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Geometrical effects in protein nucleation

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Abstract

To understand the importance of protein anisotropy and the influence of translational and rotational degrees of freedom on the nucleation event, we calculate numerically-exact values for the mean encounter time for two non-spherically symmetric molecules to form a cluster, regarded here as a precursor to nucleation. A lattice model is formulated in which the asymmetry of the molecules is accounted for by representing each as a 'dimer' in the sense that each molecule is specified to occupy two lattice sites. The two dimers undergo simultaneously translation and/or rotation, and the mean times for their encounter are determined. Exact numerical results are obtained for small lattices via application of the theory of finite Markov processes, and the results corroborated and extended to large lattices by performing Monte Carlo simulations. These calculations allow one to understand in a detailed way the interplay among geometrical anisotropy, translational and internal (rotational) degrees of freedom and system size in influencing the seminal nucleation event.

Keywords: Monte Carlo simulations; Dimers; Protein nucleation

1. Introduction

Experiments on protein crystallization have shown that the production of crystals with orientational order depends in a most sensitive way on temperature, salt concentration, pH, precipitant, and so on [1]. Moreover, it is often the case that even under optimal conditions, success is not always guaranteed. Consequently, in the past few years, there has been a developing literature on the factors, both experimental and theoretical, whose understanding might enhance the success of protein nucleation experiments. The work presented in Refs. [1–10] provides an important segway into the literature of the field.

The particular facet of the nucleation event that we wish to study in this article is the effect of anisotropy on the crystallization of molecules with short-range, hard–core interactions. The importance of protein anisotropy in crystallization has been emphasized recently [7–10], and what we present here is a statistical-mechanical (lattice) model whose elaboration allows us to study the seminal event of two protein molecules, each represented as a 'dimer' (in the sense that it occupies two lattice sites) and each simultaneously

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translating and rotating, colliding (eventually) to form a two-particle cluster. Empirically, it is known that protein-to-protein interactions possess a very weak attractive part, a fact which provides the motivation in our model for the two molecules to touch each other in order that this weak interaction is manifested. The model is detailed enough to sort out the relative importance of translational vs. internal (rotational) degrees of freedom, the relative importance of one-point or two-point collisions, and the probability (or not) that an encounter between two molecules leads to nucleation.

2. Formulation

Throughout this work we are concerned with an irreversible reaction in which two, simultaneously-diffusing asymmetric molecules of the same species A give rise to a cluster A_2, whereupon the process terminates:

$$A+A\rightarrow A_2. \qquad (1)$$

In order to incorporate the principal microscopic-level processes underlying (1), a lattice model is adopted and the dynamics defined by scheme (1) is then studied using both the theory of finite Markov processes and the Monte Carlo simulation method. This dual approach was mobilized in a recent article [11], where the simpler case of reactive encounters between two simultaneously-translating symmetric particles (viewed as 'monomers' in the lattice representation) was considered explicitly.

The dynamics of system (1) on a lattice depends on both the space dimensionality and the coordination number. In particular, it is well known that non-universal behavior associated with the generation of anomalous inhomogeneous fluctuations can be expected in low dimensions and/or poor connectivity; see, for example [12,13]. In the following we will rather be concerned with the *universal* behavior prevailing when the dimensionality exceeds a critical value, a value which, in turn, generally depends on the non-linearity of the dynamics. If process (1) involved symmetric molecules, regarded as 'monomers' occupying a single lattice site, this universal behavior would already be realized by choosing a two-dimensional space (here, a square-planar lattice). Adding a third dimension would then not modify the *qualitative* behavior, although quantitative modifications amounting essentially to the lengthening of the time scales involved would occur. In the context of the present work, we shall admit that this critical dimensionality ($d=2$) holds true as well where A, in Eq. (1), is now an asymmetric molecule, a 'dimer', occupying two sides on the lattice.

Consider for definiteness two dimers positioned on a 5×5 square-planar lattice subject to periodic boundary conditions. If one assumes that the dimers are identical, as are the two ends of each dimer, then it turns out that there are 50 symmetry-distinct, mutual orientations (configurations) of the two dimers on such a lattice.

In the Markovian approach, one considers explicitly, starting from a given initial configuration, the consequent new configurations that result from the translational (center of mass) motion and from the rotational motion of each of the molecules. The latter is here modeled as a 90° pivoting of the dimer around each of its extremities, left- or right-ward. There are thus 4 translational and 4 rotational degrees of freedom, resulting from each initial configuration, 64 possible new configurations.

