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# Simulating the interaction of lipid membranes with polymer and ligand-coated nanoparticles

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#### ABSTRACT

Nanoscience and nanotechnology are undergoing rapid expansion owing to the promise of breakthroughs in key sectors, such as energy and medicine. As for medicine, interaction of nanomaterials with biological membranes is a key issue, both for the development of drug and gene delivery vectors and for understanding the molecular basis of nanoparticle (NP) biological activity. NP-membrane interactions are often studied with the aid of molecular simulations of model membranes, allowing to overcome the limitations in temporal and spatial resolution generally encountered by experimental techniques applied to fluid membranes. In the present review we summarize the current literature on simulations of NP-membrane interactions, focusing on small polymeric and ligand-coated NPs. Open questions emerging from experiments concern the effect of NP size, surface charge, and ligand arrangement on NP partitioning into and permeation across membranes. While simulations are contributing to significant progress in this area, some challenges remain, namely regarding the representation of the complexity of biological environments and the cooperative behavior of NPs (e.g. aggregation), as well as methodological challenges to tackle the intrinsically multi-scale nature of nano-bio interactions.



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#### Introduction

Biological membranes are designed to protect living cells from the environment, while still allowing the entrance of nutrients and the exit of waste materials. They are made up of one or two monomolecular layers of lipids, proteins (embedded or bound to the surface), and carbohydrates (generally covalently linked to lipids and proteins) [1]. The lipid component, spanning a few nanometers in thickness [2], keeps together the assembly. Biological membranes perform essential cellular functions, such as communication between the cell and the outside world, and their properties and functioning depend on membrane composition [3–5]. Lipid composition typically includes hundreds to thousands of different lipid species [5,6], is specific for each organism and for each tissue and organelle within an organism, and is tightly regulated by a number of cellular mechanisms [5,7]. Negatively charged lipids are abundant in bacterial membranes [8], while the plasma membrane of animal cells contains mostly neutral lipids [5]; the difference in electrostatic charge explains the selective binding of positively charged antimicrobial agents to bacterial membranes [9]. Besides electrostatic charge, also structural, elastic, and dynamic properties of lipid membranes depend on lipid composition, and they affect membrane functioning and interaction with proteins [3,10,11]. Not surprisingly, lipid composition is tightly regulated in bacteria and higher organisms [5,12].

The interaction with nanosized exogenous materials effectively alters membrane composition and properties, including structural, dynamic, and elastic properties, and hence it can alter the normal functioning of the membrane. Due to their high surface-to-volume ratio, nanosized particles interact strongly with biological membranes, which makes them particularly interesting for technological applications requiring the delivery of natural or synthetic particles to cells, but also potentially dangerous when such delivery is not desired [13,14]. From a technological standpoint, the greatest challenge is probably to design NPs able to enter cell membranes passively (i.e. without the need for energy-consuming mechanism) and directly, avoiding endocytosis-like mechanisms, and without membrane disruption [15]. From the toxicology standpoint, understanding the molecular mechanisms of cell membrane entrance and/or damage by NPs would help designing safer materials, unable to cross cellular barriers [16,17]. In both cases, understanding the molecular-level interactions between NPs and biological membranes is paramount to make progress. Experiments probing NP-membrane interactions are more and more often complemented by molecular simulations, allowing to overcome the limitations in temporal and spatial resolution generally encountered by experimental techniques applied to fluid membranes [15,18-20]. Due to the complexity of biological membranes, simulations often consider rather simple models of biological membranes - i.e. membranes consisting of only one or a few lipid types, often without any proteins. While this is a strong simplification, it is justified in some cases because the main physical properties of biological membranes stem from lipid self-assembly. The literature in this area has grown so much over the past decade that it is now very difficult to provide a comprehensive review.

In the present manuscript, we focus on simulation studies of the interaction between model lipid membranes and a subgroup of nanoparticles, namely polymer and ligand-coated nanoparticles. Both types of nanoparticles present organic functional groups on their surface, hence they can have similar surface properties - which is important in determining their interaction with lipid membranes and can be simulated using the same methodologies (i.e. force fields, etc.) used for simulations of biological macromolecules. Such 'soft' nanoparticles are very common because relatively easy to synthesize and highly versatile. We further restrict our scope to small nanoparticles, with size comparable to the thickness of a lipid membrane, as they can be simulated using chemical detail, allowing a direct correspondence with nanoparticles used in experiments. Two types of small soft nanoparticles are most common: those with a metallic nucleus and those without. In the following, we will review critically the simulation studies published so far on these two categories of nanoparticles, highlighting the links with experimental studies. Before going through the current literature, we summarize the most important open questions pertaining to NP interaction with lipid membranes.

