Progress in ligand design for monolayer-protected nanoparticles for nano-bio interfaces

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Ligand functionalized inorganic nanoparticles, also known as monolayer protected nanoparticles, offer great potential as vehicles for *in vivo* delivery of drugs, genes, and other therapeutics. These nanoparticles offer highly customizable chemistries independent of the size, shape, and functionality imparted by the inorganic core. Their success as drug delivery agents depends on their interaction with three major classes of biomolecules: nucleic acids, proteins and membranes. Here, we discuss recent advances and open questions in the field of nanoparticle ligand design for nanomedicine, with a focus on atomic-scale interactions with biomolecules. While the importance of charge and hydrophobicity of ligands for biocompatibility and cell internalization has been demonstrated, ligand length, flexibility, branchedness, and other properties also influence the properties of nanoparticles. However, comprehensive understanding of ligand design principles lies in the cost associated with synthesizing and characterizing diverse ligand chemistries and ability to carefully assess the structural integrity of biomolecules upon interactions with NPs.

I. INTRODUCTION

Nanomedicine and nanotherapeutics have emerged in the last two decades as an important avenue in the treatment of cancers, infectious diseases, orthopedic problems, and a wide range of other conditions[1-6]; a recent report predicts the global market to reach over $350 billion by the year 2025.[7] Nanotherapeutics for *in vivo* drug delivery has long been an area of intense interest,[8, 9] as the majority of publications and patents in nanomedicine are in the field of drug delivery.[9] The ultimate goal for nanoparticle-mediated drug delivery is to deliver a therapeutic payload efficiently while minimizing adverse effects. In traditional methods of drug delivery, such as direct injection, there are a variety of barriers for the drug to ultimately reach its target. These barriers include uptake by the immune system, capture by the reticulo-endothelial system (RES) organs, protein adsorption, or difficulty penetrating cellular membranes;[10-13] however, various nanoparticle (NP) architectures have shown great promise in overcoming some, if not all, of these issues.

The architecture of many NPs used in drug delivery consists of a nanoscale core with surface functionalization or corona (Fig. 1) that may aid in targeting, bioavailability, protection from uptake, or even response to specific stimuli.[1, 3, 11, 13-15] There has been a significant attempt to develop concepts and rules for rational NP design for *in vivo* drug delivery,[1, 2, 10-19] yet the size and complexity of the design space for NP surface functionalization (ligand length, chemistry, charge, density, etc.) have led to few conclusive findings.[11-13, 17-19] At the center of the issue is the lack of fundamental understanding of the properties and processes at bio-nanoparticle interfaces.[1, 10, 14]

Given the extremely small length scales, difficulty in environmental control, as well as synthetic challenges in NP functionalization, it is often difficult, if not impossible, to directly observe NP-bio interfacial interactions experimentally.[13, 14] Hence, *in silico* studies are proving to be highly useful to describe phenomena critical to NP performance that cannot be easily observed from more traditional methods.[10-15, 18, 20-23] Simulation techniques, such as molecular dynamics (MD) and dissipative particle dynamics (DPD), are able to probe the effect of surface modification or/and environment at the atomic or molecular scale[11-14, 20, 22, 23]. Moreover, the increase in computational power over the past decade has allowed simulations to reach size and time scales previously intractable without sacrificing atomic resolution[23]. The number of possibilities for NP surface functionalization, however, may require further incorporation of modern computational algorithms to efficiently explore the design space and effectively develop design rules for effective drug delivery[18, 21].

While NP core materials may be composed of polymers (both synthetic and bio-polymers such as proteins), dendrimers, metals, ceramics, or a wide range of self-assembled organic materials[3, 5, 13], in this perspective, we focus primarily on inorganic core materials functionalized with short, synthetic ligands that can be used for drug delivery[5, 11-13, 15, 16, 18, 22]. Such nanoparticles can potentially serve as a multifunctional platform for both therapeutic and diagnostic purposes. For reviews on NPs composed of other materials, see Refs. [2, 3, 5, 13, 24]. This perspective is organized as follows: first, the properties of organic ligands and their known effect on *in vivo* biological response are discussed. Next, we focus on recent findings in the modeling of NP interactions with nucleic acids, membranes, and proteins, respectively (Fig. 1). Towards

formulation of a potential guidance for the design, study and application of organic ligand-functionalized nanoparticles in nanomedicine we organized the discussion on the properties of NPs as: (1) charge and pH responsiveness; (2) hydrophobicity and hydrophilicity, (3) ligand geometry which includes side chains, bulky groups, length, and grafting density, (4) core size and shape, and (5) other properties such as mixed monolayers, chirality and flexibility. Finally, the outlook and possible solutions for developing design rules for NP ligands via *in silico* design methodology are discussed.

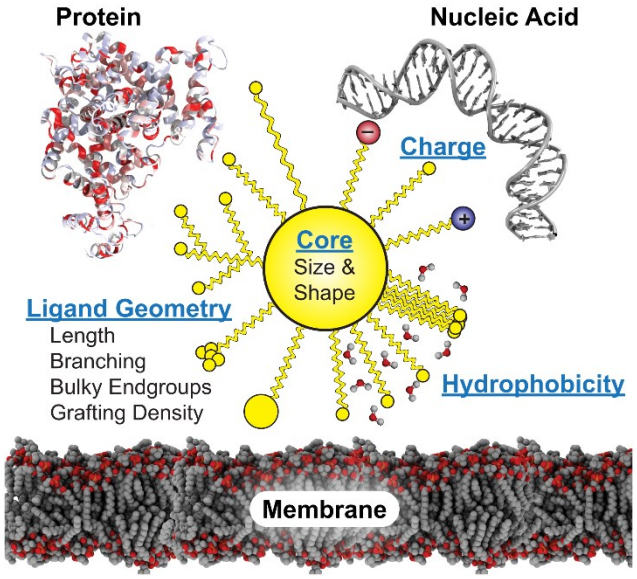

FIG. 1. Schematic representation of a ligand-functionalized metal nanoparticle interacting with DNA, a protein, and a lipid membrane.

