A study on how conformation entropy of attached macromolecules drives polymeric collapse and protein folding

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KEYWORDS

Conformational entropy, polymers of surface, protein folding

ABSTRACT

The conformation of macromolecules attached to a surface is influenced by both their excluded volume and steric forces. Here I use self-avoiding random walk simulations to evaluate the occurrence of various conformations as a function of the number of monomeric units to estimate the effect of conformational entropy of a tethered chain. Then, a more realistic scenario is assessed, which can more accurately reproduce the shape of a tethered macromolecule. The simulations presented here confirm that it is more likely for a polymer to undergo a collapse conformation rather than a stretched one, as a collapse conformation can be realized in more different ways. Also, they confirm the 'mushroom' shape of polymers close to a surface. From this simple approach, we can

estimate how conformation entropy changes with the size of the molecule. These results predict that for a model 100-units polymer close to a surface, the conformation entropy contributes with over 129 k_BT toward its collapse. This conformation entropy is higher than that of typical hydrogen bonds and even barriers that keep proteins folded. As such, entropic collapse of macromolecules plays an important role in realizing the mushroom shape of attached polymers and can be the driving force in protein folding, while the polypeptide chain emerges from the ribosome.

INTRODUCTION

Polymers are macromolecules with many repeating units, which are frequently adsorbed to surfaces. While linked to a surface, it is well known that polymers form mushroom like structures, when not densely spaced (**Figure 1A**), and brush-like structures when the surface density is high (due to steric repulsive forces) [1]. However, polymers do not tend to be fully stretched, nor do they lie flat on an inert surface, and their conformation dynamics is driven by thermal motion [2]. Seen as a particular kind of polymers, proteins are synthesized by the ribosome machinery as a sequence of 20 amino acids, following the genetic code (**Figure 1B**). The backbone of any protein is the peptide bond, formed between the carboxyl terminus of one amino acid and the amine terminus of the next. The functional groups that are not part of the peptide bond interact with each other and the water environment, to guide somehow this newly expressed polypeptide chain into a well-defined 3-dimensional (3D) structure [3]. The final conformation, known as the native fold of a protein, is formed by secondary structure elements, such as alpha helices and beta strands, encompassed by unstructured regions, and arranged in a specific order. As the polypeptide chain emerges from the ribosome channel, this system can be also seen as a macromolecule attached to a surface at one if its ends. This polypeptide chain will also not be fully stretched, nor will it lie flat on the surface of the ribosome. Also, an interesting question was first formulated by Levinthal, who realized that an algorithmic search for an typical polypeptide chain (over 100 amino acids) to find the native protein structure would take an enormous time, much longer than the time it takes the process *in vivo* (typically less than a second for a protein domain) [4]. However, this search ignores the effect of the conformational entropy on a tethered chain.

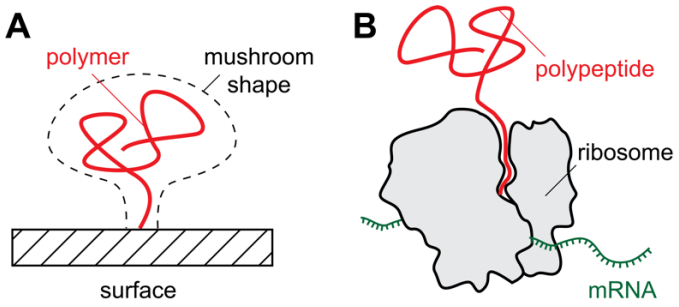

Figure 1. Polymers attached to a surface at one end. A) Schematics of a polymer attached to a hard surface and taking a mushroom-like shape. B) Schematics of a polypeptide emerging from the ribosome in its track to becoming a protein.

Entropy is introduced through the second law of thermodynamics, and is typically seen as a measure of the disorder of a system. As a system advances toward thermodynamic equilibrium, entropy increases to its highest value. In general, we can describe entropy of a system as a probability distribution among a discrete set of microstates which lead to a well-defined macrostate. The entropy $S(N)$ for N microstates of a given extension is then given by:

$$S(N) = k_B \ln (N)$$

where k_B is the Boltzmann constant. As any observed macrostate *i* at a given extension is realized from a specific number of microstates occurring with equal likelihood, the probability of each macrostate to occur is proportional to the number of microstates forming that macrostate $P_i = N/N_{total}$, where N_{total} is the total number of possible unique states forming all macrostates. From this perspective, conformational entropy can be viewed as a thermodynamic potential, as a chain will collapse in parallel to its synthesis [5]. Indeed, for tethered macromolecules, single molecule force spectroscopy techniques such as the Atomic Force Microscopy (AFM), have given us a glimpse into better understanding the forces related to the entropically-driven polymeric collapse. AFM tethers a single molecule between a surface and a cantilever and can apply forces ranging from pN to nN while changing the tether length over nm distances [6]. For example, a

macromolecule made from ~100 units requires forces of several hundred pN to extend to less than 90% its contour length [7]. AFM also measured the strength of the covalent bonds holding the polymeric backbone together, which ranged between 2 to 4 nN [8]. Interestingly, these forces are smaller than the theoretical force needed to fully extend a 100-unit polymer to its contour length [7]. Another interesting finding from single molecule measurements comes from the entropic collapse, which can contract a polypeptide chain for tens of nanometers against forces as high as 10 pN, to form the native state of a protein and generate a barrier of ~6 k_BT at 12 pN [9]. It was proposed that this collapse represents a force-induced barrier, as typically the end-to-end difference between a folded and unfolded protein is less than 1 nm [9]. Obviously, the single molecule measurements represent systems out-of-equilibrium, where the probability of each state P_i changes with force and time.

