#### Article

# Variations of the Depletion Zones around Inclusions Explain the Complexity of Brush-Induced Depletion Interactions

Published as part of Journal of Chemical Theory and Computation special issue "Developments of Theoretical and Computational Chemistry Methods in Asia".

Daeseong Yong, Ji Woong Yu, Bae-Yeun Ha,\* and Changbong Hyeon\*



such as the phase behavior of colloid–polymer or colloid-depletant mixtures and clustering of inclusions in mobile brushes. They arise from the tendency to minimize the volume of the depletion zone formed around colloidal particles or inclusions. In comparison to depletion interactions widely studied for colloidal particles or polymers in a suspension of spherical depletants, depletion interactions between nonspherical inclusions in mobile polymer brushes display complex behaviors. When the brush is shorter than the inclusion height, the inclusions in brushes experience apparent attraction; yet, such attraction is reduced or even becomes repulsive when the brush is overgrown beyond the inclusion height. Here we use the self-consistent field theory (SCFT) to calculate the



depletion zones around two cylindrical inclusions and offer a clear explanation of how these complex behaviors arise. In tall brushes, the changes of the depletion zone volume with varying intercylinder separation are opposite in sign at the upper and lower parts of cylinders. Consequently, in tall brushes, cylinders even shorter than the size of a correlation blob experience repulsion, but long cylinders attract each other. Our study reveals that brush-induced depletion interactions are decided by the complex interplay among the sizes of brushes, inclusions, and correlation blobs.

## 1. INTRODUCTION

Transmembrane proteins are often found to form nanoclusters on the cell surface.<sup>1,2</sup> The mechanisms such as membrane undulation-induced thermal Casimir-like long-range attraction,<sup>3,4</sup> helix—helix interaction,<sup>5</sup> protein sorting via hydrophobic mismatch,<sup>6,7</sup> and membrane curvature-mediated interaction<sup>8,9</sup> are generally considered to underlie the protein nanocluster formation on the cell surface. The glycocalyx, a layer of glycolipids and glycoproteins that densely coat the cell surface, has recently been shown to play vital roles in promoting the integrin nanocluster formation and integrin-mediated downstream signaling.<sup>10–14</sup>

Motivated by experimental observations on the role of glycocalyx, recent studies have adopted the ideas of Asakura and Oosawa (AO)<sup>15,16</sup> and the self-consistent field (SCF) approach<sup>17</sup> to investigate the effect of laterally mobile polymer brushes on inclusions confined to two-dimensional (2D) surfaces. Compared with the extensive studies over the past decades on depletion interactions in colloid–polymer mixtures<sup>18–31</sup> and biopolymers in a crowded environment,<sup>32–40</sup> less has been investigated on a scenario of brush polymer-induced depletion interactions. Although both the AO and SCF theories

have predicted brush-induced depletion attractions between inclusions,<sup>15–17</sup> the two theories differ from each other in that they rely on distinct physical assumptions. Furthermore, their results have not been compared on an equal footing. Recently, we have shown that the blob concept incorporated into the AO theory is essential for quantitatively explaining the potentials of mean force (PMFs) obtained for different grafting densities and brush heights from molecular dynamics (MD) simulations.<sup>16</sup> We herein show that SCFT, based on the mean-field strategy of determining the effective potential field around an ideal chain,<sup>41–44</sup> not only reproduces the scaling relations for depletion free energy against grafting density identical to those from the AO theory but also generates PMFs that align well with those from MD simulations.

 Received:
 February 27, 2025

 Revised:
 May 10, 2025

 Accepted:
 May 21, 2025

 Published:
 June 5, 2025





After describing the methods of MD simulations (Section 2.1) and SCFT (Section 2.2), we first review the basics of brush polymers, highlighting the concept of correlation blobs in the semidilute environment (Section 3.1), which is essential in the quantitative formulation of the brush-induced depletion interaction using the AO theory,<sup>16</sup> and next review the associated quasi-2D version of the AO theory (Section 3.2).<sup>15,16</sup> In Section 3.3, we show that once the excluded volume parameter,  $\Lambda$ , of SCFT, which can be related to the grafting density  $\sigma$  by mapping it to the strong-stretching theory (SST),<sup>45-48</sup> is adequately selected, the free energy profiles acquired from SCFT align well with those from MD simulations and thus the scaling relations predicted by the AO theory.

By leveraging the computational efficacy of SCFT in gaining equilibrium properties of dense polymer systems, we calculate the density profiles of brush monomers  $\phi(\mathbf{r})$  around cylindrical inclusions, representing signaling proteins on membranes, with varying brush height and intercylinder separation (Section 3.4). The density profiles are used not only to quantify the depletion free energy but also to decompose the volume of the depletion zone around the two cylinders into those around the lateral surface and above the circular face. This effort suggests that the two depletion zone volumes change in an opposite manner with the intercylinder separation. The competition between depletion interactions arising from two different regions of the cylindrical inclusions gives rise to an intriguing theoretical prediction (Section 3.5): embedded in tall brushes, two coin-like cylinders, whose height (h) is even shorter than the size of the correlation blob at the surface, repel each other mainly due to the tendency to minimize the depletion zone volume above the cylinders.

Lastly in the Conclusions (Section 4), we put brush-induced depletion interactions into the context of glycocalyx-enhanced integrin signaling, linking our theoretical prediction of cylinderheight dependent depletion interactions in tall brushes to the effect of conformational change of integrins on their clustering in cell surfaces coated with thick glycocalyces.

For clarity, we summarize the key parameters and variables used throughout the paper in Table 1.

# 2. METHODS

2.1. Molecular Dynamics Simulations and Potential of Mean Force between Two Cylinders in Polymer Brushes. Molecular dynamics (MD) simulations were performed using the Large-scale Atomic/Molecular Massively Parallel Simulator (LAMMPS).<sup>49</sup> The low friction Langevin equation was integrated at the temperature  $T = 1.0 \ \epsilon/k_{\rm B}$ , where  $k_{\rm B}$  is the Boltzmann constant and  $\epsilon$  is the Lennard-Jones parameter described below.

First, the cylindrical inclusion of diameter  $D = 10\sqrt{2}b$  and height  $h = 20\sqrt{2}b = 2D$  was modeled using a harmonically constrained composite rigid body consisting of 6500 beads. We used a harmonic potential with a force constant  $k \simeq 10^3 \epsilon/b^2$  to restrain the beads at the bottom layer (z = 0.0) of the cylinder only along the z-direction, so that the cylinders were free to move laterally.

Next, the bond potential along each polymer chain consisting of N monomers was modeled with the finite extensible nonlinear elastic (FENE) potential and the repulsion between the neighboring monomers with the Weeks-Chandler-Anderson (WCA) potential

## Table 1. Parameters and Variables Used in the Study

polymer	Ν	number of monomers comprising each brush polymer
	Ь	monomer size
	ξ	blob size, $\xi \simeq b g^{3/5}$
	g	mean number of monomers inside a blob
	R <sub>0</sub>	size of an ideal chain of length N, $R_0 = N^{1/2}b$
	$R_F$	size of a real chain of length N (or Flory radius), $R_F \simeq N^{3/5} b$
brush	n <sub>p</sub>	number of brush polymers on the 2D surface
	Α	area of the surface covered by the brush polymers
	$\sigma$	grafting density $\sigma = n_{\rm p}/A$
	H	brush height
	П	osmotic pressure
	$\phi$	monomer volume fraction in the interior of brush
cylinder	D	diameter of a cylinder
	d	center-to-center distance between two cylinders
	$\delta_c$	depletion layer thickness
	h	cylinder height
AO theory	$A_{\rm ex}$	excess area
	$V_{\rm ex}$	excess volume
	N	number of interaction units
	$F_{AO}(d)$	AO free energy (potential) at $d$
	$\Delta F_{ m AO}$	Free energy gain, $\Delta F_{AO} = F_{AO}(\infty) - F_{AO}(D)$
SCFT	$\phi(\mathbf{r};d)$	mean monomer density at <b>r</b> for intercylinder separation $d$
	$\phi_{\scriptscriptstyle b}$	bulk monomer density
	$V_p$	total volume occupied by the brush segments, $V_p = \int d\mathbf{r} \phi(\mathbf{r})$
	$u_0$	strength of self-avoidance
	$ ho_0$	mean density of a single brush segment, $\rho_{\rm 0}$ = $Nn_p/V_p$
	Λ	excluded volume parameter, $\Lambda = N\beta u_0\rho_0$
	$W(\mathbf{r})$	self-consistent field at <b>r</b> , $W(\mathbf{r}) = \Lambda \phi(\mathbf{r})$
	$F_{\rm SCF}(d)$	free energy at $d$ obtained from SCF calculations
	$V_{depl}(y)$	depletion volume at a separation $y$
	$V_{\rm depl}^{\rm top}(y)$	depletion volume on the cylinder top
	$V_{\rm depl}^{\rm lat}(y)$	depletion volume around the lateral surface of the cylinders

$$U_{b}(r_{i,i+1}) = -\frac{1}{2}k_{F}r_{o}^{2}\ln\left[1 - \left(\frac{r_{i,i+1}}{r_{o}}\right)^{2}\right] + 4\epsilon\left[\left(\frac{b}{r_{i,i+1}}\right)^{12} - \left(\frac{b}{r_{i,i+1}}\right)^{6} + \frac{1}{4}\right]$$
(1)

with  $k_{\rm F} = 30.0 \ \epsilon/b^2$  and  $r_{\rm o} = 1.5b$ . The monomers along the chain as well as in the different chains repel each other via the WCA potential, realizing the self-avoidance of polymer chains.

$$U_{\rm nb}(r) = \begin{cases} 4\epsilon \left[ \left(\frac{b}{r}\right)^{12} - \left(\frac{b}{r}\right)^{6} \right] + \frac{1}{4} \\ 0 & \text{for } r \ge 2^{1/6} b \end{cases} \quad \text{for } 0 < r < 2^{1/6} b \end{cases}$$
(2)

The beads comprising the cylinder body and the chain monomers also repel each other via eq 2. To prevent polymers from penetrating the surface at z = 0, the WCA potential (eq 2) was placed at z = -b, with r in eq 2 being replaced with the minimal distance from the surface. For a given grafting density,  $\sigma(=n_{v}/A)$  where  $n_{v}$  is the number of brush polymers in the system and A is the area of the 2D surface, the lateral dimension of the simulation box was set to  $L_x = L_y = \sqrt{n_p/\sigma}$  with periodic boundary conditions. Along the z direction, we used shrink-wrapped boundary conditions to adjust the simulation box to the brush height.