II. NP FUNCTIONALIZATION

The surface functionalization of inorganic NPs with organic molecules (ligands) mediates the interactions between NPs and biological system by increasing biodistribution by preventing agglomeration and increasing solubility and improving NP stability by preventing oxidation and leeching of the inorganic core, while partitioning it from biomolecules. Ligands also serve a more active role by binding biomolecules and loading

therapeutic compounds, such as small-molecule drugs and nucleic acids. Moreover, ligand selection can be linked to a specific biological response and possible interaction with biomolecules.

A. Charge and pH responsiveness

Electrostatic interactions between NPs and biomolecules are perhaps the most influential for biological activity. The magnitude, selectivity, and responsiveness of these interactions can be finely controlled by increasing the number of charged groups on each ligand or the proportion of charged ligands in mixed monolayer. Zwitterionic NPs, with both anionic and cationic groups, are generally more biocompatible than purely cationic or anionic NPs[25]. Titratable groups, such as amines and carboxylates, allow NP charge to vary in response to the local pH, which varies with location and disease state. For more consistent charges, groups such as sulfonates and quaternary ammonium compounds are suitable. However, the number and types of charged groups is not the only factor to consider for electrostatic interactions.

Counterion and polyelectrolyte condensation around charged NPs alters their apparent electric potential, or zeta potential, while interactions between like-charged groups alter their pKa[26]. This behavior can be exploited to engineer the zeta potentials of NP-biomolecule complexes. For example, nucleic acids can cross negatively charged membranes when complexed with cationic NPs. Analytical models with idealized geometries can predict general trends in the structure-property relationship of charged NPs and can explain behaviors such as pKa variation and like-charge attraction in polyelectrolytes.

B. Hydrophobicity

The hydrophobicity of NP ligands is known to control the shape of ligand corona and interactions with the aqueous environment. Usually, most NP ligands feature an inner alkyl chain and an outer hydrophilic region. Hydrophilic NPs have longer half-lives and lower rates of immune activation by reducing nonspecific interactions, particularly with proteins. This strategy is exemplified by poly-ethylene glycol (PEG) functionalized nanoparticles, which were a significant advancement in biocompatible NP design by minimizing interaction. Ligand designs with variable hydrophobicities have been shown to be more effective for specific applications[27] since it is known that hydrophobic regions of proteins can serve as epitopes recognition sites.[28] Ligands incorporating amino acids and derivatives provide versatile hydrophobicity with convenient synthesis. However, hydrophobic substituents represent an underexplored area of ligand chemical space.

C. Ligand geometry

Steric and free volume effects arising from ligand length, branching, bulky substituents, and grafting density influence the flexibility of the ligand corona and specific biomolecular interactions. Many biomolecules feature characteristic geometry that controls their function. For example, the DNA major groove binds transcription factors that control gene expression.[29] While these properties are often more difficult to measure dynamically than hydrophobicity and zeta potential, they are a valuable and necessary component of rational NP ligand design. Stiffer ligands may cause greater conformational change in the biomolecules, such as bending or separation, while more flexible ligands bind without causing significant conformational change.

D. Core geometry

Nanoparticle core geometry influences biological interactions as well as optical, electronic, and magnetic properties. Advancements in NP synthesis provide a wide variety of shapes, sizes, and compositions which are important metrics for direct interactions with biomolecules and the distribution of nanoparticles throughout the body. For example it is widely accepted that extravasation from the vasculature is size and shape dependent and that shear stresses in the bloodstream act differently on spherical versus nonspherical particles. The choice of an inorganic core allows overall geometry to be optimized without restricting the choice of surface functionalization.

While the therapeutic or diagnostic efficacy of any NP design is application-specific, its interactions with the range of biomolecules must be considered. Optimizing NP designs requires a more thorough understanding of how ligand properties influence behavior.

III. INTERACTIONS WITH NUCLEIC ACIDS

Efficient wrapping or packaging of DNA is essential for the gene delivery field[30], where nucleic acids are transported across cell membranes with the help of transfection vectors such as cationic nanoparticles[46], dendrimers[47], and lipids[48]. Effective NPs for nucleic acid therapies must protect NAs from chemical, biological, and physical damage, avoid immune activation, localize to the targeted tissue, and cross the negatively charged membrane. Recently, ligand-coated inorganic nanoparticles have been utilized to create nanoparticle gene delivery agents that are responsive to magnetic fields[51] or may be guided using ultrasound[52]. Nucleic acid delivery faces several unique challenges, namely, their high charge, vulnerability to degradation by endonucleases, reactive oxygen species, and acidic conditions, intrinsic immunogenicity, and the need for nuclear

translocation. Since DNA/RNA transfection is dependent on the size, shape, and surface properties of the DNA/RNA-vector complex[32], control over the NA structure is critical for creating effective transfection agents.

A. Charge

NP charge is critical for NA delivery, since NA are negatively charged biomolecules. Similar to histone octamer, charged nanoparticles are able to package DNA and affect nucleic acid conformation and function. It has been shown that transcription by T7 RNA polymerase may be inhibited by the binding of small AuNPs functionalized with tetraalkylammonium ligands to DNA[55]. Cationic NPs, such as silica nanoparticles functionalized with poly-lysine[56, 57] or AuNPs with ammonium cation ligands[35], of size similar to the histone octamer have been thought of as model histones. The proposed mechanism of DNA compaction involves wrapping of DNA around nanoparticles similar to DNA/histone packaging[56, 58]; however, the quality of compaction is difficult to assess. Overall, wrapping duplex DNA around small charged NPs (< 10 nm) requires approximately half of ligands in a mixed monolayer to be positively charged, which corresponds to a charge density of 0.07 $Å^{-2}$.{Nash, 2015 #112} Nanoparticles with lower charge resulted in NP binding to DNA without significant conformation change. Efficient wrapping is necessary for long nucleic acids but is not required for shorter nucleic acids, such as siRNAs. While NP charge stands out as design variable, interestingly, the zeta potential of charged NPs alone is not very strongly related to the packaged size of nucleic acids on NPs. For example, nanoparticles functionalized with first generation lysine dendrons were ~28-fold superior to polylysine[37].

