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Multiscale Modeling of Functionalized Nanocarriers in Targeted Drug Delivery

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Abstract

Targeted drug delivery using functionalized nanocarriers (NCs) is a strategy in therapeutic and diagnostic applications. In this paper we review the recent development of models at multiple length and time scales and their applications to targeting of antibody functionalized nanocarriers to antigens (receptors) on the endothelial cell (EC) surface. Our mesoscale (100 nm–1 μ m) model is based on phenomenological interaction potentials for receptor–ligand interactions, receptor–flexure and resistance offered by glycocalyx. All free parameters are either directly determined from independent biophysical and cell biology experiments or estimated using molecular dynamics simulations. We employ a Metropolis Monte Carlo (MC) strategy in conjunction with the weighted histogram analysis method (WHAM) to compute the free energy landscape (potential of mean force or PMF) associated with the multivalent antigen–antibody interactions mediating the NC binding to EC. The binding affinities (association constants) are then derived from the PMF by computing absolute binding free energy of binding of NC to EC, taking into account the relevant translational and rotational entropy losses of NC and the receptors. We validate our model predictions by comparing the computed binding affinities and PMF to a wide range of experimental measurements, including *in vitro* cell culture, *in vivo* endothelial targeting, atomic force microscopy (AFM), and flow chamber experiments. The model predictions agree closely and quantitatively with all types experimental measurements. On this basis, we conclude that our computational protocol represents a quantitative and predictive approach for model driven design and optimization of functionalized NCs in targeted vascular drug delivery.

Keywords

Monte Carlo; absolute binding free energy; multivalent interactions; flow enhanced adhesion; glycocalyx; cell membrane; molecular dynamics

1. INTRODUCTION

According to a recent report [1], around 10 million people in the world were newly diagnosed with cancer in 2009. Targeted drug delivery using functionalized nanocarriers (i.e., NCs coated with specific targeting ligands) has been clinically identified as a promising approach in both therapeutic and diagnostic applications in cancer treatments. However, targeting of NCs to endothelium remains an important design challenge in pharmacological and biomedical sciences. The use of functionalized NCs offers a wide range of tunable design parameters (size, shape, type, method of functionalization, *etc.*). In principle, massive experimentation in a high-dimensional tunable parameter space is needed to achieve optimal treatment in drug delivery applications [2]. Therefore a physiologically sound and computationally tractable model, which is both quantitative and predictive, can prove to be crucial in the rational design of the drug delivery using functionalized NCs.

However, the design of functionalized NCs faces many challenges due to several inherent complexities of the underlying interactions: (i) molecular and geometric parameters surrounding receptor-ligand interactions and NCs; (ii) lack of accurate characterization of hydrodynamics; (iii) physico-chemical barriers for NC uptake/arrest; (iv) uncertainty in targeting environment. Binding affinity (K_a) is a direct measure of the efficiency of targeting. Recently Muro *et al.* [3] have experimentally studied the binding affinity of functionalized NCs to ICAM-1-expressing EC surface in which they report that the binding affinity of anti-ICAM-1 coated NC to EC could be two orders of magnitude higher than that of anti-ICAM-1 binding to ICAM-1. Haun and Hammer [4] have investigated the kinetic rate constants of attachment and detachment of 210 nm NCs as a function of receptor density, ligand density on surface, and flow shear rate, and identified a time dependence of the detachment rate due to multivalent binding. Ho *et al.* [5] studied the effect of antibody surface coverage on equilibrium binding constants by measuring fractional coverage of bound NCs (80 nm in diameter) as a function of NC concentrations; by fitting their experimental data, they observed linear dependence of K_a on antibody surface coverage, leading them to conclude that the system was dominated by monovalent interactions. Despite such previous studies on NC binding, a comprehensive understanding of the determinants of NC binding to EC is still limited.

From a modeling perspective, numerical estimation of the binding affinities (or the absolute free energy of binding) is a significant challenge. First, the characterization of the underlying parameters of the model from independent experiments is non-trivial due to the complexity of the system; some parameters are even unavailable in the literature, therefore calling for their de-novo estimation using molecular dynamics simulations. Secondly, the calculation of binding affinities necessarily involves the determination of absolute binding free energies, which requires extensive sampling over conformational degrees of freedom as well as the determination of various (translational and rotational) entropy changes upon binding. Recently, inspired by the framework of Woo and Roux [6] on calculation binding affinity between a flexible ligand and a receptor, we have developed a mesoscale model to compute the absolute binding free energy of binding of NC to EC. The mesoscale model pertains to spherical NC functionalized with antibodies and binding to antigens on EC surface amidst fluid flow and glycocalyx (see Fig. 1). We discuss in the following sections, how the physical and geometrical parameters for the mesoscale model and interactions are derived from the independent experiments; this includes the properties of the flow, NC size and shape, antibody surface coverage on NC, glycocalyx resistance, and antigen-antibody interaction. In order to estimate de novo the only parameter unavailable in the literature, namely the flexural rigidity of the antigen, we perform detailed molecular dynamics (MD) simulations and fluctuation analysis. Then, using the parameters derived from experiments and MD in our mesoscale model, we compute the binding affinities of NC to EC (described

below) and compare the model predictions with experimental data. Using this model, we have investigated the effects from various tunable and design parameters under physiological conditions and compared model predictions quantitatively with corresponding experimental measurements. In all cases investigated, the quantitative model predictions agreed remarkably well with experiments, which provide a comprehensive validation of our approach. In closing, we describe some new experiments are designed based on our model predictions as well as promising avenues for extending the model as a promising numerical tool for optimal design of functionalized NCs in targeted drug delivery.