To calculate the PMF between the inclusions in brushes, the configurations of the system were collected through the umbrella sampling method along the separation (*d*) between the central axes of the two cylinders. At each sampling point, the system was first relaxed for  $10^{3}\tau$ , where  $\tau[=(mb^{2}/\epsilon)^{1/2}]$  is the natural time unit for the MD simulations, followed by a production run for  $10^{5}\tau$  under a bias potential,  $U_{\rm B}(d;d_{k}) = k_{s}(d - d_{k})^{2}/2$  with  $k_{s} = 200.0 \ \epsilon/b^{2}$  and  $d_{k} = D + 10.0b - kb/4$  ( $k = 1, \dots, 42$ ). The unbiased free energy profile was reconstructed by means of the weighted histogram analysis method (WHAM).<sup>50,51</sup>

**2.2. Self-Consistent Field Theory.** In order to obtain the mean field version of free energies of a system, the SCFT considers the partition function using a functional integral over an auxiliary field. The theory works best for many-chain systems, such as melts, blends and copolymers, <sup>41,48,52</sup> where the pressure from the surrounding chains counterbalances the interchain repulsions making the statistics of individual chains nearly ideal.<sup>41</sup>

To calculate the free energy between the inclusions in brushes separated by d, we start with the partition function of a system consisting of  $n_p$  polymer chains

$$\mathcal{Z} \propto \frac{1}{n_p!} \prod_{\alpha}^{n_p} \int \mathcal{D}[\mathbf{r}_{\alpha}] \mathrm{e}^{-\beta U[\mathbf{r}_{\alpha}(s)]}$$
(3)

Equation 3 is written in terms of the functional integrals over the space curves representing  $n_p$  polymer chains  $[\mathbf{r}_{\alpha}] \equiv \{\mathbf{r}_{\alpha}(s)|\alpha = 1,2,\dots,n_p; 0 \le s \le N\}$  along with the energy potential  $U[\mathbf{r}_{\alpha}] = U_0[\mathbf{r}_{\alpha}] + U_1[\mathbf{r}_{\alpha}]$ .  $U_0[\mathbf{r}_{\alpha}]$  denotes the chain connectivity potential

$$\beta U_0[\mathbf{r}_{\alpha}] = \frac{3}{2b^2} \sum_{\alpha=1}^{n_p} \int_0^N ds \left(\frac{\mathrm{d}\mathbf{r}_{\alpha}(s)}{\mathrm{d}s}\right)^2 \tag{4}$$

and  $U_1[\mathbf{r}_{\alpha}]$  is the sum of solvent mediated nonbonded pairwise interactions  $v(\cdots)$  between the monomers along a single chain as well as between the chains

$$\beta U_{1}[\mathbf{r}_{\alpha}] = \frac{\beta}{2} \sum_{j=1}^{n_{p}} \sum_{k=1}^{n_{p}} \int_{0}^{N} ds \int_{0}^{N} ds' \nu(|\mathbf{r}_{j}(s) - \mathbf{r}_{k}(s')|)$$
(5)

This particle (monomer)-based expression of the canonical partition function  $\mathcal{Z}$  (eq 3) can be replaced with the field-theoretic representation via the Hubbard–Stratonovich transformation.<sup>53</sup>

Specifically, we first introduce the monomer density field at position  $\mathbf{r}$ ,  $\hat{\rho}(\mathbf{r}) = \sum_{\alpha=1}^{n_p} \hat{\rho}_{\alpha}(\mathbf{r}) = \sum_{\alpha=1}^{n_p} \int_0^N ds \delta(\mathbf{r} - \mathbf{r}_{\alpha}(s))$ , and rewrite the eq 5 into a density field-based expression

$$\beta U_{1}[\mathbf{r}_{\alpha}] = \frac{\beta}{2} \int d\mathbf{r} \int d\mathbf{r}' \hat{\rho}(\mathbf{r}) \nu (|\mathbf{r} - \mathbf{r}'|) \hat{\rho}(\mathbf{r}')$$
(6)

Next, by introducing a delta functional of the density field that satisfies the relation  $F[\hat{\rho}] = \int \mathcal{D}\rho \delta[\rho - \hat{\rho}]F[\rho]$ , where  $\delta[\rho - \hat{\rho}] = \int \mathcal{D}w \, e^{i\int d\mathbf{r}w(\mathbf{r})[\rho(\mathbf{r}) - \hat{\rho}(\mathbf{r})]}$  with an auxiliary field  $w(\mathbf{r})$  that can be interpreted as the fluctuating chemical potential field at  $\mathbf{r}$ , one obtains the field-theoretic representation

 $-(\beta/2)\int d\mathbf{r} \int d\mathbf{r}' \rho(\mathbf{r}) v(|\mathbf{r}-\mathbf{r}'|)\rho(\mathbf{r}')$ 

$$\begin{aligned} \frac{1}{n_p!} \int \prod_{\alpha}^{n_p} \mathcal{D}[\mathbf{r}_{\alpha}] e^{-(3/2b^2) \int_0^N (\mathrm{d}\mathbf{r}_{\alpha}(s)/\mathrm{d}s)^2} \int \mathcal{D}_{w} e^{i\int \mathrm{d}\mathbf{r}_{w}(\mathbf{r})(|\rho(\mathbf{r}) - \hat{\rho}(\mathbf{r})|)} \\ &= Z_0 \int \mathcal{D}\rho e^{-(\beta/2) \int \mathrm{d}\mathbf{r} \int \mathrm{d}\mathbf{r}' \rho(\mathbf{r})v(|\mathbf{r} - \mathbf{r}'|)\rho(\mathbf{r}')} \\ &\int \mathcal{D}w \exp\left(i\int \mathrm{d}\mathbf{r}w(\mathbf{r})\rho(\mathbf{r}) + n_p \ln Q[iw]\right) \\ &= Z_0 \int \mathcal{D}w e^{-(1/2\beta) \int \mathrm{d}\mathbf{r} \int \mathrm{d}\mathbf{r}' w(\mathbf{r})v^{-1}(|\mathbf{r} - \mathbf{r}'|)w(\mathbf{r}')} Q[iw]^{n_p} \end{aligned}$$
(7)

where

$$Z_0 = \left[\int \mathcal{D}\mathbf{r}_{\alpha} \,\mathrm{e}^{-(3/2b^2) \int_0^N \mathrm{d}s (\mathrm{d}\mathbf{r}_{\alpha}(s)/\mathrm{d}s)^2}\right]^{n_p} / n_p! \tag{8}$$

and

$$Q[iw] = \frac{\int \mathcal{D}\mathbf{r}_{\alpha} e^{-(3/2b^2) \int_0^N ds (d\mathbf{r}_{\alpha}(s)/ds)^2 - i \int_0^N ds w(\mathbf{r}_{\alpha}(s))}}{\int \mathcal{D}\mathbf{r}_{\alpha} e^{-(3/2b^2) \int_0^N ds (d\mathbf{r}_{\alpha}(s)/ds)^2}}$$
(9)

Q[iw], introduced in eq 7, corresponds to the single chain partition function subject to a purely imaginary potential  $iw(\mathbf{r})$ , and it is obtained by noting the relations  $\hat{\rho}(\mathbf{r}) = \sum_{\alpha}^{n_p} \hat{\rho}_{\alpha}(\mathbf{r})$ ,  $\hat{\rho}_{\alpha}(\mathbf{r})$  $= \int_{0}^{N} ds \delta(\mathbf{r} - \mathbf{r}_{\alpha}(s))$  and

$$\int \mathcal{D}w \, \mathrm{e}^{-i \int \mathrm{d}\mathbf{r} w(\mathbf{r}) \hat{f}_{\alpha}(\mathbf{r})}$$
$$= \int \mathcal{D}w \, \mathrm{e}^{-i \int \mathrm{d}\mathbf{r} w(\mathbf{r}) \int_{0}^{N} \mathrm{d}s \delta(\mathbf{r} - \mathbf{r}_{\alpha}(s))} = \int \mathcal{D}w \, \mathrm{e}^{-i \int_{0}^{N} \mathrm{d}s w(\mathbf{r}_{\alpha}(s))}$$

The expression in the last line of eq 7 is obtained after carrying out functional integration over the density field  $\rho(\mathbf{r})$ . Thus, the partition function is cast into a field theory with an effective Hamiltonian that varies with the fluctuating auxiliary field *w*, i.e.,  $\mathcal{Z} \propto \int \mathcal{D}we^{-\beta H_{\text{eff}}[w]}$ .