It is important to stress that in this model the two dimer molecules are allowed to undergo these positional changes simultaneously and independently, which amounts to assuming low activation barriers for the corresponding motions. In particular, it is assumed that there is no significant hindrance to rotation. At the level of the transition probability matrix of the underlying Markov process one will then have non-vanishing entries (viz., for each initial alignment of the two dimers on the given lattice, 64 probability factors corresponding to the possible configurational changes).

In some of these simultaneous displacement events, the two dimers can collide, and if one argues that upon collision, the two dimers 'stick' to each other then, in effect, what we are considering here is the effect of geometrical anisotropy on the nucleation event. At this point, it becomes important to note that two dimers can experience

either ‘one-point’ or ‘two-point’ collisions. These terms reference the nature of the collision channels available to the two dimers. Consider, for example, the case of two dimers in a parallel configuration on the lattice. Of all the parallel alignments which are possible, consider those for which the dimers are either immediately adjacent to each other, or are separated by a single lattice spacing. Note that two alignments are possible. In the first, there is an exact ‘match’ between the ‘head’ and ‘tail’ of the two dimers; in the second, the two dimers are staggered (either up or down) by a single lattice spacing, i.e. the head of one dimer is in concert with the tail of the other dimer. If both dimers now undergo a translation toward each other, in the first alignment there will be a ‘two-point’ collision. If, on the other hand, they are staggered, collision will result in the two dimers touching each other only at a single point (‘one-point’ collision).

If one now considers rotations, there arise a myriad of new, one-point and two-point collision scenarios. For example, in the case where the two dimers are perfectly ‘matched’ but separated by a single lattice spacing, both dimers can pivot into each other, or one dimer can translate toward the other while the other is pivoting, etc. The important point is that these collision events are intrinsically local events, reflecting and emphasizing the nature (and weakness) of the attractive interactions between two protein molecules. This intrinsic difficulty is of a very different nature from that arising from density effects, which are merely related to the size of the system (lattice).

If one catalogues the total number of one-point and two-point collisions that are possible, accounting for all initial conditions and allowing for all possible translations and rotations, the number of former events far exceeds in number the latter. Even considering all possible collision events, and even on the smallest lattice considered here (the 5×5 square planar lattice), the number of possible events is a number like 100, whereas the total number of possible joint displacements is of the order of 3500. Of these 100 or so collisional events, only a small minority correspond to two-point collisions which, in view of the weakness of attractive interactions, are likely to be the ones driving nucleation. In particular, there are 6 symmetry-distinct, two-point collision events, 4 of which involve the simultaneous translation of two dimers, 1 of which involves the simultaneous rotation of the dimers, and 1 which involves one dimer undergoing a translation and the other a rotation. It is important to stress that as the system size becomes larger, these 6 events remain the only possible two-point collision events. Thus, even before one works out the dynamics of the system in detail, it is evident that nucleation is here, statistically, a relatively rare event compared to nucleation in an ordinary material. This will be borne out in the results presented below.

Once the collision rules are specified the mean encounter time can be computed as the lowest eigenvalue of the fundamental matrix associated to the transition probability matrix (and the results compared with the ones obtained via Monte Carlo simulation; see below). For a lattice of uniform connectivity, the reciprocal of the smallest eigenvalue is the overall mean walklength of the simultaneously diffusing dimers before the process terminates Eq. (1).

As noted above, effects owing to geometrical anisotropy are already present and can be studied on the 5×5 lattice. As the Markov method becomes increasingly difficult to handle with increasing system size, these calculations were augmented using the Monte Carlo simulation method. The lattice size, N, was varied from $N=5$ (the minimum size where all configurations for the collision events can be realized) to $N=21$. The further, extensive calculations reported below will reveal that this increase in system size simply dilates, in a rather straightforward way, the time scale of the nucleation event.

