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Stiffness can mediate balance between hydrodynamic forces and avidity to impact the targeting of flexible polymeric nanoparticles in flow†

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Abstract

We report computational investigations of deformable polymeric nanoparticles (NPs) under colloidal suspension flow and adhesive environment. We employ a coarse-grained model for the polymeric NP and perform Brownian dynamics (BD) simulations with hydrodynamic interactions and in the presence of wall-confinement, particulate margination, and wall-adhesion for obtaining NP microstructure, shape, and anisotropic and inhomogeneous transport properties for different NP stiffness. These microscopic properties are utilized in solving the Fokker–Planck equation to obtain the spatial distribution of NP subject to shear, margination due to colloidal microparticles, and confinement due to a vessel wall. Comparing our computational results for the amount of NP margination to the near-wall adhesion regime with those of our binding experiments in cell culture under shear, we found quantitative agreement on shear-enhanced binding, the effect of particulate volume fraction, and the effect of NP stiffness. For the experimentally realized polymeric NP, our model predicts that the shear and volume fraction mediated enhancement in targeting has a hydrodynamic transport origin and is not due to a multivalent binding effect. However, for ultrasoft polymeric NPs, our model predicts a substantial increase in targeting due to multivalent binding. Our results are also in general agreement with experiments of tissue targeting

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Conflicts of interest

There are no conflicts to declare.

measurements *in vivo* in mice, however, one needs to exercise caution in extending the modeling treatment to *in vivo* conditions owing to model approximations. The reported combined computational approach and results are expected to enable fine-tuning of design and optimization of flexible NP in targeted drug delivery applications.

1. Introduction

Targeted nanoparticles (NPs) loaded with drugs that are directed to precise locations in the body may improve the treatment and detection of many diseases.^{1–6} Targeting of NPs functionalized with antibodies to vascular endothelial surface molecules such as the intracellular adhesion molecule-1 or ICAM1 depends on several physiological factors such as anti-body density, receptor expression, cellular mechanical factors, hydrodynamic conditions such as hematocrit (HCT) density, blood flow rates, and vessel diameters.^{7,8} In addition, several design factors such as size, shape, flexibility/stiffness, and NP architecture, collectively influence targeting efficacy (*i.e.*, tissue selectivity as well as avidity), see ref. 8, 9 and references therein. However, the individual contributions of these multitude of factors are often not discernable leading to largely an empirical and exhaustive search for optimal design. In particular, there is a need for precise control of the specificity and selectivity of binding of the NPs to the target (inflamed or diseased) tissue of interest under varying pathophysiological and hydrodynamic conditions in the vasculature.

In previous studies, we and others have shown that modeling can serve as a predictive tool in designing the functionalization characteristics of rigid NPs.^{10–19} In this case the enthalpy of multivalent binding is compensated by the translational entropy loss of bound receptors on the cell surface to a large extent, as well as the entropy loss of NP translation and rotation. These studies demonstrate that modeling can play a crucial role by providing rational design principles for rigid NP on the basis of the underlying thermodynamic and hydrodynamic considerations.

Experimental and modeling studies of flexible NPs have revealed that the flexibility can introduce novel hydrodynamic effects that can be exploited in targeted drug delivery.^{20,21} We consider a new class of flexible biocompatible polymeric NP comprising a lysozyme rich core with dextran brushes, which is capable of hosting guest molecules including small hydrophobic drugs and large contrast imaging agents,^{22,23} important for biomedical applications involving diagnostic imaging and therapeutic delivery. While the carrier construct has been physically characterized and realized as a promising vehicle for drug delivery *in vivo* in mice models,²⁴ its optimization for targeting specific tissues is far from clinical translation. How precisely the internal hydrodynamics of NP relaxation is coupled to the external hemodynamics, hydrodynamic lift, effect of confinement on carrier mobility to determine NP deformability, multivalent adhesion to cells, and drug release kinetics, is not obvious, and little quantitative mechanistic analyses have been reported to date.

We hypothesize that the rules governing multivalent binding behavior for flexible NPs will be quite different from those for rigid NPs noted above on both hydrodynamic as well as adhesion aspects. Specifically, we hypothesize that on the hydrodynamic front, the shear, confinement, and interaction with HCT will collectively define the shape of the flexible NP,

which through shape-dependent hydrodynamic interactions will impact NP spatial distribution and margination. On the adhesive front, upon multivalent binding, we expect the entropy loss per receptor ligand bond to be lower for the flexible NP compared to its rigid counterpart, thereby driving larger multivalency. However, an additional contribution to entropy loss resulting from change in NP accessible conformations upon binding applies for the flexible NP, which is absent for the rigid NP. The collective effects of these entropic contributions will compensate with the enthalpy of multivalent binding leading to new rules for NP binding. We expect such findings will have a direct impact on the design of flexible NP for a given application demanding a desired selectivity and specificity of binding to target tissue, which necessitates a departure in our intuition and rational thinking when switching from rigid to flexible functionalized NP. We demonstrate here that these competing effects can be tuned by controlling the degree of flexibility by tuning the NP stiffness.