In our problem of brushes, we use a delta function to model the nonbonded potential  $v(\dots)$ , i.e.,  $v(|\mathbf{r} - \mathbf{r}'|) = u_0 \delta(\mathbf{r} - \mathbf{r}')$  with  $u_0$  denoting the strength of self-avoidance, which leads to

$$\exp(-\beta H_{\text{eff}}[w]) = \exp\left[-\frac{1}{2\beta u_0} \int d\mathbf{r} w(\mathbf{r})^2 \right] Q[iw]^{n_p}$$
(10)

The SCFT version of free energy of the system is formally acquired through the saddle point approximation as follows

$$\beta F_{\text{SCF}} = -\ln \mathcal{Z} = -\ln \int \mathcal{D}w \, \mathrm{e}^{-\beta H_{\text{eff}}[w]}$$
$$\simeq H_{\text{eff}}[w^*] = -n_p \ln Q[iw^*] + \frac{1}{2\beta u_0} \int \mathrm{d}\mathbf{r} w^*(\mathbf{r})^2 \tag{11}$$

Along with the normalized arc length  $0 \le s \le 1$ , the Q[iw] defined above is written as

$$Q[iw] = \frac{\int \mathcal{D}\mathbf{r}_{\alpha} e^{-(3/2Nb^2) \int_0^1 ds (d\mathbf{r}_{\alpha}(s)/ds)^2 - iN \int_0^1 ds w(\mathbf{r}_{\alpha}(s))}}{\int \mathcal{D}\mathbf{r}_{\alpha} e^{-(3/2Nb^2) \int_0^1 ds (d\mathbf{r}_{\alpha}(s)/ds)^2}}$$
$$= \langle e^{-iN \int_0^1 ds w(\mathbf{r}_{\alpha}(s))} \rangle_0$$
$$= \langle e^{-i \int d\mathbf{r} \hat{\rho}_{\alpha}(\mathbf{r}) w(\mathbf{r})} \rangle_0 \tag{12}$$

Note that Q[iw] corresponds to a path integral subject to the external potential  $iNw(\mathbf{r})$  along  $0 \le s \le 1$ , and for the case of



**Figure 1.** Height (*H*) and lateral fluctuations ( $\delta R_{\perp}$ ) of brush polymers (N = 100, b = 1) at s = L and s = L/2 as a function of grafting density ( $\sigma$ ). (A) Illustration of a brush along with the length scales (H,  $\xi$ , and  $\delta R_{\perp}$ ) discussed in the main text. (B) H versus  $\sigma$  (top) and  $\delta R_{\perp}$  versus  $\sigma$  (bottom). The brush forming grafting density is  $\sigma > 1/R_F^2 \approx 4.4 \times 10^{-3}$ . Thus, the grafting density,  $0.01 \leq \sigma b^2 \leq 0.08$ , corresponds to the semidilute regime that confirms the scaling behaviors of brush polymers expected from eqs 21 and 24. For  $\sigma \gtrsim 0.1$ , the lateral fluctuations display a distinct behavior  $\delta R_{\perp} \sim \sigma^{-1/2}$ .

brush polymer it can be related to the chain propagator from 0 to *s* at position **r**,  $q(\mathbf{r}, s) \propto \int \mathcal{D}\mathbf{r}_{\alpha} e^{-\beta U(\mathbf{r}_{\alpha}, 0, s)} \delta(\mathbf{r}_{\alpha}(s) - \mathbf{r})$ , and its back-propagator  $q^{\dagger}(\mathbf{r}, s) \propto \int \mathcal{D}\mathbf{r}_{\alpha} e^{-\beta U(\mathbf{r}_{\alpha}, s, 1)} \delta(\mathbf{r}_{\alpha}(s) - \mathbf{r})^{54,55}$  $Q[iw] = \frac{1}{V_{p}} \int d\mathbf{r}q(\mathbf{r}, s)q^{\dagger}(\mathbf{r}, s) \propto \int \mathcal{D}\mathbf{r}_{\alpha} e^{-\beta U(\mathbf{r}_{\alpha}, 0, 1)}$ (13)

Here,  $q(\mathbf{r},s)$  obeys the following diffusion equation under an effective potential field  $iNw(\mathbf{r})$  with the one-end grafted boundary condition  $q(\mathbf{r}, 0) = R_0 \delta(z - \epsilon)^{41,55}$ 

$$\frac{\partial q(\mathbf{r}, s)}{\partial s} = \left[\frac{R_0^2}{6}\nabla^2 - iNw(\mathbf{r})\right]q(\mathbf{r}, s)$$
(14)

where  $R_0^2 = Nb^2$ , and its back-propagator  $q^{\dagger}(\mathbf{r}, s)$  from 1 to *s*, satisfies  $-\partial_s q^{\dagger}(\mathbf{r}, s) = \left[\frac{R_0^2}{6}\nabla^2 - iNw(\mathbf{r})\right]q^{\dagger}(\mathbf{r}, s)$  with  $q^{\dagger}(\mathbf{r}, 1) = 1$ .

The concentration at position **r**, solely due to the  $\alpha$ -th polymer, is given by

$$\hat{\phi}_{\alpha}(\mathbf{r}) = \hat{\rho}_{\alpha}(\mathbf{r})/\rho_{0} = \frac{N}{\rho_{0}} \int_{0}^{1} \mathrm{d}s \delta(\mathbf{r} - \mathbf{r}_{\alpha}(s))$$
$$= \frac{V_{p}}{n_{p}} \int_{0}^{1} \mathrm{d}s \delta(\mathbf{r} - \mathbf{r}_{\alpha}(s))$$
(15)

Here,  $V_p = v_0 n_p N$ , with  $v_0 = \rho_0^{-1}$  denoting the volume of a single segment, is the total volume occupied by the entire segments comprising the brush. Then, the mean monomer concentration (or density) at **r** by the whole polymers comprising the brush, satisfying  $\int d\mathbf{r} \phi(\mathbf{r}) = V_p$ , is obtained by taking a functional derivative on Q[iw](eq 12)

$$\phi(\mathbf{r}) = n_p \langle \hat{\phi}_{\alpha}(\mathbf{r}) \rangle = -\frac{n_p}{\rho_0} \frac{\delta \ln Q[iw]}{\delta[iw]}$$
(16)

which is also related with the chain propagators as

$$\phi(\mathbf{r}) = \frac{1}{Q[iw]} \int_0^1 \mathrm{d}s q(\mathbf{r}, s) q^{\dagger}(\mathbf{r}, s)$$
(17)

When eq 14 is solved self-consistently along with eqs 13 and 17 until the effective field  $w(\mathbf{r})$  converges to  $w^*$  ( $\mathbf{r}$ ), the functional derivative of the effective Hamiltonian (eq 10) vanishes, satisfying the following key relations

$$\frac{\delta\beta H_{\text{eff}}[w]}{\delta iw} |_{w=w^*} = -n_p \frac{\delta \ln Q[iw]}{\delta iw} - \frac{iw^*(\mathbf{r})}{\beta u_0}$$
$$= \rho_0 \phi(\mathbf{r}) - \frac{iw^*(\mathbf{r})}{\beta u_0} = 0$$
(18)

yielding the self-consistent solution of SCFT<sup>54</sup>

$$\Lambda \phi(\mathbf{r}) = i N w^*(\mathbf{r}) \equiv W^*(\mathbf{r}) \tag{19}$$

where  $\Lambda \equiv N\beta u_0 \rho_0$  is defined as the excluded volume parameter in SCFT. Under this condition  $iw^*$  (**r**) becomes a real number, and the solution of **eq** 14 amounts to getting the chain propagator of an ideal chain subject to an effective external potential field proportional to the local concentration,  $\Lambda \phi(\mathbf{r})$ . Furthermore, the auxiliary field,  $iw^*$  (**r**), determined as the solution of SCF **eq** (**eq** 14), enables one to compute the corresponding free energy given in **eq** 11. For brushes with inclusions, **eq** 11 is solved for the two cylindrical inclusions separated by *d* along with the boundary conditions,  $q(\mathbf{r}, s) = 0$ and  $q^{\dagger}(\mathbf{r}, s) = 0$  for **r** inside the inclusions. Finally, **eq** 11 is cast into the following form after dividing both sides by  $\sigma R_0^2$ 



**Figure 2.** Brush-induced depletion interaction between two inclusions. (A) Illustration of depletion attraction between two proteins in polymer brush on a 2D surface. (B) Top view of the illustration. The boundaries (dotted lines) around the inclusions demarcate the depletion zone, and the pale blue circles depict the ranges of correlation blobs. The hashed area in magenta depicts the excess area,  $A_{ex}(d = D; \delta_c)$  (eq 26), gained when the two inclusions are brought together. Specified in the figures with the arrows are the height of inclusion (*h*), brush height (*H*), diameter of the inclusion (*D*), depletion layer thickness ( $\delta_c$ ), and the center-to-center distance between the two inclusion (*d*).

$$\frac{\beta F_{\text{SCF}}(d)}{\sigma R_0^2} = -\frac{A}{R_0^2} \ln Q[W] + \frac{A}{2R_0^2 V_p} \int d\mathbf{r} W(\mathbf{r}) \phi(\mathbf{r})$$
(20)

where the symbol \* for the self-consistent field  $W^*$  obtained from SCFT is dropped for simplicity.

To solve the SCF equations, we employed the Crank-Nicolson and alternating-direction implicit methods while setting the contour and grid intervals as  $\Delta s = 1/400$  and  $\Delta L = R_0/20$ , respectively. The chain propagators and partition function are computed by utilizing an open-source library.<sup>56</sup> All computations are parallelized using a GPU, with each SCFT run taking approximately an hour on an NVIDIA V100.