# Soft nanoparticles: open questions

Experiments probing the interaction of NPs with biological membranes or model membranes raised a number of questions on the effect of NP size, shape, surface charge, and ligand arrangement, as well as the role of rafts (in a biological context) and phase separation (in model membranes).

#### Role of size and shape

Size and shape have a great impact on NP uptake into cells. First of all, size is the main factor determining the mechanism of NP entry. Small NPs (<10 nm) can in principle translocate across membranes passively and directly, avoiding endocytosis-type mechanisms, with important consequences on the efficiency of cargo delivery and on toxicity [21–23]. Second, even for particles entering cell membranes using the same mechanism, NP size and shape affect the kinetics of internalization, as shown, for example, by Chan and co-workers [21,24]. Yet, two questions remain open: first, experimental samples generally possess a certain degree of polydispersity; second, samples may aggregate after preparation, even upon contact with the cell membrane, changing both size and shape. Uncertainties in NP size and shape pose difficulties for understanding the general principles of nanomaterial–membrane interactions, particularly in a physiological milieu, where it is often difficult (if at all possible) to control NP and membrane properties.

#### Role of surface charge

Hydrophobic NPs generally aggregate in aqueous solution and form larger nanoparticles. Aggregation makes NPs difficult to handle in solution, reduces the surface-to-volume ratio, and alters the mechanism of NP entrance into cells, sometimes increasing NP toxicity [25–28]. To avoid aggregation, often NPs are functionalized or coated with charged ligands. The sign of the charge greatly affects the way the NP interacts with lipid membranes, which in turn affects the mechanism of NP biological activity: positively charged NPs generally interact more strongly with membranes (which generally contain at least a small fraction of negatively charged lipids and no positively charged lipids), are internalized more efficiently, and display higher toxicity compared to negatively charged NPs [16,29,30]. Some experimental evidence indicates that different types of positively charged NPs cause the formation of transient pores in lipid membranes [29,31–33]. Attraction of cationic NPs to lipid membranes may be explained by electrostatic forces (when negatively charged lipids are present in the membrane), but the mechanisms of translocation and pore formation remain unclear.

Another way to solubilize NPs, reduce non-specific protein adsorption, and reduce NP aggregation is coating with non-charged ligands, such as zwitterionic ligands [34], polyethylene oxide (PEO or PEG) [35–37], or hydroxyl groups [38]. Zwitterionic ligands also promote internalization by passive transport [23]. In general, electrically neutral ligands appear to reduce NP interaction with membranes and cell internalization, independently of the chemistry of the NP core [39]. Similar considerations hold also for purely organic (polymeric) NPs [19]. While differences in biological activity due to NP charge have been confirmed with different materials, the molecular basis is highly debated.

#### Role of ligand arrangement

NP internalization appears to depend not only on NP size and electrostatic charge, but also on the arrangement of ligands on the NP surface. The first evidence in this direction was published by the group of Stellacci in 2008 [40], showing that 'striped' NPs (i.e. NPs in which ligands with different properties are arranged along stripes on the NP surface) can enter cell membranes directly, avoiding endocytosis, and without damage to the membrane; NPs with the same chemical composition in which ligands are distributed randomly on the surface were endocytosed [40]. The finding is particularly important, but an explanation of the molecular mechanism is still lacking.

#### Role of rafts and lipid phase

It has been reported that lipid phase state affects the interaction with NPs, and also that NPs can affect the phase state of a biological membrane. For example,

the presence of lipid rafts (i.e. functional nanoscale domains usually rich in cholesterol) is necessary for raft-mediated endocytosis; Rotello et al. showed that cholesterol-depleting agents reduce NP uptake independently of NP size and surface charge [23]. Considering experiments on non-biological membranes, cationic dendrimers were shown to induce holes only in fluid phase membranes, not membranes in the gel phase [41]. On the other hand, Granick et al. have shown that charged NPs can affect the phase state of lipid membranes, inducing the formation of gel phases in fluid membranes or fluidizing previously gelled membranes [42]. Mechanisms have been proposed to explain the interplay between NP properties and membrane phase, but evidence is rather indirect.

