

REVIEW ARTICLE OPEN

Theory, simulations and the design of functionalized nanoparticles for biomedical applications: A Soft Matter Perspective

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Functionalised nanoparticles for biomedical applications represents an incredibly exciting and rapidly growing field of research. Considering the complexity of the nano–bio interface, an important question is to what extent can theory and simulations be used to study these systems in a realistic, meaningful way. In this review, we will argue for a positive answer to this question. Approaching the issue from a “Soft Matter” perspective, we will consider those properties of functionalised nanoparticles that can be captured within a classical description. We will thus not concentrate on optical and electronic properties, but rather on the way nanoparticles’ interactions with the biological environment can be tuned by functionalising their surface and exploited in different contexts relevant to applications. In particular, we wish to provide a critical overview of theoretical and computational coarse-grained models, developed to describe these interactions and present to the readers some of the latest results in this fascinating area of research.

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INTRODUCTION

When thinking about nanoparticles’ striking behaviour, what often comes to mind are their optical, electronic, or catalytic properties. These properties are due to the sensitivity of the electronic structure at this peculiar length-scale, in-between that of molecules and bulk, macro-scale objects. In practice, understanding and rationalising these properties requires making sense of quantum effects. This can be done to various degrees of approximation, ranging from highly accurate quantum chemistry techniques¹ to density functional theory² or simplified tight-binding models.³

Crucially, the nanoscale is also the length-scale of some of the most important biological constructs. Proteins, the workhorse of our body, have dimensions from a few to a few tens of nanometres.⁴ In many cases, these proteins are functional when assembled into larger, but still nanoscale objects, such as enzymes or antibodies. Viruses are another important example of a “biological” object with nanoscale dimension. The first step of their interaction with a host cell is their attachment to the cell membrane, a complex object made of various nanoscale structures, such as the lipid bilayer, ion channels, membrane proteins, and lipid rafts, just to cite a few. We make these examples to suggest, certainly not for the first time,⁵ that understanding interactions between nanoscale objects opens up the door to controlling and designing the way nanotechnology can be interfaced with our body. In other words, it is a crucial step towards the rational design of nanoparticles-based biomedical applications.

An archetypal example of a biomedical application where nanoparticles can be of great impact is that of drug-delivery, a problem where relatively “simple”, or better said, *coarse-grained*

models have lead to important advancements in our understanding. Such models can be used not only to make sense of the vast amount of available experimental results, but also to suggest design principles for the synthesis of new systems. It is the scope of this review to present some of these models in a critical way, with the purpose to give the reader a sense of what they really describe, and hence when their use is appropriate. We also immediately point out that we do not aim here to be comprehensive, and various important works will be missed. We hope we will be excused for this deficiency as long as our main goal, to present a certain type of approach and techniques to a larger readership, will be reached.

An important preliminary question that should arise at this point is the following: When can coarse-grained models and Soft Matter Physics, a classical description, be meaningfully used to study nanoscale systems and their interactions? To answer this question, we first notice that whereas the *exact* interaction between nanoscale objects depends on that between all their atoms, and hence eventually on complex quantum-mechanical details, important features controlled by this interaction are remarkably insensitive to such details. Consider the phase diagrams of different systems: Despite their clear differences, both colloids in polymer solutions⁶ and gold nanoparticles functionalised with DNA strands⁷ display what is essentially a “gas–liquid” phase coexistence region. Whereas the exact boundaries of such phase transition depend on very specific details of the potential, its overall topology can be predicted treating these components as spheres interacting via an attractive square well (see Fig. 1). The reason why this simplification works is that once certain degrees of freedom are traced out,^{8,9} or in other

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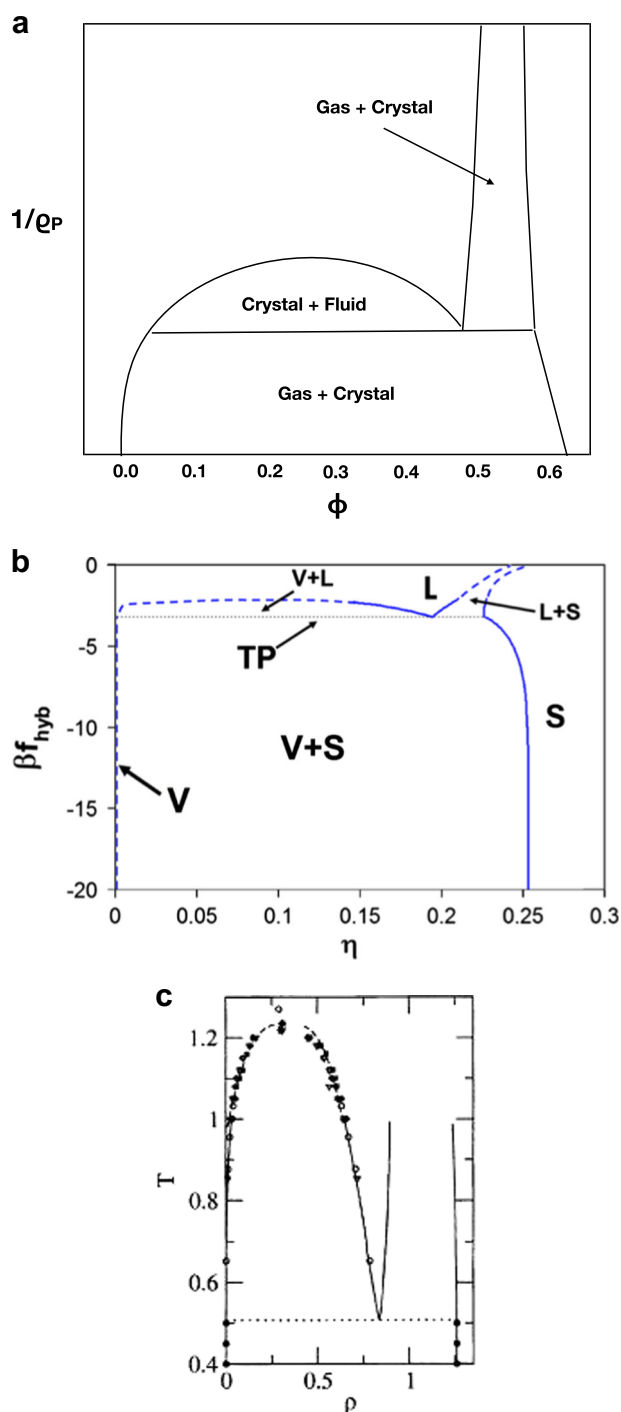


Fig. 1 Phase diagram for (a) colloids in polymer solutions as calculated via the Asakura–Oosawa model¹⁵⁴ (drawn with data from¹⁵⁵) and (b) DNA-coated colloids (taken from ref. 7 with permission from the American Physical Society). Despite being completely different systems, they display a similar topology, as captured by (c) a simple square-well potential (taken from ref. 156 with permission from the American Physical Society). A general lesson from Soft Matter Physics is that apparently unrelated systems can display a very similar behaviour, due to the common shape of the interparticle interaction potential after coarse-graining

words once their average effect is accounted for, apparently rather different systems present an interaction potential of similar shape.

An approximate description of the energetics of a system is often also enough to understand or predict important

experimental observations. In fact, a simpler description can make evident trends that would be otherwise hidden by the complexity of the true interactions. Take for example, the case of nanoparticles undergoing receptor-mediated endocytosis. A qualitative understanding of this process can be gained by a simple model accounting for the bending energy of the cell membrane and the free-energy of interaction with the nanoparticle's surface.¹⁰ Moreover, the parameters in this model can be taken either directly from experiments, or numerically calculated from the microscopic interactions between all components via coarse-graining.^{8,9} In this sense, coarse-grained models can be considered as the last step of a ladder in a multiscale approach.

One final aspect to consider is a practical one. The relevant time-scale and length-scale of many processes related to biomedical applications are simply inaccessible via atomistic simulations, and coarse-grained models represent the only possible approach. Let us take again the paradigmatic case of endocytosis. A typical gold nanoparticle of 20 nm contains hundreds of thousands of atoms. The timescale for its adsorption and endocytosis ranges from seconds to hours depending on details of the system.¹¹ A truly atomistic description is thus a computationally intractable problem. Moreover, one such simulation would provide a single point in the multidimensional parameter space (e.g., ligand length and strength, nanoparticle size, etc.), that we need to sample to obtain information about optimal design.

Going back to our initial question, it is the level of coarse-graining involved in building the model that determines the type of predictions that can be made and trusted: a model for receptor-mediated endocytosis that describes ligands and receptors as structureless particles^{10,12} cannot be expected to give accurate answers for a *specific* system, e.g., the *exact* bond strength required for internalisation. However, they can provide valuable insights in understanding how tuning the single-bond strength can qualitatively affect the overall process.

