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A Novel Model of Protein Crystal Growth: Kinetic Limits, Length Scales and the Role of the Double Layer

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A kinetic model has been designed that tries to capture some most important physico-chemical properties of crystallization from water-based electrolyte. The crystal growing process is thought to proceed in a conserved system, in which the charged-mass conservation law is obeyed. Although the model phenomenon under study proceeds in a mass-convection regime, it is readily interface-controlled. The interfacial control is identified with the role played by the double-layer, presumably of the Stern type, surrounding the object under growth. The product of supersaturation and individual biomolecule velocity in the double-layer appears to be both the controlling kinetic factor and the asymptotic (kinetic) limit being achieved by the process, *i.e.* the crystal growth rate approaches the value of the mentioned product. The first successful test of the model was carried out on data representing the lyzosyme crystal growth.

INTRODUCTION

Complex systems, especially those the investigations of which have important as well as practical consequences, are worth studying. Among others, there appears a task termed protein crystallization, which is a real challenge because it looks both cumbersome and almost hopeless for reasonable theoretical understanding.[1,2] A few simple arguments can be raised here to exemplify the expected difficulties.

First, a separation effect due to the proteins occurs in water solution, where initial steps in the separation of proteins often involve precipitation by salts such as NaCl added to the solution. Intermolecular interactions between proteins and electrolytes govern the behaviour of these processes, and an understanding of such protein interactions is important in the design and operation of all protein separation processes as well as in some other processes, *e.g.* ion-exchange.

Second, because the system under study seems to be at least a two-phase system, a molecular-thermodynamic model incorporating Coulombic and hydrophobic interactions, dispersion attraction, excluded volume and ion-excluded volume effects could be of help, *e.g.* in predicting phase equilibria for both precipitation by salts and extraction in aqueous two-phase systems. Some experimental methods like, for example, low-angle laser-light scattering, osmotic pressure measurements, and vapor pressure osmometry provide experimental information on the intermolecular forces between proteins and salts.[3]

Such measurements also enable to get quantities required for protein crystallization to occur. It has quite recently been proposed that the second virial coefficient must lie within a »crystallization realm«, where the resulting intermolecular potential is not too attractive, permitting crystals, rather than aggregates, to form.[1,4] By carrying out the measurements, an effective Hamaker

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constant, regressed from the experimental osmotic second virial coefficient, can be determined for models of the protein-protein potential of mean force. The effects of specific protein-protein interactions are usually incorporated by means of an adhesive hard sphere potential. Specific ion-protein interactions are also of importance in a number of systems.[5]

Finishing the list of tedious tasks to be done while approaching, possibly in a versatile way, the overall aspects of the phenomenon in question, let us state the following. Namely, while looking more deeply into the effect of polyelectrolyte on protein crystallization, two key parameters determine whether polyelectrolyte effects are important: the Bjerrum length and charge spacing. The Bjerrum length refers to the approximate distance within which electrostatic interactions dominate thermal motions for two charges. For example, the Bjerrum length for F-actin is of the order of 1 nm in water at 20 °C. The linear charge spacing in a polyelectrolyte, in turn, is obtained by dividing the total net surface charge by the length. For F-actin, for example, it is usually 3–4 times smaller than its corresponding Bjerrum length. (Right at this point, note also that the diluted solution is below 0.05 mg ml^{-1} for the same biomolecule.)

In the past, this was a subject of serious scientific debate[6] and it was concluded that the electric energy of an ionic solution could be determined by measuring the average distance between the ions. Also, the quantity, which measures the thickness of the ion atmosphere or, to recall something better known, the thickness of a double-layer of possibly Helmholtz type, proves to be the characteristic length. Regarding the fact that this thickness depends on the concentration of the electrolyte, the electric energy of the solution also becomes a function of this quantity. The fact that this thickness is inversely proportional to the square root of the concentration is responsible for the characteristic appearance of the limiting laws for highly diluted solutions.[7] Moreover, it is an essential characteristic of the electrolytic solution that the measure of this order is determined by the thermal equilibrium between the attracting (electrostatic) forces and temperature movement, especially while monitoring the crystal growth.