Here I analyze from an entropic perspective the behavior of a macromolecule attached at one of its ends, which is representative for polymers on a surface or proteins emerging from the ribosome (**Figure 1**). I use a simple self-avoiding random-walk approach to simulate and determine the number of equivalent conformations that a molecule can take when diffusing on a 2D and 3D lattice along Cartesian (or rectangular) coordinates. I find that entropy increases rapidly with the number of monomeric units and estimate that for a 100-units polymer to be of ~ 85.5 k_BT , and ~ 129 k_BT respectively. I then use a realistic chain model to determine the most likely conformation of a polymer and find that the conformation with the lowest entropy is in close proximity to the surface, in a collapsed state. Taken together, this study aims to improve our understanding on how entropic effects can be the driving force for the collapse of polymers close to a surface and how this collapse can play a crucial role in reducing the number of conformations during protein folding.

METHODS

Simulations were done using Igor Pro (Wavemetrics), where a chain was first randomly grown in 2D to have a desired number of units, or nodes, one step at a time. The chain was grown starting from point (0,0) in (*z*,*x*) and using a random value taken from a uniform distribution to determine the direction of the following step as either +*z*, -*z*, +*x* or -*x*. After each step, the algorithm checked (1) the hard-wall assumption: that the value along the *z* axis is positive, as a molecule cannot pass through the surface to which it is attached; (2) excluded volume assumption: that the position of the node generated does not overlap with any other previously generated nodes, as two nodes cannot occupy the same position in space. The conformations satisfying the hard-wall and excluded volume conditions were then analyzed against previously simulated paths and were collected if the generated conformation (microstate) did not appear previously. The simulation was run until no new conformations were generated. A similar approach was employed for the 3D simulations over Cartesian coordinates, where the simulation was started in (0,0,0) for (*x,y,z*) and the chain was grown with equal probability of one step along each of the three possible directions. Following data collection, the randomly generated paths were analyzed based on either the separation between the surface and the free end of the molecule (the value reached along the *z* coordinate), or the distance between the two ends, evaluated as $\sqrt{x^2 + z^2}$ for 2D search, and as $\sqrt{x^2 + y^2 + z^2}$ for the 3D search. The entropy was evaluated as $k_B \ln (N_{max} - N_{min})$, where N_{max} is the highest number of equivalent microstates for a given number of units *n*, and N_{min} is the occurrences in the state with the lowest occupancy.

For realistic molecular simulations, 1,000,000 traces were generated having 100 nodes/units and a maximum bending angle between segments of $\theta = 0.96$ rad (equivalent to $\theta =$ -0.96), where:

$$\langle \cos\theta \rangle = \frac{\langle \vec{l}_i \cdot \vec{l}_{i+1} \rangle}{{l_b}^2}$$

with $\vec{l}_i$ and $\vec{l}_{i+1}$ being two adjacent segments and l_b being the bond length. This stiffness parameter can be related to contour length l_p as [10]:

$$l_p = \frac{-l_b}{\ln\left(\langle \cos\theta \rangle\right)}$$

The value for the bending angle was chosen to match the persistence length of a polypeptide chain [11]. During simulations, the molecules were not allowed to have coordinates with negative values in *z*. Following these simulations, the generated trajectories (molecular conformations) were used to estimate the separation between the free end and the surface, as well as the molecular end-to-end length, following the same procedure, as described above.

RESULTS

To visualize how entropy shapes the conformation of a polymer chain, we commence our journey with the simple example of a 2D random walk along the *x*-*z* coordinates (**Figure 2**). In this case, the walk starts from (*x,z*) = (0,0) and proceeds in steps of one increment along either the *x* coordinate ($x+=1$ or $x-=1$) or along the *z* coordinate ($z+=1$ or $z-=1$). Due to the hard/impenetrable surface assumption, the first step cannot be -*z*, and the pathways that led to a negative value in *z* were discarded as well. Furthermore, the same position can only be occupied by a single node. **Figure 2A** shows the possible conformations for a chain made of five nodes ($n = 5$) separated over their extension in respect to the surface, l_s (which is also the final value in *z*). For any given number of nodes (monomers), the chain can only have a single fully extended conformation ($l_s = n - 1$), but can follow up many collapsed paths. In the case considered here

for $n = 5$ nodes, there are $N = 8$ possible conformations that reach an extension from the surface of 3 units, 32 microstates that have the free end 2 units away from the surface, and only 8 conformations where the final node lies on the surface (**Figure 2A**). This simple experiment reveals several interesting subtilities about a polymeric chain: 1. a molecule has a lower free energy in a collapsed state than in an extended state, as there are more equivalent conformations (microstates) closer to the surface versus a single extended state; 2. while an untethered molecule will obviously have the most populated state at an average extension of zero, due to steric effects coming from the surface, the most likely state has an extension larger than zero. Hence, the macrostate with the highest probability will be close to the surface, but not on the surface.