In practice, the type of highly coarse-grained modelling approach we will consider here focus more on understanding *trends*, typically with respect to a few design parameters of interests to experiments, and less on providing accurate numbers. Eventually, whether or not this is useful at all should be considered in light of the ability of this approach to describe experimental observations and real physical phenomena. Highly coarse-grained models have already proved to properly describe the self-assembly of functionalised nanoparticles in a large variety of systems.^{13–16} As we are about to show, understanding certain problems related to their interaction with a biological environment seems to be also treatable in a similar way.

PREDICTING AND CONTROLLING NANOPARTICLES ENDOCYTOSIS

One of the main biomedical applications of functionalised nanoparticles is that of drug-delivery.¹⁷ To this purpose, nanoparticles must be able to penetrate into cells. An important question is thus how different nanoparticles' designs affect the internalisation pathway, which for nano-sized objects typically occurs via receptor-mediated endocytosis.¹⁸ Parameters that can be tuned during synthesis are typically both geometric properties of the nanoparticles, e.g., size and shape, as well as the type and number of ligands coated on their surface.

Different aspects of receptor-mediated endocytosis have been intensively studied using both theory,^{11,19–25} and different types of simulations^{10,26–37} with coarse-grained models, often combining the two.^{10,27} A pioneering work on this problem is that of Gao et al.,¹⁹ who proposed a theoretical model to address the size dependence of this process, drawing from previous work on the growth of membrane adhesion patches.³⁸ These authors analysed the diffusion of mobile membrane receptors to the nanoparticle,

from which the wrapping time as a function of the particle size was calculated. The main ingredients in the problem are the free energy of adhesion due to ligand–receptor bond formation ΔF_{bond} , the entropy loss due to receptors localisation in the contact area $\Delta F_{\text{entropy}}$ and the bending energy cost to wrap the membrane $\Delta F_{\text{membrane}}$. The latter is calculated using the Canham–Helfrich’s Hamiltonian,^{39,40} which has been shown to correctly reproduce various important and experimentally observed features for both biological, as well as inorganic membranes.^{41–44} In one way or another, the free energy contributions considered by Gao et al. are the same ingredients found in all subsequent works on this topic. This model shows that the time to fully wrap a nanoparticle has a minimum for a specific size, and diverges above and below two limiting values, essentially meaning that such particles will never undergo endocytosis. Using a thermodynamic model based on the same free-energy contributions, but considering the number of nanoparticles that can be internalised by a cell rather than the wrapping time, Hongyan et al.²¹ and Sulin et al.²⁰ reached similar conclusions. Such trends have indeed been experimentally observed for a variety of different cells and nanoparticles types.^{20,45} In practice, the presence of a minimum and maximum radius for endocytosis can be understood considering two limits. The minimum size is given by the need to overcome the elastic energy penalty, independent on particles’ size, with the nanoparticle–membrane adhesion energy due to bond formation, which grows linearly with particles’ area. Instead, for large particles, the balance is between the same adhesion energy and the loss of entropy for receptors due to their accumulation at the nanoparticle–membrane interface, and consequent depletion from the rest of the membrane. This latter contribution depends on both receptors and ligands surface densities.¹⁹ Importantly, these models highlight which energetic contributions are required to understand the optimal uptake and how their relative magnitude can be shifted depending on parameters, such as the density of ligands or the bond strength. This latter fact can rationalise the spread in optimal values for endocytosis observed in experiments with different systems.

Also building on the work of Gao et al.,¹⁹ Decuzzi and Ferrari²⁴ proposed a model where the bond energy was specifically dependent on ligand structural properties, such as length and stiffness. These authors also augmented the free-energy with a non-specific interaction term, to account for the fact that not only ligand–receptor bonds contribute to the cell/nanoparticle interaction, but also non-specific forces, e.g., van der Waals, electrostatic, and water-mediated hydrophilic or hydrophobic forces. Finally, the effect of thermal fluctuations in the membrane was also partially introduced. Two are the main results of this model: the first is to point out that the bond stiffness plays as much of a role as the bond energy, a fact that should be considered in the design of new ligands. The interested reader can find a general statistical mechanical theory on the influence of ligand structure in binding in ref. 46. The second main result is to show that the presence of non-specific interactions can greatly alter the optimal radius for endocytosis. The latter is shifted to higher values for repulsive forces, and to lower ones for attractive ones, which is somehow expected if one considers that repulsive (attractive) non-specific forces would rescale the adhesion energy per unit area to higher (lower) values, which always favours (penalises) wrapping. The model presented in ref. 24 was later expanded by the same authors to account for the effect of shear rate to study nanoparticles adhesion and endocytosis to cells under transport, e.g., in the blood stream.²⁵ In this case, a stability map was calculated, showing that stable adhesion *without* endocytosis is also possible in this case. Moreover, it was highlighted that the bond compliance, i.e., the ability of the bond to modify an applied force, plays a major role when compared to the bond strength once flow is accounted for.

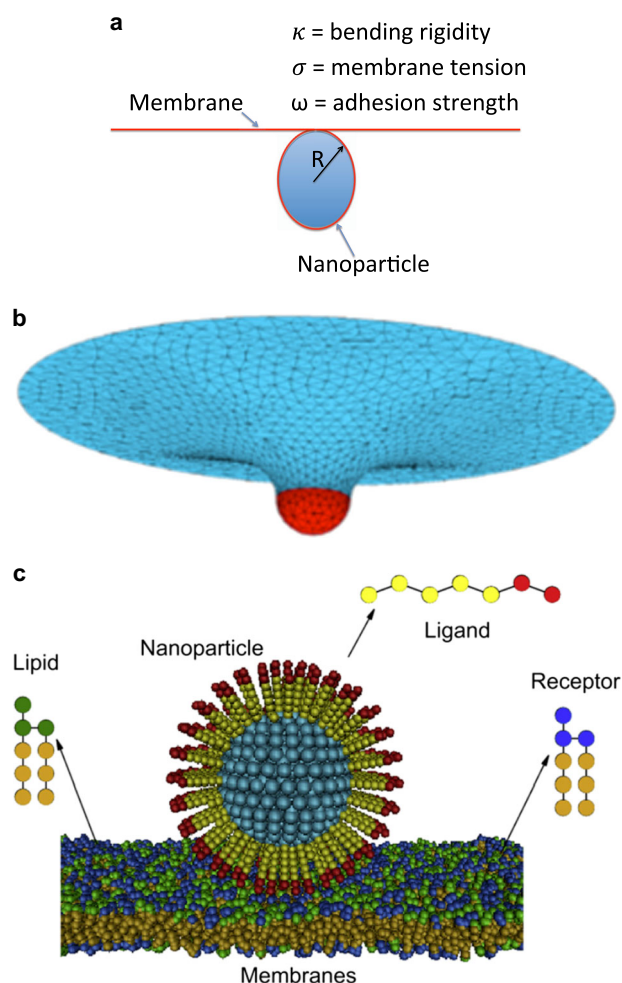


Fig. 2 Different levels of coarse-graining in models for receptor-mediated endocytosis of ligand-functionalised nanoparticles. **a** In analytical models, the wrapping geometry is assumed, and only highly symmetrical particles shape can be treated. **b** Continuum model based on a triangulated surface. (Adapted from ref. 35 with permission from The Royal Society of Chemistry.) Such surface allows to numerically discretise the continuum description defined by the Canham–Helfrich Hamiltonian.^{39,40} In this way, constraints in the wrapping geometry can be relaxed, and particles of different shape can also be studied. **c** Coarse-grained, particle-based model (taken from ref. 31). These types of models, typically studied via molecular dynamics or dissipative particle dynamics,⁵¹ directly address the true discrete nature of the system. Using this more detailed description, “molecular” features can be studied, and inhomogeneities (e.g., in ligand coverage) and arbitrary shape easily addressed

The aforementioned theoretical models necessarily make certain assumptions to simplify the problem and make it analytically (or semi-analytically) tractable. For example, ligands coverage on the nanoparticles is considered uniform and wrapping is assumed to follow specific paths. Also, in general, only very specific nanoparticles shapes, such as spherical particles or infinitely long cylinders (where wrapping is not influenced by their sharp ends) can be addressed with such models. All or part of these assumptions can be relieved by computer simulations. In order to study the effect of different shapes, Dasgupta et al.³⁴ used a computational model where the membrane is simulated via a so-called dynamically triangulated surface^{47,48} (see Fig. 2b). In essence, this is a numerical method that allows discretising the integral appearing in the Canham–Helfrich Hamiltonian^{39,40} for the membrane deformation energy for an arbitrary (but still smooth)

shape. In this way, these authors were able to study the wrapping of realistic finite-size particles, including cylindrical, elliptical, and cuboidal nanoparticles. In their model, Dasgupta et al. also considered the effect of membrane tension (neglected in the models previously described), but not the entropic penalty due to receptors' localisation. In this sense, this model might be more appropriate to study wrapping mediated by anchored instead of mobile receptors. However, this entropic term is not so important as long as the number of ligands is far less than that of receptors and no major depletion effect takes place. The results of this simulations point out that shape has a strong effect on the nanoparticle wrapping process.^{49,50} Non-spherical nanoparticles were shown to have various meta-stable wrapping states, separated by relatively high-energy barriers that increase with increasing sharpness of their edges, an aspect that can critically slow down their uptake. Moreover, it was shown that elongated particles display a complex internalisation pathway where reorientation can occur. This latter process leads to trapping in a metastable state, providing a likely explanation for experimental data that reported increased binding, but reduced endocytosis, for ellipsoidal particles compared to spherical ones.^{49,50}

