On morphological selection rule of noisy character applied to model (dis)orderly protein formations

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We propose that the main mechanism controlling the selection rule of model (dis)orderly protein formations, such as non-Kossel crystal growth and aggregation of lysozyme from aqueous solution, is an ion-channeling filter having flicker-noise properties. This filter is originated at the interfaces between growing solidlike object and its external liquid-type phase, and it can be considered as a series of voltage gated ion subchannels. The dynamics of each channel is studied by using both simulation and analytic argumentation lines, and represents a novel thought on how to utilize the presence of constructive-noise sources in protein formation, a field of utmost experimental and technological interest. © 2010 American Institute of Physics. [doi:10.1063/1.3431196]

I. INTRODUCTION

Protein crystal formation from solution is a field of vivid theoretical and practical interest in which the dissipation of energy across the free energy gradient between the solid and fluid phases can give rise to different morphologies of polymorphic crystalline outputs in the steady state.1,2

The morphological selection rules applied to our type of modeling rely on discriminating between the constant growth rate, attributable to crystal formation from metastable solution conditions and, on the contrary, nonconstant growth tempo, ascribable more to disorderly or partially ordered aggregations, such as (dis)orderly protein aggregations. In this second case, the white-noise macroion velocity-field effect near the nucleus’ surface is contrasting markedly with its flicker-noise counterpart, arising in crystal-formation conditions.

Most of the theoretical descriptions of protein aggregation concentrate only on the mature stage of the growth since the description of the nucleation stage is very challenging to carry out, although it is very important from the experimental point of view because it affects the quality of the finally obtained crystal structure.

Experimental data show that in the water-based electrolytic solution, the driving force of growth of protein crystals are hydrophobic and electrostatic interactions between its aminoacids.3 In biopolymeric solutions, electrostatic interactions are present between biomolecules and countercharged salt’s particles and precipitants with the participation of the structured water.4,5 The most electrostatically active particles are: polar aminoacids and dissociated salts—NaCl (laboratory) and/or KCl (physiological environment). These electrostatic interactions may alter the screening effects (damping of macrion’s electrostatic fields caused by the presence of counterions), which ultimately cause an electrostatic double layer (eDL) of the Stern type6 to emerge. Thus, around the growing crystal, the eDL has two distinguishable regions. (i) An internal boundary consisting of a pinned layer in which the solid phase, “perturbed” with local curvatures, is in contact with a “gas” of ions/proteins. (ii) This gas of ions/proteins is also in contact with the fluid blurred interface, playing a role of an external boundary, cf. Fig. 1.

These physical conditions suggest the identification of the eDL with an asymmetric ion channel where biomolecules, being themselves positively charged macrion’s,7,8 walk along the crystal’s border into step’s kink direction, cf. Figs. 2–4. Such a channel acts as a filter, permitting (or not) the ions to pass through and therefore controlling the late-stage crystal growth and/or aggregation of proteins.

In a previous study,7 we have analyzed the protein (viz ion) random walk of preferentially superdiffusive nature around a growing protein crystal/aggregate. We have shown that the growth results from a competition effect between the solid dynamic boundary condition ascribed to the growing crystal/aggregate, and its curvature-containing free boundary counterpart which is represented in Fig. 1 by the line separating the protein solution from the eDL ionic channel.

The diffusion coefficient characterizing macrion’s dynamics in the eDL ion channel has to contain quantitative signatures of both boundary conditions mentioned, and indicates whether the new phase grows as an orderly phase, or a converse scenario prevails. Within a suitable time interval,7,8 the time-dependent part of the diffusion function manifests this signature through a power law dependence. Using the fact that the diffusion coefficient is proportional to the macrion’s velocity autocorrelation function (VAF), in the present work we will show that the properties of the power spectrum of the VAF can be used to indicate the type of (dis)orderly aggregation of proteins that can be obtained. These results may help the experimenter to access readily our model considerations. In view of this, the present approach considers new important features of the model that

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are lacking in previous works:  

(i) By means of Monte Carlo simulations, we have made a successful attempt on deriving the flicker noise and power law properties of the VAF from a proposed simulation model of dislocation-driven crystal versus aggregate formation. 

(ii) In view of the results of the simulation, within the analytical domain we have analyzed the nonzero macroion’s mean-velocity case, cf. Eq. 19 and Fig. 7. This quantity is more detectable in the experiment and therefore permits a better connection between simulation, theory, and experiment. The analysis considers two limiting cases of the spherical-nucleus-based growing process, that is, when mass convection, or protein crowding, effects dominate and when the interfacial-field fluctuations are more pronounced. Both cases lead to very different growth dynamics. In this form, we show the compatibility of both approaches by offering a quantitative support of the analytical model.

The simulation model is based on the following considerations. The growth of the surface of the protein crystal is prompted by the dislocations and often evolves in a spiral way. As a consequence of this curvature-involving asymmetry, series of terraces can be observed along the crystal’s surface. Here, we will assume that each terrace with its surrounding eDL asymmetric ion-channel grows independently of the neighboring terraces, due to the presence of the Ehrlich–Schwoebel barriers, expected to manifest on the edges of the steps. 

In the model presented here, biomolecules that constitute the crystal, diffuse within the external layer of the eDL ion-channel driven ultimately by the hydrophobic interactions between amphiphilic residues of the biomolecules, see Fig. 1 and Ref. 12 in which the cessation-to-growth conditions have also been specified.