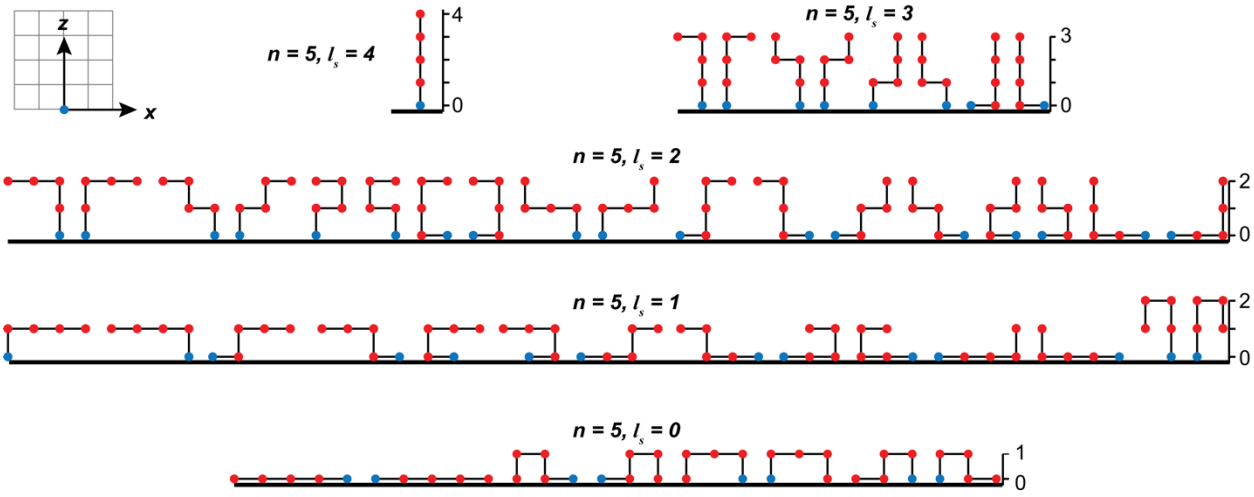

Figure 2. Diffusion of a polymer on a 2D lattice. All possible conformations of a n = 5 nodes chain arranged as a function of its extension from the surface. The highest number of equivalent states is N = 32 at an extension l_s = 2 units. The blue node shows the (0,0) position.

To better understand how the chain collapse changes with the number of units forming a molecule, we performed the same experiment for paths ranging from $n = 2$ to $n = 12$ nodes.

When the states are counted based on their final separation from the surface, l_s, a few things become apparent (**Figure 3A**): 1. the number of possible microstates N increases dramatically with the number of monomers n; 2. the location of the most populated macrostate slowly increases with the number of nodes, away from the surface (dotted line in **Figure 3A**). To quantify how this change in number of microstates, N, evolves with the number of steps, we can track the number of conformations of the most populated macrostate as conformational entropy, S (**Figure 3B**). In this case, the entropy change can be simply defined as $\Delta S = k_B \ln (N_{max} - 1)$, since there is a single fully stretched state for any given number of nodes, n. The change in conformational entropy with the number of nodes is linear, evolving with $\Delta S_{2D} = -1.5 + 0.87 \cdot n$ over the investigated range, in units of k_B.