2. Methods

A summary of the computational model and experimental methods is presented below, and detailed descriptions are made available in ESI.[†]

2.1. Computational model

The illustration shown in Fig. 1 highlights the major components and methods for the BD simulation of polymeric NP transport and adhesion to the cell surface. A coarse-grained model of the lysozyme-core/dextran-shell NP of different stiffness introduced in ref. 25 is subjected to confinement due to the endothelial surface. The initial microstructure of NP is a star polymer with 25 arms attached to a core, with each arm modeled by beads of radius a connected through four Kuhn springs in series. In order to model the conformational dynamics of the star polymer, we consider a topology where each bead is connected to other beads through bonds and cross-links modelled *via* pair potentials introduced in ref. 25, see ESI section S1.[†] That is, the NP stiffness in the model was varied by introducing additional harmonic interactions between the beads (cross-links) to create NP models with varying stiffness (models 1–5, see Table 1); see ref. 25 for details including the estimate of the NP stiffness for the different models. The stochastic and inter-bead hydrodynamic forces are included to model the internal segmental motion of these deformable carriers taking into account the hydrodynamic interactions (HI) under physiologically relevant conditions. Brownian dynamics (BD) simulations are carried out to evaluate the effects of the confining boundary and NP stiffness on the NP microstructure and transport properties. We also consider HI between pairs of NPs including long-ranged corrections,²⁶ see ESI section S1.[†]

To include the NP hydrodynamics far away from any boundaries, (*i.e.*, in the bulk), we follow the model and method for deformable NP described in ref. 25 which is based on BD simulations with HI between beads. Since the NP is close to the wall, the velocity streamlines around a bead will be perturbed by the presence of the wall. To accommodate the effect of the wall, we include the hydrodynamic lift due to the wall in the mobility of a bead which depends on its proximity to the wall. Due to the presence of other beads surrounding a given bead we consider a mobility based on contributions of all other beads in

a quasi-2D simulation box that is periodically replicated along directions parallel to the wall, see ESI section S1.1.† Other considerations included in the model are boundary effects to include the effect of the endothelial glycocalyx, margination pressure due to colloidal microparticles introduced at a volume fraction (ϕ), and multivalent adhesion between the antibodies on the functionalized NP and the diffusing cell surface receptors on the endothelial surface. These specific features included in our model are described in ESI section S1.2.† Larger microparticles offer a margination force on the beads. The force due to margination is calculated from a hydrodynamically-derived potential for an effective radius of NP computed through a method based on dynamical density functional theory (DDFT), described in ref. 27 and 28, see ESI section S1.2.† Adhesive interactions between the antibodies on the functionalized NP and the diffusing receptors on the cell surface, as well as interactions between the NP and the endothelial glycocalyx layer are treated according to models reported earlier,^{14–17,29} see ESI section S1.2.† For comparison, the adhesion between functionalized rigid spherical NP and cell surface is also computed using the procedure outlined in ref. 15–17.

Depending on inter-bead positions, radii of beads and viscosity of the solvent, this long-range interaction influences the diffusivities of pair particles approaching each other. Static and dynamic properties quantifying the NP microstructure (including structure factors $S(k)$, radius of gyration, R_g , shape factor, S , deformation and conformational entropy) are computed as functions of the NP stiffness, see ESI section S1.3.† The properties computed from BD simulations such as the microstructure and diffusivity are incorporated into a Fokker–Planck equation framework solved in MATLAB in order to determine the spatial distribution of NP along the distance from the adhering cell surface, see ESI section S1.4.† The detailed parameter sets used in our model are summarized in Table S1 in ESI.† Typical runs for the BD consisted of 10^{12} time steps. The computations for a typical trajectory noted above required 3 CPU-weeks on a single core of an Intel Xeon 2.7 GHz workstation.

2.2. Experimental methods

The polymeric as well as rigid NP synthesis, functionalization, and characterization steps are described in ESI section S2.† ICAM-expressing Chinese hamster ovary cells (CHO cells) were plated and confluent cells were assembled into confocal imaging chambers and flow adapted to mimic endothelium whose actin fibers are aligned to flow as in the human microvasculature at physiological shear stresses of 100 and 500 s^{-1} . Colloidal microparticles (8 μm PS beads) were added to the perfusate in concentrations of $\phi = 0, 0.1$ and 0.3 of the perfusate volume. NPs were introduced into the perfusate and dynamic binding studies were performed using epifluorescence microscopy. A MATLAB program was used to provide fluorescence counts, which were normalized to cell number using cell counts performed on bright-field images obtained in sequence with the fluorescence images at each of the time-points. For a comparison to NP uptake *in vivo*, we quantified endothelial targeting as a function of lung uptake of polymeric and polystyrene (PS) NPs in mice. Lung uptake is representative of endothelial targeting because the lung accounts for roughly 30% of the endothelium *in vivo*.³⁰ To focus on contributions of NP uptake in the pulmonary vasculature that are dependent on the targeting molecules (ICAM1), we report the ratio of the tissue uptake of anti-ICAM coated NP to that of IgG coated NP in lung. While measured, we do

not focus on non-targeted tissues, because the uptake of particles in the main reticuloendothelial system (RES) organ, liver, did not change with variations of the anti-ICAM surface density. Details of the experimental protocols for cell culture and *in vivo* experiments are available in ESI section S2.†

2.3. Model validation in the bulk flow

In an earlier report, our coarse-grained models for the polymeric NP (models 1–5) were examined to explore the effect of shear rate on the NP structure, transport properties and elastic properties of the NP.²⁵ The elastic properties computed for models 1–5 based on the study in ref. 25 are reported in Table 1.