The most desirable efficiency scenario is the stationarity of the growing process, in which the crystal grows with a controllable constant speed. However, it is important to stress that this growth with constant speed is obtained only when the crystal’s curvatures and other finite-size effects do not play any important role. This case corresponds to the one when the asymmetry of the channel disappears or is negligible. This situation may occur in the late-stage temporal behavior of the system, but it does not suffice to get the constant speed of the formation under examination because there is also a second condition for achieving this constant growth speed. From the deterministic point of view, the speed of the macroions within the eDL ion-channel ought to be constant. However, the velocity of the macroions is, in general, a stochastic variable. Thus, constant growth speed also implies that velocity correlations have to conform effectively to the flicker-noise power spectrum of $1/f^\alpha$, where $\alpha = 1$ prevails ($f$—a frequency). Relevant examples in which this flicker-noise is commonly present are channels of both, natural and synthetic membranes.

As mentioned before, the computer simulations designed...
are used in Sec. II to give a detailed description of this controlling mechanism. The computer model of the diffusing probe particle which tests the viscoelastic properties of the crystal’s terrace, mimics the growth of terraces on the crystal’s surface, and is presented in Sec. III. The generalized building blocks move along the terrace and are attached to the crystal in the kink positions, thus performing a semidirected motion. The step’s propagation, as a result of the macroions diffusion and attachment versus detachment events, can be related to the ion-channel currents. The computer model tests the probe particle behavior when performing random walk (RW) on the hydrophobic-hydrophilic surface. Some RW exponents can be obtained, and their values depend on the hydrophobicity of the surface tested. These results are interpreted in terms of a thermodynamic-stochastic model which is presented in Sec. III.

The paper is organized as follows. In Sec. II we discuss the computer model of crystal’s surface growth in which the (hydrophobic-hydrophilic) terraces are treated as separated and horizontal ion channels on which the probe particle performs RW, see Sec. III. Section IV is devoted to formulate an analytical model of the crystal growth which is used to explain the origin of the flicker-noise 1/f power spectrum by the stochastic analysis of the problem. This constitutes a central problem addressed by our paper. In the last section, we present a discussion of the results coming from the performed study, and conclude our overall effort.

II. FROM SPHERE-LIKE TO FACETED NON-KOSSEL CRYSTAL GROWTH AND AGGREGATION: THE EDL ION-CHANNEL FILTER

The formation of facets (flat polygon-type surfaces) is a nearly ubiquitous phenomenon in crystal growth and plays a major role in guiding the growth of protein crystals. Once a (spherical) crystal grows, the expanding crystal develops facets because some crystalline surfaces accumulate material more slowly than others. Condensing biomolecules are especially attracted to rounded surfaces (curvature effects) that are rough on the atomic scale because such areas present greater available molecular binding. This occurs because a larger area of the biomolecules is exposed to the solution, and therefore there are more places where the incoming particles could be attached. Molecularly, flat regions have fewer dangling (free biomolecules abstracting) ends and are thus less favorable attachment sites. The microscopic growth process is characterized by the surface growth mechanisms of the protein crystal faces, including dislocation growth, and the most favorably two-dimensional nucleation growth. The dislocation-driven growth occurs along screw dislocation defects on the crystal face, as it is shown schematically in Fig. 2. The addition of growth units along a dislocation growth step results in the formation of a hillock as shown in Fig. 2(a), and eventually (in reality) the growth of the face as a whole.

The growth rate of the faceted crystal is determined by the surface diffusivity of attracted biomolecules and the geometry of the steps. In this case, the average growth rate of the spiral in the direction perpendicular to the surface could be identified with the growth rate, \( V_{gr} \) of the spherical crystal, and is given by

\[
V_{gr} = \frac{dR}{dt} = \frac{v_{\text{step}} d}{\lambda_0},
\]

wherein \( v_{\text{step}} \) is a step propagation velocity (parallel to the step), \( \lambda_0 \) is the average distance between two steps and \( d \) is a steps height, as shown in Fig. 2(b). Here, \( R \) is the average radius of the (quasispherical) faceted crystal.

In the case of crystallization of charged particles (such as proteins) immersed in the electrolyte around the crystal, an EDL of the Stern type is often observed and plays a role of selection layer (filter).

The concentrations of biomolecules in the external layer of the EDL, see Fig. 1, amounts to \( \sim 2c_0 \) while in internal layer the concentration is \( \sim c_0 \). In the external (diffuse) layer of the EDL, charged biomolecules with the surrounding counterions perform a RW in the presence of the electrostatic field of the internal (solid) boundary of the EDL. Under certain favorable electrostatic conditions, the biomolecules can jump into the internal layer (pinned layer) of EDL, where they roll on the crystal surface under the influence of strong hydrophobic forces. This process occurs until they ultimately land in their minimum-energy locations (kinks).

In the terraces forming the surface of the growing crystal, the macroions walk “uphill” between two neighboring Ehrlich–Schwoebel barriers, which appear on the opposite borders of them, see Fig. 3. Thus, the EDL of a Stern type together with the existence of facets associated to Ehrlich–Schwoebel barriers, are the leading factors dominating ions’ dynamics at the interface between the crystal and its external phase, and acting as a filter; the EDL ion-channel filter.

Taking into account the structured hydrophobic-hydrophilic and simple electrostatic interactions between the macroion and the crystal’s surface, and also between the macroion and salt’s ions present in the solution, the EDL ion-channel can be considered as a series of voltage gated ion subchannels. Each subchannel stops or lets through the macroions across the channel to the neighboring local minima on the crystal’s surface, see Fig. 3.

This situation forces us to consider the surface of the crystal as a series of single compartments, that is, a sequence of multibarrier channels. Each compartment can be described as a single ion channel with two geometrical parameters, length and width, the later being typically of the size of about 2–3 protein (macroion) diameters. Other important

![FIG. 2. a) The faceting and a screw dislocation driven spiral-like growth is shown for a spherulitic crystal at times t/t0; b) A magnified detail of the staircase cross section of the spiral and its crystal-growth involving landmarks. Here, \( d \) represents steps height, \( \lambda_0 \) the average distance between two steps and \( v_{\text{step}} \) the step propagation velocity parallel to the step.](http://jcp.aip.org/jcp/doi/10.1063/1.523206)
parameter which describes the state of the channel, and that can be related to the surface diffusivity of the macroions which manifests itself in the upper terrace growth, is the step formation velocity \( v_{\text{step}} \), see Fig. 2.