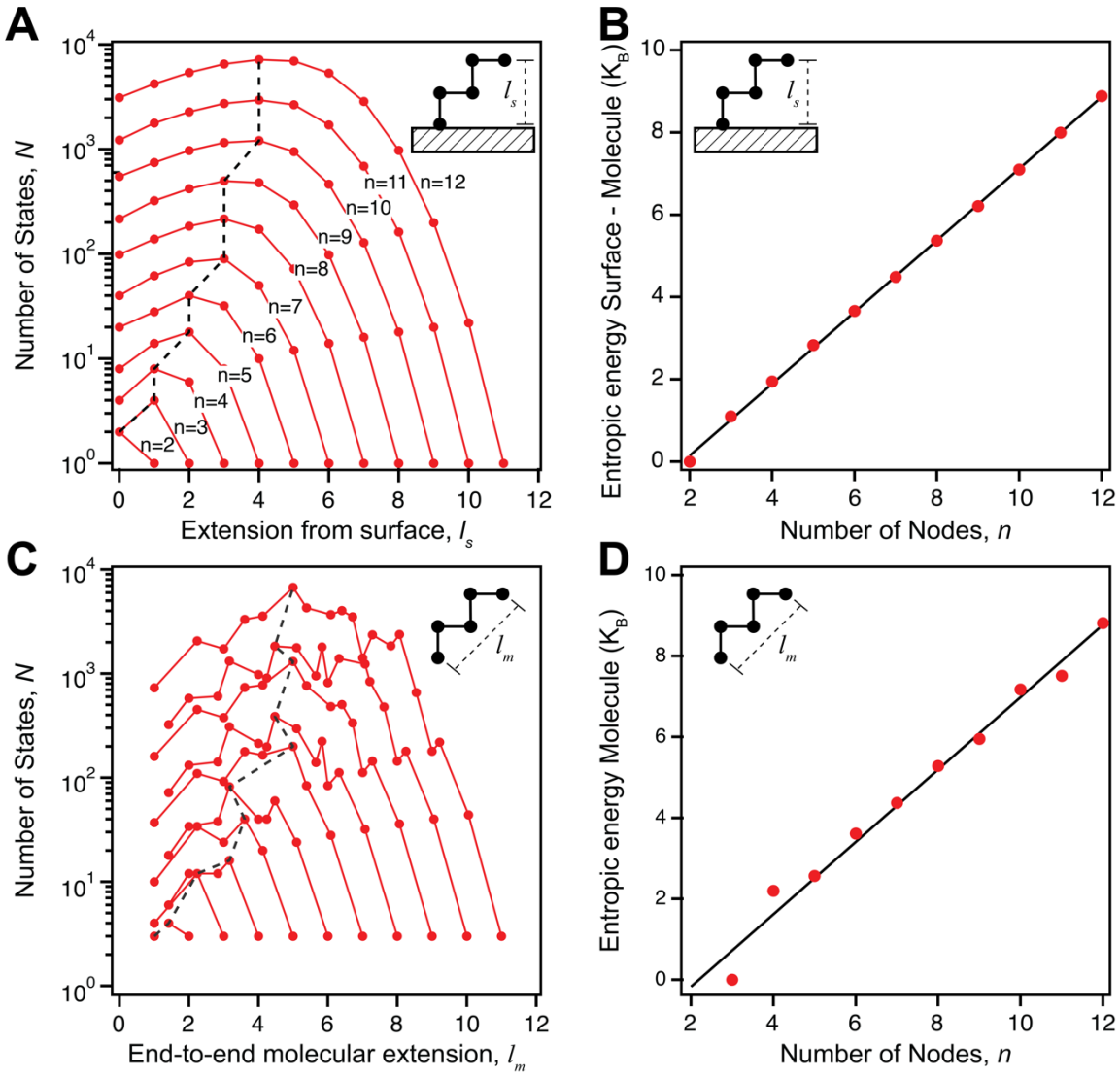

Figure 3. Entropy change of a polymer on a 2D lattice. A) Number of states as a function of extension from the surface, calculated for a chain with changing number of units, from n = 2 to n = 12. B) Calculated entropy increase from a fully stretched state to the most populated state, assuming surface-to-free-molecular-end extension equivalency. C) Number of states arranged by molecular length. D) Calculated entropy increase from fully stretched states to the most populated state, assuming end-to-end extension equivalency. The dotted line in panels A and C shows the most populate state. The continuous lines in panels B and D show linear the fit.

So far, we considered the microstates equivalency from the perspective of the free end in respect to the surface. However, the same exercise can be done from the perspective of the end-to-end separation, or molecular length l_m (**Figure 3C**). While, for example, a 2-node path will have two microstates with $l_s = 0$, which are $(x, z) = (+1,0)$ and $(x, z) = (-1,0)$, and one with $l_s = 1$, $(x, z) = (0,1)$, from the molecular length perspective all possible three microstates are equivalent and equal to one unit, $l_m = 1$. Similarly, for higher number of units, l_s will always have integer values, while l_m, which is given by $\sqrt{x^2 + z^2}$, will not. Interestingly, from a molecular microstate perspective, the number of equivalent microstates also increases rapidly with the number of monomeric units, while the location of the most populated macrostate slowly evolves with *n*. When following the same approach as for the surface-molecular end, the change in conformational entropy with the number of units can be determined (**Figure 3D**). In this case, a dependency is seen that follows the formula: $\Delta S_{2D} = -2 + 0.89 \cdot n$, also in units of k_B.