## 3. RESULTS AND DISCUSSION

**3.1. Polymer Brush.** We consider a polymer brush where  $n_p$  polymers are grafted to a 2D surface of area *A*. If the grafting density  $\sigma(=n_p/A)$  is greater than the density defined by the Flory radius of isolated chain  $(R_F \simeq bN^{\nu}$  with  $\nu \simeq 3/5$ , more precisely  $\nu = 0.588$ ), i.e.,  $\sigma R_F^2 > 1$ , the polymer chains overlap with each other, transitioning from a mushroom-like configuration to brushes (Figure 1). As a result, each chain is deformed into a string of N/g correlated blobs of size  $\xi$ , inside which self-avoidance dominates chain statistics:  $\xi \simeq \tau^{1/5} bg^{3/5} < R_F$  with  $\tau = (T - \Theta) / T$  associated with the second virial coefficient  $B_2 \sim \tau b^3$ . This consideration leads to the mean brush height *H* that obeys the Alexander-de Gennes brush scaling<sup>57–59</sup>

$$H \simeq \left(\frac{N}{g}\right) \xi \simeq N b (\tau \sigma b^2)^{1/3}$$
(21)

Since the grafting distance between the neighboring chains under the brush-forming condition is effectively set by  $\xi$ , the grafting density  $\sigma$  is related to  $\xi$  as

$$\sigma \simeq 1/\xi^2 \tag{22}$$

Importantly, as discussed in the section that follows, the correlation blob serves as the fundamental interaction unit in determining the brush-induced depletion interactions.<sup>16</sup>

Instead of using the blob concept, one can rely on the Flory argument to reach the same expression with eq 21. Specifically, the free energy of the stretched brush chain is given by

$$\beta F \simeq \frac{R_{\parallel}^2}{Nb^2} + \frac{1}{2}\tau b^3 \int c(\mathbf{r})^2 d\mathbf{r} + \cdots$$
(23)

where the first term is associated with the entropic cost of stretching an ideal chain, and the second term represents the pairwise two body interaction arising from the intrachain excluded volume interaction.<sup>41,60</sup> Throughout the paper,  $\beta = 1/k_{\rm B}T$ . To simplify the calculation, we consider a uniform concentration  $c(\mathbf{r}) \simeq (N/V)$  and  $\int d\mathbf{r} \rightarrow V$  with  $V \simeq R_{\parallel} \times d_{gr}^2 \simeq R_{\parallel}/\sigma$  where  $d_{gr}$  denotes the grafting distance that satisfies the relation  $\sigma \simeq 1/d_{gr}^2$  which casts eq 23 into  $\beta F \simeq R_{\parallel}^2/Nb^2 + (1/2) \tau b^3 N^2 \sigma/R_{\parallel}$ . Its minimization with respect to  $R_{\parallel}$ , i.e.,  $\frac{d\beta F}{dR_{\parallel}}|_{R_{\parallel}=H} = 0$ , leads to the same expression of the scaling with eq 21.

Also, note that the conformation of a single polymer chain in brushes can effectively be generated under an extensile force  $f_{\parallel}$ . The work associated with stretching the chain from the free state satisfying  $\langle \vec{R}_{ee} \rangle = 0$  to the mean height H (work  $= f_{\parallel} \times H$ ) should be balanced with the mean number of blobs  $(H/\xi)$ , each of which contributes the free energy of  $k_{\rm B}T$  so that the free energy of extension is given by  $k_{\rm B}T \times H/\xi$ . Thus, the size of blob,  $\xi$ , is associated with the extensile force  $f_{\parallel}$  as  $H \simeq Nb(b\beta f_{\parallel})^{2/3}$ . Inside the brush height is related with  $f_{\parallel}$  as  $H \simeq Nb(b\beta f_{\parallel})^{2/3}$ . Inside the brush  $(\xi < x < H)$ , the lateral fluctuation of a chain on a plane normal to the extensile force  $f_{\parallel}$  is related to H as  $\langle \delta R_{\perp}^2 \rangle \simeq k_{\rm B}TH/$  $f_{\parallel} \simeq (Nb) \ b^{2/3}(\beta f_{\parallel})^{-1/3}$ , or it can be described simply by a random walk of a blob projected on the plane  $\langle \delta R_{\perp}^2 \rangle \simeq (2/3)$  $(N/g)\xi^2$ . Thus, together with eq 21, we obtain an arc lengthdependent lateral fluctuation,  $R_{\perp}(s) \equiv \langle \delta R_{\perp}^2 \rangle^{1/2}$  ( $0 \le s \le L$  with L = bN)

$$\delta R_{\perp}(s) \simeq (2/3)^{1/2} b^{1/2} s^{1/2} (\sigma b^2)^{-(2\nu-1)/4\nu}$$
(24)

It is noteworthy that for a fixed N and  $\nu = 3/5$ , the lateral fluctuations of polymer brushes depend only weakly on the grafting density,  $\delta R_{\perp} \sim \sigma^{-1/12}$ . Since the interior of brushes is

effectively in the semidilute regime, it follows from the des Cloiseaux scaling of osmotic pressure  $\beta \Pi \sim 1/\xi^3 \sim \phi^{9/4} \sim \sigma^{3/2}$ that  $\delta R_{\perp} \sim \Pi^{-1/18}$ . However, when the grafting density is too high ( $\sigma > 0.1$ ), the lateral fluctuations are restricted by its neighbors, displaying the same scaling behavior as that of blobs ( $\xi \sim \sigma^{-1/2}$ ), i.e.,  $\delta R_{\perp} \sim \sigma^{-1/2}$ , and  $\delta R_{\perp} \sim \Pi^{-1/3}$ . These  $\sigma$ dependences of  $\delta R_{\perp}$  and H are confirmed by the MD simulations demonstrated in Figure 1B.

We note that the scaling relations discussed throughout this study rely on the assumption that there is a clear separation in length among b,  $\xi$ , and H, which are the monomer size, the blob size, and the brush height, respectively. The scaling law  $\sigma^{-1/12}$  in Figure 1B, for instance, is reliable when  $H \gg \xi \gg b$ . Under this condition, the brush system is well in the semidilute scaling regime. The data points obtained with  $\sigma < 0.01$  (mushroom phase) or  $\sigma > 0.1$  (regime of concentrated polymer solution) in Figure 1B are not deep inside this regime. Widening this regime requires simulating longer chains.

**3.2.** AO Potential for Brush-Induced Depletion Interaction. The AO theory, originally proposed for attraction between hard spheres or flat surfaces suspended in a solution of depletants,<sup>18,19</sup> can be extended to other geometry as well as to the one in two dimensions (2D).<sup>30,31</sup>

When two parallel cylindrical objects of diameter D are separated by a distance  $d \ge D$  in brushes, the two key length scales relevant for the problem are (i) the diameter of the inclusion (D); (ii) the depletion layer thickness ( $\delta_c$ ) that corresponds to the size of the polymer free region around cylinders in Figure 2B. As the distance d decreases from d > D + $\delta_c$  to  $D \le d \le D + \delta_c$  due to the depletion forces, the total volume accessible for brush polymers  $V(d_i\delta_c)$  increases from  $V_>$  to  $V_> +$  $V_{ex}(d_i\delta_c)$ 

$$V(d; \delta_c) = \begin{cases} V_{>} & \text{for } d > D + \delta_c \\ V_{>} + V_{\text{ex}}(d; \delta_c) & \text{for } D \le d \le D + \delta_c \end{cases}$$
(25)

where  $V_{>} = (A - \pi D^2/2) \min(h,H)$  and the *d*-dependent excess volume formed for  $D < d < D + \delta_c$  is given by  $V_{\text{ex}}(d; \delta_c) = A_{\text{ex}}(d;\delta_c) \min(h,H)$  with the excess area created between the two inclusions (Figure 2B)<sup>16</sup>

$$A_{\text{ex}}(d; \, \delta_c) = \frac{1}{2} \left[ (D + \delta_c)^2 \cos^{-1} \left( \frac{d}{D + \delta_c} \right) - d\sqrt{(D + \delta_c)^2 - d^2} \right]$$

$$(26)$$

Thus, in reference to the configurations of inclusions separated by  $d > D + \delta_{\sigma}$  the entropy of the system increases by  $\Delta S \propto \ln\left(\frac{V_{z} + V_{ex}}{V_{z}}\right)$  and the effective free energy (AO potential) between the cylinders at separation  $D < d < D + \delta_{c}$  is given by

$$\beta F_{AO}(d; \, \delta_c) \simeq -N \ln \left[ 1 + \frac{V_{ex}(d; \, \delta_c)}{V_{>}} \right]$$
$$\approx -N \frac{V_{ex}(d; \, \delta_c)}{A \min(h, H)}$$
$$\approx -\sigma(N/n_p) A_{ex}(d; \, \delta_c)$$
(27)

where the second line is obtained for  $V_{\text{ex}}/V_{>} \ll 1$  and  $V_{>} \approx A \min(h,H)$ . The proportionality constant denoted by N signifies

the number of statistically independent interaction units that contribute to the AO potential.