2. MODELING METHODS

2.1. Mesoscale Model Implementation

As shown in Fig. 1, we model the NC as a rigid sphere with uniformly distributed antibodies on its surface. The interactions between NC and cell surface are mimicked by the interactions between antibodies and antigens (ICAM-1s) on a flat surface. The interactions are considered through the Bell model [7], i.e., through the reaction free energy: $\Delta G_r(d) = \Delta G_0 + 0.5kd^2$, where d represents the distance between the reaction sites of the interacting antibody and antigen, ΔG_0 is the free energy change at equilibrium state ($d = 0$) and k is the interaction bond force constant.

We also account for the antigens' flexure by allowing them to bend and rotate in θ and ϕ space (see Fig. 1). Under the assumption of small flexural deformations, we model the flexure of an antigen as bending a beam from equilibrium (upright) position, thus the bending energy due to flexure can be calculated as (see Ref. [8] for details):

$$\Delta G_f = \left(2EI/L^3\right)y_L^2 \quad (1)$$

where EI is the flexural rigidity of antigens, L represents the antigen length and y_L is the distance of the antigen tip from its equilibrium upright position. For small deformations, $y_L \approx L\theta$, therefore the bending energy can be expressed as:

$$\Delta G_f(\theta) = (2EI/L)\theta^2 \quad (2)$$

Clearly the antigen flexural movement is highly orientation dependent. Hence, a configurational-biased sampling technique [9] is implemented in our model to improve the efficiency of sampling of the configurations of flexural movement. This sampling procedure obeys detailed balance and can be described in five steps as:

- (i) We first randomly select one antibody and one antigen, which are close enough to form a bond.
- (ii) Then we randomly generate 100 trial orientations $\{\mathbf{t}_1, \mathbf{t}_2, \dots, \mathbf{t}_{100}\}$, in which $\mathbf{t}_i = (\theta_i, \phi_i, b_i)$, $\theta_i = \arccos(r_i)$ is a random angle between 0 and $\pi/2$ and r_i is a uniformly distributed random number between 0 and 1, ϕ_i is a random angle uniformly distributed between 0 and 2π , b_i is a random integer between 0 (break a bond) and 1 (form a bond). For each trial $\mathbf{t}_i$, we calculate the bond energy $U^{or}(\mathbf{t}_i) = b_i(\Delta G_r(d_i) + \Delta G_f(\theta_i))$.
- (iii) The Rosenbluth factor[9] is then calculated as:

$$W_n = \sum_{j=1}^{100} \exp \left[-\beta U^{or}(\mathbf{t}_j) \right], \quad (3)$$

and then we select one trial $\mathbf{t}_n$ out of the 100 trial trials with probability

$$p(\mathbf{t}_n) = \frac{\exp \left[-\beta U^{or}(\mathbf{t}_n) \right]}{W_n}.$$

- (iv) We then calculate the old bond energy or $U^{or}(\mathbf{t}_o)$ and generate another 99 trial orientations, based on which we determine:

$$W_o = \exp \left[-\beta U^{or}(\mathbf{t}_o) \right] + \sum_{j=2}^{100} \exp \left[-\beta U^{or}(\mathbf{t}_j) \right]. \quad (4)$$

- (v)

Finally we accept the selection of $\mathbf{t}_n$ with the probability of $\min \left(1, \frac{W_n}{W_o} \right)$

To include the effect of flow, a simple one-dimensional shear flow with shear rate of S is introduced as shown in Fig. 1. We also account for the glycocalyx, which usually exists in *in vivo* environments, by adding a layer of height h above the cell surface (see Fig. 1). The flow inside the glycocalyx layer is obtained by solving the Brinkman's equation without any pressure gradient, i.e.,

$$\frac{u}{K_p} - \frac{\partial^2 u}{\partial z^2} = 0, \quad (5)$$

in which the glycocalyx is modeled as a porous medium and K_p is its Darcy permeability. Following the procedures in Ref. [10], K_p is determined by solving two-dimensional Stokes flow past an array of cylindrical fibers. The approximate expression is

$$\frac{K_p}{r_f^2} = \frac{\ln(c^{-1/2}) - 0.745 + c - c^2/4}{4c}, \quad (6)$$

where the fiber volume fraction $c = 2\pi r_f^2 / \sqrt{3} (2r_f + \Delta)^2$, the fiber radius $r_f = 6$ nm and fiber gap $\Delta = 8$ nm. By solving the Brinkman equation with the resulting value of K_p we found that the flow inside the glycocalyx layer is very small, which is consistent with the observations of *in vivo* experiments [11, 12]. Therefore, as indicated in Fig. 1, we neglect the flow inside the glycocalyx ($u = 0$ at $z \leq h$). The flow induced drag F_x and torque T_y are calculated by solving the steady-state Stokes equation for the shear flow past a sphere near a surface:

$$\begin{cases} \nabla p = \mu \nabla^2 \mathbf{u} \\ \nabla \cdot \mathbf{u} = 0 \end{cases}, \quad (7)$$

with the no-slip boundary conditions and inlet flow condition of $u = S(z - h)$, where μ is the dynamic viscosity of the fluid and S is the shear rate. In our simulations, we set $\mu = 0.001$