In the calculations, NP under shear deforms and orients along the direction of the applied shear and it was observed that the orientation and deformation under shear are dependent on the shear rate and NP stiffness. The asymmetry in the shape of NP can be quantified by calculation of the elongation index (EI) along the direction of the applied stress, $(L - W)/(L + W)$, where L and W are the long and short axis of the deformed NP. Eigenvalues of the radius of gyration tensor along the major and minor axes of the deformed NP, defined in ESI section S1.3,† are used to calculate L and W (*i.e.* $L = \sqrt{\langle \lambda_1 \rangle}$, and $W = \sqrt{\langle \lambda_2 \rangle}$) Fig. 2(a) shows the calculated EI for polymeric NP model 1 and 5 *vs.* internal stress. We note that the internal stress reported here originates from bead-to-bead interactions and stretching of the bond.²⁵ The higher value of stress in the computational analysis is due to significant crowding which enhances the viscosity. Additionally, while the applied shear stretches the polymeric NP, there is compression in the orthogonal directions causing stress to build up due to steric effects. These factors are explicitly considered in the model and method.²⁵ At high shear flow the soft NP (model 1) undergoes larger deformation and the degree of prolateness increases which manifests as a greater EI. As the shear flow is lowered, the NP EI approaches a small value closer to zero, which corresponds to near a spherical shape. Under the same conditions, the EI values for polymeric NP with larger stiffness (*i.e.*, model 5) are smaller relative to that of model 1. The experimental measurement of EI for the polymeric NP which was performed in high viscosity media is depicted in Fig. 2(b) as reported by ref. 31. The comparison of computed and experimental results in Fig. 2 indicate that model 5 matches the experimental results for the polymeric NP with respect to the dependence of EI *versus* applied stress. Interestingly, the calculated elasticity of model 5 (15 kPa) is close to the experimentally measured value of 67 kPa for the polymeric NP reported recently.³¹ We conclude that model 5 in the computation best represents the properties (elastic modulus or EM and EI *vs.* applied stress) of the polymeric NP utilized in the experiments. Nevertheless, in the ensuing calculations, we report the results for all models because NP stiffness can be modulated experimentally by altering grafting density and/or cross-linking density.

2.4. Model validation of NP margination due to suspended colloidal microparticles

During blood flow, the RBCs occupy the central domain of the vessels and this marginates the smaller particles suspended in blood including the NPs. While colloidal microparticles of similar dimensions to RBC have a different margination characteristic, they do also form a depletion (cell free) layer, albeit of smaller thickness;^{27,28} these recent studies on the colloidal suspensions show a well-defined depletion layer which is estimated around 1.3 μm .

²⁷ The cell culture experiments, described in ESI section S2,[†] were performed using 8 μm PS beads. To keep consistent with the experiments, our computational model for the margination potential is derived using hardspheres of the same diameter.

There have been several studies in the literature on the margination of NPs in the flow.^{19,32–34} Several recent computational and experimental studies, considering RBCs in flow volume show that the size of particles strongly affects the margination propensity.^{19,33,34} Cooley *et al.* carried out computational and experimental studies using PS particles spanning nano- to micro scale sizes with varying shapes in presence *versus* absence of RBC flow³⁴ and modeled the RBC as a membrane with triangulated bead-spring networks.²⁰ Their findings of 2-d simulations indicate that larger micro-particles undergo enhanced margination compared to smaller nano-particles, consistent with previous investigation by 3D simulations. The margination potential, as described in ESI Section S1.2,[†] is computed for 3 different NP diameters and the results are shown in Table 2. Although the suspension in our computational method are modeled as hardspheres to mimic the experimental protocol in ESI section S2,[†] the results of Table 2 show enhancement of margination as a function of particle size which is consistent with the findings of Cooley *et al.*³⁴

3. Results and discussion

3.1. Effect of margination and NP stiffness on NP microstructure and transport properties

Our Brownian dynamics results in the absence of physiologically relevant shear, margination and glycocalyx resistance, *i.e.*, including only the hydrodynamic confinement show that while the extent of deformation is not significantly influenced by NP stiffness, the stiffness and wall hydrodynamic interactions do alter the radius of gyration and the diffusion coefficient.³⁵ Due to the presence of the confining wall, the NP undergoes deformation and as the NP stiffness increases the NP radius of gyration decreases and as the size of the NP decreases the diffusivity of the NP parallel to the wall surface increases, while its perpendicular diffusivity decreases. Similar results were observed when there is no confinement effect.²⁵ Thus, as long as the NP is at a distance $y \gg a$ from the wall it remains spherical for all NP stiffness we considered in Table 1.