The trends observed by Dasgupta et al.³⁴ expand and confirm results from previous molecular dynamics (MD) and dissipative particle dynamics (DPD) simulations observed while studying the kinetics of nanoparticles adsorption.^{10,29,33} In both MD and DPD simulations, the equation of motion for a system of interacting particles are solved,⁵¹ which allows accounting for the discrete nature of the system. This allows overcoming some of the limitations of continuum approaches previously described, in particular, their validity when strong gradients (e.g., in surface curvature, or ligands concentration) are present. What particle-based MD and DPD simulations pay in computational complexity is given back by the possibility to easily introduce inhomogeneities, e.g., arbitrary ligand spatial distributions, as well as a more explicit description of the structure of ligands, allowing to study their effect. Furthermore, these models naturally describe the dynamics of the system, hence no assumption is necessary regarding the internalisation pathway of nanoparticles. Nevertheless, models for nanoparticles endocytosis are still highly coarse-grained compared to atomistic or molecular-based descriptions (see Fig. 3). Vacha et al.¹⁰ used an implicit-solvent model for cell membranes, previously proposed by Cooke and Deserno,⁵² where each lipid is coarse-grained into three bonded beads. The last two beads are used to represent the hydrophobic tails and attract each other. The first, representing the hydrophilic head group, is purely repulsive. The reason for using such simple model is that particles of this type naturally self-assemble in a bilayer structure, like the phospholipids in the cellular membrane. To keep the number of parameters to a minimum, nanoparticles were also represented as made of beads like those for the hydrophilic heads, with some displaying an additional short-range attraction to represent ligands. In this model, besides the nanoparticle shape, endocytosis was studied as a function of ligand–receptor bonds strength and ligands surface density. Various non-trivial results were found (see Fig. 4). Rod-shaped nanoparticles were observed to internalise more easily than spherical ones, whereas their aspect ratio seems to have little influence. Moreover, particles with sharp edges strongly adsorbed to the membrane without being internalised. Finally, endocytosis was shown to depend strongly on the strength of the ligand–receptor interaction and it was not observed below a limiting value. These trends were deduced by comparing nanoparticles of different designs at the same time-point in an MD trajectory. As the authors themselves point out, these results should be interpreted as *suggesting* that the kinetic of endocytosis is slowed down in certain cases. However, it might be possible that the simulation time was simply not enough to observe internalisation. Indeed, conclusive results can only be obtained by looking at the free-

energy of the process, a fact often overlooked when describing MD and DPD simulations of these systems. An important aspect of the work of Vacha et al. was to show that, if one considered the thermodynamics rather than the kinetics of wrapping, most results could be rationalised using essentially the same theoretical models previously described. This confirms that a continuous description is sufficient to capture the main features of nanoparticles endocytosis and provides support for the reliability of these theoretical models for the a priori design of new systems.

As stated before, particle-based simulations can be used to provide a more realistic description of ligands and allow to assess the influence of their architecture. For example, using DPD simulations Ding and Ma³¹ found out that once ligands length and flexibility are accounted for, a metastable state of “frustrated engulfment” can also be observed, where ligand depletion from the top of the nanoparticle does not allow to complete the wrapping process. Since ligand depletion is, in essence, a polarisation effect that creates an inhomogeneous density of ligands on the nanoparticle, similar results should be obtained whenever highly stretchable ligands are used. This suggests that more rigid ligands would instead help driving complete wrapping, whereas increasing receptor–ligand bond strength would not. Interestingly, it also suggests that for nanoparticles with mobile rather than grafted ligands endocytosis could be slowed down, or even completely suppressed. This was indeed recently shown by Schubertova et al.³⁶ in a systematic investigation on the effects of mobile ligands using MD simulations and the computational model developed by Vacha et al.^{10,27} These authors observed that below a certain surface concentration binding patches are formed, depleting them from the rest of the surface and hence reducing the thermodynamic drive for wrapping the nanoparticle further. Looked from another perspective, this can be rationalised as a weaker effective ligand–receptor bond strength, due to the entropic penalty of localising the pair inside the patch area.⁵³

Surface functionalisation of nanoparticles is used not only to graft targeting ligands, but also to protect their surface. For example, dense brushes of poly-ethylene-glycol (PEG), a highly bio-inert polymer, are often used to prevent the adsorption of serum protein and to reduce the non-specific uptake of nanoparticles by macrophages.⁵⁴ For this reason, Li et al.²⁸ studied the effect of PEGylation on endocytosis via a combination of DPD and self-consistent mean-field theory (SCMFT).^{55,56} The latter is widely used in the study of polymers, especially when high monomer densities are considered, since in this case determining equilibrium quantities via particle-based models requires long simulation times.⁵⁷ In, ref. 28 PEG chains are end functionalised with targeting ligands, coupling steric effects to binding. Under the conditions studied, it was found that PEGylated nanoparticles with high grafting densities can be more easily wrapped, as also observed in experiments.⁵⁴ Densities are “high” or “low” depending on the relative value at which the brush changes regime. At low PEG density, polymers are in the so-called mushroom regime (see Fig. 5a). In this case, the ligands at the end of the PEG chains do not protrude out because of the polymer tendency to coil. However, due to polymer–polymer excluded volume interactions, above a certain density a transition to the so-called brush regime occurs. In this case, polymer ends (and hence ligands) are more concentrated at the top of the brush surface (see Fig. 5b). This means that the true ligand “surface” density is not simply proportional to the grafting density and grows faster than linearly, affecting trends in internalisation as a function of ligand density. Another aspect pointed out by Li et al. is that upon endocytosis PEG is confined by the cell membrane, causing a free-energy penalty due to polymer compression. Above a certain polymer length, this contribution was found to be higher than the membrane bending energy. Inclusion of this term in a simplified theoretical model, similar to those previously described,^{19–21} was