$\text{kgm}^{-1}\text{s}^{-1}$ (dynamic viscosity of water). The Stokes equations are solved using the commercial software COMSOL and the flow induced drag F_x and torque T_y at discrete vertical points are computed through integration of the force and torque on NC surface. The calculated drag and torque are consistent with theoretical data from Goldmann *et al.* [13] when the sphere is close to the surface.

When the NC approaches to the cell surface, the binding is significantly impacted by the presence of the glycocalyx layer, especially in *in vivo* conditions. The glycocalyx layer usually extends hundreds of nanometers above the cell surface as described in many different experiments [12]. To account for the normal resistance of the glycocalyx, we add a harmonic potential $1/2k_{glyx}H^2$ per unit differential area, where H is the penetration depth of the NC into glycocalyx as indicated in Fig. 1 and k_{glyx} is the glycocalyx stiffness. Based on different models, the k_{glyx} can be assumed constant or linearly increasing with H and the values are extracted by fitting NC binding data with and without glycocalyx obtained in *in vivo* experiments [14].

Regular Metropolis Monte Carlo (MC) steps are employed for: (i) bond formation/breaking, (ii) NC translation and rotation, and (iii) antigen translation. Move (i) is selected randomly with a probability of 50%, and in the remaining 50%, the NC translation, rotation, and antigen translation are selected randomly with probability of $0.5N_{ab}/N_t$, $0.5N_{ab}/N_t$ and $(N_t - N_{ab})/N_t$ respectively; N_t is the combined total number of antibodies (N_{ab}) and antigen molecules. The simulations are run in parallel on four processors with different realizations of the same physical system. An adaptive step size for NC translation/rotation and antigen diffusion is implemented to ensure a Metropolis acceptance rate of 50%.

2.2. Calculation of the Absolute Binding Free Energy

Generally for binding of ligands (**L**) (or NC) to receptors (**R**) the binding process can be described as: $\mathbf{L} + \mathbf{R} \leftrightarrow \mathbf{LR}$, where **LR** is the ligand and receptor in binding state. At thermodynamic equilibrium, the binding affinity (or association constant) K_a is defined as:

$$K_a = \frac{[\mathbf{LR}]}{[\mathbf{L}][\mathbf{R}]} = \frac{p_1[\mathbf{R}]_{tot}}{[\mathbf{L}]p_0[\mathbf{R}]_{tot}} = \frac{1}{[\mathbf{L}]} \times \frac{p_1}{p_0}. \quad (8)$$

Here **[L]**, **[R]** and **[LR]** as the fraction of receptors with no ligand and one ligand bound respectively, so that **[R]** and **[LR]** can be expressed as $[\mathbf{R}] = p_0[\mathbf{R}]_{tot}$ and $[\mathbf{L}] = [\mathbf{R}]_{tot}$, where $[\mathbf{R}]_{tot}$ is the total receptor concentration in the whole system. We relate the fraction in Eq. (8) to the ratio of the integral of configurational degrees of freedoms in the bound state to the unbound state [6]:

$$K_a = \frac{1}{[\mathbf{L}]} \times \frac{\int_{bound} d\mathbf{l} d\mathbf{X} e^{-\beta U_{bound}}}{\int_{unbound} d\mathbf{l} d\mathbf{X} e^{-\beta U_{unbound}}}, \quad (9)$$

where U_{bound} and $U_{unbound}$ are the total potential energies of the system at bound and unbound states, $\beta = 1/k_B T$ in which k_B is the Boltzmann constant and T is the absolute temperature. **l** represents all the degrees of freedom associated with the ligand (NC) and **X** is the degrees of freedom for the remaining molecules (receptors). On a per ligand basis, the ligand concentration is $[\mathbf{L}] = 1/V_{unbound}$, where the denominator is the volume accessible to an unbound ligand. The integral associated with the unbound state ($U_{unbound} = 0$) in Eq. (9) is determined over translational degrees of freedom (yielding the volume $V_{unbound}$), and rotational degrees of freedom (yielding a factor of $8\pi^2$ in 3-dimensions).