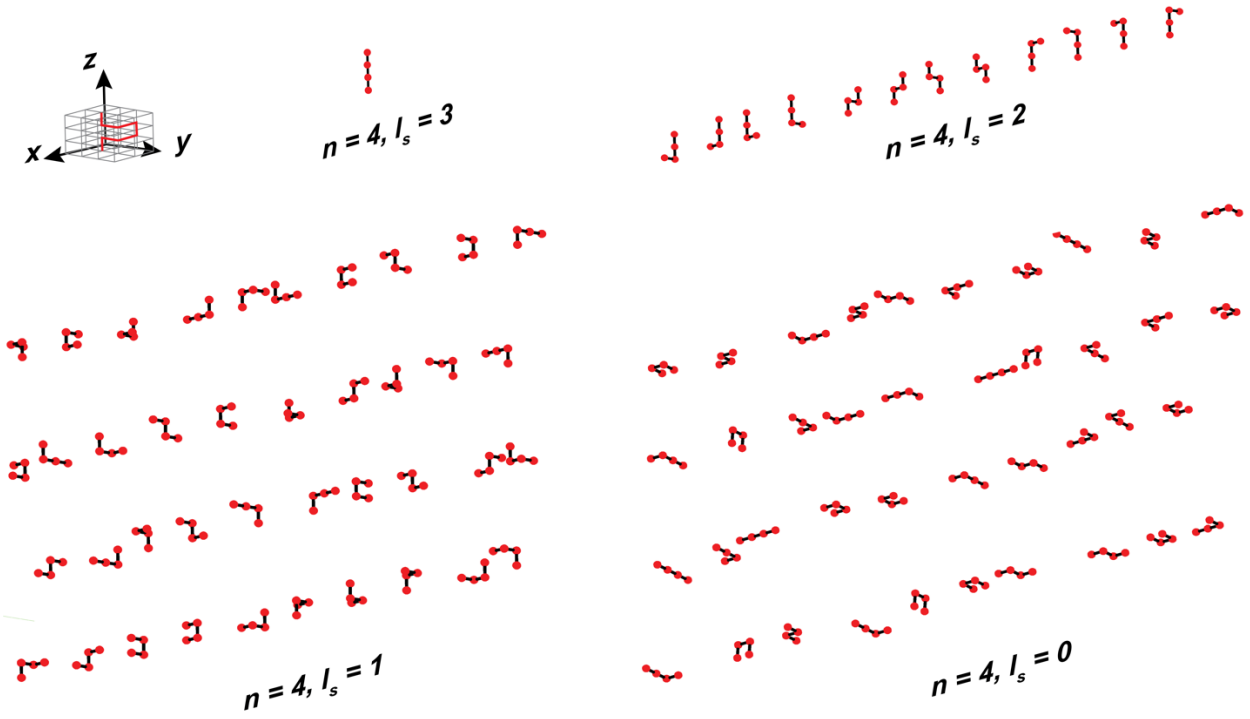

Figure 4. Diffusion of a polymer on a 3D lattice. All possible conformation of a n = 4 nodes chain arranged as a function of its extension from the surface. The highest number of equivalent microstates is N = 40 at an extension l_s = 1 and l_s = 0 units.

Next, we will follow a similar analysis in 3-dimensions (3D). In this case, the random path was generated on a lattice along Cartesian coordinates starting from $(x, y, z) = (0,0,0)$ and collecting all the states that follow both the exclusion volume and hard-surface conditions (see Methods for more detail). As seen in **Figure 4**, a chain made of four nodes ($n = 4$) can reach a single stretch conformation ($N = 1$) in respect to the surface, $l_s = 1$. However, the number of conformations rapidly increases to $N = 12$ for $l_s = 2$, and to $N = 40$ for both $l_s = 1$ and $l_s = 0$.