Importantly, the mean number of interaction units per polymer chain  $(N/n_p = 1)$ , which contribute to the depletion interaction, can vary depending on the degree of correlation between the segments, so that it is confined in the range of

$$1 \le N/n_p \le \frac{\min(h, H)}{\xi}$$
(28)

First, the lower bound  $N/n_p = 1$  corresponds to the case when a single brush polymer chain contributes to the depletion interaction as a whole. Second, one could suggest that the steric repulsion from each blob is the fundamental unit of interactions. In this case, min(h,H) / $\xi$ , corresponding to the mean number of blobs per chain that interact with the cylinder, is expected to scale with the grafting density ( $\sigma$ ) as

$$\frac{\min(h, H)}{\xi} = \begin{cases} H/\xi \simeq N/g \sim \sigma^{1/2\nu} & \text{for } H < h\\ h/\xi \sim \sigma^{1/2} & \text{for } H > h \end{cases}$$
(29)

The extent of correlation between the segments, which sets  $N/n_p$  to a value somewhere between the two bounds, is germane to the problem of brush-induced depletion interactions.

While not stated explicitly, the AO argument presented in eqs 25-27 suggests that the thickness of depletion layers ( $\delta_c$ ) decides the excess volume created between the cylinders. In what follows, we will show that  $\delta_c$  is closely related with the size of blobs formed in brushes.

**3.3.** PMFs from SCFT and Their Comparison with AO Theory and MD Simulations. The dependence of the free energy profile obtained using the SCFT (eq 20) on the grafting density ( $\sigma$ ) is not explicit. Thus, it is not straightforward to make direct comparison between the free energy profiles obtained from SCFT and those from MD simulations. Here, we map the excluded volume parameter  $\Lambda$  introduced in eq 19 to the  $\sigma$  by means of the strong-stretching theory (SST).<sup>45–48</sup> We begin the mapping by considering the optimal configuration of the chain obtained through minimization of the total chain energy  $E[\mathbf{r}_{\alpha}(s)] = \int_{0}^{1} ds \left(\frac{3}{2R_{0}^{2}}|\mathbf{r}_{\alpha}'(s)|^{2} + W[\mathbf{r}_{\alpha}(s)]\right)$ , which is a func-

E[ $\mathbf{r}_{\alpha}(s)$ ] =  $\int_{0}^{1} ds \left(\frac{1}{2R_{0}^{2}} \mathbf{r}_{\alpha}(s)\right) + w[\mathbf{r}_{\alpha}(s)]$ , which is a functional of chain conformation  $\mathbf{r}_{\alpha}(s)$ . From the calculus of variation, i.e.,  $\delta E[\mathbf{r}_{\alpha}(s)] = 0$ , we get  $3\mathbf{r}_{\alpha}''(s)/R_{0}^{2} - \partial_{\mathbf{r}_{\alpha}}W[\mathbf{r}_{\alpha}(s)] =$ 0. Any chain conformation satisfies the boundary conditions  $z_{\alpha}(1) = 0$  and  $z_{\alpha}(0) = z_{0}$ , such that the tip of the chain (s = 0)starting from the height  $z = z_{0}$  always ends at z = 0 that corresponds to the grafting point (s = 1). This is analogous to a situation for a simple harmonic oscillator, where the time it takes to reach the minimal point of potential energy is identical regardless of the initial displacement. Thus, the effective potential energy is parabolic along the z-axis,  $W(z) \propto C - Bz_{\alpha}^{2}$ , which yields the equation of motion  $3z_{\alpha}''(s)/R_{0}^{2} + 2Bz_{\alpha}(s) = 0$ , and its solution  $z_{\alpha}(s) = z_{0} \cos(\pi s/2)$  with  $\sqrt{2BR_{0}^{2}/3} = \pi/2$ . We thus find

$$W(\mathbf{r}) = \Lambda \phi(\mathbf{r}) = \frac{3\pi^2 (H^2 - z_a^2)}{8R_0^2}$$
(30)

Here the constant *C* was determined from the condition of W(H) = 0. The relations  $V_p = \int d\mathbf{r}\phi(\mathbf{r}) = A \int_0^H \phi(z_\alpha) dz_\alpha$  and  $AR_0 = V_p$  lead to



**Figure 3.** Depletion free energies from SCFT and MD simulations. (a)  $\beta F_{SCF}(x)/\sigma R_0^2$  between two cylindrical inclusions calculated by SCFT. The one by AO theory with  $N/n_p = 1$  is depicted in magenta. (b)  $\beta F_{MD}(x)/\sigma R_0^2$ . (c)  $\beta \Delta F/\sigma R_0^2$  versus  $\sigma$  (for MD) or  $\Lambda$  (for SCFT). The solid lines annotated with  $\sim \sigma^{\alpha}$  with  $\alpha = 0.10$  (H < h in A-(c)) and -1/4 (H > h in B-(c)) are the scaling relations predicted from the AO theory. For (A) H < h and (B) H > h, MD simulation results in (b) were obtained with N = 50 and N = 150, respectively.



**Figure 4.** Depletion layer. (A) Depletion layer thickness around a tall cylinder ( $h = 9R_0$ ) at height  $z = 1.9R_0$  for varying  $\Lambda$  calculated using  $\pi(R_0 + \delta_c)^2 = \pi R_0^2 + 2\pi \int_{R_0}^{\infty} r(1 - \phi(r)/\phi_b) dr$ . (B) Depicted are the monomer concentration profiles of brushes around the cylinder with  $D = 2R_0$ ,  $\phi(r)/\phi_b$ , generated from SCFT. The bottom panel shows the *z*-dependent depletion layer thickness for varying  $\Lambda$ .

$$\frac{\Lambda V_p}{A} = \frac{\pi^2}{4} \left(\frac{H}{R_0}\right)^3 R_0 \tag{31}$$

The resulting relation  $H/R_0 = (4\Lambda/\pi^2)^{1/3}$  together with eq 21 allows us to associate the excluded volume parameter  $\Lambda$  with the grafting density  $\sigma$ , establishing a one-to-one correspondence between  $\Lambda$  and  $\sigma$ .

$$\Lambda = (\pi^2/4)(R_0/b)^3 \tau \sigma b^2 \propto \sigma \tag{32}$$

The SCF calculation with  $\Lambda = 0$  generates conformations of an end-grafted phantom Gaussian chain along with the boundary condition of impenetrable cylinders. Notably, the PMF from SCF calculations between two cylinders of diameter  $D = 2R_0$  most favorably matches with the AO potential (eq 27) with  $N/n_v = 1$  and  $\delta_c = 0.45D \simeq R_0$ , namely,  $\beta F_{\text{SCF}}(x)/\sigma R_0^2 \simeq$ 



**Figure 5.** (A) Monomer density profiles of brush polymers  $\phi(\mathbf{r};d)$  around cylindrical inclusions of diameter  $D = 2R_0$  and height  $h = 2R_0$  calculated with  $H = 4R_0$  and  $\Lambda = 2\pi^2$  for varying intercylinder separations  $(d - D)/R_0 = 0.1 - 1.0$ . (B) The volume of the depletion zone visualized by calculating  $\phi_b(z) - \phi(\mathbf{r};d)$ , where  $\phi_b(z)$  denotes the mean monomer density at the bulk defined at a large distance from the cylinders.

 $\beta F_{AO}(x) / \sigma R_0^2 = A_{ex}(x;0.45D) / R_0^2$  (cyan circles and magenta line in Figure 3A,B-(a)). As briefly mentioned, brush-induced depletion interactions with  $N/n_p = 1$  signify that each brush chain contributes to the depletion interaction as a whole, and behaves like an ideal chain without any correlation.

Calculations using SCFT show that the range of interaction between the two inclusions (or depletion layer thickness) shrinks with increasing  $\Lambda$  (Figure 3A-(a),B-(a)). It could be surmised that the interchain repulsion, implicitly incorporated into the self-consistent field  $W(\mathbf{r})$ , pushes the polymer chains toward the cylinders, rendering the depletion interaction and depletion layer thickness shorter-ranged. The depletion zone around the cylinder exhibits minor variation with the height (z) from the surface (Figure 4B, the panel at the bottom).<sup>44</sup> The depletion layer thicknesses at  $z = 1.9R_0$  explicitly calculated for varying  $\Lambda$  ( $10^{-1} < 4\Lambda/\pi^2 < 10^2$ ) indicates that the scaling behavior of  $\delta_c \sim \Lambda^{-1/2}$  holds over the range of large  $\Lambda$ , i.e.,  $10 < 4\Lambda/\pi^2 < 100$  (Figure 4A). From  $\Lambda \sim \sigma$  (eq 32), it follows that  $\delta_c$ 

~  $\sigma^{-1/2} \sim \xi$ . This is, in fact, consistent with our recent MD simulation study,<sup>16</sup> which showed the depletion layer thickness around cylindrical object scales with the grafting density as  $\delta_c \sim \sigma^{-1/2}$  and that  $\delta_c/D < 1$  for all practical purposes.