The final form of the binding affinity for interactions between NC and cell surface can be expressed as (see Refs. [15, 16] for detailed derivations):

$$K_a = \frac{1}{[L]} \times T_1 \times T_2 \times T_3, \quad (10)$$

where T_1 represents the entropy loss from the bounded receptors and it can be calculated as:

$$T_1 = \frac{A_{R,b}^{(1)} \times A_{R,b}^{(2)} \times \dots \times A_{R,b}^{(N_b)}}{A_{R,ub}^{(1)} \times A_{R,ub}^{(2)} \times \dots \times A_{R,ub}^{(N_b)}}. \quad (11)$$

Here $A_{R,b}^{(n)}$ is the accessible surface area to the n th receptor in the bound state and $A_{R,ub}^{(n)}$ is the area in the unbound state. Term T_2 accounts for the NC rotational entropy loss due to binding:

$$T_2 = \frac{(N_{ab}/N_b) \Delta\omega}{8\pi^2}, \quad (12)$$

where N_{ab} is the number of antibodies per NC, N_b is the total number of bonds in equilibrium state, $\Delta\omega$ is the rotational volume of the NC in the bound state which can be determined from the distributions of Euler angles as described in Ref. [17]. The term T_3 accounts for NC translational entropy loss,

$$T_3 = \frac{A_{NC,b} \int e^{-\beta W(z)} dz}{A_{NC,ub} l_z}, \quad (13)$$

where $A_{NC,b}$ is the area for the NC translational movement in the bound state, $A_{NC,ub}$ and $A_{NC,ub} l_z$ are the area and volume accessible to the NC in the unbound state, and $W(z)$ is the potential of mean force (PMF).

To calculate the PMF $W(z)$, we choose a reaction coordinate z along which we perform umbrella sampling with harmonic biasing potentials. The umbrella sampling is performed with window size of $\Delta z = 0.05$ nm and the harmonic biasing potential in each window is chosen to be $0.5k_u(z - z_{0,i})^2$, where $0.5k_u(\Delta z)^2 = 1.0 \times 10^{-20}$ J, k_u is the harmonic force constant and $z_{0,i}$ is the location of the center of window i . The NC is slowly moved to the cell surface by updating $z_{0,i}$. A total of 200 million Monte Carlo steps are performed in each window and the histogram is stored only when there exists at least one bond. All the relevant parameters including the window size Δz , strength of the biasing potential k_u and the sampling size in each window have been tested to ensure convergence. The WHAM algorithm [18] is used to unbias and combine the histograms in different windows to form a complete PMF ($W(z)$) profile using a tolerance factor of 10^{-6} . PMF profiles for each system are averaged over four independent realizations.

2.3. Parameter Estimation

The simulation parameters are chosen to make direct contact with the experimental system in Ref. [3], the receptor (antigen) parameters are chosen to mimic ICAM-1. The ligand (antibody) parameters are chosen to mimic the murine anti-ICAM-1 antibody which binds specifically to ICAM-1. For the interactions between antibody and ICAM-1, Muro *et al.* [3]

reported the equilibrium free energy change between antibody and ICAM-1 to be -7.98×10^{-7} J at 4°C , which we set as ΔG_0 in the Bell model of our simulations. We obtain the bond spring constant $k = 1000$ dyn/cm by fitting rupture force distribution data reported from single-molecule force spectroscopy [8, 19]. Both ΔG_0 and k are assumed to be temperature independent based on which we derive the value of the reactive compliance γ (distance along the reaction coordinate to reach the transition state or point of rupture) to be ~ 0.4 nm, which agrees very well with experimental evaluations [19, 20]. The saturation antibody coverage has been experimentally determined [3] to be 220 per NC with a diameter of 100 nm. The ICAM-1 surface density on endothelial cell is set at 2000 ICAM-1 molecules/ μm^2 to be consistent with experimental report [3] of the available binding sites per endothelial cell. In the cases including the presence of glycocalyx, we follow the procedures outlined in our earlier study [8] in which we assume a glycocalyx height of 100 nm. The glycocalyx stiffness is estimated as $k_{glyx} = 3.4 \times 10^9$ J/m⁴ to be consistent with the experimental observation [14] of a 500-fold decrease in binding for 100 nm NCs in the presence of glycocalyx compared to when it is absent. Finally, we also account for the flexure of ICAM-1 (see Fig. 1); since the exact flexural rigidity EI for ICAM-1 proteins is unavailable in the literature, we estimate this value *de novo* by performing MD simulations and fluctuation analysis, which is described in the following section. A summary of model parameters is provided in Table 1.

2.4. Molecular Dynamics (MD) and Fluctuation Analysis for Flexure

Flexural rigidity (flexure) describes the receptor's resistance to bending forces, and therefore impacts both the conformational sampling of the flexible receptor as well as the binding free energy between ICAM-1 and antibody. The flexure of ICAM-1 has not been directly measured by experiment, so we turn to molecular dynamics simulation to provide a microscopic description of its fluctuations. Coarse-grained molecular dynamics (CGMD) is the preferred method for studying these fluctuations because it provides efficient means for conformational sampling and direct estimation of physical properties. We have employed MARTINI, a coarse-grained (CG) molecular dynamics force field developed by Marrink and coworkers [24, 25] to access time and length scales which would be prohibitively costly with all-atom MD. MARTINI was parameterized in a “top-down” manner designed to semi-quantitatively reproduce the structural and thermodynamic properties of lipid and alkane test systems. It has also been recently extended to include proteins [26], carbohydrates [27] and polarizable water [28]. The CG methodology used in MARTINI retains relevant degrees of freedom with a minimal set of tunable parameters and common chemical building blocks. This provides orders-of-magnitude improvements in computational efficiency without the need to re-parameterize the model for novel systems.

