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Note

How Does a Polymer Brush Repel Proteins?*

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Abstract The captioned question has been addressed by the steric effect; namely, the adsorption of proteins on a surface grafted with linear polymer chains decreases monotonically as the grafting density increases. However, there is no quantitative and satisfactory explanation why the adsorption starts to increase when the grafting density is sufficiently high and why polyethylene glycol (PEG) still remains as one of the best polymers to repel proteins. After considering each grafted chain as a molecular spring confined inside a "tube" made of its surrounding grafted chains, we estimated how its free energy depends on the grafting density and chain length, and calculated its thermal energy-agitated chain conformation fluctuation, enabling us to predict an adsorption minimum at a proper grafting density, which agrees well with previous experimental results. We propose that it is such a chain fluctuation that slows down the adsorption kinetically.

Keywords: Polymer brush; Protein adsorption; Antifouling.

Using polymer chains grafted on a surface, *i.e.*, polymer brushes or denoted as PEGylation when polyethylene glycol (PEG) is used, to repel proteins has been known and used for a long time in biomedical applications. It has been experimentally shown that the adsorption of proteins on such a polymer brush generally decreases as the grafting density increases and theoretically explained on the basis of the steric effect; namely, those grafted water-soluble chains are highly hydrated and cover the surface so that proteins are not able to reach the underneath surface. This is why such an explanation leads to a monotonic decrease of the adsorption as the grafting density increases[1–5]. However, it has also been repeatedly reported that the adsorption increases if the grafting density becomes too high[6–8] and there exists an optimal degree of PEGylation in *in-vivo* experiments[9], which is not explainable by either the steric effect or the hydrolysis of the grafted chains. As for the question why PEG still remains as one of the best to repel proteins in spite of the existence of different water-soluble polymers, it has been argued that PEG can bind water more strongly from the point of view of its electron-donor/electron-acceptor character[10]. In order to explain why there is a minimum adsorption at a certain grafting density, Vanderah *et al*.[6] tried to qualitatively attribute it to the compression and restriction of conformational mobility after a protein chain contacts with a small patch of grafted chains; namely, an increase of the total free energy, so that the rejection of the adsorbed protein chain reduces the free energy. For a surface covered with fully stretched chains, there will be much less or no conformation-related free energy change so that the adsorption increases at higher grafting densities.

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Recently, we have studied how polymer chains with different topologies are dragged through a small cylindrical pore by an elongation flow with a critical flow rate[11–15], which involves the confinement of a polymer chain inside a tube. Apparently, this kind of study has nothing to do with polymer brushes. However, if considering that each grafted chain is actually confined inside a "tube" made of its surrounding grafted chains, as schematically shown in Fig. 1, we will find that they actually share very similar underline physics. Namely, for a grafted chain has N Kuhn segments and each segment has a size of a, its unperturbed size (R_0) is $aN^{1/2}$ and its free energy (A) contains two entropy-related parts: the excluded volume and the elastic energy, *i.e.*,

$$\frac{A}{k_{\mathrm{B}}T}=\frac{a^3\dfrac{N^2}{2}}{\pi\left(\dfrac{D}{2}\right)^2 L}+\frac{L^2}{R_0^2} \tag{1}$$

where D and L are the inter-chain distance and the chain end-to-end distance, respectively. The first term reflects the segment-segment interaction inside a confined volume $\pi(D/2)^2L$; and the second term deals with the chain stretching inside a tube with a size much smaller than R_0. Note that $\pi(D/2)^2$ is the reciprocal of the grafting density (σ) and aN is the Contour length of the chain (L_{Contour}). Minimalizing A in Eq. (1) leads to an equilibrium chain end-to-end distance (L_0) as

$$L_0=a\left(\frac{N^2R_0^2}{\pi D^2}\right)^{\frac{1}{3}}=\left(\frac{a^2}{\pi D^2}\right)^{\frac{1}{3}}L_{\mathrm{Contour}}=\left(\frac{a^2}{4}\right)^{\frac{1}{3}}\sigma^{\frac{1}{3}}L_{\mathrm{Contour}} \tag{2}$$