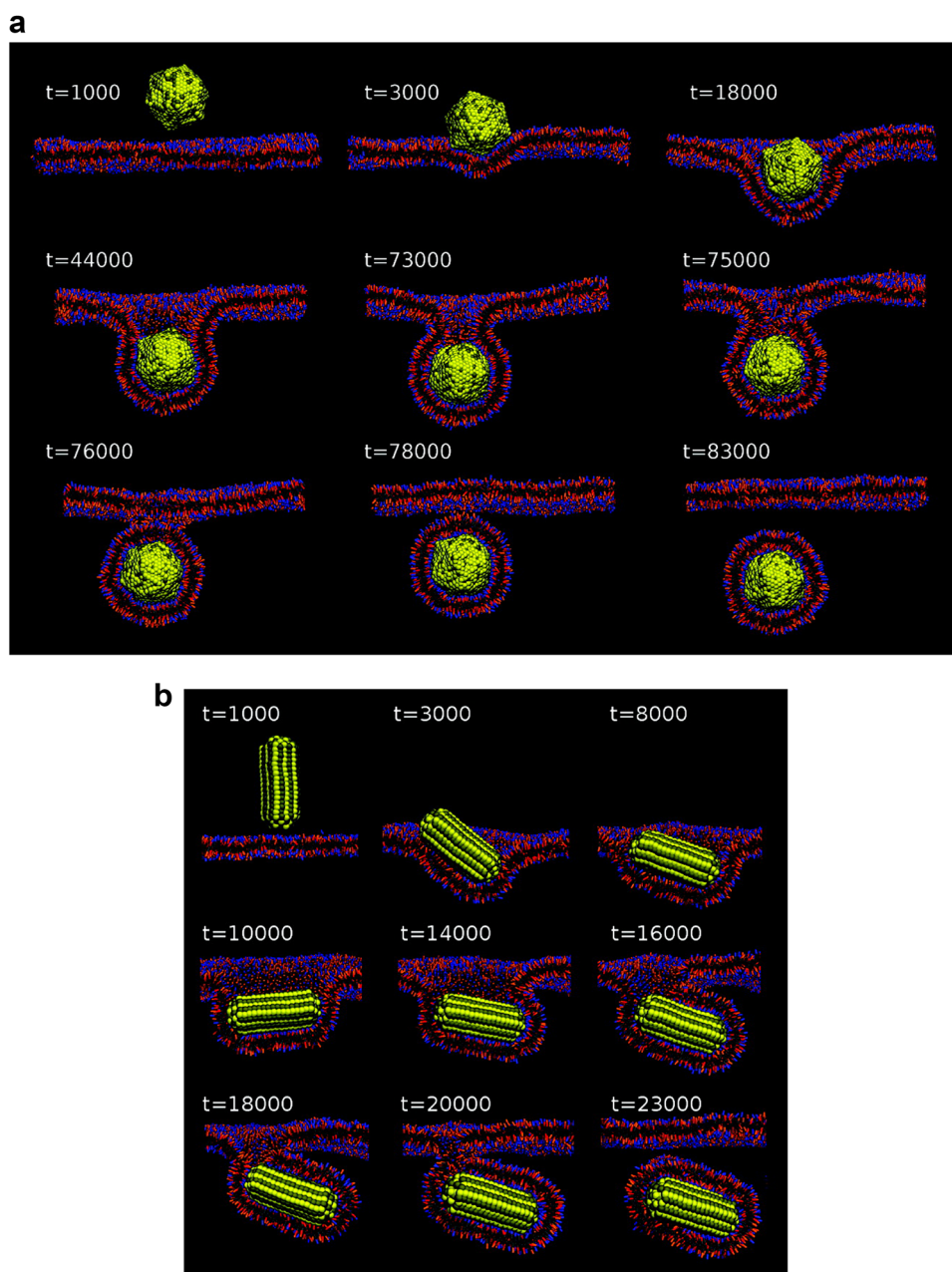


Fig. 3 Results from MD simulations of nanoparticles endocytosis from Vacha et al.¹⁰ **a** Endocytosis as a function of particle size, coverage and ligand–receptor interaction strength. Each snapshot is taken at the same time-point. **b** Time trajectory of endocytosis of a spherocylinder, revealing an internalisation pathway involving re-orientation. (Reprinted with permission from ref. 10 Copyright 2011 American Chemical Society)

critical to calculate the minimum ligand–receptor binding strength for uptake of PEGylated nanoparticles.²⁸

Another aspect addressed using coarse-grained particle-based models is that of possible cooperative effects between nanoparticles.^{26,32} Some experimental observations had shown that very small nanoparticles and nanotubes, with size below the supposed lower critical radius, were found inside cells.^{58–60} This observation seemed to contrast with both other experimental observations, as well as with predictions from theory and simulations.^{10,19–21} Gao et al. had already speculated¹⁹ that this fact could be explained by considering the formation of clusters. Using DPD simulations to study a scenario with various nanoparticles on a bilayer, Tongtao and Zhang³² indeed observed the formation of such clusters and

their uptake. This effect, dependent on membrane tension and nanoparticles average distance (i.e., surface density), was shown to occur even for mutually repulsive nanoparticles, due to membrane elasticity-induced attraction.

The simulations and theoretical models described so far considered the case of hard, non-deformable nanoparticles. However, various nanocarriers, such as liposomes⁶¹ or polymerosomes⁶² are soft, as well as partially permeable to the internalised drug. To study the differences between these carriers, Li et al.³⁷ performed DPD simulations of particles of different hardness, going from soft-polymeric nanoparticles, medium-hardness liposomes and hard, non-deformable nanoparticles. Only the latter could be easily endocytosed. Instead, for softer particles, the

process was frustrated, either due to ligands depletion from the part of the nanoparticle initially not in contact with the cell membrane or because the nanoparticle deformation leads to a metastable wrapped state. All the aforementioned features of soft nanoparticles observed in simulations were either earlier predicted²² or later rationalised^{11,23} by theoretical models. In practice, this was done by starting with the model developed by Gao

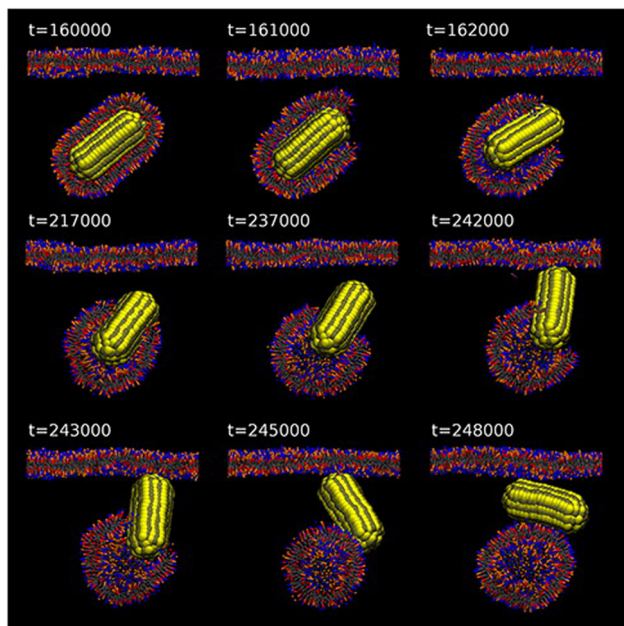


Fig. 4 Time-trajectory from an MD simulations of Vacha et al.²⁷ showing the release of a spherocylindrical nanoparticle following endocytosis upon a change in ligand–receptor interaction. (Reprinted with permission from ref. 27 Copyright 2011 American Chemical Society.)

et al.¹⁹ for hard nanoparticles and simply adding an energy term to account for nanoparticles' elastic deformation, further proving the generality of the theoretical model to describe a wide range of nanoparticles' design. It should be pointed out, however, that coarse-grained simulations cannot always be replaced by simpler theoretical models. For example, and quite interestingly, for soft polymeric nanoparticles fast drug delivery could still be observed via a further mechanism not requiring endocytosis,³⁷ which the earlier theoretical models would not be able to capture: fusion of the nanoparticle lipid membrane with the cellular one, with subsequent formation of a pore throughout which the drug could enter. This mechanism, which was found in simulations to be strongly dependent on the interaction between the hydrophobic part of the liposome and the lipid tails of the cell membrane, is known to be used by some enveloped viruses to infect cells.⁶³

Once a nanoparticle is internalised via endocytosis, it enters the cell membrane surrounded by a lipid bilayer, which must be removed if drugs have to be delivered inside the cytosol. In ref. 27 Vacha et al. used MD simulations with the same model employed in ref. 10 for studying shape effects on endocytosis to address this issue. These authors showed that also the nanoparticle *release* strongly depends on its size and shape. For spherocylinders and small spherical particles, simply reducing the ligand–receptor interaction was enough to destabilise the nanoparticle–lipid membrane complex and quickly drive the release (see Fig. 6). Such reduction in attraction can be achieved in practice by exploiting a pH-sensitive ligand–receptor pair, since the pH inside and outside a cell is different.²⁷ However, this mechanism was not enough for larger spherical nanoparticles. In this latter case, it was shown that the releasing bilayer must be under appropriate tension. If not, the formation of the pore necessary for nanoparticle's escape requires overcoming a large free-energy barrier, preventing its release at any observable timescale. This tension can occur if nanoparticles expand once inside the cell, as experimentally observed in viral capsids.^{64,65} In fact, this release mechanism is already exploited in some synthetic gene-delivery

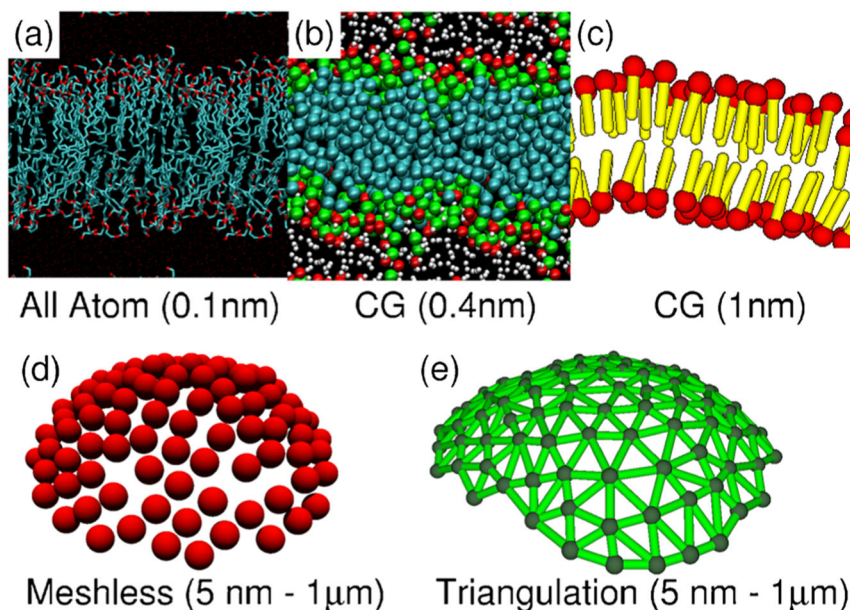


Fig. 5 Different types of coarse-grained models employed in simulations of cellular membranes. Models are ordered by their level of coarse graining, from low to high. **a** All atom simulations, where different functional molecular groups are bundled together, but still retaining chemical specificity. **b** A coarse-grained model where various groups are bundled in a single bead. **c** as in **b**, but a simpler two-particle model, one for the lipid head and one for the lipid tail, is used. **d** A “meshless” membrane, where each bead now represents a part of the surface. In this case, bead–bead interaction is parametrised to stabilise a surface of specific curvature and bending rigidity. **e** A dynamically triangulated surface used to discretise the Canham–Helfrich Hamiltonian^{39,40} for an arbitrary smooth surface. Originally published in ref. 157