B. Ligand geometry

Efficacy of NA compaction also depends on NP curvature, which is a function of the ligand length and grafting density. Experimental studies have shown that the length of alkyl substituents on quaternary ammonium ligands finely controls the degree of DNA interaction, presumably through interaction with the major groove. High concentrations of weakly charged, largely hydrophobic, ligand-functionalized nanoparticles are capable of causing DNA strand separation[38]. A related challenge in NP design is cytotoxicity, for example, the addition of bulky hydrophobic groups to NP ligands has been shown to increase cytotoxicity[32], and high concentrations of nanoparticles with ligand end groups consisting of quaternary amines[36] and hydrophobic groups may cause changes in the structure of DNA such as unwinding of the helix.

Iron oxide nanoparticle size did not influence delivery efficiency for short siRNA strands{Varshosaz, 2015 #170}. However, for the delivery of plasmid DNA, nanoparticles with sizes of 50 to 100 nm showed optimal DNA delivery, which the authors hypothesized was due to increased bending energy around the smaller nanoparticles for larger DNA strands[51].

C. Solvent and ion buffer

Solvent environment and buffer ion concentrations together with the concentration of nanoparticles can play a significant role in the structural integrity of DNA and affect the efficiency of compaction and gene transfection. All-atom MD simulations were used to investigate the effect of nanoparticle charge, concentration and solution salt concentration on the binding of histone-mimic nanoparticles to double-stranded nucleic acids [40]. Simulations showed that nanoparticles with adequate charge can bind and cause changes in conformation, or bending, to double-stranded DNA. The response of double-stranded

RNA, however, was very different and only occurred at very low salt concentrations and was coupled with damage to the nucleic acid helical structure. Results also indicated that the spatial distribution of charges in the nanoparticle ligand corona can have a critical effect on nucleic acid binding. Single-stranded nucleic acids exhibit greater flexibility in solution and the extent of self-hybridization is dependent on the length.[41] Interactions between NPs and single-stranded nucleic acids are sequence dependent with pyrimidines being more susceptible to NP-induced conformational changes than purines for both ssDNA and ssRNA.[42]

Overall, the charge and hydrophobicity of NPs are critical for interactions with nucleic acids, but ligand and charge mobility along with the shape of the corona are also important. For example, it has been shown that NPs with equal length mixed-monolayers were more efficient at siRNA transfection than NPs with more extended cationic ligands[43] (Fig. 2). The design space is further complicated by the chemistry of biological motif particularly the packaging of double-stranded RNA (dsRNA) with and without nanoparticles remains underdeveloped and is very challenging due to the higher structural rigidity of dsRNA. Challenge remains what properties of nanoparticles and ligands allow for efficient nucleic acid packaging and simultaneously can preserve the structural integrity of DNA/RNA. Detailed characterization of nucleic acid compaction with cationic NPs is now critical for further development of efficient gene carriers and for the synthetic compaction and storage of nucleic acids.

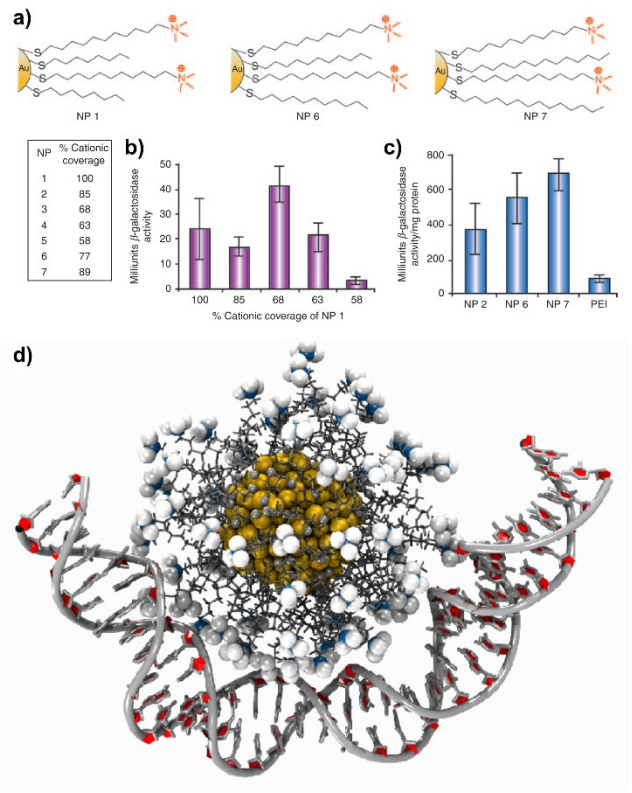

FIG. 2. Mixed-monolayer-protected gold nanoparticles for DNA delivery. (a) Schematic of the different ligand designs. (b) Percentage of charged ligands is correlated with transfection efficiency. (c) Mixed monolayers with equal ligand lengths were more efficient than those of different lengths, and all outperformed PEI. (d) Snapshot of MD simulation of NP 3 (NP 1 with ~70% charged ligands) with 37-mer DNA. (a-c) Adapted with permission from Y. Ding, Z. Jiang, K. Saha, et al., Molecular Therapy 22(6), 1075 (2014). Copyright 2014 American Society of Gene & Cell Therapy.

IV. INTERACTIONS WITH MEMBRANES

The transport of nanoparticles across cell membranes while avoiding endosomal entrapment is a prerequisite for drug delivery applications. Much progress have been achieved in understanding properties of NP that are required for efficient interactions with the membrane.