#### **Coated metal nanoparticles: applications and toxicity**

Metallic nanoparticles possess a number of unique physical properties making them attractive for applications in different areas of technology and medicine, such as imaging of biological tissues [43–45] and photothermal therapies [43,46–48]. While applications are numerous, concerns about toxicity are also important. In terms of biomedical applications, one of the most important goals is to prepare NPs that can efficiently enter cell membranes and access the cytosol (and sometimes the cell nucleus) without membrane disruption. Uncoated, 'naked' metal nanoparticles are often rather toxic and therefore not biocompatible, and tend to have short persistence time in living organisms [49]. The most common strategy to reduce toxicity and improve persistence consists in coating the nanoparticles with organic ligands or polymers [50]. This is currently a subject of great interest, but systematic studies using consistent methodology are generally not available, so the relationship between chemical composition (both for the metal and the organic coating), physical properties (i.e. particle size, shape, etc.), and toxicity remains unclear. In particular, it is unclear what properties determine the interaction of nanoparticles with cell membranes. This lack of understanding is hampering progress in the design and development of more effective nanoparticles for biomedical applications.

#### **Coated gold nanoparticles: computational studies**

The most widely used ligand-coated metallic NPs, both in experimental and simulation studies, consist of a gold (Au) core. While also several other metal NPs are commonly studied experimentally, we deem the current computational literature still immature; therefore we will restrict our report to ligand-coated gold NPs.

Gold is generally considered inert with respect to living organisms, and therefore nontoxic, at least in bulk form [49]. Also, Au NPs are easy to functionalize in different ways (Figure 1), they can be prepared in a range of sizes, their optical properties can easily be tuned, and they can efficiently convert light into heat [49]. Stable coating can be provided via reaction with thiols, forming Au-S covalent



**Figure 1.** Gold nanoparticles with different functional groups. On the left, atomistic structure of the Au-S core of a Au<sub>144</sub>SR<sub>60</sub> nanoparticle (R not shown), as predicted by Hakkinen et al.[35]. On the right, examples of ligands commonly used in experimental and simulation studies. Reproduced with permission from [20].

bonds and yielding monodisperse NPs covered with a single layer of organic ligands (often referred to as monolayer-protected Au NPs) with different surface properties [49].

Simulations of metals with classical molecular mechanics methods generally require the use of specialized force fields, typically including multi-body interactions [51]. Yet, in monolayer-protected NPs, the organic ligands shield the metal core from direct interaction with the environment. In this case, it is common practice to ignore the peculiar nature of the metal force field, and use simple pairwise potentials, common for biological macromolecules [52]. Both atomistic and coarse-grained (CG) force fields have been used in modeling ligand-coated metal NPs, depending on the particular question at hand.

A number of experimental and simulation studies focused on charged Au NPs, in which the organic ligand terminates with positively charged (e.g. amino) or negatively charged (e.g. carboxylate) groups. In this case, electrostatics plays an important role in driving the interaction between charged NPs and membranes. Considering the limitations in current CG models [53], atomistic models should be preferred, in principle, for the study of charged NPs. Akola and Vattulainen used all-atom simulations to investigate the initial stages of Au NP interaction with asymmetric lipid membranes as a function of NP charge [54,55]. They showed that cationic NPs bind spontaneously to both negatively charged and neutral (zwitterionic) lipid membranes, but binding to neutral membranes requires overcoming a 12 kJ/mol free energy barrier. Anionic NPs, instead, only bind to neutral membranes. In all cases, only the initial steps of NP-membrane interactions could be probed, due to limitations of atomistic simulations in terms of time scale. Such limitations can be overcome by the use of CG models, at the cost of lower detail and lower accuracy.

Alexander-Katz et al. used the MARTINI CG model [56,57] to simulate the entrance of cationic gold NPs (alkanethiol-protected Au NP with ammonium

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terminal groups) following membrane poration by a transmembrane potential [31]. A change in the electric field across the membrane could, in principle, be generated by the accumulation of charged NPs on the membrane surface. We notice, though, that the transmembrane potential used to porate the membrane was large (1.5 V), and it is not clear if realistic concentrations of Au NPs could generate a transmembrane potential large enough to porate a lipid membrane. The same authors also used a thermodynamic approach to describe the entry of negatively charged Au NP into membranes [58] (alkanethiol-protected Au NPs, with or without sulfonate terminal groups), in which the free energy cost of NP translocation is represented as the sum of hydrophobic, hydrophilic, electrostatic, bilayer deformation, and ligand entropy contributions:

$$\Delta G_{\rm tot} = \Delta G_{\rm phobic} + \Delta G_{\rm philic} + \Delta G_{\rm elec} + \Delta G_{\rm thick} + T\Delta S_{\rm lig}$$