NP under the influence of a suspension of colloidal micro-particles particulates experiences an additional margination potential. The time averaged mean-square-displacements (MSDs) along horizontal and perpendicular directions for different NP stiffness in the presence of margination were computed, based on which the horizontal and perpendicular diffusivities were calculated. Compared to NP perpendicular MSD where larger hindrance forces are, there is little effect on the horizontal component of MSD. This contrast is clearly evident in the calculated diffusivity (Fig. 3(a)). The perpendicular self diffusivity in the presence of margination, approaches a very small value due to the effect of confinement. The diffusivity decreases with proximity to the wall, however, it must be noted that the transient response of the NP is slow and for all NP stiffness, the diffusivity plateaus at large correlation time where the perpendicular diffusivity is zero.

In the presence of margination, the NP undergoes deformation which is quantified through the calculation of the eigenvalues of the radius of gyration tensor (see ESI section S1.3[†]).

The radii of gyration in the horizontal and perpendicular directions are defined by, $R_{gh} = \sqrt{\lambda_1 + \lambda_3}$ and $R_{gp} = \sqrt{\lambda_2}$, respectively. These quantities are plotted in Fig. 3(b). The NPs for smaller NP stiffness undergo more deformation than that of larger NP stiffness. In Fig. 3(b), the horizontal and perpendicular radii of gyration are plotted for various NP stiffness (models 1–5). We find the difference between horizontal and perpendicular radius of gyration is highest for model 1 (NP of smallest stiffness). With increasing NP stiffness, the perpendicular and horizontal radii of gyration approach each other. In the table in Fig. 3(b), we provide the values of the EI which are computed from the eigenvalues of the radius of gyration tensor along the major and minor axes of the deformed NP. For all models 1–5 considered in this study, the NP takes a shape that is similar to an oblate spheroid, and with increasing NP stiffness, the degree of oblateness becomes smaller. In conclusion, margination induces extreme anisotropy in polymeric NP and deforms it towards oblate shape, which significantly impacts the mobility perpendicular to the wall, and this effect is more pronounced at low NP stiffness.

To investigate the alterations to the microstructure beyond changes in R_g , we compute the structure factor, see ESI section S1.3.† We follow³⁶ and rescale k_1 with R_{gh} for $kR_g \ll 1$ and expand the $S(k)$ as: $S(k_1) \approx N \left(1 - \frac{1}{3} k_1^2 \langle R_{gh}^2 \rangle + \dots \right)$. In Fig. 3(c) k_1 is rescaled with $\frac{2\pi}{R_{gh}}$, *i.e.*,

$\bar{k}_1 = \frac{k_1 R_{gh}}{2\pi}$. The structure factors for various models of NP stiffness collapse into a single master curve for $\bar{k}_1 \ll 1$. The wave vector corresponding to length scale larger than R_{gh} , *i.e.*, $\bar{k}_1 \gg 1$, indicates a terrace behavior which is also reported for star polymers.³⁷ The differences in the scaled structure factors for $\bar{k}_1 > 1$ indicates the packing of beads and the internal structure of the NP due to margination and the NP stiffness. We find $S\left(k = \frac{2\pi}{R_g}\right)$ indicates the overall compressibility of the NP (under margination) which increases with decreasing NP stiffness.

The snapshots in Fig. 3(d), (middle panel, left inset), shows the NP for model 3 near wall in absence of margination. There is no visual deformation and it is spherical. The same NP in the presence of margination deforms significantly as shown in Fig. 3(d), (middle panel, right inset). To further quantify the effect of margination and NP stiffness on NP microstructure, the bead positions are collected and following,³⁸ irregular Voronoi tessellation around the beads of the NP is performed to allocate fluid volume element share to each bead. We calculate the volume and area of Voronoi elements for each bead and compare the margination effect of models 1, 3, 5 as shown in Fig. 3(d and e). Unlike the irregular Voronoi tessellation in bulk,²⁵ where the distribution on volume shows bimodal characteristic and shifts with increasing the NP stiffness, the Voronoi tessellated area (Fig. 3(d)) and volume (Fig. 3(e)) provides information about the effect of margination and distribution of each Voronoi near the wall is perturbed. However, it is evident that the distribution of area and volume for model 1, which is more deformed under margination, shows larger fractions at larger Voronoi tessellated areas and volume upon margination, indicating that NP stiffness significantly influences the microstructure for NPs under margination.

3.2. Effect of NP binding to the endothelial surface

NP binding to the endothelial surface is mediated through multivalent receptor–ligand interactions described in ESI section S1.2.† In Fig. 4(a), we plot the R_g for bound NP as a function of stiffness; the values of EI are also provided in form of a table, from which it is evident that for low NP stiffness the NP suffers from large deformation and for NP stiffness the NP resists deformation. As a comparison, we plot the EI values for the different models when NP is under wall-confinement alone, subject to margination alone, and subject to adhesion alone and find that the deformation of the NP (as measured by EI) under adhesion to be very close to that observed in the presence of margination (Fig. 4(b)).