In MARTINI, groups of four heavy atoms are mapped to one interaction site or “bead” with a standardized mass of four water molecules and an effective size of 0.47 nm. Ring-like structures have a finer mapping that retains their natural geometry. There are four types of beads: polar, apolar, nonpolar, and charged. These are further divided into classes based on hydrogen bonding propensity. Van der Waals interactions are modeled by a Lennard-Jones (LJ) potential. Differential LJ interactions between bead types produce the necessary attractive and repulsive forces between phases; this partitioning behavior is the key point of contact with experiments. The LJ potential is shifted to zero from 0.9 nm to the cutoff distance of 1.2 nm. Additionally, charged beads interact according to the Coulombic potential. Bonded interactions include a weak harmonic spring between neighbors and a harmonic angle potential between next-nearest neighbors. Neighbors are bonded by a weak harmonic spring without LJ interactions, while next-nearest neighbors interact with a harmonic angle potential. Bonded parameters for amino acids are derived from bond, angle, and dihedral distributions surveyed in the Protein Data Bank. Using the same bead types as

the lipid model, non-bonded parameters were tuned to match the partitioning free energy of amino acid analogs between water and oil phases as well as a host of all-atom model results. MARTINI has been used to study several different protein systems, including the self-assembly of G Protein-coupled receptors on a bilayer [29], the lateral diffusion of peptides in membranes [30] and membrane poration by peptides [31, 32]. Moreover, the CG methodology plays an important role in multiscale simulation methods [33]. Given MARTINI's ability to semi-quantitatively describe the properties of larger biomolecular systems, it is the method of choice for estimating ICAM-1 flexure.

Simulations were performed in GROMACS 4.5.3 [34] using the standard simulation parameters outlined above and described in detail by Monticelli *et al.* [26]. A homology model of ICAM-1 (450 residues) was constructed from PDB structures of both the D1–D2 [35] and D3–D5 domains of ICAM-1 reported by Yang *et al.* [36]. Given that the physiological dimerization state is unknown or at best ambiguous [37] we have simulated the monomer as well as dimer systems. Harmonic restraints replaced the missing dimer interface loops in the dimer simulation, but this loop was explicitly modeled (using MODELLER [38]) for the monomer simulation.

Each model was solvated in a water box with periodic boundary conditions and at least 4 nm between the protein and the box edge (200,000 water beads for the dimer and 50,000 for the monomer). Counter-ions were added to create a physiological concentration of 0.150 nM. To mimic attachment to a membrane, the C-terminal amino acids were position-restrained. All simulations were conducted in position-restrained NVT, followed by unrestrained NPT ensembles using the standard (maximum) MARTINI time step of 40 fs and the Berendsen thermostat with a pressure of 1 atm, a time constant of 1 ps, and a compressibility of $4.5 \times 10^{-5} \text{ bar}^{-1}$. The neighbor list was cut off at 1.2 nm and the LINCS algorithm was used to constrain bond angles. Simulations were run for 100 ns after an initial 10 ns equilibration period. As per recommendations by Monticelli *et al.* [26], an elastic network model (ENM) with a force constant of 500 kJ/molnm was applied to all CG beads within 0.5–0.9 nm to support/stabilize higher-order protein structure.

In order to extract flexural rigidity from CGMD simulations, we model ICAM-1 as an elastic rod represented by a parametric curve with tangent angle $\theta(s)$, where s is the contour length. For an elastic rod with small fluctuations, flexure is given by Hooke's law, such that the total bending energy is given by:

$$U = \frac{1}{2} EI \int_0^L \left(\frac{d\theta}{ds} - \frac{d\theta^0}{ds} \right)^2 ds \quad (14)$$

We remove any contributions to the bending energy by the mean curvature of the elastic rod, $\theta^0(s)$, to allow for bent native conformations. According to the analysis by Gittes *et al.* [39], we decompose the rod shape into Fourier modes (with coefficients a_n and a_n^0) and rewrite the bending energy term as Eq. (15):

$$U = \frac{1}{2} EI \sum_{n=1}^{\infty} \left(\frac{n\pi}{L} \right)^2 (a_n - a_n^0)^2. \quad (15)$$

The equipartition theorem states that each mode contributes $kT/2$ to the energy, where k is the Boltzmann's constant and T is the temperature; therefore we can calculate EI from the variance of each Fourier mode according to Eq. (16):

$$\text{var}(a_n) = \left\langle (a_n - a_n^0)^2 \right\rangle = \frac{kT}{EI} \left(\frac{L}{n\pi} \right)^2 \quad (16)$$

Eq. (17) relates flexure to the persistence length, the distance at which tangent-tangent angles become uncorrelated. Both the flexure and persistence length describe the same property: the receptor's resistance to bending.

$$L_p = \frac{EI}{kT}, \quad \cos(\theta(s) - \theta^0(s)) = \exp\left[-\frac{s}{L_p}\right] \quad (17)$$

These definitions are valid as long as the protein is elastic and experiences only thermally-driven fluctuations which are small relative to the mean curvature.