A. Charge and pH responsiveness

Net NP charge, membrane charge, and the pH of the surrounding environment are three crucial factors that can influence the interactions of NPs with cell membranes. Translocation efficiency broadly increases with net NP charge, while anionic ligands tend to decrease membrane disruption. For instance, the translocation efficiency and cytotoxicity of zwitterionic NPs is a function of pH, the resulting degree of protonation associated with the NP-bound ligands, and cell type.[44] The charge of NPs and membrane lipids influences NP-membrane interactions and translocation potential. When considering neutral or anionic membranes, anionic NPs can bind to these membranes but are unable to translocate, while cationic NPs can translocate only through asymmetric anionic membranes. In the latter case, membrane penetration efficiency was also predicted to be positively correlated with cationic surface charge density.[45] However, simulations have also revealed that zwitterionic NPs translocate only at intermediate degrees of protonation (e.g., 50% and 75% protonation as shown in Fig. 3).[46] Thus, it is evident that NP ligand charge density and the presence of charged, asymmetric membrane lipids favor NP-membrane translocation and that charge density can be regulated via pH, providing an avenue for the targeted uptake of NPs into specific cell types in more acidic, extracellular microenvironments (e.g., tumors). The influence of cell type on NP uptake could be related to differences in lipid composition and lipid symmetry, given the complex lipid bilayer compositions and asymmetries that often exist *in vivo*.[47] Thus, in order to target a specific tissue and cell type for drug and gene delivery, one must also characterize and understand the target environment to design an effective carrier.

B. Hydrophobicity

Hydrophobicity has a profound impact on the ability of a monolayer-protected NP to interact with and translocate through a lipid bilayer. NPs with uncharged, hydrophilic ligands bind to the membrane's surface with disruption, while NPs with hydrophobic ligands become embedded within the membrane. However, a combination of hydrophilic and hydrophobic ligands, in the form of a block co-polymer with a cleavable hydrophobic block, affords NP translocation.[48] This design strategy represents an interesting approach involving the presence of a sacrificial hydrophobic outer layer to encourage NP movement into the membrane and a hydrophilic inner layer to trigger release into the cytosol.

C. Ligand geometry

Geometrical and mechanical characteristics of ligands, such as ligand grafting density, length, flexibility, and the presence of bulky functional groups, introduce additional NP design considerations. Increased ligand grafting density provides a higher membrane penetration efficiency coupled with a longer translocation time.[49] Furthermore, while translocation efficiency is enhanced by longer ligands for fully cationic NPs (protonation degree of 100%), the opposite behavior has been observed for partially cationic, zwitterionic NPs (protonation degree of 50%).[46] It has also been shown, through simulation, that an asymmetric, specifically Janus, arrangement of hydrophilic and hydrophobic ligands can provide a two-fold increase in NP translocation efficiency relative to a symmetric ligand arrangement.[48] Additionally, an investigation of anionic, mixed-monolayer-protected gold NPs revealed that increased ligand flexibility decreases the barrier to membrane deformation and translocation.[50] Lastly, bulky hydrophobic ligand designs have been shown to increase cytotoxicity for cationic[27] and anionic NPs[51], but

the behavior of bulky, hydrophilic ligands is unclear. These examples present a collection of ligand characteristics that are advantageous for NP translocation.

D. NP size and shape

Nanoparticle size and shape are additional characteristics that are important during the formulation of design strategies for NP-mediated delivery applications. It was reported that NPs with a striped arrangement of unbranched ligands significantly improved internalization compared to homogenous or heterogeneous, randomly arranged ligands.[52] It has also been predicted through simulations that both rod-shaped NPs and striped NPs (i.e., possessing a equatorial band of longer NPs) can bind more efficiently to a membrane's surface than spherical NPs; this behavior was attributed to the increased surface area available for NP-membrane binding.[48] It has also been reported that a sufficiently small NP core size (e.g., ≤ 3 nm) affords an optimal penetration efficiency.[49] Thus, it is clear that sufficiently small and asymmetrically shaped NPs present useful design characteristics.

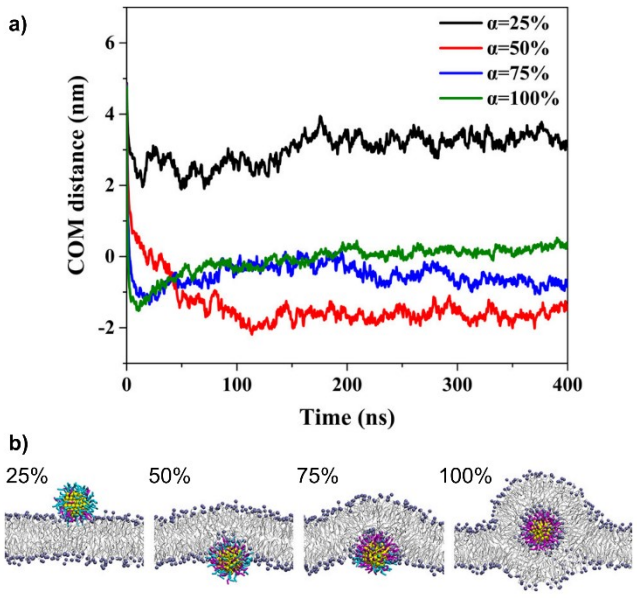

FIG. 3. Coarse-grained molecular dynamics simulation results showing the influence of nanoparticle (NP) degree of protonation (α) on the translocation of an NP through an asymmetric phospholipid bilayer (outer leaflet: 100% zwitterionic DPPC, inner leaflet: 80% DPPC + 20% anionic DPPG). The NP consisted of a 2.5-nm-diameter gold core with pH-responsive zwitterionic ligands (hydrophobic chain + anionic bead + cationic bead); pH responsiveness was afforded via protonation of the anionic bead leading to a net positive charge for a given ligand. (a) Temporal profiles for distance between the center-of-mass (COM) of the NP and the COM of the lipid membrane for four different degrees of protonation of the NP (25, 50, 75, and 100%). (b) Snapshots of the NP and lipid membrane with an increasing degree of protonation. Adapted with permission from X. Quan, D. Zhao, L. Li, and J. Zhou, Langmuir 33, 14480 (2017). Copyright 2017 American Chemical Society.