In Fig. 4(c), $S(k_1)$ is plotted against k_1 . For k_1 of the order of $\frac{2\pi}{R_{gh}}$, $S(k_1)$ shows a collapse of data sets corresponding to various stiffness. The structure factor for $k_1 \gg \frac{2\pi}{R_{gh}}$ shows the internal structure of the NP, while for $k_1 \ll \frac{2\pi}{R_{gh}}$ the value of $\frac{S(k_1)}{S(0)}$ demonstrates a plateau similar to cases without tethering. The irregular Voronoi tessellated area and volume for NP upon binding depicted in Fig. 4(d and e) also show distributions similar to those without binding but in the presence of margination. In summary, our calculations without margination or binding show that the NP for all stiffness assumes a near-spherical shape, (EI ≈ 0). However, in the presence of margination and binding, the NP undergoes extreme deformation and assumes an oblate shape, as shown in Fig. 4(a). It must be noted that the shape of the NP discussed here can also be influenced by the deformation of the NP under shear flow as noted in Fig. 2 and discussed in an earlier study;²⁵ comparing the calculated EI values, we note that for physiological shear, the shear-induced deformation of NP is comparable to the NP deformation under margination or binding.

3.3. Integration of multiple effects on spatial distribution of NP

Our analyses and results thus far have delineated the effects of wall-hydrodynamic interactions, NP adhesion, and the effects of margination on NP microstructure and transport properties. The obvious question is whether and how NP stiffness and deformability influences targeted binding. To assess the role of aforementioned factors on the NP concentration close to the endothelial surface, we integrate the combined effects of NP stiffness, margination, binding, and glycocalyx, into a combined Fokker–Planck framework for convection-diffusion processes.³⁹ We perform a systematic analysis of the obtained simulation data by solving the 1-dimensional Fokker–Planck equation to calculate and compare the NP spatial distribution near the wall-adhesion regime with experimental observations. In its formulation, we consider the effect of shear flow, an inhomogeneous diffusion coefficient in the direction normal to the flow due to wall mediated influence on the mobility, and the effect of margination due to larger suspended colloidal microparticles. The resultant concentration field of deformable NPs can be written as:

$$\frac{dc(y,t)}{dt} = \frac{d}{dy} \left(D_{\perp}(y) \frac{dc(y,t)}{dy} \right) - \frac{d}{dy} (u_{\text{lift}}(y)c(y,t)) - \frac{d}{dy} (u_{\text{avg}}(y)c(y,t)). \quad (1)$$

The left hand side represents local concentration changes of NP and the right hand side represents transport due to diffusion and convection. $u_{\text{lift}}(y)$ is a drift velocity that is directly proportional to the lift force due to shear flow, *i.e.*, $u_{\text{lift}}(y) = \frac{D_{\perp}}{k_B T} F_{\text{lift}}(y)$ ²⁷ with $D_{\perp}$ being the diffusion coefficient in the direction perpendicular to the surface which is approximated as $D_0 \beta_{\perp}$, where $\beta_{\perp}$ is defined in ESI section S1.1.[†]

In the calculation of the lift force, the r_s must be modified for non-spherical NPs, *i.e.*, $r_s = r_{\text{eq}} = (R_{g11} R_{g22} R_{g33})^{1/3}$, where r_{eq} is the radius of a sphere having the same volume as the deformable NP with oblate or prolate shape. However, beyond the shape anisotropy, we modified the lift force considering the fact that the NP stiffness influences the effective packing and consequently the porosity of the polymeric NP. This effect is incorporated by directly computing the lift on each bead and then summing over all beads by multiplying the

lift force equation by the factor $F_r = \frac{\sum_{i=1}^N F_{\text{lift},b}^i}{F_{\text{lift,eq}}}$, which is the ratio of the collective lift

force acting on all the beads of NP to the lift force acting on a spherical NP at the center of mass of all beads with $r_s = r_{\text{eq}}$ being the equivalent radius of gyration of the deformable NP. Expressing F_r as the stated ratio allows us to consider this effect as a spatially dependent factor where the distance is calculated from the center of the equivalent sphere and the confining wall. We find that the value of F_r changes from 0.007 for model 1 to 0.18 for model 5, suggesting that NPs with higher stiffness possess a smaller porosity than those with a lower stiffness.

The term $u_{\text{avg}}(y)$ in the right hand side is the average velocity in response to physiological factors including the margination due to suspended colloidal microparticles and the glycocalyx forces, *i.e.*, $u_{\text{avg}}(y) = M_{\perp} F_{\text{avg}}(y)$ with $M_{\perp} = D_{\perp}/k_B T$ being the mobility of the NP in the direction perpendicular to the wall. The solution procedure is outlined in ESI section S1.4[†] and the plots in that section (Fig. S2, ESI[†]) show the force exerted on the beads of the NP due to shear flow and physiological factors including glycocalyx and margination. The potential energy can be obtained from the applied forces which are depicted in the inset of Fig. S2.[†] The force and the corresponding potential are found to be varying with distance from the wall as well as with the stiffness of NPs. In Table 3 we provide the values of the equivalent radius of gyration (*i.e.*, r_{eq}), which clearly increases with increase in shear stress, which causes the effective margination potential between the volume fraction of hardsphere suspension and NP to be sheardependent as noted by the value of the shear enhancement due to margination factor (SEMF); the SEMF is change in value of the margination potential for the interaction of the NP with hardsphere suspension. This effect is more pronounced for model 1, leading to an enhancement in the relative binding as evidenced by the reported values of the SEMF in Table 3.