In the equation above, some of the contributions were calculated based on atomistic models, with the membrane and solvent treated implicitly. The main conclusions obtained with such approach were: (a) the most stable states present a 'snorkeling' conformation of the NP, i.e. the charged termini of the ligands 'snorkel' to stably interact with the lipid head groups while the NP core is embedded at the center of the membrane; (b) the free energy of transfer depends strongly on NP size and on the nature of the ligands, with hydrophobic ligands (alkanethiols without sulfonate groups) favoring membrane embedding [58]. This thermodynamic view has three main limitations: first, it relies on several assumptions regarding interaction and deformation energies, and therefore it remains mostly qualitative; second, it does not clarify the path for NP entrance into a membrane; third, it disregards the possibility of NP aggregation, both in the membrane and in the aqueous milieu (while aggregation can significantly affect the thermodynamics of NP translocation).

Investigating the path for NP insertion into a membrane, Alexander-Katz used all-atom simulations of lipid ribbons (i.e. stripes of lipid bilayers with highly curved edges, as found also in toroidal pores) to show that the critical step in the process is the formation of a contact between hydrophobic ligands and hydrophobic lipid chain – a statistically rare event requiring lipid chain protrusion (i.e. transient exposure of a hydrophobic lipid chain to the head group region) [59]. This finding was confirmed by simulations of spontaneous NP insertion into non-curved, defect-free bilayer membranes, upon generation of a hydrophobic NP-lipid contact by pulling a lipid acyl chain towards the NP [60].

Rossi and co-workers used CG simulations to explore the energetics of generating a snorkeling conformation for a single anionic alkanethiol-protected NP in a membrane [61] (Figure 2). They confirmed that NP translocation into membranes is a three-step process: (1) NP adhesion to the membrane, driven by electrostatic interactions; (2) formation of a hydrophobic contact between NP and lipids; (3) translocation towards the center of the membrane as NP ligands



**Figure 2.** Translocation of a gold NP into a lipid membrane. (a) Adsorption; (b–c) contact between lipid chain and NP; (d) partial embedding; (e) NP ligands bind to opposite leaflet. Reproduced with permission from [61].

flip-flop to the opposite membrane leaflet. We notice that only the first of the three processes is fast, while the second and third involve rare events, difficult to sample even with CG simulations; yet, these can be sampled in simulations only using appropriate biasing techniques. The works of Alexander-Katz [60] and Rossi [61] represent a first step in this direction, but more work is required to fully characterize the energetics of NP translocation.

One of the most intriguing questions on Au NP insertion into membranes regards the effect of ligand arrangement, and particularly the difference between a random distribution and a distribution with stripes. While experiments indicate that striped Au NPs permeate more easily [40], calculations using the thermodynamic model of Alexander-Katz show no significant difference in the free energy of transfer for different ligand arrangements [58]. Angelikopoulos and co-workers used a CG approach to the same problem, calculating the free energy of transfer for negatively charged Au NPs of 6 nm in diameter modeled as rigid spheres [62]. Calculations relied on the MARTINI CG model [56,57], and the presence of two types of ligands was accounted for using different particle types (anionic and hydrophobic) on the spherical surface. NPs with ligands arranged randomly presented hydrophobic patches (although not stripes) on the NP surface, which interact favorably with lipids, slowing down NP permeation. The main limitation of this work lies in the NP model itself, which lacks flexibility in the arrangement of the ligands, and therefore is difficult to compare with experiments on soft, monolayer-protected NP. On the other hand, free energy calculations on NPs with flexible ligands require very large sampling, as indicated by several authors [60,61,63]. To overcome this problem, Gao and co-workers used DPD simulations [64], with a model [65] featuring a level of detail and chemical specificity comparable to the MARTINI model [56,57]. They found that striped NPs present the lowest free energy barrier for crossing the membrane

due to restrictions in their rotational degrees of freedom [66]. Yet, the CG nature of the model raises questions on the possibility to interpret quantitatively the experimental data [40].

