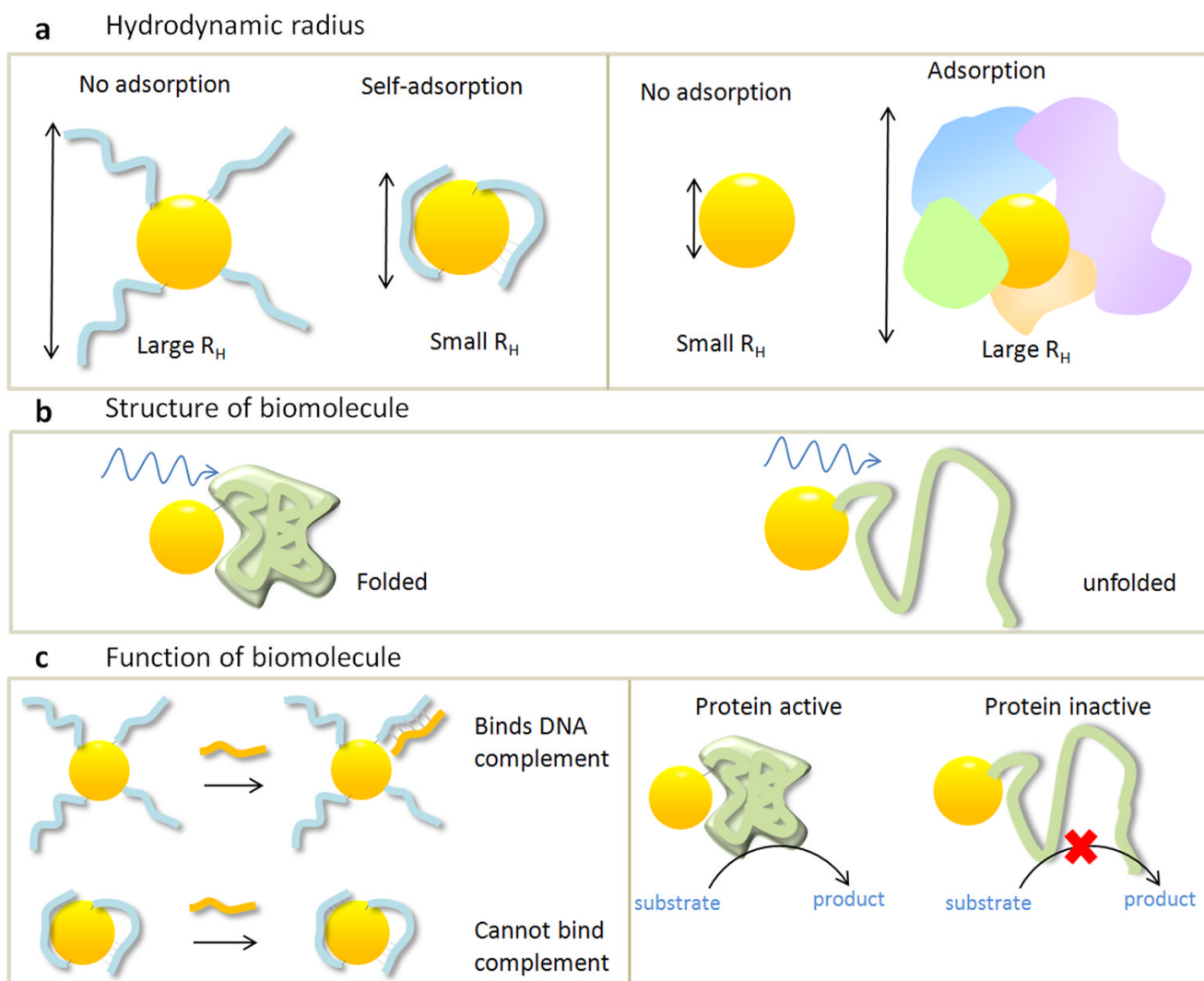


Figure 3. Characterizing NSA. a) R_H is sensitive to self-adsorption and adsorption of other species. b) Measuring the effect of NSA on secondary structure of the linked biomolecule. c) Measuring the effect of NSA on biomolecular function of the linked biomolecule.

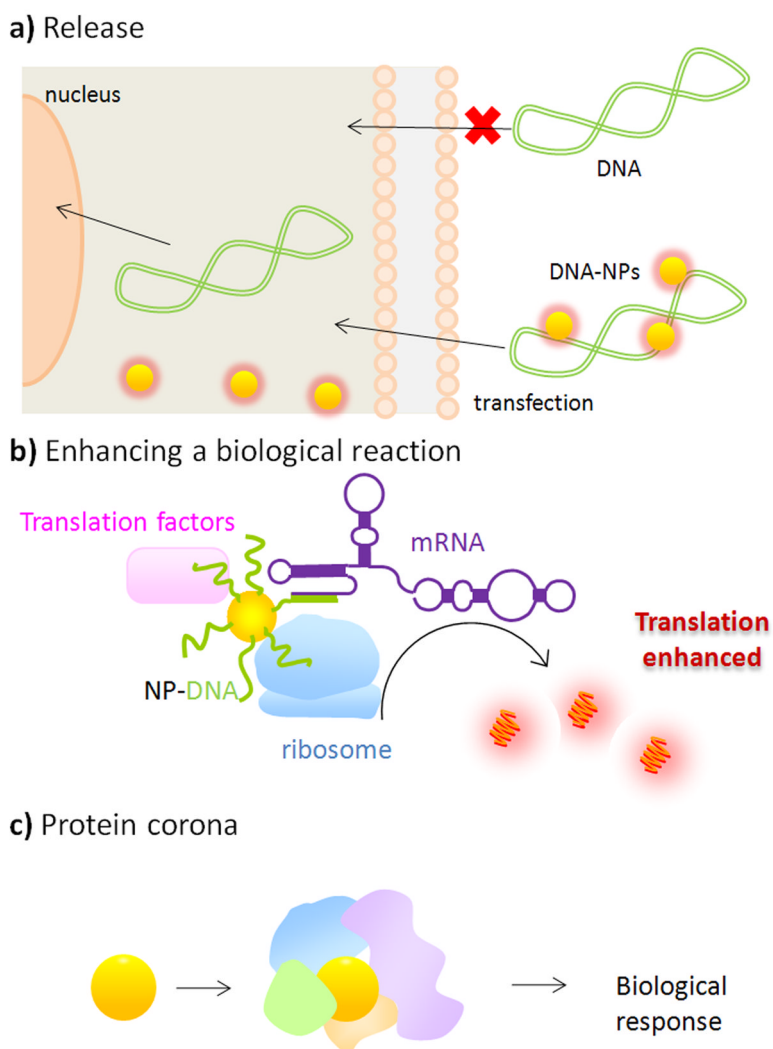


Figure 4. Utilizing NSA. a) Tunable intracellular release from NP-DNA “nanoplexes.” Adapted from [55]. b) Enhancing protein translation. From [56]. c) Protein coronas induce a biological response.