Fig. 5(a and b) show the numerical solutions of the Fokker–Planck equation (see ESI section S1.4[†]) for relative concentrations of NP close to the wall at shear stresses of 0 (top panel) and 500 s⁻¹ (bottom panel) and with ϕ level of 0 and 0.15 for different NP stiffness. This range corresponds to the flow rate in capillaries and micro-capillaries. We also note that the relative concentration is computed based on the hydrodynamic radius of NP to consider the NP size in the context of its environment.

Our calculations show that increasing ϕ as well as increasing shear enhances margination for polymeric NPs of all stiffness we investigated. However, margination for PS NPs only increase with ϕ but not shear. The difference in relative concentration between 0 and 0.15 volume fractions without flow (0 s^{-1}) and for all NP stiffness is because of the volume fraction effect which is a significant factor in the NP margination. This effect is captured in the definition of margination potential (see ESI section S1.2†) which is different for zero and non-zero ϕ .

3.4. Comparison to *in vitro* cellular and *in vivo* experiments

In the cellular experiments, to demonstrate a clear sensitivity to the mechanics of deformable NPs, 200 nm polystyrene (PS) beads and 192 nm dextran–lysosome polymeric NPs displaying identical anti-ICAM antibody surface concentration to target CHO–ICAM cells under shear at 100 or 500 s^{-1} were utilized in flow seeded with $8 \mu\text{m}$ beads at volume fractions of 0, 0.2 and 0.3 to influence NP margination. The summary of experimental results of NP binding under different shear, ϕ , and time-points is provided in Fig. 5(c). The results of experiment demonstrate that compared to rigid PS beads, the binding of deformable NPs is enhanced at high shear stress with a non-zero ϕ . The enhancement in binding of polymeric NPs upon introduction of ϕ is quantitatively consistent between the model predictions and experimental results suggesting that the complex interplay between collision interaction and NP deformability is responsible for this effect. The insensitivity of the binding of PS NP to shear is also quantitatively consistent between model and experiments. Finally, the model predictions show that the enhancement due to shear and ϕ are NP stiffness dependent, therefore identifying NP stiffness as an important design factor for tuning NP margination.

We wondered if the enhancement of polymeric NP binding under physiological fields such as shear and volume fraction of particulate suspension extends to *in vivo* studies. Based on the biodistribution studies of polymeric and polystyrene NP in mice (see ESI section S2.3†), we quantified the tissue level (percent of injected dose per gram of tissue) in lung for both polymeric and PS NPs. These results, shown in Fig. 5(d), suggest that the enhancement factor in the case of polymeric NP is larger than that for the polystyrene NP suggesting an increased efficacy in tissue uptake for polymeric NPs. In fact the relative uptake which is the ratio of percent injected dose per gram of anti-ICAM functionalized NP to that functionalized with anti IgG is the same for both types of NPs indicating that the binding enhancement is not due to the antibody functionalization presence, but due to hydrodynamic effects impacting the flexible NPs.

3.5. Effect of NP stiffness on NP avidity to cell surface

Thus far the results focussed on the effect of NP stiffness on NP margination to the vessel wall. Even though the experimental results suggest that the enhancement in binding is primarily as a result of the margination effect, we explored the effect of NP stiffness on the binding avidity mediated by multivalent interactions. First, we examined the NP avidity through the statistics of multivalent interactions, represented by the numbers of simultaneous bonds formed between the ligands on the NP and the receptors on the cell surface. Fig. 6(a–d) show the probability distribution of multivalent binding interactions, computed at

equilibrium for a functionalized NP in contact with a flat substrate. The number of multivalent bonds (m) formed by the NP is a strong function of the NP stiffness, and it may be seen that as the NP stiffness increases, it stabilizes less number of multivalent bonds.

In multivalent interactions between the NP and the cell surface mediated by receptor–ligand binding, the total enthalpy is often compensated by the entropy loss upon binding. That is, multivalent adhesive interactions while lowering the enthalpy also cause loss of configurational entropy of the polymeric NP and this competition will dictate the overall efficiency of binding. In Fig. 6(e), the quasi-harmonic vibrational entropy of the NP is plotted for various NP models, which reflects that a NP with higher stiffness resists deformation more and as a result experiences less vibrational entropy of the beads motion. We find the adhered NP shows less vibrational entropy while the unbound NP in the bulk fluid shows larger vibrational entropy. We also observe more reduction in the vibrational entropy of tethered NPs in the presence of margination due to the suspended colloidal microparticles and the glycocalyx, as the additional confining potential modulates the interaction between the NP and the cell surface. It is worthwhile to note that the multivalent interactions between receptors and ligands are due to both entropy (S) and enthalpy (H) changes upon binding.