ICAM-1 is composed of five ordered protein domains denoted D1–D5, which suggest a simple discretization in which six key residues that bookend these domains can provide a useful approximation of its contours. We selected the most intuitive candidates: 26N, 83Y, 185F, 281Y, 366L, and 450P. This yielded the best approximation of the receptor shape. We calculated flexure via Fourier mode analysis and the tangent correlation method. For the latter, the contour lengths were defined from a single reference point at the C-terminal residue to the midpoint between residues forming the bending angle.

3. RESULTS AND DISCUSSION

3.1. Calculation of Flexure from CGMD

Fig. 2 depicts the CG monomer and dimer structures with elastic network bonds. Flexure estimates are summarized in Table 2. We used both the Fourier mode analysis from Eq. (15) and tangent-tangent correlation methods from Eq. (17) to estimate flexure from 100 ns segments of ICAM-1 monomer and dimer CGMD trajectories. Not surprisingly, the dimer is more rigid than the monomer, owing to the harmonic restraints used to mimic poorly-resolved dimer interface loops; in particular, we estimate a monomer flexure of 800 pN•nm² and dimer flexure of 20,000 pN•nm². In addition to the systems described here, we tested the sensitivity of these results by simulating similar systems with additional restraints, different ENM parameters, and different loop-modeling methods.

There is up to 10-fold disagreement between flexure calculated via the tangent correlation method and the first mode of Fourier decomposition. We can attribute this to the approximations inherent to both the calculation method and the ICAM-1 model itself. In the tangent correlation calculation, the errors are relatively large, indicating large variation in $\Delta\theta(s) = [\theta(s) - \theta^0(s)]$, and the receptor was discretized by only five angles. The Fourier mode analysis does not have this limitation, and each mode should provide an independent estimate of flexure. However, due to the limited resolution and sampling of our discretized curves, only the lowest modes are reliable.

Our model could be improved by using a fitted, heterogeneous elastic network model which has been parameterized to match finer, all-atom MD simulation as per methods developed by Lyman *et al.* [41] Since the dimerized structure was used as a template for both the monomer and dimer starting structures, it is also likely that our model does not accurately capture the true conformation of the monomeric form. The equilibrium between monomer and dimer has also not been resolved by our model. Despite these approximations, our CGMD calculations provide a useful *de novo* estimation of the flexural rigidity and

persistence length. To place our calculation in the biological context, we compare the computed value of persistence length for ICAM-1 (current study) to those for common rod-like biomolecules estimated from experiments and simulations, see Table 3.

3.2. Calculation of Potential of Mean Force (PMF) of NC Binding to EC

Following Ref. [15], here we calculate the PMF profiles for NCs with diameter of 100 nm and the number of antibody $N_{ab}=162$ (which corresponds to 74% of saturation coverage). Due to the large experimental uncertainties in flexural rigidity of ICAM-1 receptors, we explore three values of ICAM-1 flexural rigidities at 1400, 7000 and 35000 pN•nm², which lie in between that of glyco-proteins (700 pN•nm²) and actin filaments (15–70×10³ pN•nm²), see Table 3. These values also span range of the ICAM-1 flexural rigidities evaluated using CGMD described in the previous section.

Fig. 4(a) shows the calculated PMF profiles with different ICAM-1 flexural rigidities at temperature of 27 °C, as clearly indicated, for all three cases three firm bonds form ($N_b = 3$) with a similar characteristic free energy well of around $-32 k_B T$ in equilibrium state when projected along the reaction coordinate z . Fig. 4(b) shows the spatially averaged distribution of the bound ICAM-1 relative to the center of NC for the case with $EI = 7000$ pN•nm², and this is basically an annulus distribution, based on which the outer (r_o) and inner radius (r_i) is estimated; all the area terms in Eq. (11) are directly related to r_o and r_i . In particular, $A_{R,ub}^{(1)}=A_{R,ub}^{(2)}=\dots=A_{R,ub}^{(5)}=\pi r_o^2$, $A_{R,b}^{(1)}=\pi r_o^2$, $A_{R,b}^{(2)}=\pi(r_o^2-r_i^2)$, $A_{R,b}^{(3)}=A_{R,b}^{(4)}=A_{R,b}^{(5)}=A_{NC,b}=(r_o-r_i)^2$. As indicated in Fig. 4(d), the ICAM-1 distributions are nearly the same for different flexural rigidities. Fig. 4(c) shows the distribution of the flexure angle (θ) at equilibrium state for different flexural rigidities. As expected, the average flexure angle increases with decreasing EI . Based on the PMF profile in Fig. 4(a), the NC translational entropy loss (Eq. (13)) is calculated and as stated above the rotational entropy loss can be evaluated based on the Euler angle distributions. Finally the binding affinities (K_d) and the dissociation constants ($K_d=1/K_a$) is determined and listed in Fig. 4(d). There is a slight increase in binding with smaller EI ; but the effect is small and within the statistical errorbars. In a similar fashion [16], the binding affinity between single antibody and ICAM-1 is also be calculated and as listed in Table 4, both the interactions of NC-cell surface (for 100 nm NCs) and antibody-ICAM-1 and found consistent with corresponding experimental measurements under similar conditions.