From the AO theory and the condition  $\delta_c/D < 1$  which approximates  $A_{\rm ex}(D;\delta_c)$  (eq 26) to  $D^2(\delta_c/D)^{3/2}$ , it is anticipated that  $\beta \Delta F_{\rm AO}/\sigma R_0^2$  scales as

$$\frac{\beta \Delta F_{AO}}{\sigma R_0^2} = \left(\frac{N}{n_p}\right) \frac{A_{ex}(D; \,\delta_c)}{R_0^2} \sim \left(\frac{N}{n_p}\right) \frac{D^2 [\delta_c/D]^{3/2}}{Nb^2}$$
(33)

Thus, for H < h, the relations  $N/n_p = H/\xi \simeq N/g \sim N\xi^{-1/\nu}$ (eq 29) incorporated into eq 27 lead to

$$\beta \Delta F_{\rm AO}^{H < h} / \sigma R_0^2 \sim \xi^{-1/\nu} D^{1/2} \sigma^{-3/4} \sim D^{1/2} \sigma^{(2-3\nu)/4\nu} \sim \sigma^{0.10}$$
(34)

pubs.acs.org/JCTC



**Figure 6.** (A) Free energy gain as a function of relative brush height (H/h) calculated for several values of cylinder height  $(h = 0.1R_0, 0.5R_0, 1.0R_0, 1.5R_0, and 2.0R_0)$  when *H* is varied from 0 to  $5R_0$  (*H* varied to  $10R_0$  for  $h = 2.0R_0$ ). (B) Depletion zone volumes above the circular face and around the lateral surface of cylinders. (C, D) Depletion zones around cylinders with  $H = 4R_0$  at two different separations,  $y[=(d - D)/R_0] = 3$  (left) and y = 0 (right) (see also SI Movies S1, S2, S3, S4, and S5). (C) for  $h = 2R_0$  and (D) for  $h = 0.1R_0$  yield attraction and repulsion, respectively. (E)  $V_{depl}^{top}(y)$  (solid lines) and  $V_{depl}^{tat}(y)$  (circles) as a function of separation (y) for various *h*'s while the brush height is fixed to  $H = 4.0R_0$ . (F)  $V_{depl}(y) = V_{depl}^{top}(y) + V_{depl}^{tat}(y)$  calculated for varying *h*'s, normalized by the value at large separation ( $V_{depl}(\infty)$ ). Calculated separately as well are the normalized depletion zone volumes above the circular face ( $V_{depl}^{top}(y)/V_{depl}^{top}(\infty)$ ) and around the lateral surface of the cylinders ( $V_{depl}^{tat}(y)/V_{depl}^{tat}(\infty)$ ).

with  $\nu = 0.588$ . Both the dependences of  $\beta \Delta F_{\rm SCF} / \sigma R_0^2$  and  $\beta \Delta F_{\rm MD} / \sigma R_0^2$  on  $\sigma$  are consistent with each other and in good agreement with the prediction made by eq 34 (Figure 3-A(c)).

For H > h, the segment of a brush polymer directly in contact with the inclusion is limited by the inclusion height, *h*. Thus, the mean number of blobs exerting the depletion force on the inclusion is given  $N/n_p \simeq h/\xi$ , and the scaling relation of  $\beta \Delta F_{AO}/\sigma R_0^2$  against  $\sigma$  predicted from the AO theory reads

$$\beta \Delta F_{\rm AO}^{H>h} / \sigma R_0^2 \sim (h/\xi) D^{1/2} \xi^{3/2} \sim h D^{1/2} \xi^{1/2} \sim \sigma^{-1/4}$$
(35)

This accounts for the results from MD simulation and SCFT in the range of  $0.01 \le \sigma \le 0.05$ . Notably, the profiles of  $\beta F(x)/\sigma R_0^2$ from SCFT exhibit a repulsive barrier around  $x \le 1.2$  at  $\Lambda = 16\pi^2$ (Figure 3B-(a)) is consistent with the PMF from MD simulation at high grafting densities,  $\sigma b^2 = 0.08$ , 0.09 (Figure 3B-(b)). As discussed in studies on colloid–polymer mixtures, excluded volume interactions among depletants are responsible for these repulsive barriers,<sup>23–25</sup> whose height gets larger for long brush polymer chains overgrown beyond the inclusion height (H > h), especially at high grafting density.

Note that the scaling relation in eq 35 derived from the AO theory appears to deviate from the data at high grafting density  $\sigma$ , especially for tall brushes (H > h) (Figure 3B-(c)). The deviation can be attributed to the emergence of a different scaling regime at sufficiently high grafting density  $\sigma$ . In this case, the brushes are in the dense regime rather than in the semidilute regime.<sup>61</sup> This is paralleled by constrained lateral chain fluctuations at high  $\sigma$  shown in Figure 1B.

**3.4.** Monomer Density Profiles around Cylindrical Inclusions. In terms of the computational cost, SCF calculations are far more effective than MD simulations in calculating equilibrium properties of dense polymeric systems. It is straightforward to extend the calculations of the monomer density profiles around a single cylinder (Figure 4) to those around two cylinders. The mean monomer density profiles  $\phi(\mathbf{r})$ , calculated using eq 19, suggest how the depletion zone changes with the varying separations between the two cylinders (Figure 5A).

Given the mean monomer density profile  $\phi(\mathbf{r})$  demonstrated in Figure 5A, it is also straightforward to directly visualize the profile of the depletion zone (Figure 5B). By subtracting  $\phi(\mathbf{r})$ from the height-dependent bulk density  $\phi_b(z)$ , which is defined as the one at a large distance from the cylinders, one can calculate the profile of the depletion zone as

$$\phi_{\text{depl}}(\mathbf{r}; d) = \phi_b(z) - \phi(\mathbf{r}; d) \tag{36}$$

and quantify the volume of the depletion zone via the integration of eq 36

$$V_{\rm depl}(d) = \int \phi_{\rm depl}(\mathbf{r}; \, d) d\mathbf{r}$$
(37)

A few remarks are in place regarding the monomer density profiles (Figure 5A) and the corresponding depletion zones with varying intercylinder separation (Figure 5B). (i) The mean monomer density is nearly constant over  $0 < z \leq h/2$ , but it decreases at greater  $z(\geq h/2)$  until it vanishes at the tip of the brush ( $z \approx H$ , see Figure 5A). (ii) The brush polymers are depleted further in the space between the two cylinders than in other regions when their separation is reduced to  $y[=(d - D)/dt^2]$  $R_0 ] \lesssim 0.4$ , suggesting that the imbalance in osmotic pressure starts being created at this separation. (ii) The spatial variation of the mean monomer density  $\phi(\mathbf{r})$  (eq 16) offers visualization of the depletion zone. The depletion zones are found on top of the circular faces as well as in the space between the two cylinders (Figure 5B). Of particular note is that the volume of the depletion zone above the circular face of the cylinders is greater when the two cylinders are nearly in contact (y = 0.1)

than when they are well separated (y = 1.0) (Figure 5B), showing an opposite trend to the depletion zone around the lateral surface of the cylinders.

Thus, given that the depletion forces are built in the direction that minimizes the total volume of depletion zone, the variations in the depletion zone volume in the upper and lower parts of cylinders with their separation are characterized with opposite signs, giving rise to intriguing outcomes discussed in the next section.

**3.5. Nonmonotonic Variation of Depletion Interaction** with Increasing Brush Height. Our recent study on brushinduced depletion interaction using MD simulations has demonstrated nonmonotonic variation of depletion attraction with increasing brush height.<sup>16</sup> In contrast to MD simulations, SCFT does not involve any sampling issue and can be used to analyze the aforementioned nonmonotonic variation systematically.

Figure 6A demonstrates the brush-height dependent free energy gain (eq 20) calculated using SCFT at  $\Lambda = 2\pi^2$  for varying cylinder heights  $(h = 0.1R_0 - 2R_0)$ . The following points are noted. (i) The free energy gain changes nonmonotonically with brush height (H/h) for all values of *h*, and saturates at  $H/h \gg 1$ . (ii) The crossover point of nonmonotonic free energy gain, marked with blue triangle in Figure 6A, displays its dependence on the cylinder size (*h*). (iii) In sufficiently tall brushes  $(H/h \gg$ 1), the free energy gain is positive for short cylinders  $(\beta \Delta F / \sigma R_0^2)$ > 0. See the curves of  $h = 0.1R_0$ ,  $0.5R_0$  in Figure 6A), indicating that for coin-like flat cylinders whose cylinder height is even shorter than the surface blob size  $(h < \xi \simeq \sigma^{-1/2})$ , the cylinders repel each other when they are brought in contact. The net repulsion results from the fact that the depletion attraction directly acting on their lateral surface is insignificant in comparison to the depletion repulsion on the top. (iv) For tall cylinders ( $h = 2.0R_0$  in Figure 6A), on the other hand,  $\beta \Delta F / \sigma R_0^2$ saturates to a negative value in sufficiently tall brushes  $(H/h \gg$ 1), which indicates that the cylinders experience attraction.

The origin of the cylinder height-dependent variations of free energy gain are quantitatively understood by means of the depletion zone,  $\phi_{depl}(\mathbf{r};d)$  (eq 36), visualized around the cylinders via 2D projection at two different separations y = 3and 0 (Figure 6B–D), as well as by means of the depletion zone volume,  $V_{depl}(y)/V_{depl}(\infty)$  (see eq 37), calculated as a function of separation y (Figure 6E,F). The depletion zones around the cylindrical inclusions, decomposed into the top and lateral parts, clarify how the volume of each part, i.e.,  $V_{depl}^{top}(y)$  and  $V_{depl}^{tat}(y)$ , changes with the interinclusion separation (Figure 6E). When the two cylinders are brought together,  $V_{depl}^{lat}(y)$  decreases, whereas  $V_{\text{denl}}^{\text{top}}(y)$  increases (Figure 6E). These opposing trends of volume variation compete each other, giving rise not only to the nonmonotonic change in the free energy gain with growing brush height, but also to a variation in the crossover point in the curves (see the blue triangles in Figure 6A). It is also of particular note that in comparison with  $V_{depl}^{lat}(y)$  whose magnitude is expected to increase linearly with the cylinder height (h), the variation of  $V_{depl}^{top}(y)$  remains relatively insensitive to h (Figure 6E). For coin-like flat cylinder with  $h = 0.1R_0$  whose height is even smaller than the size of correlation blob  $(h < \xi \ll H)$ , it is observed that  $V_{\text{depl}}^{\text{lat}}(y)/V_{\text{depl}}^{\text{lat}}(\infty) \sim 1 (\text{or} \log(V_{\text{depl}}^{\text{lat}}(y))/V_{\text{depl}}^{\text{lat}}(\infty))$  $\sim 0$ ) over the entire range of y. This signifies that the lateral depletion force exerted to the cylinder becomes negligible, and that the depletion interaction exerted to these short cylinders is dominated by the change in the depletion volume on the cylinder top.