For a given polymer chain, both a and L_{Contour} are constants so that L_0 is only related to the grafting density (σ). Replacing L in Eq. (1) with L_0 leads to the minimum free energy (A_{min}) as

$$\frac{A_{\mathrm{min}}}{k_{\mathrm{B}}T}=\left(\frac{27a}{16}\right)^{\frac{1}{3}}\sigma^{\frac{2}{3}}L_{\mathrm{Contour}} \tag{3}$$

Eqs. (2) and (3) can also be obtained from the "blob" argument as follows. After being confined inside a small cylinder "pore" with a diameter of D, a grafted chain can be divided into n_{b} inter-connected "blobs" and each blob has N_{b} Kuhn segments, a diameter of D and an energy of $k_{\mathrm{B}}T$. Therefore, $D=aN_{\mathrm{b}}^{1/2}$ and $L_0/D=n_{\mathrm{b}}=N/N_{\mathrm{b}}$.

$$L_0=D\frac{N}{N_{\mathrm{b}}}=DN\left(\frac{a}{D}\right)^2=\pi^{\frac{1}{2}}\left(\frac{a}{2}\right)\sigma^{\frac{1}{2}}L_{\mathrm{Contour}} \tag{4}$$

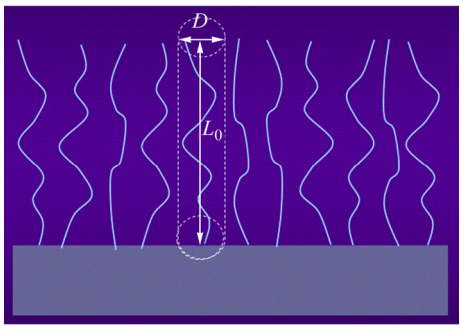

Fig. 1 Schematic of each grafted chain as a molecular spring confined inside a cylindrical tube made of its surrounding grafted chains on a surface

For a fully covered surface, the grafting density is between two extreme cases: the grafted chains are just touched each other without any chain stretching and each one has a size of R_0; and the chains are fully stretched

so that the interchain distance is a. Namely, $\sigma_{min} = 1/R_0^2$ and $\sigma_{max} = 1/a^2$. Therefore, the minimum free energy is in the range $N^{1/5} \leq A_{min}/k_BT \leq N$, depending on the grafting density. For a typical grafted PEG chain with a molar mass of ~2 kg/mol, $\sim 2k_BT \leq A_{min} \leq \sim 25k_BT$, where we know that $a \sim 1$ nm and each Kuhn segment contains about two monomers.

As expected, when A_{min} is too high, the thermal energy (one k_BT) is not able to agitate the grafted chain to undergo a large fluctuation of the chain end-to-end distance (L) so that it behaves like a rigid "spring"; and on the other hand, if A_{min} is too low, the thermal fluctuation induces a large relative change of L but its absolute change is small because initially $L_0 \sim R_0$. Therefore, there exists a proper minimum free energy, *i.e.*, a proper grafting density, at which the fluctuation of L reaches a maximum. Intuitively, we are able to speculate that A_{min} should be around 10 k_BT so that the thermal fluctuation can induce ~10% of the free energy change. A simple calculation for the typical grafted PEG chain shows that such a 10% free energy change can lead to ~25% fluctuation of L. Surprisingly, our estimations quantitatively agree well with literature results. Here, three of them, covering different polymer brushes, are listed as follow.

Example 1: By grafting PEG chains on a thermally sensitive poly(*N*-isopropylacrylamide) (PNIPAM) microgel, one can use the temperature change to shrink or swell the microgel[16] (alternate the surface area on which a given number of chains are grafted) so that the grafting density can be varied by a simple change of the solution temperature. Figure 2 shows that as the temperature increased from 25 °C to 32 °C, the grafting density and the grafted PEG layer thickness (here is the change of the hydrodynamic radius) increased ~6.5 and ~1.9 times, respectively. Surprisingly, such measured grafting density dependence of the grafted polymer layer thickness quantitatively agrees with Eq. (2) without any adjustable parameter except using a well-known experimentally measured value of $a \sim 1$ nm.