Coarse-grained models for Nanoparticles / Cell-Membrane endocytosis				
Model type	Description	Pros	Cons	Useful to
Analytical [14,27-33]	Continuum	<ul style="list-style-type: none"> Direct insight into role of each parameter 	<ul style="list-style-type: none"> Only simple NPs shapes treatable Endocytosis mechanism must be assumed 	<ul style="list-style-type: none"> Derive qualitative conclusions & simple design rules / rules-of-thumb
	Numerical [20,41]	Continuum	<ul style="list-style-type: none"> Can treat complex NPs shapes Computationally efficient 	<ul style="list-style-type: none"> Mechanisms involving discrete nature of system (e.g. [43]) not accessible
Particle-based [13,23,34-40,42,43]	Discrete. Explicit representation of groups of atoms	<ul style="list-style-type: none"> Endocytosis pathway arises naturally from system dynamics 	<ul style="list-style-type: none"> Computationally expensive Results highly system specific 	<ul style="list-style-type: none"> Check assumptions of coarser descriptions Obtain accurate & detailed microscopic picture

Fig. 6 Table summarising differences, advantages and disadvantages of different approaches to coarse models for nanoparticles' endocytosis

systems.⁶⁶ Again, it is important to understand for nanoparticles' design that particles are classified as "small" or "large" in relation to the lower critical size required for endocytosis, which depends on the strength of adhesion per unit area.¹⁰

Overall, it should be clear that receptor-mediated endocytosis can be described and well understood by theory and simulations with highly coarse-grained models, whose results should be taken into account when designing functionalised nanoparticles. In this regard it is also important to highlight that whereas here we only discussed the role of endocytosis, there are many other important aspects that should be considered in drug delivery,^{67,68} quite prominently nanoparticles' immunogenicity, or nanoparticles' filtration by organs, such as the liver and the kidneys. Whereas the latter depends mainly on properties, such as nanoparticles size and shape that can be captured within simplified models,⁶⁷ immunogenicity is often dependent on subtler chemical details. In this case, we need to recognise and stress that such aspects cannot be addressed by the kind of coarse-grained modelling discussed here and a proper treatment requires more accurate descriptions.

CONTROLLING NANOPARTICLES BINDING SELECTIVITY

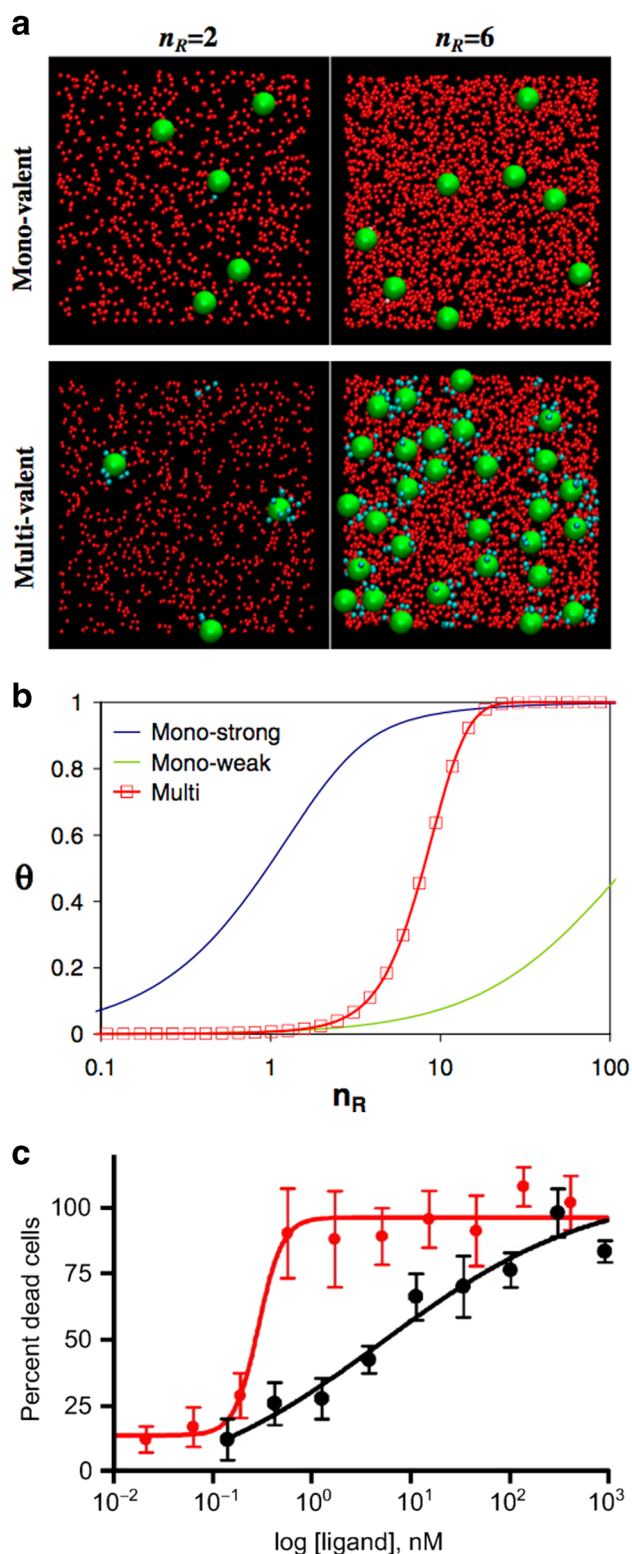
Selectivity is an aspect of tantamount importance for drug delivery. In fact, one of the most promising aspects of nanoparticles is the possibility to deliver their payload on-demand to specific cells. This is particularly relevant in the case of cancer, where non-selective chemotherapy can lead to highly debilitating, if not fatal, consequences.

Due to their size, nanoparticles inherently possess some sort of selectivity towards cancer tissue, an effect typically ascribed to the so-called enhanced permeability and retention (EPR) effect.⁶⁹ This effect takes advantage of the fact tumours often display a leaky structure compared to healthy tissue, with pores of a few

nanometres where particles can enter.⁶⁷ In this way, nanoparticles with sizes below 50 nm, which would be excluded from normal tissue, can accumulate in tumours. The EPR effect provides some degree of selectivity, but is far from perfect. Moreover, it can only be exploited in solid tumours but not in "liquid" ones, such as leukaemia or lymphoma, where cells do not form solid aggregates.

A different and more general solution is to functionalise nanoparticles with ligands that can target specific membrane receptors over expressed in tumour cells. Until recently this strategy has not had much success, for which various reasons have been brought forward.⁶⁸ Whereas it is widely thought that problems, such as immunogenicity are chemistry dependent and might be difficult to study using the kind of coarse-grained models we discuss here (although some recent results are starting to question this assumption⁷⁰), other aspects of selectivity can well be captured within this modelling approach. In fact, selective targeting via functionalised nanoparticles is an area where modelling has predicted very interesting and sometimes counter-intuitive behaviours,⁷¹⁻⁷³ whose exploitation might be extremely useful in the design of targeting systems, as we are about to discuss.