## 4. CONCLUSIONS

In the conventional AO theory<sup>18–21</sup> the depletion interaction is formulated based solely on the configurational entropy of depletants associated with their accessible volume. At low density, the depletants are effectively regarded as penetrable ideal gas, whose pressure is proportional to its density, following the van't Hoff relation,  $\beta \Pi \sim \phi_d$ , where  $\phi_d$  is the volume fraction of depletants. Such a picture that ignores the correlations or interactions among the depletants may be reasonable when the depletants are small and dilute.

In contrast, for brush systems, overlap between brush chains leads to deformation of each chain into a string of blobs, and the monomer concentration in each blob remains independent of chain length and is given by  $\phi \simeq g/\xi^3 \simeq \xi^{1/\nu-3} \sim \sigma^{(3-1/\nu)/2}$ . Thus, for the brush-induced interactions, considerations based on a semidilute polymer solution characterized with the correlation length  $\xi$  and the osmotic pressure  $\beta \Pi \simeq 1/\xi^3 \simeq \sigma^{3/2} \simeq \phi^{9/4}$  are better suited than, for example, a perturbative approach used in the scaled particle theory (SPT).<sup>62</sup> It is noteworthy that the AO potential derived in eq 27 is equivalent to a free energy gain upon creating an excess volume  $V_{\rm ex}(d_i\delta_c)$  in the interior of a semidilute medium characterized with osmotic pressure  $\beta \Pi \sim 1/\xi^3$ , namely,  $\beta F_{\rm AO}(d_i\delta_c) = -\beta \Pi \times V_{\rm ex}(d_i\delta_c) = -1/\xi^3 \times \min(h,H)$   $A_{\rm ex}(d_i\delta_c)$ .

In the SCFT, which is characterized with the diffusion equation for chain propagator in an effective field, the blob concept is not explicitly incorporated into the formulation. Nevertheless, similarly to the Flory argument (eq 23), the theory still captures the essence of brush-induced depletion interactions that align well with the MD simulation results (Figure 3), reproducing the scaling feature of depletion layer thickness  $\delta_c \sim \Lambda^{-1/2} \sim \sigma^{-1/2}$  for large  $\Lambda$  (Figure 4).

Finally, our finding that taller cylinders in an overgrown brush environment experience stronger depletion attraction (Figure 6) bears relevance to integrin nanocluster formation which is deemed essential for signalings.<sup>10</sup> The thickness of glycocalyx ranges between O(10) nm and  $O(1) \mu$ m depending on the cell types.<sup>63,64</sup> Upon activation, integrins expand their structure from the bent-closed to the extended-open conformation, <sup>63,65</sup> such that the integrins' height above the membrane surface increases from  $\sim 5$  to  $\sim (20-25)$  nm. Given the relative height of glycocalyx brush to active integrins  $H/h \sim 50$ , the stability of integrin nanoclusters is determined effectively at the limit of H/h $\gg$  1 in Figure 6A. In addition to the suggestion that glycocalyxinduced membrane curvature formation is a critical mechanism giving rise to integrin nanoclusters,<sup>10,14,66</sup> our study highlights the brush-induced depletion forces between integrin proteins upon activation as another key mechanism.

## ASSOCIATED CONTENT

## **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jctc.5c00334.

- $(h = 0.1R_0)$  (Movie S1) (MOV)
- $(h = 0.5R_0)$  (Movie S2) (MOV)

$$(h = R_0)$$
 (Movie S3) (MOV)

$$(h = 1.5R_0)$$
 (Movie S4) (MOV)

 $(h = 2R_0)$ , visualizing how the depletion zone around two cylinders of  $h = 2R_0$  and  $D = 2R_0$  in brush polymers with  $H = 4R_0$  changes when the intercylinder distance is varied (Movie S5) (MOV)

## AUTHOR INFORMATION

## **Corresponding Authors**

- Bae-Yeun Ha Department of Physics and Astronomy, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada; ● orcid.org/0000-0003-2089-6759; Email: byha@uwaterloo.ca
- Changbong Hyeon Korea Institute for Advanced Study, Seoul 02455, Korea; orcid.org/0000-0002-4844-7237; Email: hyeoncb@kias.re.kr

#### Authors

Daeseong Yong – Korea Institute for Advanced Study, Seoul 02455, Korea; orcid.org/0000-0001-6360-6622

Ji Woong Yu – Korea Institute for Advanced Study, Seoul 02455, Korea; orcid.org/0000-0001-8479-401X

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jctc.5c00334

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This study was supported by the National Research Foundation of Korea (NRF) grant, NRF-2022R1C1C2010613 (DY), funded by the Korea government (MSIT), the KIAS individual Grants, AP091502 (J.W.Y.) and CG035003 (C.H.), and by Natural Sciences and Engineering Research Council of Canada (B.-Y.H.). We thank the Center for Advanced Computation in KIAS for providing the computing resources.

## REFERENCES

(1) Lang, T.; Rizzoli, S. O. Membrane protein clusters at nanoscale resolution: more than pretty pictures. *Physiology* **2010**, 25, 116–124.

(2) Baumgart, F.; Arnold, A. M.; Leskovar, K.; Staszek, K.; Fölser, M.; Weghuber, J.; Stockinger, H.; Schütz, G. J. Varying label density allows artifact-free analysis of membrane-protein nanoclusters. *Nat. Methods* **2016**, *13*, 661–664.

(3) Goulian, M.; Bruinsma, R.; Pincus, P. A. Long-range forces in heterogeneous fluid membranes. *Europhys. Lett.* **1993**, 22, No. 145.

(4) Machta, B. B.; Veatch, S. L.; Sethna, J. P. Critical Casimir Forces in Cellular Membranes. *Phys. Rev. Lett.* **2012**, *109*, No. 138101.

(5) Ben-Tal, N.; Honig, B. Helix-helix interactions in lipid bilayers. *Biophys. J.* **1996**, *71*, 3046–3050.

(6) Milovanovic, D.; Honigmann, A.; Koike, S.; Göttfert, F.; Pähler, G.; Junius, M.; Müllar, S.; Diederichsen, U.; Janshoff, A.; Grubmüller, H.; Risselada, H. J.; Eggeling, C.; Hell, S. W.; van den Bogaart, G.; Jahn, R. Hydrophobic mismatch sorts SNARE proteins into distinct membrane domains. *Nat. Commun.* **2015**, *6*, No. 5984.

(7) Schmidt, U.; Guigas, G.; Weiss, M. Cluster formation of transmembrane proteins due to hydrophobic mismatching. *Phys. Rev. Lett.* **2008**, *101*, No. 128104.

(8) McMahon, H. T.; Gallop, J. L. Membrane curvature and mechanisms of dynamic cell membrane remodelling. *Nature* 2005, 438, 590–596.

(9) Reynwar, B. J.; Illya, G.; Harmandaris, V. A.; Müller, M. M.; Kremer, K.; Deserno, M. Aggregation and vesiculation of membrane proteins by curvature-mediated interactions. *Nature* **2007**, *447*, 461– 464.

(10) Paszek, M. J.; DuFort, C. C.; Rossier, O.; et al. The cancer glycocalyx mechanically primes integrin-mediated growth and survival. *Nature* **2014**, *511*, 319–325.

(11) Offeddu, G. S.; Hajal, C.; Foley, C. R.; Wan, Z.; Ibrahim, L.; Coughlin, M. F.; Kamm, R. D. The cancer glycocalyx mediates intravascular adhesion and extravasation during metastatic dissemination. *Commun. Biol.* **2021**, *4*, No. 255. (12) Kanyo, N.; Kovacs, K. D.; Saftics, A.; Szekacs, I.; Peter, B.; Santa-Maria, A. R.; Walter, F. R.; Dér, A.; Deli, M. A.; Horvath, R. Glycocalyx regulates the strength and kinetics of cancer cell adhesion revealed by biophysical models based on high resolution label-free optical data. *Sci. Rep.* **2020**, *10*, No. 22422.

(13) Buffone, A., Jr; Weaver, V. M. Don't sugarcoat it: How glycocalyx composition influences cancer progression. *J. Cell Biol.* **2019**, *219*, No. e201910070.

(14) Shurer, C. R.; Kuo, J. C.-H.; Roberts, L. M.; Gandhi, J. G.; Colville, M. J.; Enoki, T. A.; Pan, H.; Su, J.; Noble, J. M.; Hollander, M. J.; et al. Physical principles of membrane shape regulation by the glycocalyx. *Cell* **2019**, *177*, 1757–1770.

(15) Tom, A. M.; Kim, W. K.; Hyeon, C. Polymer brush-induced depletion interactions and clustering of membrane proteins. *J. Chem. Phys.* **2021**, *154*, No. 214901, DOI: 10.1063/5.0048554.

(16) Yu, J. W.; Yong, D.; Ha, B.-Y.; Hyeon, C. Depletion Interaction between Cylindrical Inclusions in Polymer Brushes. 2024, arXiv:2411.10607. arXiv.org e-Printarchive. https://arxiv.org/abs/ 2411.10607.

(17) Spencer, R. K. W.; Ha, B.-Y. How a Polymer Brush Interacts with Inclusions and Alters Their Interaction. *Macromolecules* **2021**, *54*, 1304–1313.