3.3. Effects of Glycocalyx and Flow

In Ref. [16], we investigated the effects of glycocalyx and shear flow (hydrodynamics) on binding between NC and cell surface. The numerical implementations are described in section 2.1. The PMF profiles for different values of the glycocalyx thickness were computed, based on which the binding affinities were also calculated. Then we reported the ratio of the binding affinity without glycocalyx to that with glycocalyx as a function of glycocalyx thickness. The results indicated an exponential dependence. In other words, glycocalyx with thickness of 55~70 nm only reduces the binding by about 2 fold although that with a thickness of 100 nm can actually reduce the binding by a factor of 500. This exponential dependence was confirmed in our simulations with different particle sizes (100 nm vs. 200 nm particles) and different glycocalyx models (constant k_{glyx} vs. linear increasing k_{glyx}), and helps to rationalize the large differences between *in vitro* [47] and *in vivo* [14] experimental results of glycocalyx-mediated NC binding.

In Ref. [16], the effect of flow was also discussed by introducing a 1-dimensional shear flow with a shear rate S (see Fig. 1). From the PMF profiles at different shear rates, it was found that the free energy first decreases below a threshold S then increases. As a result, the corresponding dissociation constant (K_d) shows a biphasic trend with shear rate, in other

words, below a threshold shear rate the flow actually enhances NC binding by reducing K_d , while above the threshold the flow reduces the binding by promoting NC detachment. Consistent with experimental measurements of particle rolling [48], we have shown that this behavior was insensitive to the particle size. Through inspection of the numerical details, we found the multivalent interactions were the key to this shear enhanced NC binding phenomenon, which has been extensively studied experimentally due to its important implications in many disease related processes [49]. In summary, we have shown that the shear enhanced binding arises solely due to multivalent interactions. This effect is hence distinct from the so-called “catch” bonds which are thought to be based on a two-state conformational change in the receptors caused by shear, see Whitfield *et al.* [50].

We note that in considering the binding of 100 nm NC under *in vivo* conditions, our model in the presence of glycocalyx (Equation 7) predicts that the flow is practically zero. In this situation, the flow does not have any effect on the binding of 100 nm NCs which are fully immersed within the glycocalyx layer. Hence, the PMF in the presence of glycocalyx can be recovered from PMF in the absence of the glycocalyx through an analytical correction:

$$W(z)_{\text{glyx,h}} = W(z)_{\text{glyx,h}=0} + \int 1/2k_{\text{glyx}}H^2dA, \text{ where } H=z-h.$$

3.4. Comparison with Experiments

In Table 3, we compared the dissociation constants (K_d) computed from our model with *in vitro* measurements [3] under similar conditions for both interactions of NC-cell and single antibody-ICAM-1. Both interactions agree within reasonable uncertainties. We then studied the effect of the antibody surface coverage (σ_s) on NC [15], and interestingly we observed a threshold at about 45% coverage, below which the NC binding precipitously dropped below that of single antibody-ICAM-1 interactions. Intriguingly, this threshold was almost exactly reproduced in *in vivo* measurements of the endothelial targeting of NCs in the lung of mice at different antibody coverages; detailed description of the comparison can be found in Ref. [15]. The individual PMF profiles at different σ_s revealed an integer reduction in the multivalency (from three to two) at the threshold, and as a consequence, the binding free energy reduced by about $6 k_B T$. The significance of this result is that for 100 nm NCs, there is an optimal antibody coverage (at ~45%), above which the NCs can effectively bind to endothelial cells. We further demonstrated the accuracy of our model by comparing our model predictions with AFM force measurements. The AFM rupture force measurements of NC unbinding yielded a mean rupture force of 316 pN over 89 trials. We predict a mean rupture force of ~215–230 pN derived from our computed PMF profiles, which is again in close agreement with experiments (see Ref. [15] for details). Furthermore, our most recent reports of the effects of particle size, glycocalyx and shear flow [16], have all yielded model predictions in close agreement with experimental observations [47, 48, 51].