To implement the Monte Carlo algorithm, we note, again, that the two dimers can move either by a translation or a rotation at each discrete time step. These eight configurational changes (four translations and four rotations) have been assigned equal probabilities. Since each ‘dimer’, as a whole, performs an unrestricted random walk on the lattice, our system has well defined ‘self-averaging’ properties; see, for example [14]. Therefore, a Gibbs averaging over randomly sampled initial conditions will give an appropriate approximation

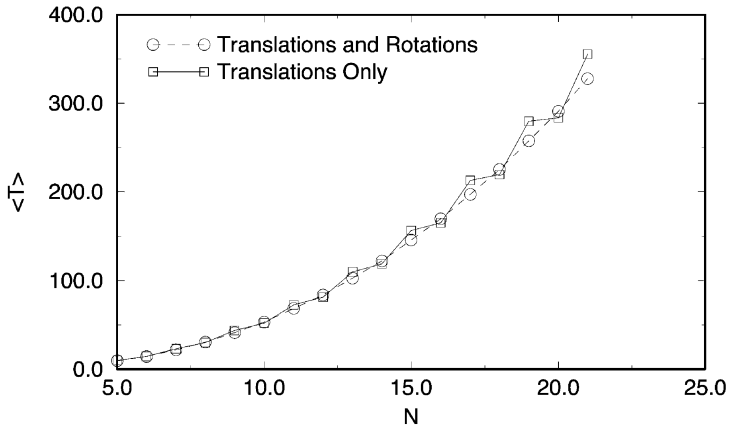

Fig. 1. Average encounter time vs. system size, where N is the linear dimension of a square-planar lattice subject to periodic boundary conditions. The dashed line displays the results for translations and rotations, and the full line shows the results for the case of translations (only). The circles and squares represent the actual data points.

of the average over all configurations of the system, for a sufficiently large sample. After each time step the system is updated in a synchronous fashion and we observe whether the two 'dimers' have collided or not. Here one can distinguish whether or not both ends of each dimer were involved in the collision or whether the participating dimers only touched each other in a head-to-head/tail-to-tail or head-to-tail collision. When one or the other kind of collision occurs, the process terminates, and the time taken, i.e. the number of updates is recorded as the 'encounter time'. Repeating the process over sufficiently many realizations (from approx. 10^6 to 10^7, in practice) one obtains, finally, a statistically-reliable, average encounter time, $\langle T \rangle$.

3. Results and conclusions

The principal results of our study can be extracted from the graphs displayed in Figs. 1–3. In Fig.

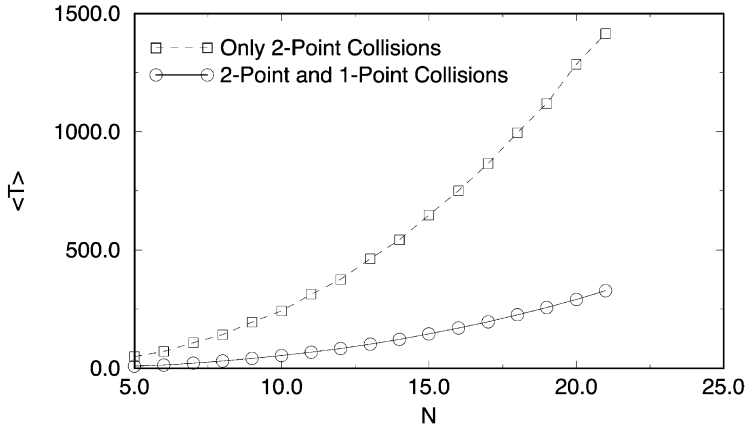

Fig. 2. Average encounter time vs. system size. The lower curve is for the case where both 1-point and 2-point collisions are considered, and the upper curve is for the case of 2-point collisions only. As noted in Section 2, the upper curve is the one that most probably reflects the time scale of the nucleation event.

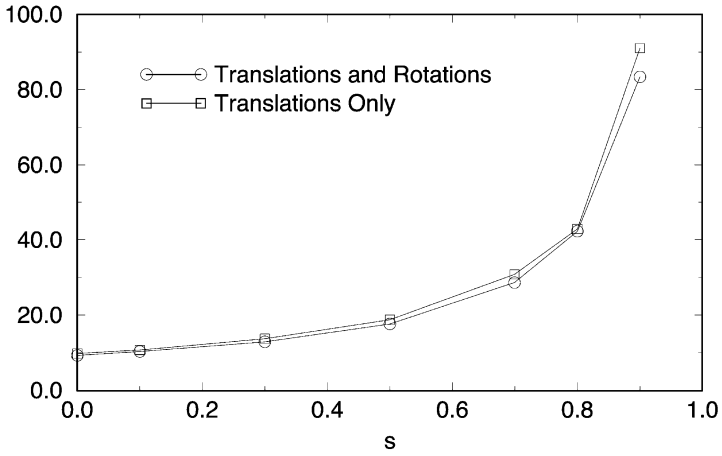

Fig. 3. Average encounter time vs. survival probability (see text). Both the upper curve (translations only) and the lower curve (translations and rotations) can be fitted by a functional dependence which goes as $1/(1-s)$, with *R*-values greater than 0.9992.