(18) Asakura, S.; Oosawa, F. On interaction between two bodies immersed in a solution of macromolecules. *J. Chem. Phys.* **1954**, *22*, 1255–1256.

(19) Asakura, S.; Oosawa, F. Interaction between Particles Suspended in Solutions of Macromolecules. *J. Polym. Sci.* **1958**, *33*, 183–192.

(20) Vrij, A. Polymers at interfaces and the interactions in colloidal dispersions. *Pure Appl. Chem.* **1976**, *48*, 471–483.

(21) De Hek, H.; Vrij, A. Interactions in mixtures of colloidal silica spheres and polystyrene molecules in cyclohexane: I. Phase separations. *J. Colloid interface Sci.* **1981**, *84*, 409–422.

(22) Shaw, M. R.; Thirumalai, D. Free polymer in a colloidal solution. *Phys. Rev. A* **1991**, *44*, No. R4797.

(23) Mao, Y.; Cates, M.; Lekkerkerker, H. N. W. Depletion force in colloidal systems. *Phys. A* **1995**, 222, 10–24.

(24) Mao, Y.; Cates, M.; Lekkerkerker, H. N. W. Depletion stabilization by semidilute rods. *Phys. Rev. Lett.* **1995**, *75*, No. 4548.

(25) Biben, T.; Bladon, P.; Frenkel, D. Depletion effects in binary hard-sphere fluids. *J. Phys.: Condens. Matter* **1996**, *8*, No. 10799.

(26) Mao, Y.; Cates, M.; Lekkerkerker, H. N. W. Theory of the depletion force due to rodlike polymers. J. Chem. Phys. 1997, 106, 3721–3729.

(27) Hanke, A.; Eisenriegler, E.; Dietrich, S. Polymer depletion effects near mesoscopic particles. *Phys. Rev. E* **1999**, *59*, No. 6853.

(28) Marenduzzo, D.; Finan, K.; Cook, P. R. The depletion attraction an underappreciated force driving cellular organization. *J. Cell Biol.* **2006**, *175*, 681–686.

(29) Binder, K.; Virnau, P.; Statt, A. Perspective: The Asakura Oosawa model: A colloid prototype for bulk and interfacial phase behavior. *J. Chem. Phys.* **2014**, *141*, No. 140901.

(30) Lekkerkerker, H. N. W.; Tuinier, R.; Vis, M. Colloids and the Depletion Interaction; Springer Nature, 2024.

(31) Miyazaki, K.; Schweizer, K.; Thirumalai, D.; Tuinier, R.; Zaccarelli, E. The Asakura-Oosawa theory: Entropic forces in physics, biology, and soft matter. *J. Chem. Phys.* **2022**, *156*, No. 080401, DOI: 10.1063/5.0085965.

(32) Zhou, H. X.; Rivas, G.; Minton, A. P. Macromolecular crowding and confinement: biochemical, biophysical, and potential physiological consequences. *Annu. Rev. Biophys.* **2008**, *37*, 375–397.

(33) Minton, A. P. Implications of macromolecular crowding for protein assembly. *Curr. Opin. Struct. Biol.* **2000**, *10*, 34–39.

(34) Ellis, R. Macromolecular crowding: obvious but underappreciated. *Trends Biochem. Sci.* 2001, 26, 597–604.

(35) Cheung, M. S.; Klimov, D.; Thirumalai, D. Molecular crowding enhances native state stability and refolding rates of globular proteins. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4753–4758.

(36) Elcock, A. H. Models of macromolecular crowding effects and the need for quantitative comparisons with experiment. *Curr. Opin. Struct. Biol.* **2010**, *20*, 196–206.

(37) Soranno, A.; Koenig, I.; Borgia, M. B.; Hofmann, H.; Zosel, F.; Nettels, D.; Schuler, B. Single-molecule spectroscopy reveals polymer effects of disordered proteins in crowded environments. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 4874–4879.

(38) Kang, H.; Pincus, P. A.; Hyeon, C.; Thirumalai, D. Effects of Macromolecular Crowding on the Collapse of Biopolymers. *Phys. Rev. Lett.* **2015**, *114*, No. 068303.

(39) Kang, H.; Toan, N. M.; Hyeon, C.; Thirumalai, D. Unexpected Swelling of Stiff DNA in a Polydisperse Crowded Environment. *J. Am. Chem. Soc.* **2015**, *137*, 10970–10978.

(40) Jeon, C.; Hyeon, C.; Jung, Y.; Ha, B.-Y. How are molecular crowding and the spatial organization of a biopolymer interrelated. *Soft Matter* **2016**, *12*, 9786–9796.

(41) de Gennes, P. G. Scaling Concepts in Polymer Physics; Cornell University Press: Ithaca and London, 1979.

(42) Joanny, J. F.; Leibler, L.; de Gennes, P. G. Effects of polymer solutions on colloid stability. *J. Polym. Sci., Polym. Phys. Ed.* **1979**, *17*, 1073–1084.

(43) Kim, J. U.; O'Shaughnessy, B. Nanoinclusions in dry polymer brushes. *Macromolecules* **2006**, *39*, 413–425.

(44) Matsen, M. W.; Kim, J. U.; Likhtman, A. E. Finite-N effects for ideal polymer chains near a flat impenetrable wall. *Eur. Phys. J. E* 2009, 29, 107–115.

(45) Milner, S. T.; Witten, T. A.; Cates, M. E. Theory of the grafted polymer brush. *Macromolecules* **1988**, *21*, 2610–2619.

(46) Kim, J. U.; Matsen, M. W. Finite-stretching corrections to the Milner-Witten-Cates theory for polymer brushes. *Eur. Phys. J. E* 2007, 23, 135–144.

(47) Kim, J. U.; Matsen, M. W. Repulsion exerted on a spherical particle by a polymer brush. *Macromolecules* **2008**, *41*, 246–252.

(48) Matsen, M. W. Self-Consistent Field Theory and Its Applications; Wiley Online Library, 2006.

(49) Thompson, A. P.; Aktulga, H. M.; Berger, R.; Bolintineanu, D. S.; Brown, W. M.; Crozier, P. S.; In't Veld, P. J.; Kohlmeyer, A.; Moore, S. G.; Nguyen, T. D.; et al. LAMMPS-a flexible simulation tool for particle-based materials modeling at the atomic, meso, and continuum scales. *Comput. Phys. Commun.* **2022**, *271*, No. 108171.

(50) Kumar, S.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A.; Rosenberg, J. M. The weighted histogram analysis method for freeenergy calculation on biomolecules. I. The method. *J. Comput. Chem.* **1992**, *13*, 1011–1021.

(51) Grossfield, A. WHAM: the weighted histogram analysis method. 2012.

(52) Fredrickson, G. The Equilibrium Theory of Inhomogeneous Polymers; Oxford University Press, 2006.

(53) Amit, D. J.; Martin-Mayor, V. Field Theory, the Renormalization Group, and Critical Phenomena: Graphs to Computers; World Scientific Publishing Company, 2005.

(54) Fredrickson, G. H.; Ganesan, V.; Drolet, F. Field-theoretic computer simulation methods for polymers and complex fluids. *Macromolecules* **2002**, *35*, 16–39.

(55) Matsen, M. W. Self-Consistent Field Theory and Its Applications. In *Soft Matter: Polymer Melts and Mixtures*; Gerhard Gompper, M. S., Ed.; Wiley, 2006; Chapter 2; pp 87–178.

(56) Polymer Field Theory Simulations with Python. https://github. com/yongdd/langevin-fts. (Accessed: September 04, 2024).

(57) Alexander, S. Adsorption of chain molecules with a polar head a scaling description. *J. Phys.* **1977**, *38*, 983–987.

(58) de Gennes, P. G. Conformations of polymers attached to an interface. *Macromolecules* **1980**, *13*, 1069–1075.

(59) de Gennes, P. G. Polymers at an interface; a simplified view. *Adv. Colloid Interface Sci.* **1987**, *27*, 189–209.

(60) Liu, L.; Pincus, P. A.; Hyeon, C. Compressing Θ-chain in slit geometry. *Nano Lett.* **2019**, *19*, 5667–5673.

(61) Moh, L. C. H.; Losego, M. D.; Braun, P. V. Solvent quality effects on scaling behavior of poly (methyl methacrylate) brushes in the moderate-and high-density regimes. *Langmuir* **2011**, *27*, 3698–3702. (62) Reiss, H.; Frisch, H. L.; Lebowitz, J. L. Statistical Mechanics of

Rigid Spheres. J. Chem. Phys. 1959, 31, 369–380.

(63) Luo, B.-H.; Carman, C. V.; Springer, T. A. Structural basis of integrin regulation and signaling. *Annu. Rev. Immunol.* **2007**, *25*, 619–647.

(64) Xiao, T.; Takagi, J.; Coller, B. S.; Wang, J.-H.; Springer, T. A. Structural basis for allostery in integrins and binding to fibrinogenmimetic therapeutics. *Nature* **2004**, *432*, 59–67.

(65) Li, J.; Jo, M. H.; Yan, J.; Hall, T.; Lee, J.; López-Sánchez, U.; Yan, S.; Ha, T.; Springer, T. A. Ligand binding initiates single-molecule integrin conformational activation. *Cell* **2024**, *187*, 2990–3005.

(66) Kuo, J. C.-H.; Paszek, M. J. Glycocalyx curving the membrane: forces emerging from the cell exterior. *Annu. Review Cell Dev. Biol.* **2021**, 37, 257–283.