On the Protein Crystal Formation as an Interface-Controlled Process with Prototype Ion-Channeling Effect

Jacek Siódmiak · Jan J. Uher · Ivan Santamaría-Holek ·
Natalia Kruszevska · Adam Gadomski

Received: 11 October 2007 / Accepted: 9 April 2008 / Published online: 29 May 2008
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Abstract A superdiffusive random-walk action in the depletion zone around a growing protein crystal is considered. It stands for a dynamic boundary condition of the growth process and competes steadily with a quasistatic, curvature-involving (thermodynamic) free boundary condition, both of them contributing to interpret the (mainly late-stage) growth process in terms of a prototype ion-channeling effect. An overall diffusion function contains quantitative signatures of both boundary conditions mentioned and indicates whether the new phase grows as an orderly phase or a converse scenario occurs. This situation can be treated in a quite versatile way both numerically and analytically, within a generalized Smoluchowski framework. This study can help in (1) elucidating some dynamic puzzles of a complex crystal formation vs biomolecular aggregation, also those concerning ion-channel formation, and (2) seeing how ion-channel-type dynamics of non-Markovian nature may set properly the pace of model (dis)ordered protein aggregation.

Keywords Protein solution · Protein crystal · Electrostatic double layer · Ion channel · Enhanced diffusion

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J. Siódmiak (✉) · N. Kruszevska · A. Gadomski
Department of Modeling of Physicochemical Processes,
Institute of Mathematics and Physics, University of Technology and Life Sciences,
Kaliskiego 7, 85-796 Bydgoszcz, Poland
e-mail: siedem@utp.edu.pl

J. J. Uher
ZSMiO, Konopnickiej 6, 43-300 Bielsko Biała, Poland

I. Santamaría-Holek
Facultad de Ciencias, Universidad Nacional Autónoma de México Circuito
exterior de Ciudad Universitaria, 04510 D.F., Mexico City, Mexico

1 Introduction

The growth of protein crystals is a complex process. The most difficult, both for experimentalists and for theoreticians, is complexity of the material designed for crystallization and complex composition of the solution in which the growth takes place [1, 2]. Most of the theoretical descriptions of this process concentrate only on the mature stage of the growth because the description of the nucleation stage is very difficult to carry out. However, the nucleation stage plays an essential role in the process because it determines the quality of the final structure [3, 4]. Experimental data show that, in the water-based electrolytic solution, the driving force of the growth of protein crystals is hydrophobic interactions between their amino acids [3]. Moreover, electrostatic interactions must also be taken into account. In biopolymer solutions, the most electrostatically active particles are polar amino acids and dissociated salts: *NaCl* (laboratory) and/or *KCl* (physiological environment). Electrostatic interactions are present between macromolecules, countercharged salt particles and precipitants with the participation of the structured water [5, 6]. This kind of interaction triggers screening effects, which ultimately cause an electrostatic double layer (eDL) of the Stern type to be formed [7]. Thus, around the growing crystal, the eDL is formed, where outermost boundaries are made up of a perturbed (with local curvatures) solid vs “gas” depletion-zone interface, acting as an internal boundary, and a “gas” depletion-zone vs fluid (blurred) interface, playing the role of the external boundary. In this case, eDL can be identified with an asymmetric ion channel, where macromolecules walk into the direction of the step’s kink. Such a channel can be thought of as a filter, permitting the ions to pass through, and thus controlling the late-stage crystal growth and/or aggregation of proteins. Omitting processes occurred in the bulk, we can reduce the modelling only to the nearest surroundings of the crystal surface, thus describing the growth as an interface-controlled process; cf., especially, Sections 2, 3, and 6 for more detailed discussions and comments.

The surface of the protein crystal, the growth of which is prompted by the dislocations, very often evolves in a spiral fashion. As a consequence, the series of terraces can be observed on the crystal’s surface. Assuming that each terrace can grow independently of the neighboring terraces, due to the presence of the Ehrlich–Schwoebel barriers on the edges of the steps, each terrace (or asymmetric ion channel) can be considered separately. In the model presented here, macromolecules that constitute the crystal diffuse in the external layer of the eDL, equivalent to the ion channel filter, from which they go into the internal layer and attach to the crystal’s surface or perform, presumably, some Lévy-type flights directly to the spots of their accretion, which are usually placed in the kink position. The most desirable scenario is that in which the crystal grows with a constant speed. The speed of the growth of the crystal or aggregates appears to be the main contribution to the growth selection rule and can be obtained when the macromolecules, feeding the crystal, move more or less with constant/nonconstant velocity within the eDL. Practically, macroions do not move independently and, to obtain growth with constant speed, the velocity correlations of the macroions have to behave as a power-law in time [8], and the correlation strength must be quite weak. Fortunately, such correlations are characteristic of power spectra such as that of $1/f$ (flicker) noise in the eDL (ion channel). Such noise is, in general, present in membrane channels, both in natural and in synthetic membranes [9–11].

We present two computer models. The first one is a 2D model for protein crystal growth performed on a square lattice with vertical orientation [12]. The second one is a 2D model with a horizontal orientation that describes the dynamics of a diffusing probe particle, testing the viscoelastic properties of the crystal’s terrace. The former mimics the growth

of terraces on the crystal's surface. The generalized building blocks (bricks) move, or rather roll, on the terrace and are attached to the crystal in the kink positions (semidirected movement) [12]. The step's propagation, as a result of the macroions' diffusivity and attachment vs detachment events, can be related to the ionic channel's current. The second computer model tests the probe particle behavior, where the particle's performs a 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. The clustering dynamics on the crystal's terrace has additionally been examined based on a supporting computer simulation, exploiting mainly its hydrophilic path. The combination of these two models can be considered as a quasi-3D simulation of the crystal growth.