#### **Polymeric nanoparticles**

As in the case of ligand-coated NPs, polymeric NPs also present organic moieties on the surface – e.g. hydrocarbon moieties with hydroxyl, carboxyl, amino, or amide functional groups; hence their surface properties can be rather similar to the surface properties of ligand-coated metallic NPs. Surface properties are an important determinant of NP interaction with both biological membranes (lung membranes and cell membranes) and model lipid membranes.

Polymeric nanoparticles are interesting in many areas of medicine as drug delivery vectors thanks to increased efficacy of the drug, lesser side effects, and the possibility of controlled release [67,68]. Several experimental studies have been published on the interaction between polymers and lipid membranes (see Refs. [69,70] for recent reviews), while simulation studies are fewer and more recent, largely due to the limitations of length and time scale in molecular simulations [19]. Here, we review modeling studies on the interaction between lipid membranes and different classes of polymers: dendrimers, linear-charged polymers, polyethylene oxide (PEO) and its derivatives, and industrial polymers.

#### Dendrimers

Dendrimers are star-shaped polymers, with a central core linked to a large number of arms. Each arm contains a branching point, and the number of branching points is often referred to as a 'generation' (G1, G2, G3, etc.). Branching allows a very precise control of the molecular weight [71], and allows fine control of electrostatic properties – which, in turn, makes dendrimers good candidates for biological applications [72]. Simulations of dendrimers have been covered by other recent reviews [73], while here we focus on one more precise aspect: the interaction with membranes.

The most popular dendrimers for biological applications are polyamidoamines (PAMAM) [32,33,74–78]. It is known from experiments that PAMAM dendrimers can penetrate directly lipid membranes [74] and, if charged, they can also damage the membrane via pore formation [32]. The interaction with membranes depends essentially on three factors: electrostatic interactions (between the dendrimer termini and lipid head groups), hydrophobic interaction (between the dendrimer core and the lipid tails [76]), and size of the dendrimer.

Early simulations of PAMAM dendrimers in the presence of lipid bilayers were performed at the atomistic level [71] and showed that charged dendrimers adsorbed onto lipids more favorably than neutral ones. Besides electrostatic interactions, also hydrophobic interactions play an important role in dendrimer binding [77]. Limitations in sampling were significant in atomistic simulations, since membrane permeation and even membrane equilibration after perturbation by inclusions take place on time scales difficult to access until a decade ago. To overcome these limitations, Lee and Larson used a CG approach [79-83] based on the MARTINI force field [56,57]. Their models reproduced structural properties of the dendrimer (e.g. radius of gyration) in an aqueous environment [79] and were used to investigate further the charge-dependence of the dendrimerlipid interaction. The CG simulations showed that decreasing the charge on the dendrimer (by acetylation) progressively reduces the interaction with the membrane, so that neutral G3 and G5 dendrimers do not insert in the bilayer, while charged equivalents do – a finding later confirmed by NMR experiments [76]. Pore formation was observed for large charged dendrimers only, such as G5 and G7, particularly upon clustering of dendrimers on the bilayer surface [80]. The simulation results are consistent with the trends observed experimentally [32]. The same authors also investigated the effect of dendrimer PEGylation (i.e. attaching polyethylene glycol – PEG – to the dendrimer surface) on their interaction with membranes [84]. It is known from experiments that PEGylation is more effective than acetylation at reducing nonspecific binding of dendrimers to membranes, making PAMAM dendrimers more biocompatible. Simulations confirmed that PEGvlation reduces pore formation by screening dendrimer charges, preventing direct interaction with lipid head groups and with other dendrimers, hindering aggregation [84].

#### Linear polyelectrolites

Polyelectrolytes have applications as drug vectors [85], sensors [86,87], transfectants [88], and biocidal agents [89]. Polycations can form stable complexes with nucleic acids (also known as polyplexes) that can be internalized via endocytosis [90]. In most cases, the mechanism of interaction between polyelectrolytes and lipid membranes is not well understood.

Polyethylenimine (PEI) is one of the most studied polycations, and an effective transfectant [90]. Its protonation state is controlled simply by pH, and the protonated form (low pH) is known to be cytotoxic [91]. Choudhury et al. studied the interaction of linear PEI with zwitterionic (POPC) membranes using atomistic MD simulations [92]. In simulations, both the protonated form (extended) and the unprotonated form (compact) interacted stably with the membrane surface, without affecting membrane structural properties. Despite the short time scale, the simulations also indicated that protonated PEI can penetrate the bilayer core, locally reduce membrane thickness, and promote pore formation, in agreement with experiments [91].