1 is plotted the average encounter time as a function of system size, where N is the linear dimension of the lattice. Results for the 5×5 lattice ($N=5$) were calculated using both the Markov method and Monte Carlo simulations. When one considers both translations and rotations, the (numerically-exact) Markov value is 9.331 whereas the Monte Carlo result is 9.344. For the case of translations only being considered, the Markov value is 9.750, while the Monte Carlo result is 9.931. The near coincidence of these results calibrates the accuracy of the Monte Carlo simulations (where 10^7 iterations were used in each simulation). Extending the Monte Carlo simulations to large lattices ($N=21$) then generates the curves displayed in Fig. 1.

The value reported above (9.750) for the case of two dimers undergoing simultaneous translations on a 5×5 square-planar lattice can be compared with the value (26.073) reported earlier [11] for the case of two monomers undergoing simultaneous displacements on the 5×5 lattice. The (near) factor of three differences in these two values reflects the compression of the available diffusion space available to two dimers. One anticipates that this difference between dimer and monomer values should decrease with increase in system size; indeed, one finds that for 21×21 square-planar lattices subject to periodic boundary conditions, the dimer value is 355.7, whereas the monomer value is 680.3, roughly a factor of two. Note, however, that if one considers dimers undergoing only two-point collisions on the same lattices, the respective values are dramatically larger. For the 5×5 lattice the value is 49.8, while for the 21×21 lattice the value is 1415.5. Since the onset of nucleation depends critically on two-point collisions leading to a cluster, the (near) factor of five differences between these values and those noted above emphasizes once again the relative rareness of this event.

Both with and without considering internal degrees of freedom, one finds a generally monotonic increase in the average encounter time with increase in N. Specifically, the increase is faster than linear with N, emphasizing the fact that nucleation on smaller lattices is more efficient, as expected. The further feature which is evident in Fig. 1 is that for the case of translational degrees of freedom (only) being considered, there is an 'even/odd' lattice dependence which becomes more pronounced with increasing lattice size.

Specifically, one finds that the encounter times for two dimers undergoing translations only on lattices of odd size (i.e. $(2k+1)\times(2k+1)$ lattices) are slightly greater than for the case where all degrees of freedom are considered, whereas the times are slightly smaller on lattices of even size

(i.e. $(2k)\times(2k)$ lattices). The observation of 'even/odd' lattice effects when translations only are considered mirrors a result noted earlier [11], where monomers undergoing simultaneous displacements on square-planar lattices of different parity were studied in detail. Here, the strictly monotonic increase in encounter times when both translations and rotations are considered reflects the fact that introducing rotations opens up reaction channels that are excluded when translations only are considered, thus bypassing constraints imposed by lattice parity.

The curves displayed in Fig. 2 display the dramatic difference in efficiency when 1-point and 2-point collisions vs. 2-point collisions (only) are considered. In both collision scenarios, the encounter event can be a consequence of the two dimers simultaneously translating into contact, simultaneously pivoting into contact, or one dimer rotating and the other translating into contact. Thus, in this calculation the full panorama of encounter possibilities is considered. The data show convincingly that, statistically, the event driving nucleation, a 2-point collision, is a relatively rare event, much less probable than two dimers touching at a single point. Furthermore, one finds that the difference between 1-point and 2-point collisions vs. 2-point collisions only becomes more pronounced the larger the system size.

The final series of calculations takes the problem one step further. In a collision, there will always be a finite probability that the two dimers do not 'stick', i.e. that they simply bounce off each other with no complex being formed. Since one anticipates that this effect will be a major factor in understanding nucleation in concentrated systems (here, small lattices), we present in Fig. 3 detailed results for the 5×5 lattice. Previous theoretical work [15] suggests that there will be a reciprocal dependence of the encounter time on the probability (s) that two dimers will survive such a collision, and this functional dependence is clearly displayed in the present calculation. Both for the case where internal degrees of freedom (rotations) are considered and for the case where translations (only) are assumed, there is (roughly) a factor of two increase in the encounter time when the survival probability increases from $s=0$ (nucleation assured) to $s=0.5$, and an increase by a factor of ten when s increases to $s=0.8$.

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