The paper is organized as follows: First, the analytical model of the crystal growth, possibly in its simplest (spherical) form, is presented. The growth with constant speed is obtained only when the asymmetric channel becomes symmetric, i.e., when the crystal's curvatures, etc. [13], do not play any role, which is the case of late-stage behavior of the system as a whole. However, it does not suffice to obtain the constant speed of the formation. It is necessary to add a second condition. From the deterministic point of view, the speed of eDL macroions must be constant. From a stochastic (more realistic) point of view, the velocity correlations have to conform to the flicker-noise power spectrum ($1/f$). Next, the computer model of the crystal's surface growth is presented, in which the terraces that are formed during the growth are treated as separate ion channels, and the dynamics of restructuring their constituent parts is also introduced. Then, the computer model in which the probe particle performs RW on the hydrophobic–hydrophilic surface is presented, and some RW exponents, the values of which naturally support the $1/f$ noise context, are derived. Finally, conclusions coming from the performed study are presented and some perspective towards linking crystal, to ion channels is proposed.

2 Late-Stage Crystal Growth as an Interface Controlled Process

For clarity, we will consider the growth of a spherical object by aggregation of particles from the external environment; see Fig. 1. The possible limitations of the spherical approximation applied to growth phenomena in complex milieus are discussed in [14].

When particles in solution aggregate, they become a part of the object and migrate in an eDL of the Stern type [7]. This double layer is characteristic of macroions' depletion and surrounds the growing object. The particles bond to the surface of the object in locations corresponding to the minimum-energy configuration. Let us assume that the nucleus density $C(\vec{r})$ is homogenous, $C(\vec{r}) = C = \text{const}$ [15], and that the concentration field of the surrounding particles is denoted by $c(\vec{r})$, with r representing the position of a particle. $\Sigma(t)$ defines the time-dependent surface of the object. As shown in Fig. 1, at time t_1 , the object has volume $V(t_1)$ with the surface $\Sigma = \Sigma(t_1)$, which increases to $V(t_2)$ with surface $\Sigma = \Sigma(t_2)$ for $t_2 > t_1$.

The rate of change of the mass in the volume $V(t_2)$ equals the net mass flux through the surface $\Sigma(t_2)$:

$$\frac{\Delta m}{\Delta t} = \int_{\Sigma(t_2)} \mathbf{j}[c(\vec{r})] \cdot d\mathbf{S} \quad (1)$$

Fig. 1 Schematic representation showing two snapshots during the growth of a spherical object. On the left-hand side, the *solid line* indicates the surface $\Sigma(t_1)$ of the object with volume $V(t_1)$ at time t_1 evolving towards the state with surface $\Sigma(t_2)$ and volume $V(t_2)$ at a time $t_2 > t_1$, indicated by the *dashed line*. Correspondingly, on the right-hand side, the *solid line* indicates final state of the object with $\Sigma(t_2)$ and $V(t_2)$ coming from the initial one indicated by the *dashed line*

where $\Delta m = m(t_2) - m(t_1)$; $\Delta t = t_2 - t_1$; $\mathbf{j}[c(\vec{r})]$ is the flux of particles, which depends on $c(\vec{r})$; and $d\mathbf{S}$ is an inward normal to the surface $\Sigma(t_2)$ [15]. We can write down the mass conservation law for the growing object [7, 16]:

$$\frac{d}{dt} \int_{V(t)} [C(\vec{r}) - c(\vec{r})] dV = \int_{\Sigma(t)} \mathbf{j}[c(\vec{r})] \cdot d\mathbf{S} \quad (2)$$

where the limit $\Delta t \rightarrow 0$ is taken and we have expressed the flux of particles in the form: $\mathbf{j}[c(\vec{r})] = c(\vec{r})\mathbf{v}(\vec{r})$, where $\mathbf{v}(\vec{r})$ is the incoming matter velocity vector field [15, 17].

Let us then assume that, initially at $t = 0$, the growing object is an ideal sphere of radius R . At time $t > 0$, the radius of the growing sphere is equal to $R = R(t)$. Because of the symmetry, the evolution Eq. (2) considerably simplifies and ultimately takes the form [13]:

$$\frac{dR}{dt} = v_{mi}\sigma_R, \quad (3)$$

where v_{mi} is the velocity of the incoming macroion and

$$\sigma_R = \frac{c_s(1/R)}{C - c_s(1/R)}, \quad (4)$$

where σ_R is the dimensionless supersaturation parameter ($1/\sigma_R$ is the near-surface solubility) and C is defined as above. The curvature-dependent concentration

$$c_s(1/R) = c_0(1 + \Gamma_1 K_1 + \Gamma_2^2 K_2 + a.t.) \quad (5)$$

is mainly 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. Γ_1 is the so-called Gibbs–Thomson or capillary constant and $K_1 = 2/R$ is twice the mean curvature [15]. Γ_2 is a Tolman length [18] defined as the difference between the radius of the surface of tension and the radius of the equimolar dividing surface, and $K_2 = 1/(r_{mi}R)$ is a Gaussian curvature, with r_{mi} the radius of the crystal building unit. For the lysozyme protein, these parameters take the following values: $\Gamma_1 = 10$ nm, $\Gamma_2 = 3.5$ nm, and $r_{mi} = 1.5$ nm. It is convenient to stress that the Tolman length depends on the packing coefficient of the crystal, and it can be related to the

superficial density at the surface of tension [18]. Additionally, K_2 refers to possible variations of the (crystal) surface tension with local curvatures, such as those created by sparsely located molecular rows [14]. Finally, notice that (5) can be generalized by taking into account *a.t.* corrections coming from elastic and nonequilibrium effects; see, for example, [13, 17, 19].

The foregoing deterministic equation in its stochastic representation takes the form [8]:

$$\frac{dR}{dt} = V(t)\sigma_R \quad (6)$$

where now $V(t)$ represents the stochastic velocity of the incoming macroions. The statistical properties of $V(t)$ are determined by thermal fluctuations, that is, it has zero mean, $\langle V \rangle = 0$, and its correlation function is given by $\langle V(t)V(s) \rangle = K(|t-s|)$, where the bracket stands for an average over noise. Thus, nonzero time-dependent correlations K within the macroion velocity field are assumed [7]. In [8], one may find an extensive theoretical survey of some important K functions, and their impact on the growth rate.