III. DIFFUSING PROBE-PARTICLE METHOD BY COMPUTER EXPERIMENT: TOWARD VAF PROPERTIES OF A SINGLE CRYSTAL’S TERRACE

A simulation model of the eDL ion-channel has been done by mimicking the crystal’s terrace surface in terms of a \( N \times N \) lattice, where \( N \) is a terrace length. Each site of the lattice has been provided with randomly distributed \( H \) (hydrophobic) and \( P \) (polar) sites, see Fig. 4. The hydrophobicity parameter \( p \) of the terrace, defined as \( p = \#P / (\#P + \#H) \), where \# the number of sites of a given type, has been changed in the range from 0 (only H sites) to 1 (only P sites).

The neutral testing walker started from the right hand side of the \( N \times N \) terrace, with \( N_{\text{max}} = 1000 \).

For each setup of the simulation 1000 runs have been performed. The time, \( t_{tr} \), needed for the random walker (RW) to traverse the terrace and reach its left end when always starting from its right border can be scaled in terms of the power law

\[
N \sim t_{tr}^{\nu}
\]

and as expected, either with elastic/reflecting or with periodic boundary conditions, the diffusion exponent \( \nu \) approaches the value equal to 1/2. Then, according to the eDL ion-channel proposed, the presence of the Ehrlich–Schwoebel barriers at the ends of the terrace was modeled by introducing a small (few lattice constants) lasting drift on the RW. This drift favors a bit longer glides over the surface in the left-hand side direction. Notice that here, lattice constants are equivalent to the equilibrium distance between centers of mass of the proteins constituting the crystal. The drift was obtained by privileging the movement in the left-hand side direction. The probability of such a move in this direction was twice bigger than in the right direction. Moreover, if the direction of motion was chosen as left, the glide over the surface was carried out over the four sites (the length of the flight is set equal to four lattice constants). The longer flights correspond to the Levy’s flight toward the Ehrlich–

![FIG. 3. The eDL ion-channeling effect occurring between Ehrlich–Schwoebel barriers which appear on the opposite borders of the crystal’s terrace. The length of the “direction arrows” reflect the movement-direction probability, focusing the movements on the kink positions characterized by the local minimum energy.](image1)

![FIG. 4. The model of the crystal’s terrace used in computer experiment with diffusing H/P-flickering probe particle launched along its planar surface. Grey squares: H-hydrophobic sites, white squares: P-hydrophilic sites. For better visualization \( N \) is taken smaller \( (N=15 \) on this picture) than in the simulation \( (N_{\text{max}}=1000) \). The walk-delays (dwell times), added to the overall simulation time, for all combinations of a possible contacts between walking particle and the terrace are shown.](image2)
Schwoebel barrier which always appears at the border of crystal’s terrace. Finally, a H-P changing property (“flicker”) walker has been allowed to move randomly along the H-P matrix, additionally residing four times units when the strongest H-H interaction occurred (penalty=4), two times units when H-P or P-H interaction took place (penalty=2), and finally 0.1 time units (penalty=0.1) when the weakest P-P interaction resulted, see Fig. 4. The residence times correspond to the energetically determined transition probabilities, characteristic of Monte Carlo simulations, cf. Ref. 14. The acceptance/non-acceptance rules involve the standard probabilistic Boltzmann factor (for \( T=\text{const} \)), as expected for such a MC realization.\(^8\) For hydrophobicity \( p=0.6 \) and particle’s flight equal to four lattice constants, the RW exponent has then been obtained as \( \sim 1 \). RW exponents for flight lengths equal 2–6 for \( p=0.6 \) and for no long flights, and for \( \#P = \#H \), are presented in Table I.

An important conclusion following from our simulations is that, in order to obtain a RW exponent approximately equal to 1, that is, when the eDL ion-channel is in the open state (what is realized by the superdiffusive RW of the particles along the multibarrier channel), the semidirected and constrained motion is needed. Such constrained motions observed on the surface of the terrace between two neighboring

<table>
<thead>
<tr>
<th>Flight length</th>
<th>( p = \frac{#P}{#P + #H} )</th>
<th>( \nu )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
<td>0.4951</td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>0.9457</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>0.9697</td>
</tr>
<tr>
<td>4</td>
<td>0.6</td>
<td>1.0139</td>
</tr>
<tr>
<td>5</td>
<td>0.6</td>
<td>0.9789</td>
</tr>
<tr>
<td>6</td>
<td>0.6</td>
<td>1.0568</td>
</tr>
</tbody>
</table>

Ehrlich–Schwoebel barriers are similar to those occurring in membrane typical ion channels, where the difference of the concentration of the ions on both sides of the channel forces ions to pass through it. To our knowledge, this is the first time that this effect has been reported. Thus, the Ehrlich–Schwoebel barriers play a role similar to that of the concentration gradient in membrane ion channels.