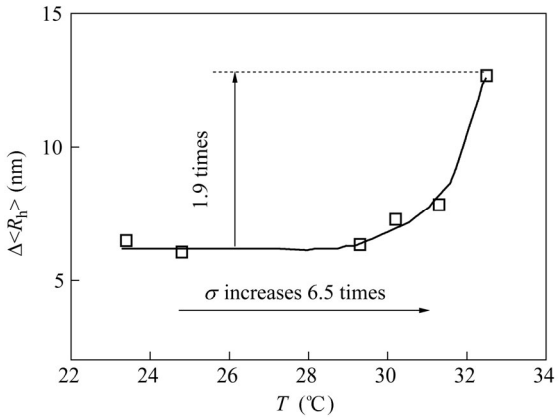

Fig. 2 Temperature dependence of thickness change of grafted polyethylene glycol (PEG) layer on a thermally sensitive poly(*N*-isopropyl acrylamide) spherical microgel (Part of Fig. 4 in Ref. [17])

Example 2: Liu *et al.*[8] used the Langmuir-blodgett trough to compress a monolayer of poly(styrene)$_{108}$-*b*-poly(ethylene oxide)$_{114}$ diblock copolymer (PS-*b*-PEO) chains on the water/air interface to increase the "grafting" density of PEO in the water phase and studied the adsorption of different proteins on such a PEO brush. As shown in Fig. 3, the adsorption first decreases as the grafting density (σ) increases in the range $0.02\ \text{nm}^{-2} < \sigma < 0.1\ \text{nm}^{-2}$ (note that here σ^{-1} is plotted); and then starts to increase with σ when the grafting density is higher ($\sigma^{-1} < 10\ \text{nm}^2$); namely, there exists a minimum adsorption at $\sigma^{-1} = 10\ \text{nm}^2$. Malmsten *et al.*[17] also found that for grafted PEG chains with a molar mass of 5000, the adsorption reaches a minimum when σ reaches 0.1 nm^{-2}. Putting $\sigma \sim 0.1$ nm^{-2}, $N \sim 50$ and $a \sim 1$ nm into Eq. (3), we found that $A_{min} \sim 13\ k_BT$, fairly close to our expected value of ~10 k_BT.

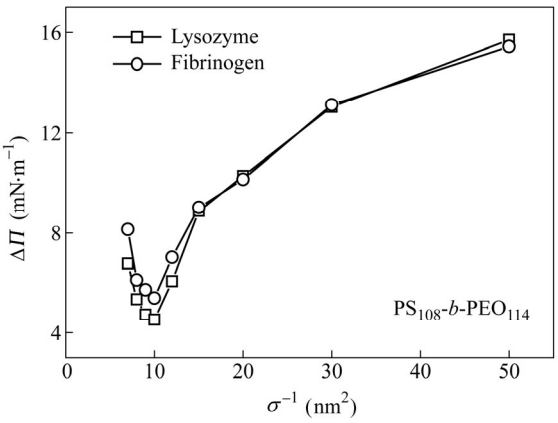

Fig. 3 Grafting density (σ) dependence of adsorption of two proteins (lysozyme and fibrinogen) in terms of surface pressure change ($\Delta\Pi$), (Higher $\Delta\Pi$ means higher adsorption.) (Part of Fig. 4 in Ref. [8]).

Example 3: Li and Huang[9] used the PEGylated lipids (M = 2 kg/mol) to strip and insert into the outer bilayer of the liposome-polycation-DNA (LPD) particles coated with two lipid bilayers and found that when the stripping (inserting) density reached 10.6 mol%, the PEG arranged in the brush mode displayed neutral surface charge and minimal protein binding when mixed with serum, resulting in little RES uptake; and thus showed high tumor accumulation. On the basis of the LPD size and the stripping density and by assuming that those inserted PEG chains form a polymer brush on the LPD surface, we know that $\sigma^{-1} \sim 5$ nm^2 and $L_{\text{Contour}} \sim 25$ nm. Using Eq. (3), we can easily estimate $A_{\min}$ to be ~10 k_BT, just as what we expected.

The next question is how the thermally agitated conformation fluctuation of the grafted chain affects the adsorption of proteins in aqueous solutions. First, we should ask whether it affects the final adsorption extent. For a given type of grafted polymer chains and a given protein solution, the final protein adsorption extent ($P_{\text{ad},\infty}$) should be only governed by the interaction (attraction) energy between the polymer and protein (ΔE); namely, $P_{\text{ad},\infty} = 1-\exp(-(\Delta E/k_BT)$. In literature, previous experimental results from different laboratories repeatedly showed that there clearly exists a minimum adsorption at an optimal grafting density, *i.e.*, after the surface is fully covered by the grafted chains, the protein adsorption decreases as the grafting density increases but further increase of the grafting density eventually leads to an increase of the protein adsorption.