4. FUTURE OUTLOOK

In conclusion, using a general multiscale framework, a powerful mesoscale model has been developed to study the targeting of functionalized NCs to cell surfaces. The model and the simulation methodology uniquely yield the absolute binding free energy of binding between NC and cell surface and has been rigorously validated through comparison with broad and independent experiments, namely, *in vitro*, AFM, and *in vivo* endothelial targeting in mice; the three-level agreement between modeling results and experimental measurements establishes the thermodynamic, mechanical and physiological consistency of the model, which we believe can now be employed for predictive simulations or as a guide for future experiments. The coarse-grained MD simulations, while subject to the well appreciated limitations provides crucial constraints on the allowable parameter values of flexural rigidity, thereby providing important structural insight as well as molecular input to the mesoscale model. Scenarios we envision for the near future include the effect of ICAM-1

density on targeting in normal vs. inflamed endothelium in cardiovascular disease models, nanocarrier internalization subsequent to binding mediated by membrane mechanics, and the effect of particle size and shape in binding and internalization, and targeting NC to complex disease states such as cancer. With further improvements to our model (such as the incorporation of membrane microenvironment and mechanics [52–56]), we believe that our current multiscale platform can be further enhanced to guide pharmacological design.

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Figure 1.

A two-dimensional schematic of the mesoscale model. The adhesion is through the interactions between antibodies on NC and ICAM-1s on the EC surface. The ICAM-1 flexure is taken into account by allowing it to bend and rotate in θ and ϕ . Simple 1-D shear flow and glycocalyx are also included as shown.

Figure 2.

Coarse-grained representation of CGMD models. This shows the ICAM-1 monomer (a) and ICAM-1 dimer (c) structures at the end of 100 ns simulations. In (a,c) the beads represent MARTINI backbone and sidechain, while lines denote elastic network bonds. The red filled circle overlays show the positions of key residues which were used for flexure calculations. Position restraints were used to fix the C-terminal membrane-adjacent residues, found at the bottom of these structures. Figures were generated with VMD 1.8.3. [40]. In (b,d), the dynamics of the molecules are shown by overlaying snapshots equally sampled from 0 ns (blue)-100 ns (red). The flexure and persistence length calculations are performed as outlined above through a fluctuation analysis of the coordinates representing these snapshots.

Figure 3.

Calculation of flexure; two calculation methods were used to estimate flexure (top- tangent method and bottom- Fourier method). The logarithm slope of the tangent correlation function (top) is the inverse of the persistence length defined by Eq. (17). Fourier mode analysis (bottom) from Eq. (15) provides independent estimates of flexural rigidity for each mode; however, only the lowest modes are accurate.

Figure 4.

The PMF profiles at different ICAM-1 flexural rigidities. (a) PMF profiles, (b) ICAM-1 distribution, (c) flexure angle (θ) distribution and (d) the outer/inner radius (r_o/r_i) of ICAM-1 annulus distribution and the dissociation constants (K_d).

Table 1

Summary of Model Parameters and the corresponding references.

Parameter	Value	Reference
Simulation surface area	1 μm^2	[8]
Simulation height	0.5 μm	[8]
NC diameter	100 nm	[3]
Antigen length	19 nm	[21]
Antibody length	15 nm	[22]
Number of antigens	2000	[3]
Number of antibodies per NC	162 (74% of saturation)	[3]
ΔG_0	-7.98×10^{-20} J	[3]
Bond spring constant, k	1000 dyn/cm	[19]
Antigen flexural rigidity	(1400–35000) pN·nm ²	[10]
Glycocalyx height	100 nm	[23]
Glycocalyx stiffness, k_{glyx}	3.9×10^9 J/m ⁴	[14]

Table 2

Summary of flexure estimates by CGMD. This table shows flexure estimates for both Fourier mode analysis and tangent correlation functions, across the monomer and dimer systems. Each monomer in the dimer system is reported separately, though they average to 18,000 pN·nm². Though Fourier analysis provides an independent estimate for flexure from each mode, all but the first are considered unreliable.

System	Method	Flexural Rigidity (pN·nm ²)	Persistence Length (nm)
Monomer	1 st Fourier Mode	800 [*]	200
	Tangent Correlation	500	100
Dimer (monomers A / B)	1 st Fourier Mode	12,000 / 24,000	3,000 / 5,000
	Tangent Correlation	2,000 / 2,000	500 / 400

^{*} We have also run simulations of ICAM-1 monomer in a straight conformation (as opposed to a bent conformation in the crystal structure). In this case, a straightened monomer configuration yields a EI value within a few fold of the bent monomer configuration derived from the crystal structure (data not shown).

Table 3

Common biomolecule persistence lengths derived from both experiment and theory. It is important to note that most of these systems show different persistence lengths under different experimental conditions.

Theory	Expt.	System	Persistence Length
	•	DNA [42, 43]	5 nm (ss), 50 nm (ds)
	•	P-selectin [44]	20 nm
•	•	Collagen [45]	50 nm
•		Glyco-proteins [10]	160 nm
•		ICAM-1 (this work)	200 nm – 3 μ m
	•	Actin [10, 39, 41]	0.5 – 20 μ m
	•	Microtubules [39, 46]	0.5 – 10 mm

Table 4

The dissociation constants for different interactions. A comparison between model predictions and experimental measurements. (Results are from Ref. [15])

Dissociation constant ($K_d = 1/K_a$)	Model	<i>In vitro</i> Experiment [3]
Antibody-ICAM-1 interaction	3.2 nM	8.5 nM
NC-cell interaction	18.2 pM ($EI=7000 \text{ pN}\cdot\text{nm}^2$)	77 pM