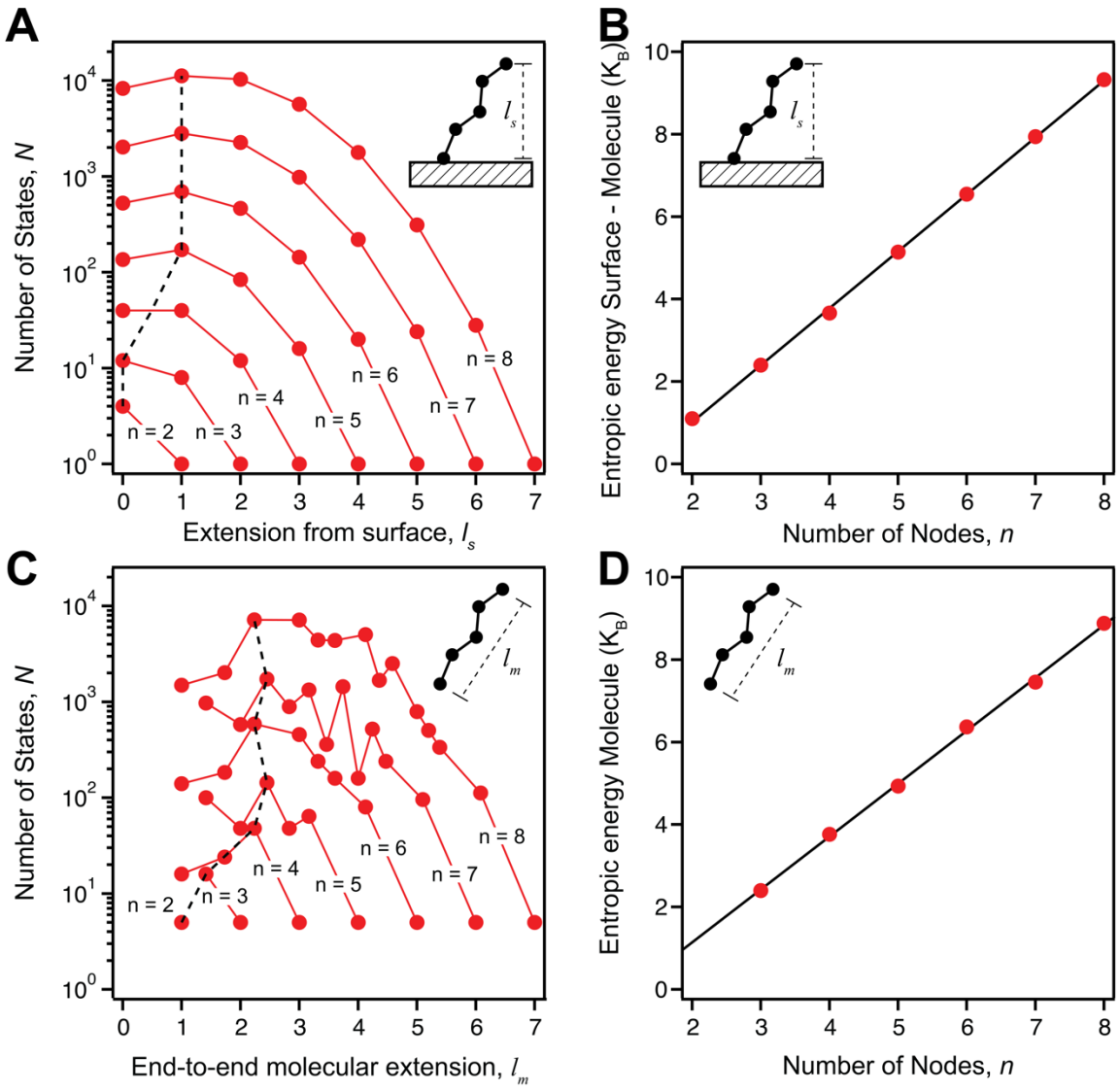

Figure 5. Entropy change of a polymer on a 3D lattice. A) Number of microstates as a function of extension from the surface, calculated for a chain with changing number of units, from n = 2 to n = 8. B) Calculated entropy increase from fully stretched state to the most populated state, assuming surface-to-free-molecular-end microstate equivalency. C) Number of microstates arranged by molecular length. D) Calculated entropy increase from fully stretched state to the most populated state, assuming end-to-end extension microstate equivalency. The dotted line in panels A and C shows the most populate state. The continuous lines in panels B and D show linear the fit.

As it was the case with the 2D search, the number of possible conformations increases rapidly with the number of monomers, *n* (**Figure 5A**). Interestingly, the location of the most likely macrostate increases much slower (dotted lines in **Figure 5A** versus **Figure 3A**). From the number of states of the most populated surface-molecule separation, one can estimate how conformation entropy changes (**Figure 5B**). In this case, the change follows $\Delta S_{3D} = -1.7 + 1.4 \cdot n$ in units of k_B.

A similar analysis can be done from a molecular perspective (**Figure 5C** and **Figure 5D**). As it was the case with the 2D search, a one-step path will have now four conformations for $l_s = 0$, which are $(x, y, z) = (\pm 1, 0, 0)$ and $(0, \pm 1, 0)$ and one with $l_s = 1$, given by $(0, 0, +1)$. The same $n = 2$ will generate five paths with molecular length of 1 unit, $l_m = 5$. Hence, when the conformation entropy is computed from a molecular perspective, it will be given by $\Delta S_{3D} = k_B \ln (N_{max} - 5)$. As seen in **Figure 5C**, the location of the most probable state evolves only slightly with the number of monomeric units, *n.* The change in conformation entropy is given in this case by: $\Delta S_{3D} = -1.4 + 1.3 \cdot n$ in units of k_B (**Figure 5D**).

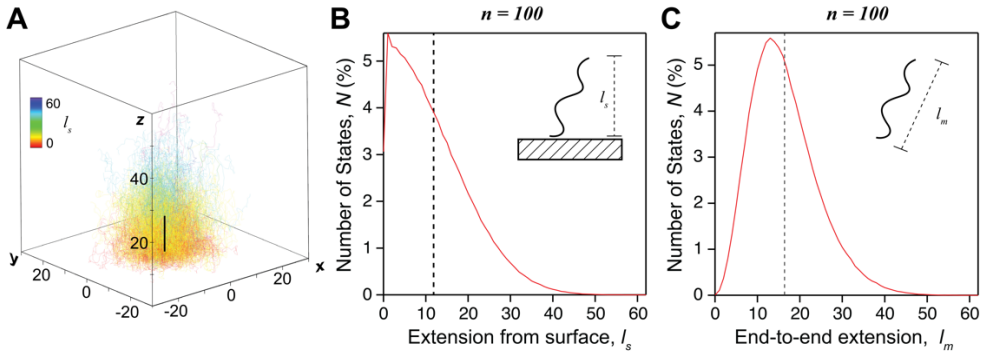

Figure 6. Freely diffusing polymer attached to a hard surface. A) Gizmo showing 1000 randomly-picked traces (from total of 1,000,000 simulated) of a polymer with 100 units, color coded based on their surface-to-free-molecular-end separation. The thick black line represents the average over these traces. B) Change in the number of states as a function of extension from the surface. The most populated state is at molecular extension of 1 unit, and the average at ~11.6 units (dotted vertical line). C) Change in the number of states

as a function of molecular extension. The most populated state is at molecular extension of 13 units, and the average at ~16.4 units (dotted vertical line).