In [13] (see also references therein), combined with the basic argument presented in [15], one may find an indirect proof that (6) also comes from applying the Gibbs' (nonnegative) entropy production equation to our open thermodynamic system; the conceptual framework of the so-called mesoscopic nonequilibrium thermodynamics [13] is, in principle, fully immersed in its well-known Glansdorff–Prigogine counterpart, except that it (1) pays more attention to the close-to-equilibrium states and (2) addresses, based only upon a minimalistic set of assumptions, cf. [13], and references therein, the problem in terms of the below-mentioned Fokker–Planck and Smoluchowski dynamics. Summing up, in part, one may firmly say that linear nonequilibrium thermodynamics underlies the rationale presented in the study below; the question of whether we are striving to examine the system exclusively near equilibrium is legitimate but could not, because of an explicit time involvement in the below-stated equations, be answered entirely positively [4]. (For a more extensive discussion of the problems of such a type, the reader is advised to consult Kurzyński [20].)

Equation (6) is a Langevin-type equation, taken in its Stratonovich representation. Fortunately, it has its Fokker–Planck and Smoluchowski counterpart, which, with corresponding initial and boundary conditions [21, 22], reads as follows:

$$\frac{\partial}{\partial t} P(R, t) = -\frac{\partial}{\partial R} J(R, t), \quad (7)$$

where $P(R, t)$ stands for a probability density of finding a spheroidal crystal of radius R at time t , and the matter flux reads as follows:

$$J(R, t) = -\left(D(R, t) \frac{\partial P(R, t)}{\partial R} + \beta D(R, t) \frac{\partial \Phi}{\partial R} P(R, t) \right), \quad (8)$$

where $\beta = \frac{1}{k_B T_s}$ is the inverse thermal energy and T_s is system's temperature. After using (4), the free energy is given by $\Phi = -\beta^{-1} \ln \left[\frac{c_s(1/R)}{C - c_s(1/R)} \right]$, which, for $t \gg t_0$, leads to $\Phi = -\beta^{-1} \ln \left[\frac{c_0}{C - c_0} \right]$. Note that the presence of the gradient $\frac{\partial \Phi}{\partial R}$ in (8) implies that the supersaturation parameter σ_R is the main driving force of the process. For lysozyme crystal growth, this has been validated experimentally; see, for example, [7, 14]. The diffusion coefficient takes the form

$$D(R, t) = \frac{D(t)}{\sigma_R^2} \quad (9)$$

with

$$D(t) = \int_0^t K(s) ds. \quad (10)$$

In (9), σ_R^2 represents the square of the near-surface solubility, as in Eq. (4), within the experimentally validated range of, e.g., lysozyme crystal growth data [7, 14].

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 [23–25]. However, there exists a large amount of experimental data, which indicates a constant growth rate, $V_{gr} = \frac{dR}{dt}$, of the crystal as the case expressing the most favorable (toward steady state) tendency for the process to go on, mostly when $t \gg t_0$ is readily fulfilled [14, 24]. Therefore, our plausible choice [8, 13] of the velocity correlation field must be a power-law correlation, i.e., $D(t) \sim t^{1-\gamma}$, with a correlation exponent $\gamma \rightarrow 0$, because such very weak correlations assure electrostatically enhanced, and weakly coupled to the field ($\gamma \rightarrow 0$), quite long jumps of the macroions towards their ultimate accretion spots on the crystal's surface [7]. This kind of correlation reproduces the asymptotic solution of (3) well, i.e., $R \sim t$ [8], equivalent to $V_{gr} \rightarrow \text{const}$, a kinetic criterion truly revealing the ordering of the non-Kossel protein crystal [5].

Let us consider the velocity correlation function of the ions, K . In general, we can decompose this quantity in a set of harmonic oscillators in the form [26]:

$$K(t) = \int s(\omega) \cos(\omega t) d\omega \quad (11)$$

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

- (a) If we assume that $K(t) \simeq (1+t)^{-\gamma}$, then the Fourier cosine transform of (11) yields a cumbersome function of ω that involves hypergeometric functions [27] of $-\omega^2$ plus terms that contain power laws of the form $|\omega|^{-1+\gamma}$.
- (b) If we assume an asymptotic form of $K(t) \propto t^{-\gamma}$ for $t \gg t_0$, then, by taking the Fourier cosine transform, one obtains

$$s(\omega) \propto \frac{2}{\pi} |\omega|^{-1+\gamma} \cdot \Gamma[1-\gamma] \sin\left[\frac{\pi\gamma}{2}\right] \quad (12)$$

with $0 < \gamma < 1$ (to make the integral transformation valid) and valid up to a given Debye cutoff frequency, i.e., it is of the form of the generalized Debye spectrum [26]. Then, it is plausible to assume that the velocity correlation of the ion is responsible for a $1/f$ noise in the eDL.

The total diffusion function as a natural and primary measure of the degree of non-Markovianity, $D(R, t)$, for a few different values of γ , is shown in Fig. 2. For $\gamma = 0$, the channel (eDL) is fully open and is characterized by $1/f$ noise, while for $\gamma \neq 0$, the channel is in an intermediate, viz., open/closed state. Finally, when diffusion function is independent of time, $D(R, t) = \text{const}$, which is equivalent to a Markovian process, in which the channel is characterized by a Gaussian (white) noise [9, 11].

The $1/f$ (flicker) noise is generally observed in ion channels and is identified with the motion of subunits constituting the channel walls [9–11]. In our case, it emerges when (1)

Fig. 2 The total diffusion function, $D(R, t)$, (9), represented asymptotically by its t -dependent part $D(t)$ as a measure of the degree of non-Markovianity: $\gamma = 0$, the channel is open and is characterized by $1/f$ noise [9, 11] with the power spectrum $s(\omega) \sim \omega^{\gamma-1}$ (asymptotic form of (12) with $\omega > 0$); $\gamma \neq 0$, the channel is in open/nonopen state; $D(t) \rightarrow \text{const}$, Markovian process, channel is characterized by a Gaussian (white) noise: it is closed. (Note that $D(R, t) \rightarrow D(t)$ when $t \gg t_0$ because the supersaturation $1/\sigma_R$ suppresses to a constant.)

adequate algebraic velocity correlations appear and (2) the presence of local curvatures at the crystal's surface is either screened (e.g., by the corresponding electric field) or somehow smoothed out because of the large dimension of the crystal, or both factors combined together—note that we always consider the asymptotic behavior of the process.