Overall, the experimental and simulation-based studies collectively suggest that a sufficiently high degree of ligand charge, increased ligand grafting density, increased ligand flexibility, a sufficiently small NP core, asymmetric NP shape, and asymmetric membranes are characteristics that typically favor NP-membrane interaction and improve the efficiency with which NPs are able to translocate through a lipid membrane. However, as mentioned, these input-output associations and correlations may be complicated by the interplay between the input NP design characteristics. Therefore, thorough multi-factor investigations are needed to delineate how combinations of different factors contribute to resulting outcomes (e.g., NP translocation/uptake, membrane disruption, and cytotoxicity).

V. INTERACTIONS WITH PROTEINS

Upon exposure to a biological environment, proteins adhere to a NP surface, forming complex and dynamic layers of proteins termed the protein corona.[53] The formation of the protein corona is time and protein concentration dependent process (Fig. 4) that ultimately replaces the synthetic identity of the nanoparticle with a specific bio-

identity[53, 54] Studies have shown that the uncontrolled formation of the protein corona can influence the uptake of the nanoparticles by the reticuloendothelial system organs and affect the targeting capabilities of the nanoparticle, reducing nanoparticle accumulation at the targeted sites.[55] [56]

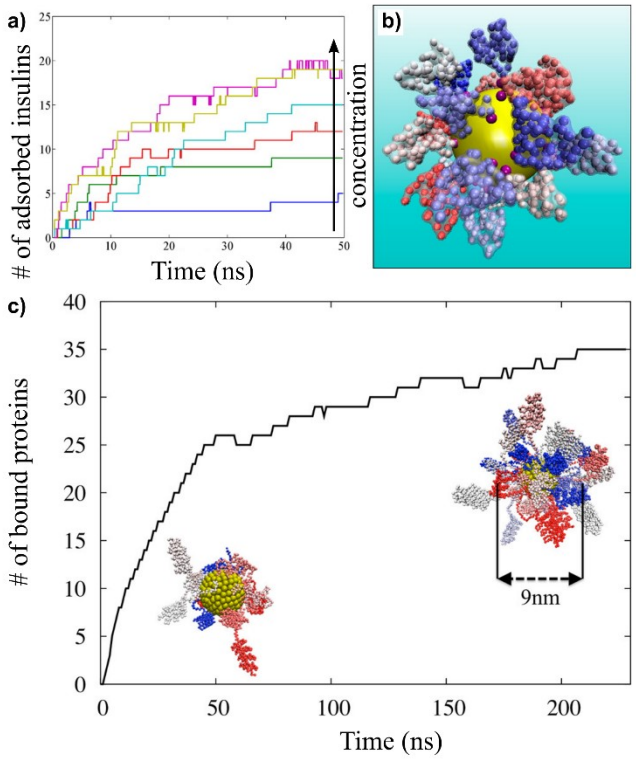

FIG. 4. Coarse-grained MD simulations of protein adsorption onto the surface of an NP. (a) Temporal profiles showing that insulin adsorption increases with higher insulin concentration. (b) An image of insulin adsorption to a citrate-coated gold nanoparticle. (c) The temporal profile of the protein corona on a gold NP. (a-b) Adapted with permission from F. Tavanti, A. Pedone, and M. C. Menziani, J. Phys. Chem. C 119(38), 22172 (2015). Copyright 2015 American Chemical Society. (c) Adapted with permission from S. Deyev, G. Proshkina, A. Ryabova, et al., Bioconjugate Chem. 28(10), 2569 (2017). Copyright 2017 American Chemical Society.

A. Charge

A promising approach to prevent nonspecific protein adsorption involves charged NP ligands.[46, 57, 58] Specifically, zwitterionic ligands have been shown to prevent nonspecific protein adhesion more effectively than common nonionic ligands, such as poly(ethylene glycol) (PEG), due to their ability to create a strong hydration layer via electrostatic interactions.[46, 57] Understanding the protein corona formation and subsequently predicting protein adhesion with zwitterionic ligands has a significant challenge of taking the electrostatic repulsion and attraction into effect and understanding how the protein binding affinity, orientation, and conformation will change as a result. Specifically, understanding how a protein is affected by the spatial distribution of charges created by the zwitterions will be difficult.

B. Hydrophilicity

NP surface functionalization with hydrophilic molecules such as PEG is a widely applied strategy to prevent nonspecific protein adsorption to the nanoparticle surface. However, it has been reported that over 70 different serum proteins heterogeneously adsorb onto PEGylated nanoparticles.[53, 54, 57, 59-66] Molecular dynamics studies have been used to probe interactions between proteins and hydrophilic polymer ligands such as PEG and demonstrated that the affinity of each amino acid to PEG can vary[59]. In a similar study, it was concluded that the solvent-accessible surface area of each amino acid at the protein surface dictates PEG-protein interactions.[60] Ultimately, these studies resulted in a simple model that can predict protein-PEG affinity but relies on the assumption that the protein does not undergo any conformational changes upon binding. The model was developed for short PEG molecules freely solvated in water and did not capture the entropic effects of tethered chains.[59, 60] Thus, further work should be aimed at developing

models that can include factors such as grafting density, PEG length and nanoparticle shape and size, which impact ligand conformational freedom, and thus protein adsorption and protein corona formation.[54, 61, 62, 67]

C. Ligand geometry

Proteins interactions are sensitive to ligand conformation, particularly with regards to functional groups. Decreasing ligand free volumes by increasing grafting density decreased protein adsorption for PEGylated NPs.[54] The different spatial distributions of charged and nonpolar groups in stereoisomers of penicillamine altered the amount and orientation of adsorbed bovine serum albumin in experimental and computational studies. The sensitivity of these interactions to ligand geometry is significant for the design chiral and achiral ligands. Researcher must consider not only the composition of NP ligands but also the conformations that they will adopt in biological environments.[58]