Next, we will follow the random walk approach for a search which is not restricted to a lattice (**Figure 6**). This search better resembles a realistic chain and a persistence length close to that of a polypeptide was chosen (see *Methods* for more details). In this case, for a molecule made of 100 monomers, the generated trajectories can be plotted and color-coded based on their separation from the surface (**Figure 6A**). As can be seen in this figure panel, representing only 1000 of the total generated traces, most of the molecules are in close proximity to the surface (red vs purple when following a rainbow color scheme for l_s). If we bin the separation from the surface of the free molecular end in increments of one unit, the most likely state has an occupancy of only 1 unit away from the surface, while the average state is ~11.6 bond-length away (**Figure 6B**). From the molecular end-to-end length, the most likely state is 13 bond-length away, while the average is ~16.4 units (**Figure 6C**). From both perspectives, the macrostate with the highest population (and hence the lowest entropic energy) is close to the surface. This result agrees qualitatively with the predictions arising from the lattice simulations (dotted lines in **Figures 3** and **5**). These results further strengthen the concept that a macromolecule has the natural tendency to collapse, in order to minimize its conformational energy. Furthermore, it was shown experimentally for an unfolded peptide chain has a gyration radius of $R_g \sim 2.5$ nm [12], which is equivalent to an end-to-end distance of ~18 units (considering the size of an amino acid as 0.34 nm and that $l_m = R_g\sqrt{6}$).

DISCUSSION

There are many instances where macromolecules are bound at one end to a surface, while having the other end either freely diffusing, or tethered to a measuring probe (such as an AFM cantilever

or a trapped micrometer-sized bead). In the introduction, we looked at two examples, a polymer attached to a flat surface and a polyprotein being produced by the ribosome, in the process of becoming a protein (**Figure 1**).

Estimating the number of microstates leading to a certain macrostate represents a straightforward way to find the most-likely polymeric conformations and to estimate the entropic energy gain when a molecule collapses close to a surface. Computationally it is not possible to determine all the possible equivalent conformations (microstates) producing a certain extension. Due to this limitation, we focus on diffusion over a lattice, which will provide the lower bounds for the contribution of conformation entropy to chain collapse. Polymer elasticity models, such as the freely-jointed chain [2] and worm-like chain [13] predict that infinite force/energy is required to fully stretch a polymer chain, and hence cannot be used to estimate the entropy from a fully stretched to a collapsed state. Due to the self-avoiding condition, there is no straightforward formula to estimate the number of unique states that correspond to a given extension. Hence, here a random-walk search approach over a lattice was used to determine the number of possible microstates for molecular diffusion, leading to a specific macrostate. In the present case, macrostates can be defined as either from the perspective of the separation of the free molecular end from the surface, or from the end-to-end separation between the first and last nodes of a molecule. At high enough monomeric units, the two perspectives produce similar entropy values. In both cases, the entropy can be then calculated as the logarithm of the number of microstates. Interestingly, for both the 2D and 3D lattice searches, and for both reference points, the entropy increases linearly with the number of monomeric units. This relation allowed us to estimate the conformation entropy for larger polymers. Furthermore, the most occupied state, and hence the state with the lowest conformational entropy, was found to be close, but not directly lying on the surface. This result neatly explains the mushroom-like conformation that polymers take close to an inert surface, where they are neither extended, not fully attached to the surface. However, the stem of the mushroom shape is surprisingly short, and the cap surprisingly compact.

Diffusion of a more realistic chain was then followed. Finding all the possible states for a randomly diffusing polymer is currently an unsurmountable task, given that the total possible number of conformations must be achieved. Hence, this approach is better suited to determine the most likely polymer shape, and not to evaluate the conformational entropy difference between an equilibrium state and the highest energy/lowest entropy state (which is the fully stretched state, with zero entropy). A more relevant result here is the insight that these simulations provide a window into the probability distribution between macrostates. Here we find that the most stable conformation is only one monomeric unit away from the surface when considering surface-free end interaction, with an occupancy of ~5.6% and the average state is at ~11.6 units away. When considering the end-to-end interaction, the most energetically favorable conformation has a separation of ~13 units with an occupancy of also ~5.6% and an average state of ~16.4 units. To put things in perspective, for 100-amino acids protein, where the unit size is ~0.34 nm/amino acid, the entropic collapse alone would bring the ends of the polypeptide chain to ~4.4 nm and ~4 nm away from the surface of the ribosome. Considering that the average size of a protein is ~4 nm, the path from the collapsed state to the folded structure is relatively short.