It is noteworthy that, according to [28], and references therein, the superdiffusion coefficient $D(t)$ obeys an extended fluctuation–dissipation relation of the Einstein–Smoluchowski type, namely:

$$D(t) = \beta^{-1} \mu(t). \quad (13)$$

The time-dependent mobility, $\mu(t)$, is then straightforwardly related with the macroion velocity by: $\mu(t) = \frac{j_E}{v_{mi}(t)}$, (formally: $j_E = |\mathbf{j}_E|$), where $v_{mi}(t)$, as above, and $j_E = |\mathbf{j}_E|$ is the flux density of the macroions, viz., proteins. It is the same flux as the one involved in Ampère's law, describing the electric current's flow; it is related to the current's intensity, i , by

$$i = \int_{\Sigma} \mathbf{j}_E \cdot d\mathbf{S}. \quad (14)$$

The flux from (2) can be considered nearly the same as the one from (14)—the only physical difference appears be that, in the former, macroions have to be taken into account as hydrated (and, as carrying all the microions' crowd with them, mainly due to the mass conservation effect), whereas, in the latter, only the actual eDL ions should preferentially be taken into account.

As is shown in [29], selectivity of the ion channels is one of their basic properties. It is mainly related to the fact that electrical and diffusive currents exhibit several asymmetries. Indeed, our model of channeling in the eDL system also possesses a structural property involving asymmetry and, as a result, selectivity. It is seen in the general current-intensity integral formula (typically involved in Ampère's law of electrodynamics), cf. (14), in which the integration goes over the virtually perturbed, due to the boundary conditions, sphere's surface that, in the asymptotic time limit, $t \gg t_0$, the sphere is nearly ideal (owing 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. Otherwise, it is in a nonopen state.

3 From Sphere-like to Faceted Crystal Growth

Of course, the spherical idealization of the crystal or aggregate is not entirely suitable for revealing certain relevant kinetic–thermodynamic details of the crystal formation process, especially at the molecular level [12, 15].

The formation of facets – flat crystalline surfaces – is a nearly ubiquitous phenomenon in crystal growth. Faceting plays a major role in guiding the growth of protein crystals [1, 5, 25].

Once a (spherical) crystal grows, the expanding crystal develops facets because some crystalline surfaces accumulate material more slowly than others. Condensing macromolecules are especially attracted to rounded surfaces (curvature effects) that are rough on the atomic scale because such areas present greater available molecular binding. Molecularly flat regions – the facet surfaces – have fewer dangling 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 2D nucleation growth. In dislocation-driven growth, growth occurs along screw dislocation defects on the crystal face. The addition of growth units along a dislocation growth step results in the formation of a hillock, as shown in Fig. 3c, and eventually, the growth of the face as a whole.

The growth rate of the faceted crystal is determined by the surface diffusivity of attracted macromolecules 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 [12, 30], cf. (3),

$$V_{gr} = \frac{v_{step} \cdot d}{\lambda_0}, \quad (15)$$

Fig. 3 **a, b** Facet formation during the growth of spherical crystals, **c** screw dislocation defects on the crystal face initiate the growth in spiral fashion, **d** cross section of the spiral and its characteristic features (d , step height; λ_0 , average distance between two steps; v_{step} is a step propagation velocity parallel to the step)

Fig. 4 Surface of the growing protein crystal showing the eDL, which can be partially considered as an asymmetric multibarrier ion channel

wherein v_{step} is a step propagation velocity parallel to the step, λ_0 is the average distance between two steps and d is the height of a step.

4 A Prototype Channeling Effect Along the Crystal's Terrace

Experimental data show that, ultimately, the driving force of growth of protein crystals is hydrophobic interactions between its amino acids in the aqueous solution [3]. In the laboratories, as well as in physiological conditions, the protein crystal grows from the solution in which electrostatically active particles are dissolved, and therefore, electrostatic interactions must be taken into consideration. The most electrostatically active particles are polar amino acids and dissociated salts *NaCl* (in laboratory) and/or *KCl* (in physiological environment) [1, 2].

In the case of the crystallization of charged particles (like proteins) in electrolyte around the crystal, an eDL is formed, where outermost boundaries are made up of a perturbed (with local curvatures) solid vs a “gas” (depletion zone) interface (internal boundary), and a “gas” (depletion zone) vs fluid (blurred) interface (external boundary). In this case, eDL can be identified with an asymmetric¹ ion channel, where macromolecules walk into the direction of the step’s kink; see Fig. 4. Thus, eDL plays the role of the selection layer (filter).

The concentrations of macromolecules in the external layer of eDL amounts to $\sim 2c_0$, while in the internal layer, the concentration gives $\sim c_0$ [7]. Mostly positively charged macromolecules with the surrounding counterions, which are in the external layer of eDL, perform RW, but they “feel” the internal (also charged) boundary of the eDL. Under favorable electrostatic conditions, they can jump into the internal layer of eDL, where they roll on the crystal surface under the influence of strong hydrophobic forces until they ultimately land in their minimum-energy locations, viz., kinks.

We can see that the surface of the growing crystal is not flat but is formed as a sequence of terraces, Fig. 4. Moreover, macroions walk “uphill” the terraces between two neighboring Ehrlich–Schwoebel barriers [25], which appear on the borders between terraces, towards the

¹The asymmetry invoked is related to the different physical natures of both interfaces by which the eDL, and thus the ion-channel-resembling object, is confined.

Fig. 5 A prototype channeling effect between Ehrlich–Schwoebel barriers, which appear on the outskirts of a crystal’s terrace

step kink, where the macroions could be incorporated into the crystal. This situation forces us to consider the surface of the crystal as a series of single compartments, cf. multibarrier channel [31]. Furthermore, each compartment can be described as a single ion channel, as illustrated in Fig. 5. Each of the channels is characterized by the two geometrical parameters: length and width of the channel, the latter being typically of the size of about 0.67 protein (macroion) diameters [7]. There is also one parameter that describes the capacity of the channel. In this case, the capacity of the channel can be related to the surface diffusivity of the macroions, which manifests itself in the upper terrace growth, v_{step} , (Fig. 3d). Taking into account the structured hydrophobic–hydrophilic and simple electrostatic interactions [6] between the macroion and the crystal’s surface, and also between the macroion and salt ions present in the solution, the 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. 5.