Since bacterial membranes are negatively charged, electrostatic interactions play a major role in the bactericidal action of polycations. Hill et al. performed atomistic MD simulations of polyelectrolyte oligomers and a model bacterial membrane consisting of both zwitterionic (DOPE) and negatively charged (DOPG) phospholipids [93]. Simulations showed that individual cationic oligomers can bind the lipid bilayer surface, driven by the electrostatic interactions with the lipid phosphate groups. Occasionally an oligomer inserted more deeply in the membrane, as observed for PEI [92], without destabilizing membrane structure. Simultaneous interaction of two oligomers, instead, led to the formation water pores.

In the case of zwitterionic membranes, it is not entirely clear what drives the interaction between lipid membranes and polyelectrolytes. Kepczynski et al. studied the interaction of POPC membranes with a strong polycation, poly(allyl-N,N-dimethyl-N-hexylammonium chloride) (P3) using simulations and experiments [94]. P3 decamers caused significant perturbation of membrane structure and pore formation. Pore formation was confirmed experimentally, as the presence of P3 caused an increase in calcein release from the POPC liposomes. As already observed for dendrimers, the interaction of polymer hydrophobic side chains with lipid acyl chains appeared to play an important role in polymer partitioning within the membrane and subsequent membrane perturbation.

#### Polyethylene oxide and poloxamers

PEO, also known as PEG, is a chemically inert, water-soluble polymer. Considered largely biocompatible, PEO has found industrial applications in drug delivery: PEO-coated (PEGylated) liposomes (in which PEO is covalently linked to the lipids) are significantly more stable towards leakage than their non-PEGylated counterparts, and persist intact in living organisms for longer times – hence the nickname 'stealth liposomes' [95–97]. Since the interaction of the polymer with the lipids is determined by grafting, we will not review here the large body of literature on the properties of stealth liposomes. We will focus instead on the interaction of lipid membranes with 'free' PEO chains (i.e. not covalently linked to lipids). The amount of simulation studies on PEG and PEG-containing copolymers available in the literature is vast and the present review can only be largely incomplete. We will focus particularly on CG simulations, since they can explore time and length scales relevant for the interaction with membranes.

The group of Klein has been one of the pioneers in modeling PEG and PEGpolyethylene surfactants [98] (often referred to as  $C_iE_j$ , where C indicates a  $CH_2$ –  $CH_2$  unit from ethylene, E indicates the ether group O– $CH_2$ – $CH_2$  from PEO, and the subscripts indicate the number of repeating units). In  $C_iE_j$  surfactants, the length of the hydrophobic and hydrophilic moieties determines the topology of the self-assembled aggregate (see Ref. [99] and references therein). Simulations showed that, in the presence of a lipid bilayer, the hydrophobic moiety of  $C_iE_j$ molecules partitions into the membrane core, while the hydrophilic moiety (i.e. PEG) remains in the interface region [100], as expected simply based on polarity. The simulations did not show significant membrane deformations or destabilization – possibly due to the relatively short length and time scales.

While other models of PEG [99,101] and C<sub>i</sub>E<sub>i</sub> surfactants [99] have been developed, the study by Klein remains the only one in the literature, to the best of our knowledge, describing the interaction of C<sub>i</sub>E<sub>i</sub> with lipid membranes on relatively large scales. Another type of PEG derivative, poloxamers, have received much more attention. Poloxamers are triblock copolymers (type ABA) with PEG as the terminal blocks and polypropylene oxide (PPO) as the central block. They are also known as Pluronics and Synperonics and are commercially available [102]. Poloxamers are amphiphiles, since PEO is more polar than PPO, and they can self-assemble in water to form micelles or other self-assembled structures, depending on the length of the PEO and PPO chains [103]. A major application of poloxamers is in drug delivery (hydrophobic drugs can be embedded in poloxamer micelles) [102], although high doses of the polymer can be toxic. In particular, poloxamers with higher affinity for biological membranes are also more hemolytic and more cytotoxic [104]. Due to the medical interest of the polymer, studies on the relationship among composition, biological activity, and interaction with membranes have been numerous [102].

Atomistic simulations by Nawaz et al. [105], reaching time scales of hundreds of nanoseconds, showed for different types of poloxamers significant alterations of lipid structure and dynamics, particularly for copolymers with shorter PEO blocks. No pore formation could be observed, probably because pore formation is a cooperative process requiring the interaction with multiple polymer chains over time scales longer than those typically accessible with atomistic descriptions.