In the approach taken here, we looked only at the free energy change arising from entropic contributions of the polymeric chain, and disregarded other interactions such as solvation effects [14], steric forces [2], attractive van der Waals, attractive and repulsive electrostatic interactions [15, 16], electronic structure [17] and crowding-induced interactions [18]. To quantify the effect of conformation entropy on the overall polymeric collapse, and rather the energetic cost for a polymer to be maintained fully extended, a direct approach is to analyze the energies associated with it. Here we use as units k_BT, as typically one such unit is considered at the boundary between an interaction and a randomly driven process [19]. To put things in perspective, a hydrogen bond has an average energy of 2-7 k_BT, a protein requires between 8-25 k_BT to cross its folding barrier, while a covalent bond, which typically forms the backbone of a polymer, is > 100 k_BT (**Table 1**). Relations were obtained on how conformational entropies vary with the size of a

molecule while using simulated 2D and 3D trajectories along Cartesian coordinates. We can now use these relations to estimate the entropic conformational energies for a typical 100-units polymer. For our most simple considered case - diffusion along a 2D Cartesian lattice - we find that the entropic energy difference is $\Delta E_{2D} = \Delta S_{2D} \cdot T = 85.5\ k_B T$ from a surface to free molecular end equivalency, and $\Delta E_{2D} = 87\ k_B T$ from an end-to-end molecular correspondence (**Figure 3**). For the same 100-units chain, these energies are even greater when considering a 3D diffusion along Cartesian coordinates, at $\Delta E_{3D} = \Delta S_{3D} \cdot T = 138\ k_B T$ from a surface-molecule analysis and $\Delta E_{3D} = 129\ k_B T$ for an end-to-end interaction (**Figure 5**). As can be seen from the comparison with other types of energies, these values already exceed the energy separating the folded and transition states of a protein and are comparable with the bond energies of the covalent interactions that form the backbone of a polymer or polypeptide (**Table 1**). Hence entropic collapse contributes significantly the free energy, effectively preventing polymers from stretching far away from a surface.

Table 1 – Typical energy for various interactions involving polymers and proteins.

Type of interaction	**Energy ($k_B T$)**
Van der Waals bonds (per interaction) [19, 20]	0.1 - 1
Hydrophobic effect (per -CH2- group) [21]	~2
Rotation along C-C bond [20]	~ 4
Trans-gauche barrier alkanes [20]	~ 6
Hydrogen bond [20, 22]	2 - 7
Solvation polypeptide [23]	6 - 18
Folded protein [24, 25]	8 - 25
Covalent bonds [8, 20]	100 - 200
Chain entropy (100 units)	*> 129*

Levinthal proposed his apparent paradox more than 90 years ago [4]. From this perspective, a random 100-amino acids polypeptide chain is not able to sample all its possible conformations in a timely manner, to become a protein. This realization led to the proposal of a funnel energy landscape [3], where the free energy ΔG decreases as the conformational entropy decreases as well (since $\Delta G = \Delta H - T\Delta S$, with ΔH representing the enthalpic contribution that must decrease faster than the entropic increase to maintain an overall negative value for ΔG). The high values associated with entropic collapse make it very tempting to consider that conformational entropy might be the main driver behind dimensionality reduction in protein folding. Indeed, we recently proposed such an idea, without having an accurate estimate of the values of the entropic energies [24]. Here we determined the lower bounds for these values. Obviously, as a peptide chain is being produced by the ribosome, it continuously undergoes an entropic collapse, and would not stay as a straight stick that suddenly contracts. Hence, the conformational entropy associated with the polymeric collapse is better viewed as a potential free energy of the folding process, or as an energetic cost, if such a process would not take place. Nevertheless, this view adds an additional component to the funnel view describing the folding energy landscape of a protein [24]: one that starts from a high-free-energy / low-entropy extended state, passes through a collapsed state (with maximum conformation entropy), and ends up in a well-defined folded 3D structure, with low entropy.

CONCLUSIONS

Conformational entropy must be of great importance for polymeric collapse and protein folding. When considering a macromolecule, a subtle thing is that the probability that a polymeric chain extends fully is the same as the probability of any other conformation. But estimates of the entropic energy driving polymeric collapse are lacking and how this phenomenon might play a significant role in the behavior of tethered biomolecules is poorly understood. Here, through iterative search, I provide an estimate of the minimum conformational entropy gained through macromolecular

collapse. Given the energy cost that an average polypeptide chain would encounter to be fully stretched, which is larger than the free energy separating the folded and unfolded states, the conformational entropy seems to have a decisive role in determining the mushroom-like shape of polymers and in the protein-folding process. Interestingly, at the two extremes of the protein folding are states with low entropy, at one end is the fully stretched polymeric state that would have an energy too high to be ever achieved, and at the other is the highly ordered native state of the protein, which is conformationally restricted by the hydrogen bonds that hold together the secondary structure elements of the molecule.

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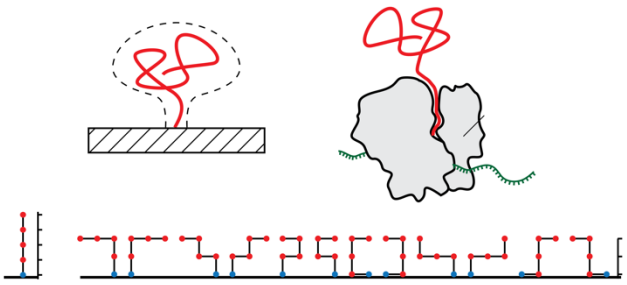

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