The channel can therefore be characterized by a RW of $\langle r^2(t) \rangle \propto t^\alpha$, where the channel is in a partly or fully open state when $\alpha > 1$ (especially $\alpha = 2$, i.e. $D(t) \propto t^1$ corresponds to a fully open channel, cf. the preceding section. Such a RW is said to be a superdiffusive. Otherwise, the channel is closed, i.e., when $0 < \alpha \leq 1$ and we obtain a normal or subdiffusive RW.

The above qualitative view is consistent with the analytical argumentation presented above in equations (8–12). In addition, it is also confirmed in the following section by a supporting Monte Carlo (MC) simulation, as shown in Fig. 6.

5 Diffusing Probe-Particle Spectroscopy by Computer Experiment Applied to Testing Viscoelastic Properties of the Crystal’s Terrace

A clear motivation for performing the numerical studies presented below comes from the experimental investigations revealing that protein self-assembly at the surface of an aqueous solution has the same structure as in the crystal structure, wherein the structure is assumed to be obtained under the so-called layer-by-layer (epitaxial) growth mode [32]. Moreover,

Fig. 6 Growth-rate fluctuations observed during lysozyme crystal growth obtained in computer simulation [12]. Positive growth rate values refer to the channel in the open state (growth), whereas negative growth rate values refer to the channel in the nonopen state (no growth observed, or melting processes occur). Note the similarity to the open-closed ion channel fluctuations observed in patch clamp experiments, such as those described in [35]

it has unambiguously been shown that protein (lysozyme) crystallization conditions are a result of competitive surface-integrated-to-volume-diffusion explicitly time-dependent kinetics, somehow postponing the thermodynamic part of the process [33], dealt thoroughly in the study below at both analytic and numerical levels.

Because, in our case, the channeling effect means that if the macroions perform a (linear) superdiffusive RW in the eDL then the ion channel is open (in other cases, it is in a nonopen state), and the superdiffusive behavior of ions in a channel should be investigated in more detail, e.g., by the probe particle, which performed a drifting RW on the hydrophobic–hydrophilic surface mimicking the crystal’s terrace. To test a RW along a crystal terrace made up of hydrophobic (H) and polar (P) sites (for a general method, see [34]), we begin with a special preparation of an $h = 20$ columnar deposit (h -height), mimicking a crystal’s terrace. Then, the H–P properties of the $h = 20$ deposit have been mapped onto a plane, constituting this way a planar terrace of the crystal. At each point of the terrace, either the H or P property has been assigned by applying a majority rule to each site labelled by “ i ”: a $site(i) = H$ iff $\#H$ at site/column “ i ” is greater than $\#P$ at the same site/column (where $\#$ denotes the number of sites of a given type). If $\#H = \#P$, then a random sampling is decided for the proper assignment to “ i ”. Afterwards, the terrace’s surface is restructured to get a hydrophilic track (minimum delay passage track), built up of P sites (a replenishment of P sites has become effective) because the surface is always exposed towards water. A model of the crystal’s terrace used in this simulation is presented in Fig. 7. Grey sites represent (H) hydrophobic sites, whereas white sites represent (P) hydrophilic sites.

The model of a crystal face (terrace) is real in the sense that (1) it contains topological defects, mainly point-like and linear, that can be modelled by the 2D percolation concept, with its site- and bond-involving types, respectively, and (2) the molecular-packing arrangements are said to be optimal when they are realized with suitable deposition rate, with more loosely than tightly arranged groups of biomolecules contributing to the arrangement [22]; the latter

again shows the hydrophilic path to be a decisive factor. For more experimental details concerning topology of the protein's crystals, especially those that show the orientation of a single protein on the crystal's surface (the majority of proteins on the crystal's surface are turned with their hydrophilic side to the solution), see [36, 37].

Along the $N \times N$ terrace (with $N = 100$), an RW of a neutral testing walker has been carried out, starting from the left-hand side of the terrace. For each simulation, 1,000 runs have been performed. First, the $\frac{1}{2}$ diffusion exponent, either with elastic/reflecting or with periodic ("infinite") boundary conditions, or with both of them applied together, has been approached by using a scaling law of

$$N \sim T^\nu, \quad (16)$$

where T is the time needed for the random walker to reach the right end of the terrace when always starting from its left counterpart. ν is the RW exponent, measured as the slope of the graph in the $\log - \log$ plot of N vs T .

Then, a few small lattice constants for lasting drift has been superimposed on the RW, favoring a bit longer glides over the surface in the right-hand-side direction. The drift was obtained by privileging of the movement in the right-hand-side direction. The probability of the movement in this direction was twice greater than in the left-hand-side direction. Moreover, if the movement direction was chosen as right, the glide over the surface was carried out over the four sites (the length of the flight is equal to four lattice constants). Finally, a H-P changing-property ("flicker") walker has been allowed to move randomly along the H-P matrix, additionally residing four time units when H-H interaction occurred (penalty = 4), two time units when H-P or P-H interaction took place (penalty = 2), and

Fig. 7 The prerequisite of the crystal's terrace, invented in the vein of [33], used in a computer experiment with diffusing probe particle launched on its planar surface. *Grey squares*: H-hydrophobic sites; *white squares*: P-hydrophilic sites. A hydrophobic (*black*)/hydrophilic (*white*) random walker (hydrophobicity/philicity is randomly chosen after each step with probability equal to 0.5) starts from the left-hand side of the surface. A direction of walk is randomly chosen, with the probability equal to 0.25 for each direction. The flight's length is equal to one lattice constant for left, up, and down directions, whereas for the right direction, the flight's length is determined by the superimposed drift, in this case, equal to 4 lattice constants. A delay sensed by the walker, descended from the hydrophobic/hydrophilic interactions between "flicker" walker and the HP surface, is realized by the residing, in a given place, four time units when H-H interaction occurred (penalty = 4), two time units when H-P or P-H interaction took place (penalty = 2), and 0.1 time units (penalty = 0.1) when P-P interaction resulted