In a pioneering experimental work, Carlson et al.⁷⁴ observed that using multivalent binding, i.e., binding through recognition of a target via multiple ligands, it was possible to achieve high selectivity towards receptors over expression. More precisely, they showed that both divalent and decavalent antibodies were able to bind to cells, and cause their death, only when their antigen was expressed above a certain threshold (see Fig. 7). This was in stark contrast with what they observed when using doxorubicin, a potent chemotherapeutic molecule, conjugated to a single ligand optimised for binding the specific receptor whose over expression they were trying to target. In this case, cell death was observed for cells bearing a broad range of receptor concentrations on their surface. In order to understand this behaviour, Martinez-



Veracoechea and Frenkel⁷¹ developed a coarse-grained model of nanoparticles adsorption and studied it via Monte Carlo (MC) simulations. In this highly coarse-grained model, the cell surface and the nanoparticles are described as an impenetrable flat surface and hard spheres, respectively, whereas ligands and receptors are represented as polymers using the blob model of Pierleoni et al.⁷⁵ The latter arises from describing a polymer as a

Fig. 7 Comparison of theory and simulations with experimental data on the super-selectivity of multivalent nanoparticles. **a** Monte Carlo simulations, showing how adsorption rapidly increases for multivalent nanoparticles (bottom) but not monovalent ones (top). **b** Adsorption from a simple theoretical model considering the statistical mechanics of non-interacting ligand–receptor pairs (**a**) and (**b**) reprinted with permission from ref. 71). **c** Number of dead cells for multivalent (red) and monovalent (black) targeting as a function of receptors concentrations. (Reprinted with permission from ref. 74 Copyright 2007 American Chemical Society.) Multivalent constructs are able to selectively kill cells only when ligands are above a certain concentration, but not below. For monovalent drugs, a broader toxicity is observed. Assuming that adsorption and subsequent endocytosis of nanoparticles would lead to cell death, theoretical, simulations and experimental data all nicely agree with each other

simple self-avoiding walk and thus contain only excluded volume interactions. In order to simulate the reversible binding and unbinding between ligands and receptors an MC move was used, first-proposed in ref. 76, where blobs are allowed to connect/disconnect with a (free-)energy change that depends on experimental binding constant K_{bind} and on the entropic cost of binding due to ligands' stretching, a concept generalised in ref. 46 for arbitrary ligands. K_{bind} encodes the potency of the ligand towards the targeted receptor, and is all that is retained about the chemical specificity of the system. Remarkably for such a simple model, the simulations in ref. 71 are able to nicely reproduce the trends observed in the experimental data of Carlson et al.⁷⁴ and perfectly capture the effect of multivalency (see Fig. 7). More precisely, it was shown that whereas the amount of adsorbed monovalent nanoparticles slowly varies as a function of receptor concentration, that of multivalent ones rapidly increases above a specific value. Armed with this model, other important aspects of the system were studied, in particular, the influence of ligands number and that of ligand–receptor bonds strength. The main message from these simulations was that “super-selectivity”, i.e., a rapid, super-linear increase in the adsorption probability, arises *exclusively* for multivalent particles and can never be observed for monovalent targeting. Moreover, super-selective behaviour requires weak single bonds and is more efficient as the bond-strength decreases. Hence, multivalency-enhanced selectivity is based on a design principle, which is the opposite of the typical biochemical approach to optimising a ligand to bind as strongly as possible to a specific receptor. This latter approach is perfectly valid if one aims to distinguish between targets displaying *different types* of receptors. However, the results in ref. 71 imply that such strategy does not allow to tell apart targets which differ for receptors' concentration.

A further important aspect of the work in ref. 71 was to show that trends observed in simulations could be easily understood without recurring to simulations at all, for which an analytical model considering the statistical mechanics of a system of reversible bonds is sufficient. That such a simplification still works suggests that super selectivity is an emergent feature purely due to multivalency.⁷⁷ No further complexity is required, in particular, no cooperativity between binding of different ligands needs to be evoked. The robustness of this effect also suggests it can be exploited using very different systems and not just nanoparticles, as long as the basic ingredient, the possibility of multiple uncorrelated ligand–receptors bonds, is satisfied. A strong indication in this sense comes from the work of Dubacheva et al.,^{73,78} which showed that the same model used for nanoparticles can be easily expanded to also describe over-expression targeting via functionalised polymers. These latter works presented an extensive comparison between theory,

simulations and experiments. In particular, ref. 73 reports a systematic investigation of the effects of the design parameters of the system, and showed that once a few input parameters of the theory were obtained from fitting of experimental data, it was able to very well predict results for new system's designs.

The aforementioned works on multivalent targeting focused on systems where a single type of receptor is present. However, in drug-delivery nanoparticles can meet different cell types on their way to their target, all expressing their own receptors. What happens when ligands can spuriously bind, albeit with a weaker strength compared to their intended target receptor, to others? In, ref. 72 Angioletti-Uberti analysed this problem combining the model of Martinez-Veraceochea and Frenkel⁷¹ with a generalised expression for ligand–receptor-mediated interactions able to treat arbitrary receptor distributions.⁷⁹ Whereas multivalency is a necessary requisite to observe super selectivity, this study showed that multivalent nanoparticles suffer from greater sensitivity to non-specific binding, exactly because of the possibility to form multiple bonds, albeit weak, with non-targeted receptors. In other words, binding to targets that do not show the targeted receptor is strongly increased compared to monovalent particles. In the same study, one possible way to reduce this effect was proposed, i.e., to use what have been dubbed “protective” receptors.⁷² These receptors, grafted on the nanoparticle itself, compete with those on cells for binding ligands. This competition can be made favourable for the targeted receptors, but not others, by a careful choice of the bond strength and the number of protective receptors.⁷²

Another recent work where multiple receptors have been considered is that of Curk, Dobnikar and Frenkel¹² These authors discuss a targeting scenario where one wishes to design nanoparticles to selectively target cells based on the presence of a receptor population. In other words, they aimed to determine the conditions under which multivalent nanoparticles bind as selectively as possible to targets with a specific ratio between different receptor types. In practice, this means that the binding energy needs to grow as quickly as possible when moving away from the target population. Under relatively broad conditions, their model shows two very interesting and non-trivial features. First, that the highest selectivity is achieved when each ligand–receptor pair has exactly the same bond strength, regardless of the fraction of receptors present. Secondly, and quite strikingly, that this optimal strength is independent on the system specifics, and was calculated to be equivalent to bonds of energy $\Delta G_{\text{bond}} \approx -1.3k_{\text{B}}T$ ($k_{\text{B}}T$ being the thermal energy), which are quite weak bonds. In practice, achieving such low bond energy using typical ligands might be complicated and would require a careful optimisation of the ligands used. Another interesting route would be to exploit multiple types of protective receptors, since as shown by Angioletti-Uberti in ref. 72 their presence is equivalent to rescaling the effective bond strength. Moreover, this strategy has the additional advantage to rescale such energy without affecting the difference in strength between targeted and untargeted receptors, thereby avoiding to increase the effects of non-specific interactions and losing selectivity. In order to substantiate their theoretical findings, Curk, Dobnikar and Frenkel also performed Monte Carlo simulations for two different systems. The first was the study of nanoparticles' adsorption on a flat, hard surface, using the same computational model as in ref. 71 but including multiple ligands and receptor types. In order to simulate a situation closer to the intended application of the theory, the second problem studied was the endocytosis of a functionalised nanoparticle by a membrane. In this latter case, nanoparticles were described using a simple patchy-particle⁸⁰ model to simulate the presence of ligands, whereas the cell membrane was described via a model, first proposed by Yuan et al.,⁸¹ where each lipid is coarse grained into a single sphere (see Fig. 3d). Some lipids are augmented with an attractive interaction to the particle

patches via a directional, short-ranged bond, as represented using the Kern–Frenkel potential.⁸⁰ Importantly, simulations for both these systems fully validated the highly nontrivial results from the theoretical model.

At this point, we would like to highlight that various properties of multivalent systems appear to be captured by considering the statistical mechanics of non-interacting ligand–receptor pairs, pioneered by Bell for specific biological systems^{82,83} and more recently generalised to arbitrary scenarios.^{79,84–86} These properties include not only the peculiar binding selectivity of multivalent systems, but also their high-binding strength, exploited to detect targets at very low concentrations using weak ligands.⁸⁷ However, a large amount of the experimental work on multivalency still discusses these properties in terms of complex models, invoking binding cooperativity between different ligand–receptor pairs.^{87,88} In this regard, it is worth noticing that claims of co-operativity should be always carefully checked. In fact, the latter often come from analysis of experimental data using theoretical models like Scatchard's or Hill's plots. However, as pointed out by Ercolani almost 15 years ago,⁸⁹ Scatchard's or Hill's equations only make sense when applied to binding of monovalent ligands to multivalent receptors, but are theoretically unfounded otherwise, and their use can lead to misinterpretation of data. With this remark, we do not want to suggest that all works on multivalency claiming co-operativity are wrong. Rather, we argue that in analysing experimental data on multivalent systems a simpler explanation not invoking co-operativity should first be considered.

PREDICTING AND CONTROLLING PROTEIN ADSORPTION

Once in the bloodstream, bare nanoparticles are quickly covered by a thick layer of proteins, the so-called protein corona.⁹⁰ It is this protein layer that determines the biological activity of nanoparticles rather than the type of nanoparticle itself,^{91,92} with important consequences. For example, it was shown that proteins can cover and shield targeting ligands, preventing targeting via ligand–receptor recognition.⁹³ Protein adsorption also controls the pathophysiology of nanoparticles⁹⁴ and can reduce their toxicity.⁹⁵ For these reasons, it should be clear that control of this phenomena is of great interest when considering applications.