In order to analyze more deeply the implications of the existence of the eDL ion-channel, we have recorded the transition times of the probe particles through the H/P terrace. Notice that knowing these transition rates is equivalent to knowing the single-channel currents that are usually measured in the study of ionic currents through single channels in lipid bilayer membranes.\(^15\) The probe particle covered a terrace of \( N=1000 \) with \( p=0.6 \), and the length of flight was two lattice constants. The obtained results are shown in Fig. 5(a). The signal presents large fluctuations with respect to the mean value of 1032 Monte Carlo steps. To perform an frequency analysis of the signal a MATLAB\textsuperscript{\textregistered} tool (periodogram) has been used. Similarly to the case of the open-channel noise\(^15\) the power spectral density of the transition time noise, presented in Fig. 5(b), changes from 1/\( f \)-noiselike behavior to that of white noise, what has been first foreseen insightfully by Łuczka et al.\(^17\) The region in which the main trend line reveals the 1/\( f \)-type noise.\(^{17}\) With \( \alpha=0.987 \) obtained from the power law fitting of the PSD data is observed for the frequency range from 3 to 300 Hz. Locally \( \alpha \) varied form 0.8 for 100 Hz<\( f <300 \) Hz to 1.5 for \( f <100 \) Hz. It must be mentioned, that the slope value \( \alpha=1 \pm 0.2 \) of the power spectrum of single ion is commonly recognized as the 1/\( f \)-type noise.\(^13\) Some exceptions of this rule are caused by the fact that the protein channels get embedded at different locations in the bilayer membrane in different experiments with a little variation in conformation. In the case of the protein diffusing on the crystal’s terrace some fluctuations of \( \alpha \) are caused by the local curvatures which play the significant role during the

\[ \text{FIG. 5. a) The transitions times recorded for the particle probing terrace of } N=1000, p=0.6 \text{ and for particle’s flight equal } 2 \text{ lattice constants. b) Power spectral density of the signal for which the main trend line changes from } 1/\text{f}^{\alpha} \text{-like behavior, for the frequency range of 3 to 300 Hz, and reveals a white noise profile, for higher frequencies, what has been also predicted by our analytical model (cf. Section IV) and what has been first foreseen by Łuczka et al.}^{17} \] (For comparison, see also Refs. 15 and 20.)

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early stage of the crystal growth and also by the patchy structure of the terrace surface, cf. Fig. 4.3 The frequency region above 300 Hz is a transition region with minimal slope [cf. random signal on Fig. 5(b)], i.e., white noise, occurs. A similar behavior, namely, the one for which the main trend line appears to be very reminiscent of the 1/fα type behavior (with α close to one) for low-frequency power spectral density (see Fig. 5(b)), has been observed for all other combinations of N, p, and for all values of the flights lengths. These results are also interesting from the perspective that depending on the peculiarities of the VAF, different morphologies of the growing object may be obtained. This will be discussed in more detail in the following section.

IV. LATE-STAGE CRYSTAL GROWTH AS AN INTERFACE–CONTROLLED PROCESS INVOLVING ION-CHANNEL-FILTERING EFFECT: A THEORETICAL MODEL

The results obtained by means of computer simulations and their similarity with those of experiments in membrane ion-channels, can be analyzed within the context of a theoretical model that we present in this section.

According to Table I and Fig. 5(b), the exponent characterizing the RW of the macroion on crystal terraces depends on the hydrophobicity parameter p, that is, ν=ν(p). It takes values around 1 for p=0.6 and smaller for p<0.6. This interesting result can be incorporated in the theory through a power law dependence of the VAF or, equivalently, through the dependence on time of the diffusion coefficient of the macroions in the eDL ion-channel. Before analyzing this point in detail and with the aim to formulate a meaningful physical model, let us first focus on the fact that the rate of entropy production is the basic rule making selection among possibly different morphologies since the growth rate characterizing crystal and/or aggregate’s formation emerges as a result of the energy dissipation associated to the corresponding free energy gradient.

Our computer–simulational results give quantitative support to a growth selection-dissipation rule proposed by Hill, and given by the following growth pace Vg:

\[ V_g = L_H \Delta \chi, \]

where \( L_H \) is a dissipation indicator that characterizes the growth rate of different morphologies (preferably crystalline). \( \Delta \chi \) is a complex driving force of the crystal (aggregate) formation. Notice that Eq. (3) is a linear force-flow law typical of nonequilibrium thermodynamics which follows from the entropy production analysis.

In accordance with the computer model, we will consider the post-nucleation stage of the growth of a spherical crystal by aggregation of particles from a bath of Brownian particles, for example, the proteins in solution indicated in Fig. 1. The aggregated particles become a part of the crystal and migrate, walking around the object under growth in the eDL of the Stern type previously described and characteristic of macroions’ depletion. The particles bond to the surface of the crystal in locations corresponding to the local minimum-energy configuration.

In these conditions, let us assume a homogenous nucleus/crystal density \( C(\vec{r})=C=\text{const} \) (0 < \( |\vec{r}| < R \), where \( R \) is nucleus’ radius), and a surrounding concentration field \( c(\vec{r}) \) as a function of the position \( \vec{r} \) (\(|\vec{r}| > R \)) and \( \Sigma \) defines the surface of the object (\( |\vec{r}|=R \)). At time \( t_1 \) the object has the volume \( V(t_1) \) with surface \( \Sigma(t_1) \) which increases to \( V(t_2) \) and \( \Sigma(t_2) \) for times \( t_2 > t_1 \), cf. Fig. 1. The rate of change of the charged mass in the volume \( V(t_2) \) equals the net mass flux through the surface \( \Sigma(t_2) \):

\[ \frac{dm}{dt} = \int_{\Sigma(t_2)} j[c(\vec{r})] \cdot dS, \]

where \( (dm)/(dt) \) is the rate of change of mass when \( t_1 \to t_2 \) and \( j[c(\vec{r})]=c(\vec{r})v(\vec{r}) \) is the current of particles and \( v(\vec{r}) \) is the incoming biomolecular-matter velocity vector field. Here, \( dS \) is an inward normal to the surface \( \Sigma(t_2) \). The mass conservation law for the growing object then reads6,26

\[ \frac{d}{dt} \int_{V(t)} [C(\vec{r}) - c(\vec{r})] dV = \int_{\Sigma(t)} j[c(\vec{r})] \cdot dS. \]