Roccatano and co-workers studied the interaction of poloxamers with lipid membranes using a CG approach [106], focusing on the relationship between copolymer composition and cell membrane binding. They developed CG models (compatible with the MARTINI force field [56,57]) for three different Pluronics with different hydrophilic–hydrophobic balance and different length. Simulations involved either individual chains or few copies of poloxamer chains – a situation relevant at polymer concentrations below the critical micelle concentration. Shorter chains inserted only partially in the lipid bilayer, while longer chains were able to cross the membrane entirely, reducing membrane thickness and increasing the area per lipid, consistent with experiments [107].

Milano and co-workers took an original approach for the description of poloxamers interacting with membranes, developing a CG model based on a self-consistent field model [108]. In this model, the calculation of pairwise interactions is replaced with the calculation of forces between individual particles and an external field – which allows for a significant speed-up of the calculation. Simulations with this model showed self-assembly of polymer chains to form stable micelles in the absence of the lipid membrane, while, in the presence of a lipid bilayer, individual polymer chains were released from the micelle and entered the bilayer, until the micelle dissolved. Incorporation of hydrophobic drugs into

the polymer micelles stabilized the micelle, while polar drugs (quickly released from the micelles) had substantially no effect on micelle stability [108].

#### **Industrial plastics**

Industrial plastics (e.g. polyethylene, polypropylene, polystyrene (PS), etc.) are produced at a pace of 280 million tons per year and steadily increasing [109]. The vast majority of the production is in bulk form, but nanoparticles are also commercially available, with size down for 20 nm – significantly larger than the thickness of a biological membrane. However, most likely smaller nanoparticles are generated by physical and chemical degradation, taking place after disposal over time scales between tens and hundreds of years [110]. Micrometer-sized particles have been observed for a long time in all sorts of marine animals [111–113], and it is difficult to imagine any reasons why plastic degradation would stop at the micrometer level.

The permeation of nanoplastics through cell membranes and their effect on the properties of cell membranes have been recently studied by experiments [114– 116] and simulations [117,118]. Notman and co-workers studied the interaction of cross-linked PS nanoparticles (between 1.3 and 3.7 nm in radius) with model membranes [117] (Figure 3). They used the MARTINI force field [56,57] and the model of PS developed by Rossi et al. [119], and characterized the permeation kinetics and thermodynamics. Results showed that, for smaller nanoparticles (up to 2.8 nm in radius) the free energy gain in the system upon insertion of the nanoparticle in the membrane grows approximately linearly with the area of the nanoparticle [117]. Monticelli and co-workers also simulated the interaction of PS nanoparticles with membranes using the MARTINI force field [56,57,119], considering NPs with size below 8 nm in diameter, but without assuming crosslinking within the particles [118]. They observed spontaneous entry of PS NPs into lipid membranes, and analyzed the effects of the polymer on the properties of lipid membranes as well as the effect of the membrane on the polymer (Figure 3). In the absence of cross-linking, polymer particles (which are solid in water) become substantially liquid upon transfer into the lipid environment - a remarkable result not completely unexpected, since the membrane interior resembles an alkane environment, and alkanes are reasonably good solvents for PS (cyclohexane is a theta-solvent at 307 K [120]). Dissolved as individual chains, polymers cause major perturbations in all membrane properties: area per lipid, lipid and protein diffusion, and elastic moduli. PS chains also have a striking effect on membrane phase behavior – potentially relevant for biological membranes, where cholesterol-rich domains play an important role in many physiological functions [121,122]. A ternary lipid mixture (with saturated lipids, unsaturated lipids, and cholesterol), displaying a temperature-dependent phase separation between a liquid-ordered (Lo) phase and liquid-disordered (Ld) phase, was used to mimic the phase behavior of biological membranes. PS chains partitioned



**Figure 3.** Polystyrene NPs (PSNP) in membranes. Left panel: snapshots (side view) from simulations of cross-linked PSNPs (in blue) at different distances from the center of a lipid membrane (head groups in yellow and red, acyl chains in gray). Reproduced with permission from [117]. Right panel: snapshots (top view) from simulations of non cross-linked PSNPs (purple) in a lipid membrane (cyan), after 5  $\mu$ s of simulation (top panel) and after 10  $\mu$ s of the same simulation (bottom panel); if not cross-linked, PS chains melt in the membrane at room temperature. Reprinted with permission from [118], Copyright (2014) American Chemical Society.

selectively to Ld phases and significantly stabilized Ld–Lo phase separation [118]. Based on a comparison among simulations with other molecules affecting phase separation, these authors proposed that the mechanism of stabilization depends on the exclusion of cholesterol from the Ld phase [123]. Since the driving forces for domain formation in biological membranes are similar, they hypothesized that similar effects may be operating also in cell membranes – a prediction amenable to experimental verification but not tested to date.