D. NP size and shape

Overall NP geometry also influences protein adsorption independent of ligand chemistry. Recent work by Garcia-Alvarez et al. demonstrated that the protein corona on PEGylated star shaped gold nanoparticles contained different proteins than the protein corona on PEGylated gold nanorods of the same size. In addition, the researchers demonstrated that increasing the size of each nanoparticle shape resulted in a difference of proteins found in the corona.[62] Another study conducted by Walkey et al. demonstrated that NP size affects the nanoparticle-protein interactions on spherical gold nanoparticles.[54] These studies show that morphology and size of the nanoparticle can significantly alter the nanoparticle's function *in vivo*. Future studies should focus on

increasing the understanding of the driving forces responsible for the altered protein corona composition.

Overall, while the general composition of the protein corona can be measured experimentally, many features such as orientation and the binding site of ligands on the protein surface require computational work.[60, 68] Given that ligand functionalization is necessary for biocompatibility and fine control over NP interactions, future studies should focus on exploring diverse ligand designs, not bare NP surfaces.[53] In addition, further understanding is needed on how protein adsorption will change as a function of pH, ionic environment, protein concentrations, and shear stress. Recently, it was shown that dynamic flow causes a shear stress on the nanoparticle that influences protein adsorption to PEG and tannic acid on gold nanoparticles. Currently, it is unknown how shear flow affects all protein-nanoparticle interfaces.[53] Understanding how amino acid composition affects protein corona formation as a function of each of these parameters creates a number of possible combinations that cannot be fully screened using traditional methods. For this reason, computational models are essential for designing nanoparticles with efficient, targeted drug delivery.[69]

VI. POSSIBLE SOLUTIONS

Inorganic NPs have shown great potential as versatile drug and gene delivery platforms. Experimental and computational studies have demonstrated that hydrophobicity and charge are critical factors in controlling the biocompatibility and efficiency of NPs. However, more work is needed to understand the role that NP shape and surface patterning have on their biological properties. Complex ligands, such as highly branched or multivalent, and mixed-monolayers have shown improved efficiency, but the reasons for

this are still unknown. Further, the interactions between even simple NPs and the broader biological milieu are poorly understand, including responses to multivalent ions, pH, temperature, and small molecules.

The use of *in silico* tools provides a way for quick, inexpensive screening of potential ligand designs. Simulations can predict the performance of both the final structure, precursors, and assembly conditions (such as solvent choice), thereby accelerating synthesis efforts. However, the vast design space of functionalized nanoparticles and the continuous increase in available computational power calls for tools that go beyond simple statistical models to uncover complex and non-intuitive design principles. While machine-learning (ML) tools, such as artificial neural networks have long been a focus in computer science, their use in the design of biomimetic materials has been less widespread. The availability of open-source software packages has made these tools accessible to the broader materials science community, but fundamental challenges centered on data generation, organization, and analysis, the shape of the hypothesis space, and interactions with experimental work requires a tailored approach to the use of ML tools in *in silico* materials design. The improvements in search efficiency will be necessary to generate sufficient high-quality data for training ML models. Further improvements can be made by using a multiscale, multiresolution model. We believe that tight integration of ML tools into the simulation workflow will become an essential part of future high-throughput *in silico* materials design.

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1. Petros, R.A. and J.M. DeSimone, *Strategies in the design of nanoparticles for therapeutic applications.* Nature Reviews Drug Discovery, 2010. **9**(8): p. 615-627.
2. Prasad, M., et al., *Nanotherapeutics: An insight into healthcare and multi-dimensional applications in medical sector of the modern world.* Biomedicine & Pharmacotherapy, 2018. **97**: p. 1521-1537.
3. Torchilin, V.P., *Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery.* Nature Reviews Drug Discovery, 2014. **13**(11): p. 813-827.
4. Wagner, V., et al., *The emerging nanomedicine landscape.* Nature biotechnology, 2006. **24**(10): p. 1211-1217.
5. Zazo, H., C.I. Colino, and J.M.J.M. Lanao, *Current applications of nanoparticles in infectious diseases.* Journal of Controlled Release, 2016. **224**: p. 86-102.
6. Zhang, L., et al., *Nanoparticles in Medicine: Therapeutic Applications and Developments.* Clinical Pharmacology & Therapeutics, 2008. **83**(5): p. 761-769.
7. Inc., G.V.R., *Market research report, Nanomedicine Market Analysis by Products, (Therapeutics, Regenerative Medicine, Diagnostics), by Application, (Clinical Oncology, Infectious Diseases), by Nanomolecule (Gold, Silver, Iron Oxide, Alumina), Segment Forecasts*. 2017.
8. Morigi, V., et al., *Nanotechnology in Medicine: From Inception to Market Domination.* Journal of Drug Delivery, 2012. **2012**: p. 1-7.
9. Wagner, V., et al., *Nanomedicine : Drivers for development and possible impacts.* European commission joint research centre, 2006: p. 45-53.
10. Ding, H.-m. and Y.-q. Ma, *Computer simulation of the role of protein corona in cellular delivery of nanoparticles.* Biomaterials, 2014. **35**(30): p. 8703-8710.
11. Li, Y., et al., *Cell and nanoparticle transport in tumour microvasculature: the role of size, shape and surface functionality of nanoparticles.* Interface Focus, 2016. **6**(1): p. 20150086-20150086.
12. Li, Y., M. Kröger, and W.K. Liu, *Shape effect in cellular uptake of PEGylated nanoparticles: comparison between sphere, rod, cube and disk.* Nanoscale, 2015. **7**(40): p. 16631-16646.
13. Shen, Z., M.-P. Nieh, and Y. Li, *Decorating Nanoparticle Surface for Targeted Drug Delivery: Opportunities and Challenges.* Polymers, 2016. **8**(3): p. 83.
14. Decuzzi, P., *Facilitating the Clinical Integration of Nanomedicines: The Roles of Theoretical and Computational Scientists.* ACS Nano, 2016. **10**(9): p. 8133-8138.
15. Zhao, J. and M.H. Stenzel, *Entry of nanoparticles into cells: the importance of nanoparticle properties.* Polymer Chemistry, 2018. **9**(3): p. 259-272.
16. Chaudhary, A., et al., *Effect of surface chemistry and morphology of gold nanoparticle on the structure and activity of common blood proteins.* New Journal of Chemistry, 2016. **40**(6): p. 4879-4883.
17. Chauhan, V.P. and R.K. Jain, *Strategies for advancing cancer nanomedicine.* Nature Materials, 2013. **12**(11): p. 958-962.
18. Kinnear, C., et al., *Form Follows Function: Nanoparticle Shape and Its Implications for Nanomedicine.* Chemical Reviews, 2017. **117**(17): p. 11476-11521.