A general approach towards control of protein adsorption is surface functionalisation with polymers.^{54,96} In this regard, both cooperative and competitive interactions between proteins and between polymers and proteins play an important role.⁹⁶ As for nanoparticles' endocytosis, accessing the timescale and length-scale to describe any realistic scenario relevant for applications, via atomistic simulations is still largely a computationally intractable problem, especially because of the timescales required to equilibrate the system. Hence, highly coarse-grained models have been developed, mostly based on a continuum description of the inhomogeneous density fields of both polymers and proteins.^{97–106} Complementary approaches have been introduced based on the SCMFT of polymers^{55–57} or on classical density-functional theory (DFT).^{107,108} These theories allow to include, albeit in an approximate way, those enthalpic and entropic contributions known to affect polymer conformations and polymer–protein interactions, and hence describe the capability of different coatings to influence adsorption. Among such contributions, one typically considers the elastic energy of the polymer, as well as the interaction between polymer chains and the solvent, often within a Flory-type description.^{109,110} In a similar way, the interaction between the polymer chain and proteins, as well as that of the grafting surface with both the polymer and the protein can also be included.⁹⁷ Within a mean-field approach, electrostatic interactions are usually introduced via a Poisson–Boltzmann description^{111–113} or on a coarser level assuming local electro-neutrality.¹⁰⁶ The inclusion of electrostatic interactions is quite important considering that under physiologically relevant

conditions most proteins and various biocompatible polymers used in applications are charged.⁹⁶ Essentially, these types of models can be expected to reproduce trends in how polymer parameters, such as grafting densities, length or flexibility, but also their architecture or the presence of charged groups, affect protein adsorption.

Pioneering work in this field has been made by Szleifer and co-workers, which in a series of papers^{97–100,114–116} systematically addressed how the thermodynamics and the kinetics of protein adsorption are affected by the presence of polymer brushes. Considering equilibrium properties, it was found that a higher grafting density better prevents protein adsorption due to steric repulsion and the competition between monomers and polymer to adsorb on the surface, a result also suggested in an earlier work of de Gennes.¹¹⁷ It was also shown that flexible polymers present a more effective barrier than rigid ones. Perhaps less intuitively, it was observed that although adsorption decreases with increasing molecular weight, a much larger effect is played by the character of the surface. In fact, for polymer-repulsive surfaces protein adsorption is almost independent above a certain polymer length for all but the shortest chains, whereas in the opposite case it keeps decreasing for all polymer length considered. Moreover, whether or not an attractive polymer–surface interaction helps to prevent protein adsorption was found to depend on the polymer molecular weight. For short polymers, polymer-repulsive surfaces perform better, whereas for longer polymers the opposite is true.

The surface influence on protein adsorption at equilibrium was rationalised by looking at the effective potential felt by proteins due to the different polymer density field observed. Knowledge of this potential was also used to discuss the kinetics of adsorption, either in a qualitative way,^{97,100} or by fully solving the equations for the dynamical evolution of the system.^{115,116} These equations can be obtained by noting that the Szleifer model can be thought of as a mean-field DFT model and the dynamical equations derived within the framework of dynamic DFT.^{118,119} (A more heuristic derivation in the context of a generalised diffusion equation can also be found in ref. 106) In essence, the underlying assumption is that the system obeys Brownian motion. Interestingly, the design principles for kinetic control of protein adsorption are opposite to those for controlling equilibrium. The underlying physical mechanism derived from modelling of various systems^{115,116} is the following: a polymer-attractive surface better prevents equilibrium adsorption because monomers accumulate close to it. However, mass conservation causes a depletion away from the surface, reducing the kinetic barrier for protein approach. As a result, even if a smaller amount will be adsorbed, it will also be adsorbed orders of magnitude faster.⁹⁹ Another important conclusion from refs. 115,116 was that contrarily to what observed for thermodynamics, polymer molecular weight strongly affects adsorption kinetics. This is mainly because in the presence of a barrier protein adsorption becomes an energetically activated process with an exponential dependence on the barrier height, which increases with molecular weight.¹¹⁶

The previous studies also highlighted two other important effects for applications. The first is that due to the coupling between protein conformation and steric effects polymer coatings can be used to selectively adsorb specific conformations.^{97,99} This behaviour, yet to be confirmed by experiments, is consistent with what was also observed in a more detailed particle-based model of Rubenstein et al.¹²⁰ The second important feature is that whereas flexible polymers are better at preventing protein adsorption, a combination including rigid ones could benefit targeting systems where polymers are end functionalised with ligands.¹⁰⁰ The reason is that for flexible polymers their functionalised ends are partially located inside the brush and not on its surface, making binding by receptors more difficult, but the opposite is true for rigid polymers. Grafted polymers do not only affect the amount of protein adsorbed, but also their

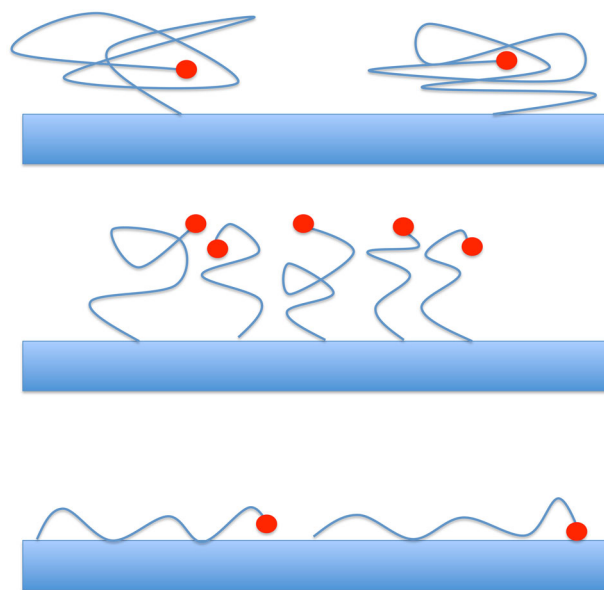


Fig. 8 Grafted polymers in different conformations. Top and middle panels: polymers grafted on a repulsive surface in the mushroom (top) and brush (middle) regime. The distribution of monomers, as well as that of the polymer ends is rather different in the two cases, leading to different properties for this brush. Bottom: as in the top panel, but for a polymer-attractive surface

adsorption mode. This aspect was investigated by Halperin¹⁰³ and Halperin and Kröger^{104,105} using both analytical theory,¹⁰³ based on the Alexander-de Gennes¹²¹ and Pincus¹²² models of brushes, as well as the full SCFMT equations. At variance with the Szleifer model, these works do not consider the protein density field explicitly but only look at the brush density and relate it to the protein adsorption energy. Within this simplified description, different adsorption modes can be captured, i.e., primary adsorption (at the grafting surface), secondary adsorption (on top of the brush), as well as ternary adsorption (within the brush). These three modes are observed depending on protein sizes and protein–polymer interaction (Fig. 8). Importantly, reduction of protein adsorption in these three different modes is achieved by controlling different parameters (e.g., grafting density for primary and brush thickness for secondary). This fact should be taken into account in the development of polymer coatings that have to be used in real blood plasma since the latter contains more than a thousand protein types.

All the aforementioned works deal with protein adsorption deal on planar brushes, i.e., polymers grafted on a planar surface. However, curvature effects can become important whenever nanoparticles' radius is much smaller than the polymer length. Another aspect not considered in these works is the effect of electrostatic forces. A semi-analytical model of protein adsorption on a spherical charged brush was first proposed by Biesheuvel et al. in ref. 123 In this model, polymer conformational effects (e.g., elasticity)^{124,125} are described using classical models for neutral brushes derived from analytical approximations to the full SCFMT equations, whereas electrostatic and excluded-volume effects were accounted for different levels of approximations. In, ref. 123 a simplified “box” model was proposed, where proteins are assumed to be in one of two environments, either adsorbed or in the bulk solution. In this model, polymer–polymer interactions were treated within the Flory–Huggins approach,^{109,110} whereas protein–protein interactions were calculated considering proteins as hard spheres using the Carnahan–Starling functional.¹²⁶ Polymer–protein interactions in this model arise only through electrostatics, calculated assuming local electro-neutrality.

In, ref. 127 the same authors proposed a more accurate description by considering the full, non-homogeneous spatial fields of polymers and proteins and treating electrostatics by solving the full Poisson–Boltzmann equations.¹²⁸ In this latter model, all excluded volume interactions were also described on an equal ground by also treating polymer beads as spheres (of a different size compared to proteins) and using the Boublik–Mansoori–Carnahan–Starling–Leland¹²⁹ functional that extends the Carnahan–Starling description to mixtures of poly-disperse spherical particles. Besides these, other types of interactions, e.g., hydrophobic or van der Waals, were not included. Finally, a key feature included in both ref. 123 and ref. 127 was to consider charge regulation, i.e., that proteins adjust their charge in accordance to the local pH, which can differ between the brush and the bulk solution if they have a difference in electrostatic potential. This aspect is crucial. In fact, the main result from Biesheuvel et al. was to show that, as also observed in experiments,¹¹² proteins with the same charge of the brush can still be adsorbed, as long as pH is close to their isoelectric point and the ionic strength (related to salt concentration¹²⁸) is sufficiently low. This happens because under such conditions proteins reverse their charge upon entering the brush.