In studying the late-stage growth process, we may assume \( t=0 \) as the instant when the radius \( R_0 \) of the growing crystal (aggregate) obeys the relation \( R_0 \geq R_c \), where \( R_c \) is the critical radius of the nucleus which distinguishes between nucleation and growth stage; for lysozyme protein \( R_c = 27 \) nm. At time \( t > 0 \), the radius of the growing sphere is equal to \( R = R(t) \). Because of the symmetry, the evolution Eq. (5) considerably simplifies and ultimately takes on the (scalar) form

\[ \frac{dR}{dt} = v_{\text{int}} \sigma_R, \]

where \( v_{\text{int}} \) is the characteristic velocity of incoming macroions and

\[ \sigma_R = \frac{c_s(R)}{C - c_s(R)} \]

is a supersaturation parameter that consists of two important terms: the one involved in the numerator that contains the fraction of not yet folded near-surface protein chains, and the denominator, in which the portion of the folded chains incorporated by the crystal’s structure manifests. These ingredients point to the fact that we examine a postnucleational growing stage of crystal vs aggregate formation. The mainly curvature-dependent concentration

\[ c_s(R) = c_0(1 + \Gamma_1 K_1 + \Gamma_2^2 K_2 + \Delta \Gamma_{\text{eq}}) \]

can be derived under the assumption of local thermodynamic equilibrium at the boundary. \( c_0 \) is an equilibrium concentration for the planar surface, practically for \( R \gg R_0 \) prescribed at the boundary, where \( R_0 \) is the radius of the growing sphere at an initial observation time \( t=0 \). \( \Gamma_1 \) is the so called Gibbs-Thomson or capillary length, which is usually of the size of 10 nm for lysozyme crystal. \( K_1 = 2/R \) is twice the mean curvature, cf. Ref. 8 and references therein, and \( K_2 \) is a Tolman length27 defined as the difference between the radius of the surface of tension and the radius of the equimolar dividing surface (for lysozyme protein \( \Gamma_2 = 3.5 \) nm) and
depends on the packing conditions of the crystal. $K_2 = 1/(r_mR)$ is a Gaussian curvature—a term introducing finite-size protein-molecule effect and $r_m$ is the radius of the crystal building unit (for lysozyme protein $r_m=1.5$ nm). Finally $\Delta \Gamma_{\text{neq}}$ indicates other possible contributions such as positive or negative (auxetic) elastic and nonequilibrium terms $^{24,25,28}$

After substituting Eqs. (7) and (8) with $\Delta \Gamma_{\text{neq}}=0$ and $\Gamma_2^2K_2=\frac{\partial^2}{(1/r_m)}(1/R)$ in our Eq. (6), which represents the growth pace we obtain

$$\frac{dR}{dt} = v_m \bar{\sigma}_R,$$

with $\bar{\sigma}_R = \frac{[\bar{c}_g(R)]/[C-\bar{c}_g(R)]},$ where $\bar{c}_g(R) = c_i(1+2\Gamma(1/R))$ is a curvature $(1/R)$ dependent concentration and $\Gamma = \Gamma_1 + \frac{1}{2}(2r_m)$.

The foregoing deterministic Eq. (9) can be written in its stochastic representation by considering the effects of thermal fluctuations on macroion velocity $v_m \rightarrow v_m + V(t)$ where $V(t)$ represents a fluctuating velocity. Equation (9) thus takes the form of a multiplicative stochastic equation $^{13}$

$$\frac{dR}{dt} = v_m \bar{\sigma}_R + V(t)\bar{\sigma}_R,$$

where $\langle V(t)\rangle=0$ and $\langle V(t)V(s)\rangle=K(|t-s|) \neq 0$ meaning, that the nonzero time dependent correlations $K(t)$ within the fluctuating part of the macroion velocity are assumed. $^5$ In Ref. 17, one may find an extensive theoretical survey of some important $K(t)$ involving problems and their impact on the growth rate; some extension of the time-correlations involving case has been proposed in Ref. 23.

Equation (10) is a Langevin type equation taken in its Stratonovich representation and has its Fokker–Planck and Smoluchowski counterpart $^{21,29}$ for the probability density $P(R, t)$ of finding a crystal of radius $R$ at time $t$, which reads as follows:

$$\frac{\partial}{\partial t} P(R, t) = \frac{\partial}{\partial R} \left( -v_m \bar{\sigma}_R P(R, t) + \frac{D(R, t)}{k_BT} \frac{\partial \Phi}{\partial R} P(R, t) 
+ \frac{D(R, t)}{k_BT} \frac{\partial P(R, t)}{\partial R} \right).$$

(11)

This equation contains two force contributions on its right-hand side. The first one comes from the constant part of macroion’s velocity which, for sufficiently high temperatures can be neglected. The second contribution is related to the previously mentioned free-energy gradient, where the free energy $\Delta \Phi = \Delta U - T \Delta S$ is given by the entropic contribution $T \Delta S = -k_B T \ln \bar{\sigma}_R$. This relation means that the difference in two subsequent free-energy values of two corresponding physical states, in this case mainly dominated by an entropic barrier, appears to be an important characteristic of the biomolecular diffusive formation of interest. $^{6,29}$ This entropic barrier, whose asymmetry depends on the curvature-dependent concentration $\bar{c}_g$, may then be interpreted as the eDL ion-channeling filter, which in turn controls the mechanism for the morphological selection rule. From Eq. (11) it follows that the thermodynamic force driving the system, $(\partial \Phi)/(\partial R)$, is derived from the solubility parameter $\bar{\sigma}_R$, that contains two important ingredients, the local curvature and the surface tension. Both factors determine the ratio between the folded/unfolded protein chains on the surface.