19. Stylianopoulos, T. and R.K. Jain, *Design considerations for nanotherapeutics in oncology.* Nanomedicine: Nanotechnology, Biology and Medicine, 2015. **11**(8): p. 1893-1907.
20. Chu, X., F. Aydin, and M. Dutt, *Modeling Interactions between Multicomponent Vesicles and Antimicrobial Peptide-Inspired Nanoparticles.* ACS Nano, 2016. **10**(8): p. 7351-7361.
21. Li, Y., et al., *Multiscale modeling and uncertainty quantification in nanoparticle-mediated drug/gene delivery.* Computational Mechanics, 2014. **53**(3): p. 511-537.
22. Li, Y., M. Kröger, and W.K. Liu, *Endocytosis of PEGylated nanoparticles accompanied by structural and free energy changes of the grafted polyethylene glycol.* Biomaterials, 2014. **35**(30): p. 8467-8478.
23. Nash, J.A., et al., *Advances in Molecular Modeling of Nanoparticle–Nucleic Acid Interfaces.* Bioconjugate Chemistry, 2017. **28**(1): p. 3-10.
24. Bao, G., et al., *USNCTAM perspectives on mechanics in medicine.* Journal of The Royal Society Interface, 2014. **11**(97): p. 20140301.
25. García, K.P., et al., *Zwitterionic-Coated "Stealth" Nanoparticles for Biomedical Applications: Recent Advances in Countering Biomolecular Corona Formation and Uptake by the Mononuclear Phagocyte System.* Small, 2014. **10**(13): p. 2516-2529.
26. Wang, D., et al., *How and Why Nanoparticle's Curvature Regulates the Apparent pK a of the Coating Ligands.* J. Am. Chem. Soc, 2011. **133**: p. 2192-2197.
27. Chompoosor, A., et al., *The Role of Surface Functionality on Acute Cytotoxicity, ROS Generation and DNA Damage by Cationic Gold Nanoparticles.* Small, 2010. **6**(20): p. 2246-2249.
28. Van Oss, C.J., *Hydrophobic, hydrophilic and other interactions in epitope-paratope binding.* Molecular Immunology, 1995. **32**(3): p. 199-211.
29. Pabo, C.O. and R.T. Sauer, *Transcription factors: structural families and principles of DNA recognition.* (0066-4154 (Print)).
30. Yin, H., et al., *Non-viral vectors for gene-based therapy.* Nature Reviews Genetics, 2014. **15**: p. 541-555.
31. Sokolova, V. and M. Epple, *Inorganic Nanoparticles as Carriers of Nucleic Acids into Cells.* Angewandte Chemie International Edition, 2008. **47**(8): p. 1382-1395.
32. Kim, S.T., et al., *The Role of Surface Functionality in Determining Nanoparticle Cytotoxicity.* Accounts of Chemical Research, 2013. **46**(3): p. 681-691.
33. McIntosh, C.M., et al., *Inhibition of DNA Transcription Using Cationic Mixed Monolayer Protected Gold Clusters.* Journal of the American Chemical Society, 2001. **123**(31): p. 7626-7629.
34. Zinchenko, A.A., et al., *Single-Chain Compaction of Long Duplex DNA by Cationic Nanoparticles: Modes of Interaction and Comparison with Chromatin.* The Journal of Physical Chemistry B, 2007. **111**(11): p. 3019-3031.
35. Zinchenko, A.A., K. Yoshikawa, and D. Baigl, *Compaction of Single-Chain DNA by Histone-Inspired Nanoparticles.* Physical Review Letters, 2005. **95**(22): p. 228101.
36. Goodman, C.M., et al., *DNA-binding by Functionalized Gold Nanoparticles: Mechanism and Structural Requirements.* Chemical Biology & Drug Design, 2006. **67**(4): p. 297-304.