0.1 time units (penalty = 0.1) when P–P interaction resulted. The modification reflects the hydrophobic interactions, which, in the case of H–H contact, are no less than two times stronger than in the case of the H–P interaction, and much stronger when P–P contact appears [3]. For hydrophobicity, defined as $p = \frac{\#P}{\#P + \#H}$, set equal to 0.6, and the particle executes a flight over a distance of four lattice constants, the RW exponent has then been obtained as ~ 1 ; see Fig. 8a. RW exponents for flight lengths equal to 0.33 for $p = 0.6$ and no long flights occur for $\#P = \#H$ as presented in Fig. 8b. It is seen that, to obtain an RW exponent approximately equal to 1, i.e., the channel in the open state (which is obtained by the superdiffusive RW of the particles along the multibarrier channel), the semidirected and constrained movement is needed. Additionally, the interior (or, the wall) of the channel must be more hydrophilic than hydrophobic. Such a constrained movement is observed in the typical ion channels [10], where the difference of the concentration of the ions on both sides of the channels forces ions to pass through the channel, as well as on the surface of the terrace between two neighboring Ehrlich–Schwoebel barriers.

An interesting result of the computer simulations presented so far appears to be that, when an HP (or even neutral) molecule passes smoothly along a crystal's terrace, a hydrophilic (water-containing) route has to be assured. It is due to the observation that the penalty of all nonhydrophilic interactions (those except P–P) is too high as to facilitate any smooth passage of a random walker. On the contrary, it rather hampers such a passage. To test this interesting physical premise, we have developed additional procedures to examine the passages under such a dynamic, particle-matrix interaction context [34]; see Fig. 9. Thus, we have designed, looking a little bit more at the dynamic aspect of the process, a supporting computer simulation in which we have placed 400 HP chains on the $N \times N$ terrace, where $N = 100$. Each of the chains consists of 10 monomers. The hydrophobicity of the chains, constituting the whole matrix, was chosen to be $p = 0.3, 0.5$, and 0.7 in consecutive MC runs. Moreover, we have permitted the HP chains to fold and aggregate to the other chains. As a result of these two competing processes (folding vs aggregation), 2D micelles can be formed; see [38] and references therein. The set of the HP chains, while scattered on the neutral surface/terrace, can be treated as a more or less porous material, in which the percolation patches [39] can be detected easily.

Fig. 8 **a** RW exponent ν for $p = 0.6$ and the particle's flight covers a distance of 4 lattice constants; **b** RW exponents for flight lengths equals 0.33 for $p = 0.6$; only short flights are observed when $p = 0.5$. Note that all "drifted" RW exponents presented here belong to ca. [0.95, 1.05], which proves that, to bring the channel into the open state, a minimum drift is needed

Fig. 9 Three subsnapshots of the HP microstructures after 94,000,000 MC steps (MCs) [38]. The structure consists of 400 chains, each of which is made up of 10 monomers. The density of the HP matrix is $\rho = 0.4$, which means that 60% of available square-lattice points are left empty. *Black dots* represent hydrophobic residues (H-type), whereas *grey dots* represent hydrophilic residues (P-type). The HP-chain hydrophobicity, p , causes, when varying, some systematic changes in the estimated HP-matrix degree of crystallinity $\chi = 0.07, 0.26$, and 0.37 , from left to right: $p = 0.7$ (left), $p = 0.5$ (middle), $p = 0.3$ (right), respectively. The mean transition time of the random walkers, t_{tr} , is a p -dependent parameter, $t_{tr} = t_{tr}(p)$, telling us that hydrophobicity, $p = 0.3$, even if with apparently big holes in the microstructure, does not allow a smooth passage of the walker; on the contrary, the hydrophilic case of $p = 0.7$ easily allows smooth passage for the walker

We have used similar techniques such as the one described for the flat HP crystal surfaces to examine viscoelastic properties of such porous materials. The diffusing probe particles were let through such a viscoelastic environment.

In the beginning of the simulation, the probe particles were located at random positions, on the left-hand side edge, and on the top edge of the surface. Next, we added a similar drift to the system. The drift was obtained on the assumption that the probabilities of the movement of the particle toward the right and bottom directions is two times greater than toward the two remaining directions. A change in hydrophobicity (p) of the HP planar environment results in a similar change in the pore (hole) sizes—it is a result of HP-chain clustering and possible crystallization as well. This simulation shows, in a preliminary way, existence of the random-walker transition time, t_{tr} , being proportional to T (the traversing time defined above), as a function of p , $t_{tr} = t_{tr}(p)$. (Both t_{tr} and T cannot be entirely compared with the “analytic” time t .) It indicates that, astonishingly, larger pores do not assure easier passage of the random walker through the matrix, see Fig. 9, because, typically, a larger cluster demands longer RW pathways than its smaller counterpart. We came then to an apparently surprising conclusion that not the average diameter of the pore but just the (large) magnitude of the cluster decides ultimately whether the walker passes through the pore smoothly or reluctantly. The prevailing tendency shown by the simulation, reflecting a physical scenario on the crystal’s terrace, has to be confirmed by longer MC runs. A certain match of the characteristic time t_{tr} and T , if this were computationally attainable, would indicate an appropriate scale of the process, thus pointing finally to its superdiffusive character with $\nu \sim 1$ or $\nu > 1$.

6 Conclusions and Perspective

In this study, we have offered both numerical and analytical approaches allowing us to analyze in detail two important factors controlling, in a fairly hybrid-type way, the initial

and mature stages in crystal growth, viz., multibarrier ion channels and the non-Markovian nature of the underlying dynamics.

An important result coming from our model is that the eDL, which electrostatically screens the growing crystal, can be described as a multibarrier ion channel. In the typical ion channels, as well as in the eDL, ions and/or macroions are transported. In the first case, a noisy ionic current appears. In the case of eDL, the superdiffusive transport of macroions, and possibly other ions, through the internal eDL macroion-depleted zone, manifests itself by the fact that not a disordered aggregate but a strongly ordered object, viz., the crystal, grows. If the processes were subdiffusive or diffusive, the result would be a disordered aggregate. Also, in the model of the eDL, the $1/f$ noise emerges explicitly in the analytic way, (12), when the purely asymptotic form of the macroion-velocity correlation function $\propto t^{-\alpha}$ appears. This was convincingly demonstrated in that our system behaves as an ion channel, not only deterministically/phenomenologically but also, and above all, in the stochastic, noise-involving domain. In other words, such a noise emerges exclusively when the tendency towards constant growth rate, $V_{gr} \rightarrow \text{const}$, prevails, i.e., when – presumably – the crystal growth is truly subjected to a special type of noise in the macroions' velocity field, termed the flicker or $1/f$ noise. Otherwise, the noise is not flicker noise, and more or less disordered protein aggregates may be formed from the aqueous solution [5, 12, 13].