The diffusion function $D(R, t)$ appearing in Eq. (11), is a natural measure of the degree of non-Markovianity, which expresses memory effects, and takes the form

$$D(R, t) = D(t)\bar{\sigma}_R^2,$$

with the temporal dissipation factor $^{1}$

$$D(t) = \int_0^t K(s) ds.$$

(13)

There is a quite substantial lack in the literature of biomolecular crystal growth that concerns a determination of the velocities of macroions near the crystal surface. $^{6,11,17,18,30}$ Fortunately, there exists a large amount of experimental data indicating a constant growth rate, $V_{fr} = dR/dt$, of the crystal as the case expressing the most favorable tendency of the growing process toward a stationary state. $^{14,30}$ However, the results obtained with our simulations suggest that a plausible choice $^{17,24}$ of the velocity correlation field, expressed by now in terms of the diffusion coefficient, can be the power law correlation

$$D(t) \sim t^{-\gamma},$$

(14)

with a correlation-dissipation exponent $\gamma \rightarrow 0$ ($0 < \gamma < 1$), having a meaning of the solution-interaction exponent of Flory–Huggins type accounting mainly for polymer-solvent and polymer-polymer interactions in the system $^{8,31}$ (cf. delays of the movements of the probe-particle, Sec. III). A supporting rationale staying behind the choice made by Eq. (14) points to the physical fact that in the limit of $\gamma \rightarrow 0$ the diffusivity given by Eq. (14) increases algebraically in time which is equivalent to an algebraic decay in time expressed by the effective viscosity in the narrow channel-type zone surrounding the growing object. This is true because the object still grows so that this zone tends to grow as well, making this way the solution more diluted and homogeneous, thus suggesting in a natural way the local-viscosity effect to drop in time, cf. Ref. 6.

The total diffusion function, $D(R, t)$ is shown in Fig. 6 for a few different values of $\gamma$. For $\gamma=0$, the channel (eDL) is fully open and is characterized by $1/f$ noise. For $\gamma \neq 0$, the channel is in an intermediate viz. open/close state. Finally, when diffusion function is independent of time, $D(R, t) = \text{const}$, implies that the channel is characterized by a Markovian (memoryless) process with white noise $^{14,16}$

It should be noted that if our correlational choice represented by Eq. (14) would be appropriate, then such a plausible conjecture naturally introduced the nondissipative (enthropic) part of the system’s behavior. Thus, the only departure from otherwise dissipative-in-nature (entropic) model that we would like to offer here is given by the correlational proposal, Eq. (15). This kind of correlation reproduces well the asymptotic solution of Eq. (6), i.e., $R \rightarrow t$, equivalent to $V_{fr} \rightarrow \text{const}$ a kinetic criterion fairly revealing the expected matter ordering within the non-Kossel protein
crystal in late-stage growing conditions.\textsuperscript{1,4} In addition, it should be realized that there is no explicit enthalpic part involved in the Boltzmann-type (time-independent) free energy $\Phi \propto \ln[(C-c_0)/c_0]$—therefore our rationale about incorporating such correlational proposal looks plausible.\textsuperscript{6}

It should be noted that, if our correlational choice represented by Eq. (14) would be appropriate, then it imposes some particular dependence on the velocity correlation function of the ions, $K$, involved in Eq. (13). In fact, we can decompose this quantity in a set of harmonic, thus decoupled, oscillators in the form\textsuperscript{32}

$$K(t) = \int s(\omega) \cos(\omega t) d\omega,$$

(15)

where $s(\omega)$ is the power spectrum of the noise (or noise density of states of the thermal bath). Thus

(a) If we assume that $K(t) \approx (1+t)^{-\gamma}$, then the Fourier cosine transform of Eq. (15) yields a cumbersome function of $\omega$ that involves hypergeometric functions of $-\omega^2$ plus terms that contain power laws of the form $|\omega|^{-\gamma}$.\textsuperscript{12}

(b) If we assume an asymptotic form of $K(t) \approx t^{-\gamma}$ for $t \gg t_0$ then, by taking the Fourier cosine transform one obtains

$$s(\omega) \approx \kappa(\gamma)|\omega|^{-\gamma-1},$$

(16)

with an oscillating in $\gamma$ domain prefactor $\kappa(\gamma) = (2/\pi)\Gamma[1-\gamma]\sin[(\pi \gamma)/2]$, with $0 < \gamma < 1$ (in order to have valid the integral transformation). Equation (16) is valid up to a given Debye cutoff frequency, i.e., it is of the form of the generalized Debye spectrum.\textsuperscript{32}

Using Eq. (9), we may now evaluate the influence of macroion velocity fluctuations, reflecting the state of the eDL ion-channel, on the average growth-speed $V_{gr} \approx (dR)/dt$, see Eq. (1). The evolution equation for the average radius $R(t) = \int R(t)dR$ is obtained by taking the time derivative and then substituting Eq. (11). After integrating by parts and assuming that $P(R,t) \approx \delta(R(t)-R)$,\textsuperscript{32} one obtains

$$\frac{d}{dt}R(t) = V_{gr}(t) = v_{mi}\overline{\alpha}_R[R(t)] + D(t)\frac{\partial}{\partial R(t)}\overline{\sigma}_R[R(t)].$$

(17)

Looking at the above equation one anticipates a novelty associated with appearance of an additional fluctuation-involving term, having also included a local change of $\overline{\sigma}_R$ in terms of $R$, the effect itself of appreciable sensitivity to virtual changes of local temperature and/or electrostatic fields.\textsuperscript{12}

Substituting now the expression for $\overline{\sigma}_R$, Eq. (9), and taking the limit $R(t) \rightarrow \tau R_0$ where the factor $\tau$ characterizes the actual size of the crystal with respect to its initial size, we obtain that the growth-speed takes the form

$$V_{gr}(t) = v_{mi}\overline{\sigma}_R(\tau R_0) + \frac{2c\Gamma\overline{\sigma}_R}{c_0} + \frac{2\Gamma}{(\tau R_0 - 2\overline{\sigma}_R)^3}D(t),$$

(18)

wherein $\overline{\sigma}_R = c_0/(C-c_0)$. From Eq. (16) it follows that velocity fluctuations increase the growth velocity with respect to the purely convective case. In addition, Eq. (18) can be used to provide an estimate of the time dependence of the growth-speed $V_{gr}$. This can be done in two interesting cases.