The decrease of the protein adsorption was generally attributed to the hydration of the grafted chains, implying the decrease of ΔE, while the increase of the protein adsorption after passing through the adsorption minimum was related to the dehydration of the grafted chains at a very higher grafting density. However, even at the highest experimentally reachable grafting density, the grafting layer still contains 70%–80% of water so that it is not as dry as one thought. In reality, after the grafted chains are fully covered a surface, further increase of the grafting density forces each chain to stretch out and the proteins can only interact with the grafted chains not the original surface. As the grafting density increases, a protein chain with a given size should interact with more grafted chains during the adsorption so that ΔE would increase with the grafting density. Therefore, the adsorption decreases as the grafting density increases before the protein adsorption reaches its minimum is not explainable by the change of ΔE.

On the other hand, previous results in literature revealed that the adsorption took different time periods to reach its maximum, ranging from minutes to days. It is interesting to see that at the optimal grafting density the adsorption kinetics was much slower[8, 17], as shown in Fig. 4, which makes us to realize that the thermally agitated conformation fluctuation actually slows down the protein adsorption. Kinetically, the adsorption needs a characteristic time (τ) for a given pair of protein and polymer chain to interact with each other. The chain fluctuation makes the chain-protein interaction time shorter than "tau" so that the adsorption does not occur most of time, *i.e.*, increasing τ in $P_{\text{ad},t} = P_{\text{ad},\infty}[1-\exp(-t/\tau)]$, where $P_{\text{ad},t}$ is the adsorption extent at a given time t. In

previous adsorption experiments, the adsorption extent was normally measured after a given time (t). Therefore, the lower apparent adsorption is actually due to the slower adsorption kinetics; *i.e.*, for a given observing time (t), a slower adsorption process with a longer τ has a lower measured apparent adsorption extent than a faster adsorption process even they have an identical $P_{ad,\infty}$.

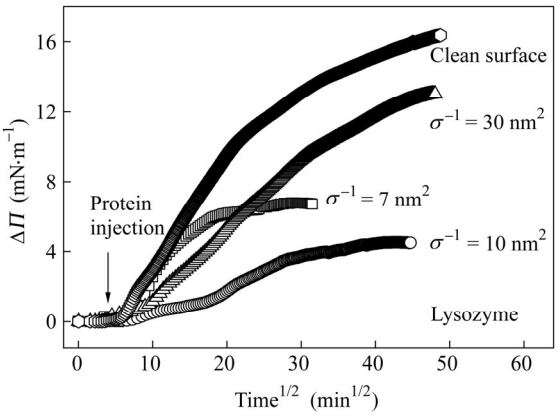

Fig. 4 Adsorption time dependence of adsorption of lysozyme in terms of surface pressure change ($\Delta\Pi$) (Higher $\Delta\Pi$ means higher adsorption.) (Part of Fig. 2 in Ref. [8]).

In summary, after considering each grafted elongated chain as a molecular spring confined inside a small cylindrical tube made of its surrounding grafted chains, we are able to estimate its free energy at its equilibrium state with an end-to-end distance longer than its unperturbed end-to-end distance. For a given polymer, both the end-to-end distance and the free energy of each grafted chain increase with the grafting density (the chain confinement). The thermal energy agitates the conformation fluctuation of each grafted chain around its equilibrium state so that its end-to-end distance randomly varies with time over a length as large as ~10 nm, generating a waving and dynamic soft surface. In a real adsorption experiment, the adsorption extent ($P_{ad,t}$) at a given time t is governed thermodynamically by the interaction (attraction) energy between the polymer and protein (ΔE) and also kinetically by the characteristic adsorption time (τ); namely, $P_{ad,t} = P_{ad,\infty}[1-\exp(-\Delta E/k_BT)][1-\exp(-t/\tau)]$, where $P_{ad,\infty}$ is the adsorption extent at $t = \infty$. It is this kind of thermally agitated conformation fluctuation of each grafted chain that increases τ, not much ΔE, so that the adsorption is kinetically slowed down, leading to a lower apparent adsorption extent at a finite observation time.

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