In fact, our discussion of the role of flicker noise in Section 2 and Fig. 2 offers a new thermodynamic–kinetic condition for protein crystallization to occur. Let us put it explicitly when based directly on Fig. 2: Crystallization occurs when the temporarily formed asymmetric channel around the crystal is open; otherwise, the channel is not open and only biomolecular aggregation can be anticipated. This is an essential, though still hypothetical, novelty thus proposed for further experimental testing. A certain intriguing nature of this novelty can then be realized when compared with crystallization criteria based either exclusively on the notion of the (negative, narrow-range) second virial coefficient, that means a thermodynamic, binary-interaction involving criterion [1, 2, 14], or on a purely kinetic, recently developed argument, coming from the surface-integrated vs bulk-diffusion competitive relationship [33]. Note, however, that our model can be compared to the latter upon realizing that, instead of the bulk far-distant diffusion, we purposely take into account its interfacial counterpart [13]. This type of random, virtually directional motion, sometimes resembling a flight of a macroion once it becomes electrostatically attracted, should not be confused with the surface diffusion indicated in [33]. The surface-integrated dynamics, in turn, could very likely be related with the local, curvature-dependent thermodynamic solubility conditions offered by our type of modeling that also indicates quite univocally when a protein crystal and when an amorphous plaque will be formed, cf. Fig. 2, and the formalism of Section 2.

Our computer model of lysozyme crystal growth supports the analytical results, mainly in conceptual accord with [33]. The noise-involving growth of the crystal was clearly shown. Emphasis was put on the phenomena taking place in the asymmetric double layer, cf. Fig. 6, and in the selected degree of non-Markovianity, cf. Fig. 3. The results obtained are consistent with those reported in [9, 11], and support a dynamic channeling effect while the crystal grows.

It follows from our approach that the latter expresses a disordered protein aggregate grown from the aqueous solution, whereas the former indicates, by applying the stochastic kinetic criterion of $V_{gr} \rightarrow \text{const}$. [35], that a non-Kossel [14, 23] protein crystal eventually emerges. Thus, it can also be concluded that examining the late-stage soft-matter crystal growth, such as that of the lysozyme type [12], could additionally shed more light on

the ion-channel properties, perhaps eventually leading to the development of systems having the properties of both ion channels and (protein) crystals. This has already been started experimentally by a German group, based on kapton foils, etc., i.e., nanotemplates with pores of truncated-cone shape [40], cf. [9, 10] as well. Another concurrent, but also experimental, evidence for such a possibility is described in [41].

In our model of late-time biocrystal growth we have, in principle, employed the free boundary condition, being an extension of the well-known Gibbs–Thomson thermodynamic condition. Extending it implies anticipation of curvature-dependent surface tension in the Buff–Tolman sense [18, 42]. Our condition at the “edge” between the crystal and its surroundings is, however, not entirely free (or, the one equipped with a sharp interface): it also involves a signature of diffuse interface by imposing the dynamic boundary condition, with the moving macroions. Therefore, our quasihydrodynamic [43] model also looks realistic, and in addition – it resembles the prototype of an ion channel working in close-to-equilibrium conditions. Last but not least, we emphasize that the open vs nonopen scenario of ion passage through the channel is qualitatively in excellent accord with another (fractal-type) diffusion-reaction picture of an ionic-wave propagation through biomembrane channels [44, 45].

Acknowledgements The authors wish to thank Prof. Zuzanna Siwy from the University of California, Irvine, USA, for fruitful discussion. A. G. would like to take the opportunity to thank the ESF Programme Stochastic Dynamics: Fundamentals and Applications (STOCHDYN) for supporting his visit to the Nanoworld Institute of the University of Genoa (April 2007).

References

1. Ducruix, A., Giege, R.: *Crystallization of Nucleic Acids and Proteins, a Practical Approach*. Oxford University Press, New York (1992)
2. McPherson, A.: *Crystallization of Biological Macromolecules*. Cold Spring Harbor Laboratory Press, New York (1999)
3. Miyazawa, S., Jernigan, R.L.: Estimation of effective interresidue contact energies from protein crystal structures: quasi-chemical approximation. *Macromolecules* **18**, 534–552 (1985)
4. Nicolis, G., Basios, V., Nicolis, C.: Pattern formation and fluctuation-induced transitions in protein crystallization. *J. Chem. Phys.* **120**, 7708–7719 (2004)
5. Vekilov, P.G., Lin, H., Rosenberger, F.: Unsteady crystal growth due to step-bunch cascading. *Phys. Rev. E* **55**, 3202–3214 (1997)
6. Holyst, R.: Some features of soft matter systems. *Soft Matter* **1**, 329–333 (2005)
7. Gadomski, A., Siódmiak, J.: Kinetics of protein crystal growth in mass convection regime. *Cryst. Res. Technol.* **37**, 281–291 (2002)
8. Łuczka, J., Niemiec, M., Rudnicki, R.: Kinetics of growth process controlled by convective fluctuations. *Phys. Rev. E* **65**, 051401.1–9 (2002)
9. Siwy, Z., Fuliński, A.: Origin of $1/f^\alpha$ noise in membrane channel currents. *Phys. Rev. Lett.* **89**, 158101.1–4 (2002)
10. Bezrukov, S.M., Winterhalter, M.: Examining noise sources at the single-molecule level: $1/f$ noise of an open Maltoporin channel. *Phys. Rev. Lett.* **85**, 202–205 (2000)
11. Fuliński, A., Grzywna, Z., Mellor, I., Siwy, Z., Usherwood, P.N.R.: Non-Markovian character of ionic current fluctuations in membrane channels. *Phys. Rev. E* **58**, 919–924 (1998)
12. Siódmiak, J., Gadomski, A.: Computer model of biopolymer crystal growth and aggregation by addition of macromolecular units—a comparative study. *Int. J. Mod. Phys. C* **17**, 1037–1054 (2006)
13. Gadomski, A., Siódmiak, J.: On the elastic contribution to crystal growth in complex environments. *Phys. Status Solidi B* **242**, 538–549 (2005)
14. Chernov, A.A.: Crystal growth science between the centuries. *J. Mater. Sci. Mater. Electron.* **12**, 437–449 (2001)
15. Gadomski, A., Grzywna, Z., Łuczka, J.: An evolution equation applied to the perturbed sphere growth. *Chem. Eng. Sci.* **48**, 3713–3721 (1993)