Up to this point, we focused on polymer brushes, but other types of architectures can be considered. Another important type is that of nanogels or microgels, where polymers are grafted both to the nanoparticle surface, as well as being crosslinked to each other.¹³⁰ This type of system has a wide range of interesting properties that makes it widely studied as drug carriers, in particular, controlled degradability and release kinetics.¹³¹ Moreover, it has been shown that certain enzymes can remain functional once adsorbed,¹³² which could be interesting to cure diseases related to the presence of a malfunctioning enzyme, or lack of a functional one.¹³³ Rather than being used to prevent proteins adsorption as in the case of brushes, with polymer gels, one aims to control it, as well as to control their release. Several models containing similar ingredients to that of Biesheuvel et al.^{123,127} have been developed to describe protein adsorption in gels.^{106,111,134,135} Johanson et al.¹¹¹ built a model to study lysozyme adsorption in large nanoparticles (500 nm in radius) of polyNIPAM-co-acrylic-acid. As in ref. 123 electrostatics effects are introduced approximately by again enforcing local electro-neutrality, a valid approximation when the system is coarse-grained to distances larger than the Debye screening length.¹²⁸ This constraint generates a global electrostatic potential, the so-called Donnan potential, that influences the chemical potential of all charged species in the system.^{128,136} This work concluded that the largest role in determining the strong protein adsorption in this system, was the interaction between electrostatics and entropic effects. Under the study conditions, the high charge of this protein (+9e) makes it equivalent to a multivalent ion. Upon adsorption in the gel, one multivalent ion can replace many mono-valent counterions, releasing them in the bulk solution where their ideal-gas entropy increases, leading to an overall decrease in the free-energy of the system.

In models based on the concept of the Donnan potential or the more accurate Poisson–Boltzmann description, electrostatic effects only depend on the value of charges and are thus non-specific. Excluded volume effects are also non-specific as they only account for protein size. Hence, as long as proteins have the same charge and volume, these models will predict the same adsorption profiles, contrarily to experimental observations. Recently, Yigit et al.¹³⁴ demonstrated how electrostatic contributions can be separated from system-specific terms arising, e.g., from hydrophobic or van der Waals interactions. This was done by fitting advanced binding models to experimental binding isotherms built from titration calorimetry.^{96,134,135} As in the model of Biesheuvel et al.,¹²³ Yigit et al.¹³⁴ also built a two-state model considering the chemical potential of proteins either inside the gel or outside in

the solution. The energetic contributions considered are electrostatic ones due to the coupling of the protein monopole to the global Donnan potential, as well as the Born self-energy change arising from the different dielectric properties of the two environments. Moreover, excluded volume interactions between proteins and between proteins and polymer are also accounted for. Finally, all protein-specific contributions are lumped in a single term. Interestingly, this splitting allows to model, in good agreement with experimental data, adsorption of *mixtures* of proteins from knowledge of constants derived from fitting of single-type adsorption experiments.¹³⁵

This thermodynamic model has been recently expanded in two ways. On the one side, Angioletti-Uberti et al.¹⁰⁶ reformulated the model within a classical DFT framework.¹⁰⁷ In this way, not only the full spatial distribution of proteins can be described, but more importantly, the machinery of dynamic DFT¹¹⁸ allowed to study the adsorption kinetics. An important result was to show that accounting for all interactions within the system is absolutely necessary to obtain a decent agreement with available experiments.¹³² This point, previously also made by Szeifer in studying adsorption on brushes,^{115,116} might seem obvious. However, it is in stark contrast with the widespread use of the simple diffusion equation to study adsorption and desorption from gels, not just in the case of proteins but more generally for drugs.¹³⁷ Use of the simple diffusion equation is equivalent to assuming that the gel behaves as an inert background. If one insists on not including energetic effects, comparison with experiments requires either using unphysical values for diffusion coefficients, or using completely empirical (and not physically justified) models to reproduce the kinetics. Moreover, these model's parameters must be re-fit to experimental data each time a new condition, e.g., pH or salt concentration, is considered.

The other direction in which the Yigit et al. model¹³⁴ was modified was that taken by Adroher-Benítez et al. in ref. 113. These authors improved the model by solving the full Poisson–Boltzmann equations for the electrostatic potential within the system. Even more importantly, the effect of the charge distribution on the protein's surface was included, in contrast with all previous studies described here where a spherically distributed charge is assumed. To keep the problem tractable, Adroher-Benítez et al. included this effect only at the dipole level, although in principle higher-order multipole corrections can also be calculated. Importantly, dipoles couple to the electric field, i.e., to the *gradient* of the electrostatic potential, unlike monopoles that couple to the electrostatic potential itself. In these nanogels, electrostatic gradients are relevant only at the interface with the solution and decay exponentially away from it with a decay length equal to the salt-dependent Debye screening length.¹¹³ Hence, dipoles favour adsorption at the interface rather than inside the gel. By looking at the competition of different terms and at the effective potential for adsorption arising, Adroher-Benítez et al. were able to show that there exist various adsorption states, similar to what was described by Halperin and Kröger in polymer brushes. This important fact should be taken into account when considering applications, in particular, because the release kinetics can be strongly affected by the initial adsorption profile.

CONCLUSIONS AND OUTLOOK

In this review, we showed how highly coarse-grained models, where chemical specificity is omitted or compounded into effective input parameters, can be used to study selected features of functionalised nanoparticles relevant to their biomedical applications. In particular, we restricted our view to those models inspired by Soft Matter Physics, here intended in a broad sense including polymers. By reducing the complexity of the interactions while retaining the basic ingredients to describe the correct physics involved, this type of models focus on understanding

general mechanisms and trends rather than concentrating on a very specific system realisation. For this reason, we deliberately left out that vast area of “bottom-up” approaches where, starting with the highest possible complexity in terms of interactions (say, a DFT or quantum chemistry calculation), one uses systematic coarse-graining techniques to build a computationally more tractable model but still aims at retaining chemical specificity. These include multi-scale models where different levels of coarse-graining are retained for different part of the system.¹³⁸ For recent reviews on this type of approaches with a focus on soft and biological matter we suggest refs. 139–141.

Despite our omissions, we hope this review will serve to spur a broader appreciation of these “simple” models and their results, especially among biomedical scientists whose aim is to design applications. In particular, our goal will be reached if one have obtained a sense of what can be done with these models, as well as about their limits.

Before we conclude, we would like to discuss three different paths forward. On the theory and modelling side, much work is still required to build up models even more directly linked to the real biological system, and assess the effect of such more detailed description. For example, it is known that endocytosis of biological entities, e.g., viruses, is mediated by the self-assembly of certain proteins, such as clathrin on the cytosol side, or by the presence of lipid rafts.¹⁴² Although endocytosis of nanoparticles can also happen following “passive” paths not directed by these mechanisms, it would be interesting to address them. In this regard, a coarse-grained description could be done by modifying the Canham–Helfrich Hamiltonian to account for heterogeneous bending/stretching modulus or different local spontaneous curvatures induced by proteins.¹⁴³ In the same way, much of the work on exploiting multivalent targeting still requires more studies to understand the effect of spurious interactions present in the biological environment, e.g., due to protein binding, which can affect targeting selectivity⁹⁴ and sometimes completely destroy it.⁹³ The same is true regarding protein adsorption, where more care should be taken in building theoretical models to address the fact that blood plasma is a multi-protein mixture with hundreds of different types interacting with each other, from which cooperative and competitive effects could arise. Whereas improvement on the theory and modelling side is certainly needed to increase their impact and reliability, we would like to point out a few biomedical applications of nanoparticles where we believe the current approaches gives robust enough predictions that could already be used as to provide effective guidelines. One is surely that of bio-sensing, especially in those cases where target detection is measured via its ability to drive self-assembly via ligand–receptor bond formation,¹⁴⁴ e.g., functionalised nanoparticles for DNA¹⁴⁵ or enzyme¹⁴⁶ recognition. Another is the fast advancing field of nanoparticles as anti-viral drugs,^{147–150} where multivalent particles are used that mimic, and compete with, cell receptors. Both these applications can benefit from the same approaches and techniques used to understand targeting selectivity,^{70–72,79} and indeed some initial step in that direction have already been taken.^{147,151–153}

In general, the take-home message that we would like to deliver is that a deeper integration of theory and computational modelling in the design stage of specific system would be welcome. In this regard, we notice that although inspired by the will to explain experimental results, theoretical models have also made interesting predictions, especially concerning targeting selectivity.^{12,71,72} Their validation and exploitation for the design of applications could lead to important advancements and have a strong impact in biomedical nanotechnology.

Data availability

All relevant data are available from the author.

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ADDITIONAL INFORMATION

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