The entropy–enthalpy compensation in the case of flexible NP arises in interesting ways mediated through multivalent receptor–ligand binding interactions. To calculate the binding free energy (*i.e.*, $F = H - TS$), we consider the binding energy averaged over all possible bonding configurations of the bound NP (to compute H). Changes in the conformational entropy of flexible NPs and those in the translational entropy of mobile receptors, yield the total entropy change upon binding, which will also have significant contributions to the overall binding free energy. The conformational entropy of NPs is calculated from the quasiharmonic frequencies of the NP motion (see ESI section S1.3†) and the translational entropy of N_{ant} receptors is computed through the Sackur–Tetrode equation.⁴⁰ The Sackur–Tetrode equation for molecules with mass m is given by

$$S_{\text{transl}} = k_B \left[\sum_{i=1}^{N_{\text{ant}}} \ln \left(\frac{2\pi m k_B T}{N_{\text{ant}} h^2} \sigma_{xi} \sigma_{yi} \right) + 2 \right].$$

Here h is the Planck constant and σ_{xi} and σ_{yi} are

the principal root-mean-square fluctuations for the center of mass of a receptor. Fig. 7(a–c) shows plots of the enthalpy, entropy, and free energy of binding per receptor–ligand bond as a function of NP stiffness. Panel (a) shows that NPs with more flexibility (*i.e.* lower stiffness) can permit optimal configurations for binding interactions that do not strain the bonds, and as a result have the most favorable enthalpy. However, more flexible NPs also show higher entropy loss upon binding interactions (Fig. 7(b)). Strong avidity would result if the gain in enthalpy significantly exceeds the loss of entropy.⁴¹ The computed values of the free energy are depicted in (Fig. 7(c)). The overall avidity is determined by the total free energy which is the product of free energy per bond and the multivalency. We depict the results for total enthalpy, entropy, and free energy in Fig. 7(d–f). Our results confirm that the enthalpy–entropy compensation in the case of polymeric NPs is substantially more pronounced than that for rigid NP. Intriguingly, our results confirm that the overall free energy of binding for model 5 (our model for the experimentally realized NP) is about the same (within statistical error) to that of the rigid PS NP confirming our conclusion in

sections 3.3 and 3.4 that the enhancement in cellular and *in vivo* targeting was due to primarily a hydrodynamic effect. However, notably, our model makes the new prediction that for ultra-soft NPs (models 1–3), avidity due to multivalent binding can be significantly more enhanced as reflected by the lower values of the total binding free energy.

4. Discussion

Using coarse-grained Brownian dynamics simulations to compute the structural and dynamic properties of a deformable core–shell polymer based NP, we explored the effects of wall-confinement, physiological factors including the glycocalyx layer, margination due to colloidal microparticles, and NP synthesis factors such as the NP stiffness and binding on NP microstructure (shape, structure factor, deformation) and transport properties (anisotropic diffusion coefficient), and NP margination (*i.e.*, radial distribution of NP concentration). Due to the presence of the endothelial surface and margination due to colloidal microparticles, the NP undergoes deformation. We quantify the effect of deformation by comparing the horizontal and perpendicular diffusivities and the diagonal elements of the radius of gyration (R_g) tensor of the NP. Upon binding to the endothelial surface, intra-NP segmental dynamics are hindered and the entropy of the NP changes. We calculated the NP's configurational entropy, and structure factors to quantify the effects of NP stiffness, wall-confinement, and hydrodynamic interactions (HI) on transport properties such as diffusion coefficient.

The effects of margination due to suspended particulates and interaction with glycocalyx is more pronounced as it alters the accessible area and volume distributions associated with the polymeric segments of the NP for all NP models (see Fig. 3). This translates into significant anisotropy in the NP structure and shape, and significant influence on the anisotropic transport properties such as the perpendicular diffusion coefficient (Fig. 3). The effects of anisotropy are large for low NP stiffness, but decrease with increasing NP stiffness.

We also investigated the NP microstructure in the presence of the above hydrodynamic effects as well as in the presence of multivalent binding interactions. Upon NP binding to the endothelial surface, the shape anisotropy, area and volume distributions of polymeric segments showed similar trends with increasing NP stiffness (Fig. 4). We hypothesized that the change in microstructure upon hydrodynamic and physiological interactions will impact the conformational entropy associated with the intra-NP segmental chain relaxation. To test this hypothesis, we computed the conformational entropy of the polymeric NP. Our results (Fig. 6) showed that the conformational entropy of the free NP decreased with increasing NP stiffness, but the effect of binding alone, and binding in the presence of margination and glycocalyx is to further decrease the conformational entropy associated with chain segmental relaxation. While the above analyses centered on Brownian dynamics was sufficient to quantify the effects of HI, physiological forces such as margination, glycocalyx, and binding, and design factors such as NP stiffness on NP microstructure, we sought to additionally investigate NP spatial distribution in blood vessels under the influence of the physiological and hydrodynamic forces discussed above. Therefore, we developed an integrative framework to compute the spatial concentration of NP by solving a 1-dimensional Fokker–Planck equation with external forces and spatially inhomogeneous

mobilities derived from our Brownian dynamics results. Our results for NP distribution close to the wall in the presence and absence of shear and in the presence and absence of volume fraction of hardsphere suspension (ϕ) computed from the solutions to the Fokker–Planck equation are found to be in good agreement with those determined experimentally. In particular, the NP distribution close to the wall is significantly influenced by the ϕ , and this effect is more pronounced for low NP stiffness compared to high NP stiffness or rigid polystyrene NP. Our analysis of the enthalpy, entropy, and free energy of binding revealed that for the model corresponding to the experimentally realized polymeric NP, enhancement in targeting due to shear and ϕ can be attributed primarily to hydrodynamic effects and not due to multivalent binding. In contrast, for the ultrasoft polymeric NP models, our analysis revealed that multivalent binding can lead to significant enhancement to the overall avidity.