#### Perspectives

Considering the rapid pace of the progress in nanomaterials and nanotechnology, as well as the technological and economic relevance of their applications, it is safe to predict that the study of NP interaction with biological materials will continue growing in the decades to come. The role of simulations in the design of new materials has become more and more prominent during the past 10 years, and we expect it to grow also in the area of biomedicine. In the following, we list the

main challenges and open questions the simulation community will have to face, in our opinion, in order to make more significant contributions to burgeoning field of bionanotechnology.

# Scientific challenges

# Complexity of biological systems

Biological systems generally present a very high degree of complexity – for instance, biological membranes typically contain hundreds or thousands of different lipid and protein species, asymmetrically distributed between the leaflets, and laterally organized in dynamic nanoscale domains [5]. Yet, simulations typically deal with extremely simplified models – for example, biological membranes are often still modeled using one or a few lipid types, with no proteins, no asymmetry, and no lateral heterogeneity. The vast majority of the simulations studies on NP-membrane interactions reviewed here describe single component lipid membranes. Sometimes the simplification is well justified, as one wishes to dissect the different contributions to a certain phenomenon; in other cases, using more complex model systems would add to the relevance of nano-bio interactions. Progress in simulating membrane complexity has recently been reported [124–128], paving the way to more realistic simulations of NP-biomembrane interactions.

#### Complexity of NP behavior in realistic environments

In experimental conditions, NPs can aggregate reversibly or irreversibly depending on environmental factors, sometimes on length and time scales beyond those accessible by standard atomistic or CG simulation techniques. Aggregation can stabilize the NP in a certain environment compared to another one, altering and even reversing the thermodynamic balance of NP distribution (e.g. partitioning within a membrane can become unfavorable for large aggregates, see [129]), and it can change the mechanism of interaction with membranes (e.g. passive transport vs. endocytosis) and the extent of membrane perturbation. These issues have been touched upon by few investigators [130–134], but remain difficult to solve as aggregation–disaggregation equilibria are concurrent with NP-membrane interactions and they are established on long time scales, difficult to access with chemically detailed simulation models. Another factor contributing to the complexity of NP behavior is the occurrence of chemical modifications to the NP, e.g. oxidation and degradation. This is often relevant in experimental conditions, but has not been considered so far.

# Methodological challenges

#### Time and length scales

A number of very relevant scientific questions on NP interactions with membranes require simulations on length and time scales beyond the reach of chemically detailed models. For example, calculations on receptor-mediated NP endocytosis have been possible only through the use of highly theoretical models, based on assumptions regarding the energy cost of membrane deformation, the energy gain upon ligand-receptor binding, etc. [135–141]. The gap between theoretical models and experiments on actual NPs can be filled via the use of multi-scale approaches, in which detailed simulations provide the input for simulations with coarser models. Similar considerations are valid for calculations on the thermodynamics of partitioning of NPs in membranes, which are challenging due to difficulties in sampling rare events. The latter may also benefit from the use of enhanced sampling algorithms [142–144], as recently shown by Rossi and co-workers [145].

#### Accuracy

Accuracy is an unavoidable concern in all molecular simulations, and particularly for simulations of lipid membranes, for which efforts towards the development of accurate force fields have been very fragmented. Detailed structural information is only available on few lipid types, and no consensus exists on the target properties to consider in the parameterization of new force fields. Progress in the quality of lipid force fields would certainly increase the reliability and credibility of studies on NP-membrane interactions.

# A political challenge

Most of the progress in nanomaterials is substantially driven by economic factors, pushing towards the development of new technologies to increase the added value of industrial manufacturing and (possibly) societal benefits. In this context, fundamental science issues receive less attention. With reference to NP-membrane interactions, we notice that most simulation studies attempt to make progress on issues relevant for new technologies, while few attempts to understand the molecular basis of biological activity or the mechanisms of toxicity of NPs. Devoting resources to such fundamental science questions is a political challenge for funding bodies and the entire scientific community.

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