**Small fluctuations.** In this case we assume $D(t) \rightarrow 0$ and expand Eq. (18) up to first order in $(2\Gamma)/(\tau R_0) < 1$, which is smaller that 1 for all times. This approximation leads to the result $V_{gr} \approx v_{mi}\overline{\sigma}_R \sim \tau^{-1}$, with the superscript $c$ indicating that only convective contributions were considered. As expected, this result indicates that for large crystals (late stage) the growth-speed is determined by the convective part.

**Vanishing convection.** In this case we assume $v_{mi} \sim 0$ and, as in the previous case we expand Eq. (18) up to first order in $(2\Gamma)/(\tau R_0)$. The result obtained together with Eq. (17) give us an approximate differential equation for $R(t)$ from which we may determine $\tau = \tau(R)$. After substituting $t = \tau(R)$ into Eq. (18) one finds that at short times an amplitude of the overall speed is

$$V_{gr} \approx \tau^{1+\gamma}(\gamma-2),$$

(19)

where now the $f$ indicates that only fluctuating contributions...
were considered, see caption to Fig. 7. It is interesting to notice that for 0 < \gamma < 1 we obtain that (1 + \gamma)/(\gamma - 2) lies in the range between (-1/2, -2), implying that in the case of superdiffusion of macroions the crystal (aggregate) tends to stop its growth in slower form than in the case of pure diffusion or convection. These expressions for \( V_{gr} \) are consistent with the behavior of \( R(t) \) observed in the Markovian case, cf. Ref. 28, in which the crystal (aggregate) initiates its growth with a velocity larger than the limiting value \( v_{mi}\delta_0 \) and decays to it monotonically.

From Eq. (11) one may also obtain that in the late stage (long times limit) the growth velocity behaves as \( V_{gr} = v_{mi}\delta_0 \). This result can be obtained directly from Eq. (10) by noting that at long times fluctuations in the crystal (aggregate) become less important and then, the late stage by satisfying \( t \gg t_1 \) leads to \( \Phi = -k_B T \ln[\frac{c_0}{(C-c_0)}] \), cf. Eq. (1) in Ref. 30. In this case, \( \delta_R = \frac{c_0}{(C-c_0)} \), is independent of \( R \), and represents a quasiequilibrium (eqq) part of the dimensionless supersaturation parameter\(^{28}\) given by Eq. (7). For convincing the reader about reliability and applicability of Eq. (10), this long-time argument enables one to derive the measured values of the growth pace of a (non-mutant) lysozyme crystal\(^{16}\). The Frenkel-type velocity of the lysozyme molecule, \( v_{mi} \), is typically of the order of \( v_{mi} \sim 10^{-2} - 10^{-1} \) cm s\(^{-1}\),\(^{6}\) while \( c_0/(C-c_0) \sim 10^{-4} - 10^{-3} \) (characteristic of non-Kossel crystals, full of aqueous-solution components promoting the nucleation stage), thus reliably estimating the speed of the lysozyme crystal formation to be on average \( [(dR)/(dt)]_{eqq} \sim 10^{-6} \) cm s\(^{-1}\)\(^{2,4}\) because \( [(dR)/(dt)]_{eqq} \sim [c_0/(C-c_0)]v_{mi} \) ultimately applies.

**V. DISCUSSION WITH A CONCLUDING ADDRESS**

In this paper, we have proposed a mechanism that is virtually able to control crystal (aggregate) growth after nucleation has finalized. We argue that the eDL of a Stern type together with the existence of facets, giving rise to Ehrlich-Schwoebel barriers and the hydrophobic-hydrophilic nature of sites at the molecular-aggregate surface, are the leading factors dominating macroions’ dynamics at the interface between the crystal/aggregate and its external phase. These factors act as a certain eDL ion-channel filter which is useful to elucidate the (in)optimality of growing (molecular-aggregation) complex conditions expressed by the system.

The study was performed by means of simulation and theoretical viz analytic models. We argued that in the asymptotic behavior of the growth process, local curvatures at the crystal’s surface are screened by the electrostatic fields due to the presence of counterions. Our simulations give good evidences that this screening, associated to the Ehrlich-Schwoebel barriers, is able to induce algebraic velocity correlations in the interfacial region, thereby controlling the
growth speed of the crystal. In view of this, we performed an analytical study of crystal growth assuming power law velocity correlations or, equivalently, a power law dependence upon time of the diffusion coefficient.

The computer model was based on the consideration that the growth of the surface of the model protein crystal is prompted by dislocations appearing at crystal’s surface, which lead to a local spiral growth. As a consequence of this curvature-involving asymmetry, series of terraces can be observed along the crystal’s surface. Charged biomolecules that eventually constitute the crystal, perform a RW over this hydrophilic-hydrophobic terrace embedded in the eDL, that is, they diffuse within the external layer of the eDL ionic-channel driven ultimatively by the hydrophobic interactions between amphiphilic residues of the biomolecules, see Fig. 1. This process constitutes the ion-channel filter wherein macroions (proteins, charged particles, aggregates) walk toward the internal layer of the eDL and attach eventually to crystal’s surface.