16. Cabrera, N., Levine, M.M.: On the dislocation theory of evaporation of crystals. *Philos. Mag.* **1**, 450–458 (1956)
17. Gadomski, A., Łuczka, J.: Some remarks concerning spherulitic growth. *Int. J. Quant. Chem.* **52**, 301–308 (1994)
18. Tolman, R.C.: The effect of droplet size on surface tension. *J. Chem. Phys.* **17**, 333–337 (1949)
19. Gadomski, A., Łuczka, J.: Convection-driven growth in fluctuating velocity field. *Acta Phys. Pol. B* **24**, 725–731 (1993)
20. Kurzynski, M.: *The Thermodynamic Machinery of Life*. Frontiers Collection. Springer, Berlin (2006)
21. Reguera, D., Rubi, J.M.: Nonequilibrium translational-rotational effects in nucleation. *J. Chem. Phys.* **115**, 7100–7106 (2001)
22. Gadomski, A., Rubi, J.M.: On the two principal curvatures as potential barriers in a model of complex matter agglomeration. *Chem. Phys.* **293**, 169–177 (2003)
23. Chernov, A.A.: *Modern Crystallography III: Crystal Growth*. Springer, Berlin (1984)
24. Chernov, A.A.: Crystals built of biological macromolecules. *Phys. Rep.* **288**, 61–75 (1997)
25. Pimpinelli, D.K., Villain, J.: *Physics of Crystal Growth*. Cambridge University Press, New York (1998)
26. Vainstein, M.H., Costa, I.V.L., Morgado, R., Oliveira, F.A.: Non-exponential relaxation for anomalous diffusion. *Europhys. Lett.* **73**, 726–732 (2006)
27. Gadomski, A., Łuczka, J.: Kinetics of temperature or pressure field induced phase transformation in lipid bilayers. *Acta Phys. Pol. B* **28**, 1827–1842 (1997)
28. Málaga, C., Mandujano, F., Santamaria-Holek, I.: Mesoscopic constitutive relations for dilute polymer solutions. *Physica A* **369**, 291–300 (2006)
29. Kosińska, I.D., Fuliński, A.: Asymmetries and anomalous phenomena in ionic transport through nanochannels. *Acta Phys. Pol. B* **38**, 1631–1645 (2007)
30. Liu, X.Y., Boek, E.S., Briels, W.J., Bennema, P.: Prediction of crystal growth morphology based on structural analysis of the solid-fluid interface. *Nature* **374**, 342–345 (1995)
31. Heinemann, S.H., Sigworth, F.J.: Open channel noise V. Fluctuating barriers to ion entry in gramicidin A channels. *Biophys. J.* **57**, 499–514 (1990)
32. Jia, Y.W., Liu, X.Y.: Self-assembly of protein at aqueous solution surface in correlation to protein crystallization. *Appl. Phys. Lett.* **86**, 023903–024100 (2005)
33. Jia, Y.W., Liu, X.Y.: From surface self-assembly to crystallization: prediction of protein crystallization conditions. *J. Phys. Chem. B* **110**, 6949–6955 (2006)
34. Santamaria-Holek, I., Rubi, J.M., Gadomski, A.: Thermokinetic approach of single particles and clusters involving anomalous diffusion under viscoelastic response. *J. Phys. Chem. B* **111**, 2293–2298 (2007)
35. Sakmann, B., Neher, E.: Patch clamp techniques for studying ionic channels in excitable membranes. *Annu. Rev. Physiol.* **46**, 455–472 (1984)
36. Karle, J.: Some developments in anomalous dispersion for the structural investigation of macromolecular systems in biology. *Int. J. Quant. Chem.* **7**, 357–367 (1980)
37. Li, H., Perozzo, M.A., Konnert, J.H., Nadarajah, A., Pusey, M.L.: Determining the molecular-packing arrangements on protein crystal faces by atomic force microscopy. *Acta Crystallogr. D* **55**, 1023–1035 (1999)
38. Kruszevska, N., Gadomski, A.: Towards a 2D model biopolymer polycrystalline aggregation based on Smoluchowski dynamics supplemented by computer experiment. *Acta Phys. Pol. B* **38**, 1819–1835 (2007)
39. Stauffer, D., Aharony, A.: *Introduction to Percolation Theory*. Taylor and Francis, London (1994)
40. Siwy, Z., Apel, P., Baur, D., Dobrev, D.D., Korchev, Y.E., Neumann, R., Spohr, R., Trautmann, C., Voss, K.: Preparation of synthetic nanopores with transport properties analogous to biological channels. *Surf. Sci.* **532–535**, 1061–1066 (2003)
41. Pechkova, E., Nicolini, C.: Accelerated protein crystal growth by protein thin film template. *J. Cryst. Growth* **231**, 599–602 (2001)
42. Buff, F.P.: The spherical interface. I. Thermodynamics. *J. Chem. Phys.* **19**, 1591–1594 (1951)
43. Babin, V., Holyst, R.: Evaporation of a sub-micrometer droplet. *J. Phys. Chem. B* **109**, 11367–11372 (2005)
44. Gadomski, A.: Diffusion-reaction approach applied to the ionic wave propagation through biomembrane channels. *Cell. Mol. Biol. Lett.* **1**, 273–289 (1996)
45. Liebovitch, L.S., Fischbarg, J., Koniarek, J.P., Todorova, I., Wang, M.: Fractal model of ion-channel kinetics. *Biochim. Biophys. Acta Biomembr.* **896**, 173–180 (1987)