5. Conclusion

In summary, NP targeting to the endothelial cell surface and in the presence of physiological factors allows for a tunable exploration of binding by optimizing NP stiffness (experimentally tuned by either changing grafting density or crosslinking density in the polymer phase). But the mechanisms that govern the exact binding behavior are a result of delicate and subtle balance between hydrodynamic interactions, physiological (glycocalyx, binding enthalpic and entropic, and volume fraction) forces, which dictate deformation and microstructure, hydrodynamic lift, and consequently the spatial distribution of NP. Our results show that new classes of flexible NPs are governed by design principles that are quite distinct from rigid and regular-shaped NPs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
A schematic of the computational components and methods for the BD simulation of flexible NP transport and binding to the cell surface.

Fig. 2.
Elongation indices extracted from (a) simulation and (b) experiments for lysozyme-dextran NPs.

Fig. 3.

(a) Horizontal and perpendicular self-diffusivity of NP in presence of margination. (b) Radius of gyration along '11'-'33' plane (horizontal to surface) ($\square$) and '22' direction (perpendicular to surface) ($\circ$). The inset shows the elongation index (EI) of NPs. (c) static structure factor $S(k_1)$ in the presence of margination. (d)–(e) Distributions of Voronoi tessellated areas ($P(A)$) and volumes ($P(V)$) for different NP models (stiffness) in presence of margination. The inset of (d) depicts the snapshots from simulation which show deformation of NP near wall without margination and with margination. Insets in (e) show side views of irregular Voronoi tessellation around the beads of the NP; here the gray spheres represent the beads and the purple lines represent the skeleton of the Voronoi tessellated lattice.

Fig. 4.

(a) Radius of gyration along ‘11’ or ‘33’ plane (horizontal to endothelial surface) ($\square$) and ‘22’ direction (perpendicular to endothelial surface) ($\circ$) and (b) comparison of EI of polymeric NP in presence of confinement, margination and binding. (c) Static structure factor $S(k_1)$ upon binding of the polymeric NP to the endothelial surface; panel (a) also shows the elongation index (EI) of NPs. (d)–(e) Distributions of Voronoi tessellated areas ($P(A)$) and volumes ($P(V)$) for different NP models (stiffness) in presence of binding. Insets in (e) show irregular Voronoi tessellation around the beads of the NP; here the gray spheres represent the beads and the purple lines represent the skeleton of the Voronoi tessellated lattice.

Fig. 5.

(a)–(b) Results of numerical solution for NP relative concentration near the wall *versus* NP stiffness, hardsphere volume fraction (ϕ) and shear flow. The concentration at the wall is reported as the average concentration in the first 100 nm adjacent to the wall surface. Panel (a) depicts the relative concentration in the absence of shear flow, whereas (b) represents the concentration at shear stress of 500 s^{-1} which enhances the concentration of deformable NP at non-zero ϕ for polymeric NP and reduces the concentration of PS NP near the cell. The NP concentration is scaled to the concentration far from the wall, *i.e.*, $C(y=62a) = 1 \text{ nMol}$. (c) Results of experiment for NP binding *versus* ϕ and shear flow. The binding is scaled to PS binding at 100 s^{-1} . (d) *In vivo* result for uptake of NP in lung.

Fig. 6. (a)–(d) The probability density of number of simultaneous ligand–receptor bonds for a polymeric NP, with $N_{\text{ant}} = 162/\text{NP}$, bound to the cell membrane. (e) Quasi-harmonic entropy: comparison between bulk and near wall.

Fig. 7.

Plots of enthalpy, entropy, and free energy of binding as a function of NP stiffness. Panels (a)–(c) show enthalpy ($H_{\text{perbond}}/k_B T$), entropy (S_{perbond}/k_B), and free energy ($F_{\text{perbond}}/k_B T$) per receptor-ligand bond and panels (d)–(f) show total enthalpy ($H/k_B T$), entropy (S/k_B), and free energy ($F/k_B T$) of binding.

Table 1

Computed stiffness of the NP

Model	1	2	3	4	5
EM (kPa)	0.43	1.44	4.02	7.6	15.02

Table 2

Effect of particle effective size on the margination potential (ϕ_{marg}). The margination potential is reported as the average margination potential over an interval [80 100] nm near wall region

Effective diameter of NP (D_{eff})	100 nm	500 nm	2 μm
$\phi_{\text{marg}}/\phi_{\text{marg}} (@ D_{\text{eff}} = 100 \text{ nm})$	1	4.6	167

Table 3

Effect of shear flow on the extent of deformation and margination potential determined using data from Sarkar *et al.*²⁵

Model	1	2	3	5
r_{eq}/a (@ $\tau = 0.3$ dyne per cm^2)	8.23	6.3	5.39	4.56
r_{eq}/a (@ $\tau = 2$ dyne per cm^2)	11.34	7.63	5.7	4.6
$\text{SEMF} = \phi_{\text{marg}} (@ \tau = 2) / \phi_{\text{marg}} (@ \tau = 0.3)$	1.15	1.064	1.015	1.002