In particular, we have recorded the transition times of the probe particles through the H/P terrace, see Fig. 5(a). These transition times are equivalent to knowing the single-channel currents that are usually measured in the study of ions currents through single channels in lipid-bilayer membranes. The signal associated to the transition times has a power-spectrum density that changes from 1/f-noise-like behavior to that of white noise, see Fig. 5(b).

In a way similar to that of membrane ion channels, such as plasma-membrane ion channels, our eDL ion-channel filter ascribed purposely to the interfacial, inhomogeneous ion-plasma involving region, might either promote or discourage the (dis)ordered aggregation of proteins, in particular the formation of lysozyme non-Kossel crystals.

The power spectrum obtained is important because it may characterize the selectivity of our eDL ion channel filter and can also be used to connect with theory. The selectivity of ion channels is one of their basic properties, and it is mainly attributed to the fact that electrical and diffusive currents exhibit several asymmetries. Our eDL ion-channel filter possesses such a structure involving asymmetry of selective nature too. This can be seen in the general current intensity integral formula, typically involved in Amper’s law of electrodynamics, and describing the ion current’s flow \( j_E \) related to the electric current’s intensity, \( i \), by

\[
i = \int \frac{1}{2} j_E \cdot dS,
\]

in which the integration goes over sphere’s surface that may be perturbed due to changeable boundary conditions. If, which appears to be a signature of the late-stage of the process, the sphere is nearly ideal (referring to the crystal’s maximum symmetry group), any influence of crystal-surface local curvatures, being most probable spots for engaging the macroions, can be neglected, and the channel is open. It implies that in Eq. (20), \( j_E \) is consistent with \( j \) of Eq. (4). This flux, involved in Amper’s law, can in turn be related to the diffusion coefficient by the fact that \( D(t) \) obeys an extended (due to explicit dependence on time \( t \)) fluctuation-dissipation relation of Stokes–Einstein type, namely,

\[
D(t) = k_BT \mu(t),
\]

in which the time dependent macroion mobility \( \mu(t) \) is then straightforwardly related with the macroion velocity by \( \mu(t) = \frac{1}{j_E} \left[ v_{mi}(t) \right] \), where, formally \( j_E = \left[ j_E \right] \), where \( j_E \) is the flux density of the macroions through the filter, as mentioned before. Let us recall however that in the part concerning the analytical study, the growth units are characterized mainly by their velocity, \( v_{mi} \), so we are not capable of saying that the growth unit is constituted by the single macromolecule, or by a small cluster e.g., a tetramer, upon interaction with both the solution and with the crystal’s surface. Based on nonstochastic (mean-field) version of our modeling, it should also be emphasized that the macroion velocity, \( v_{mi} \), when considered as a relevant parameter of the model, is fully derivable from basic principles of diffusion in weak-plasma (interfacial) regime.

This nonequilibrium statistical-mechanics model is based on the model crystal versus aggregate growth assumptions when one looks for a relationship between the probability density function (\( P(R,t) \)), undergoing Smoluchowski stochastic dynamics, and the free energy \( \Phi(R) \) reflecting the existence of the activation barriers. [Another interesting but open issue can be formulated as of whether there existed a unique interrelation between the Ehrlich–Schwoebel barrier from Fig. 3, and its more analytic, Kramers-type counterpart, assigned to the Smoluchowski equation, Eq. (11), cf. Ref. 6.] The model clearly reveals the latter “nonclassical pathway” characteristic of the prototype (macro)ion-channeling and the flicker-noise, as the one by which the overall (dis)orderly protein aggregation is likely realized. It would indirectly imply that the putative (because not experimentally checked thus far) emergence of flicker-noise ion channel(s) could be considered as another type and not of first-order stage in nucleation-growth transition from the protein solution. Thus, also in conceptual accord with Ref. 38, based on functional-density calculations, there will appear a metastable zone in the crystallization degree vs density diagram, cf. Ref. 40, for direct numerical indication, which experimental fact stands for a clear evidence that some peculiar dynamic action close to crystal surface manifests.

Our model, with its non-Markovian context staying behind it, cf. Refs. 6 and 7, supports fairly all numerical, theoretical and what important, also experimental evidences provided. Referring to the theoretical part of them only, it would be intriguing to interconnect our flicker (self-similar in frequency) channeling view with manifestation of the Ostwald rule of stages for nucleation, and presumably, subsequent growth stages, in the sense that the free-energy barriers associated with the metastable intermediate states are expected to be lower in the described situation than those for a direct transition from the protein (gas) solution to solid-object (crystal or aggregate) formation; this picture is also valid for inorganic crystals.

To our knowledge, the model system proposed here is novel an it could help interpret and comprehend many kinetic-thermodynamic peculiarities assigned customarily to the growth layer of a protein (dis)orderly aggregate in terms

\[
D(t) = k_BT \mu(t),
\]
of the indicated fluctuational framework.49 These peculiarities may even markedly change the optimal growth rate of the aggregate. Therefore, the still very ongoing conception of ion-channel dynamics is worth testing, mainly within the late-time growing context described, and hopefully to our problem as well, certainly of relevance from technological point of view. In this sense, our study could be termed as an address to experimenter, viewed in terms of asymmetric nanochannel dynamics, quite analogous to the one performed in Ref. 43. To date, a control of the flicker noise (arising from the density fluctuations) and the corresponding signal-to-noise ratio in biomolecular lysozyme type systems, examined in terms of Raman spectroscopy, appears to be a real but recently accomplished challenge,44 with potential application in pharmacology.45

According to the rationale coming out, after accepting the Ostwald picture,46 crystals may grow via a certain cascade of (meta)stable states: Our ion channels may represent such states, whereby if they are more stable they would yield a crystal, with the ion channel equipped with flicker type noise; when they are more unstable (and, subjected to some white-noise effect), in turn, they are capable of producing aggregates, thus more disorderly forms, rather.

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