37. Ghosh, P.S., et al., *Efficient Gene Delivery Vectors by Tuning the Surface Charge Density of Amino Acid-Functionalized Gold Nanoparticles.* ACS Nano, 2008. **2**(11): p. 2213-2218.
38. Railsback, J.G., et al., *Weakly Charged Cationic Nanoparticles Induce DNA Bending and Strand Separation.* Advanced Materials, 2012. **24**(31): p. 4261-4265.
39. Rahme, K., et al., *PEGylated gold nanoparticles: polymer quantification as a function of PEG lengths and nanoparticle dimensions.* RSC Advances, 2013.
40. Nash, J.A., et al., *Characterization of Nucleic Acid Compaction with Histone-Mimic Nanoparticles through All-Atom Molecular Dynamics.* ACS Nano, 2015. **9**(12): p. 12374-12382.
41. Singh, A., et al., *Effect of Oligonucleotide Length on the Assembly of DNA Materials: Molecular Dynamics Simulations of Layer-by-Layer DNA Films.* Langmuir, 2010. **26**(22): p. 17339-17347.
42. Nash, J.A., et al., *Binding of single stranded nucleic acids to cationic ligand functionalized gold nanoparticles.* Biointerphases, 2016. **11**(4): p. 04B305.
43. Sandhu, K.K., et al., *Gold Nanoparticle-Mediated Transfection of Mammalian Cells.* Bioconjugate Chemistry, 2002. **13**(1): p. 3-6.
44. Mizuhara, T., et al., *Acylsulfonamide-Functionalized Zwitterionic Gold Nanoparticles for Enhanced Cellular Uptake at Tumor pH.* Angewandte Chemie (International ed. in English), 2015. **54**(22): p. 6567-6570.
45. Quan, X., et al., *Molecular Understanding of the Penetration of Functionalized Gold Nanoparticles into Asymmetric Membranes.* Langmuir, 2017. **33**(1): p. 361-371.
46. Quan, X., et al., *Understanding the Cellular Uptake of pH-Responsive Zwitterionic Gold Nanoparticles: A Computer Simulation Study.* Langmuir, 2017. **33**(50): p. 14480-14489.
47. Fadeel, B. and D. Xue, *The ins and outs of phospholipid asymmetry in the plasma membrane: roles in health and disease.* Critical reviews in biochemistry and molecular biology, 2009. **44**(5): p. 264-77.
48. Liu, Y., et al., *The Configuration of Copolymer Ligands on Nanoparticles Affects Adhesion and Uptake.* Langmuir, 2016. **32**(39): p. 10136-10143.
49. Ding, H.-m., W.-d. Tian, and Y.-q. Ma, *Designing Nanoparticle Translocation through Membranes by Computer Simulations.* ACS Nano, 2012. **6**(2): p. 1230-1238.
50. Van Lehn, R.C. and A. Alexander-Katz, *Fusion of Ligand-Coated Nanoparticles with Lipid Bilayers: Effect of Ligand Flexibility.* The Journal of Physical Chemistry A, 2014. **118**(31): p. 5848-5856.
51. Gao, J., et al., *Aromaticity/Bulkiness of Surface Ligands to Promote the Interaction of Anionic Amphiphilic Gold Nanoparticles with Lipid Bilayers.* Langmuir, 2016. **32**(6): p. 1601-1610.
52. Verma, A., et al., *Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles.* Nature Materials, 2008. **7**: p. 588-595.
53. Caracciolo, G., O.C. Farokhzad, and M. Mahmoudi, *Biological Identity of Nanoparticles In Vivo: Clinical Implications of the Protein Corona.* Trends in Biotechnology, 2017. **35**(3): p. 257-264.

54. Walkey, C.D., et al., *Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake.* Journal of the American Chemical Society, 2012. **134**(4): p. 2139-2147.
55. Liu, J., et al., *PEGylation and zwitterionization: Pros and cons in the renal clearance and tumor targeting of near-IR-emitting gold nanoparticles.* Angewandte Chemie - International Edition, 2013. **52**(48): p. 12572-12576.
56. Deyev, S., et al., *Synthesis, Characterization, and Selective Delivery of DARPin–Gold Nanoparticle Conjugates to Cancer Cells.* Bioconjugate Chemistry, 2017. **28**(10): p. 2569-2574.
57. Cheng, G., et al., *Molecular Understanding on the Underwater Oleophobicity of Self-Assembled Monolayers: Zwitterionic versus Nonionic.* Langmuir, 2017. **33**(7): p. 1732-1741.
58. Wang, X., et al., *Probing Adsorption Behaviors of BSA onto Chiral Surfaces of Nanoparticles.* Small, 2018: p. 1703982-1703982.
59. Settanni, G., J. Zhou, and F. Schmid, *Interactions between proteins and poly(ethylene-glycol) investigated using molecular dynamics simulations.* Journal of Physics: Conference Series, 2017. **921**(1): p. 012002.
60. Settanni, G., et al., *Protein corona composition of poly(ethylene glycol)- and poly(phosphoester)-coated nanoparticles correlates strongly with the amino acid composition of the protein surface.* Nanoscale, 2017. **9**(6): p. 2138-2144.
61. Ding, H.M. and Y.Q. Ma, *Design strategy of surface decoration for efficient delivery of nanoparticles by computer simulation.* Scientific Reports, 2016. **6**: p. 26783.
62. García-Álvarez, R., et al., *In vivo formation of protein corona on gold nanoparticles. The effect of their size and shape.* Nanoscale, 2018. **10**(3): p. 1256-1264.
63. Schöttler, S., et al., *Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers.* Nature Nanotechnology, 2016. **11**(4): p. 372-377.
64. Gref, R., et al., *'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): Influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption.* Colloids and Surfaces B: Biointerfaces, 2000. **18**(3-4): p. 301-313.
65. Dobrovolskaia, M.A., et al., *Protein corona composition does not accurately predict hematocompatibility of colloidal gold nanoparticles.* Nanomedicine: Nanotechnology, Biology, and Medicine, 2014. **10**(7): p. 1453-1463.
66. Pozzi, D., et al., *Effect of polyethyleneglycol (PEG) chain length on the bio–nano-interactions between PEGylated lipid nanoparticles and biological fluids: from nanostructure to uptake in cancer cells.* Nanoscale, 2014. **6**(5): p. 2782-2782.
67. Tavanti, F., et al., *Computational Insight into the Interaction of Cytochrome C with Wet and PVP-Coated Ag Surfaces.* Journal of Physical Chemistry B, 2017. **121**(41): p. 9532-9540.
68. Kelly, P.M., et al., *Mapping protein binding sites on the biomolecular corona of nanoparticles.* Nature Nanotechnology, 2015. **10**(5): p. 472-479.

69. Schwaminger, S., et al., *Experimental characterization and simulation of amino acid and peptide interactions with inorganic materials.* Engineering in Life Sciences, 2018. **18**(2): p. 84-100.