A more theoretically-oriented reader is certainly aware that such context needs not to be supported by trivial tools. Thus, it has been recently proposed to attempt revisiting the interesting problem of electrostatic interactions between charged (colloidal) spheres dispersed in aqueous electrolytes. (One can rely on a certain analogy between protein and colloid water dispersions, *cf.* Ref. 8 for crystal growth, or Ref. 9 for general information). Even qualitative features of the inter-sphere interaction are still under study and the complete task has not been satisfactorily solved. It has to be noted here that the long-accepted theory of Derjaguin, Landau, Verwey and Overbeek (DLVO) predicts a purely repulsive electrostatic interaction between pairs of like-charged spheres (which can be a problem appearing in our study as well). This intuitively satisfying prediction is at odds, however, with a large and rapidly growing stream of experimental evidence that (colloidal) electrostatic interactions include a long-ranged attractive component, at least under some circumstances.[8]

In this paper, we wish to propose a simple model of protein crystallization. It is based on the following scheme:

(i) Take the charged-mass conservation law into account and apply it to the simplest possible case of spherical symmetry.

(ii) Choose the boundary condition in the form of the Gibbs-Thomson condition, but do not forget the »physical truth« that for the mature stage of the growing process the concentration prescribed at the boundary crystal *versus* surroundings approaches equilibrium, since the problem slowly but surely becomes curvature independent.

(iii) Make sure that the Poisson-Debye-Hückel (PDH) electrostatic scenario controlling the random walk of biomolecules along the crystal surface is readily manifested inside the double layer, causing the walk to proceed in an »intermittent« or decelerated way (in snapshots from one step, *via* an »electrostatic break« reserved for electrostatics, to another).

(iv) Realize that the growth rate is exclusively determined by what takes place in the double layer enclosing the crystal under growth, where the competition effect between the thermal movement of biomolecules and electrostatic repulsion-attraction and/or screening events control the kinetics of the process; in other words, our model does not see what happens outside the external strand of the double layer, which is considered to be the Stern (not Helmholz) type:[10] the first layer is just a »static« one, being pinned by electrostatic forces to the crystal surface, whereas the second appears to be always of diffusive nature.

Since we have introduced the Poisson-Debye-Hückel context in the double layer enclosing the crystal, by having a time-independent velocity involved as a prefactor in our growth equation, we also immediately know the limits of our modelling, provided that the described processes are realized in a diluted regime. We simply know the limits of the so-called weakly nonideal plasma or »strong« electrolytes,[11,12] so we provide:

$$\kappa_D^3 c_0 << 1 \,, \tag{1}$$

which yields a characteristic plasma parameter χ_p to obey:

$$\chi_p << 1/\sigma \tag{2}$$

where $\chi_p = Q_p^3 c_0^{1/2} \beta^{3/2}$, and where all the symbols used will be explained later on. Since $1/\sigma$ is much greater than one, our approximation seems to be very reasonable

(because $\sigma << 1$), so that a diluted solution regime and the PDH context match very well.

The paper is structured as follows. In the first section (Hanging-drop Method), we give some consideration of the hanging-drop method. In the following section (Charged-mass Conservation Law), we introduce the charged-mass conservation law, whereas in the third section (Physical Length Scales) we inspect more closely the screening effect and the role played by the double layer. Also in the third section, there is a description of the physical scales important for understanding the phenomenon under study. Next, we provide some information about the asymptotic kinetic limit of the process as well as some characteristic crystal length, which does arise while considering a stochastic perturbation of the speed of the process (Asymptotic Kinetic Limit of the Crystal Growth Rate) as a competition effect between the noise intensity and the crystal growth rate.[13] We also provide a comparison with the modelling that nowadays seems to be the most successful in describing the kinetics of protein crystal growth,[1,14] (Comparasion with Seminal Literature Data). Finally, in the last section we provide the a final address.

HANGING-DROP METHOD

A few methods are used to produce crystals. To make inorganic crystals we can choose the Czochralski method and its modification, or methods characteristic of the growth from undercooled melt. These are the most popular methods for a cheap production of good-quality monocrystals.

To produce organic crystals *e.g.* protein crystals, the most effective method is the method named »crystal growth from solution«. This method is similar to growth from the undercooled melt, where the difference in temperature is the driving force of the growth. For the growth from solution, the origin of the driving force appears to be the difference of concentrations inside and outside the nucleus. Moreover, we cannot use the high temperature method, because high temperature can destroy the structure of organic macromolecules.

One of the most popular methods used by experimentalists to produce organic crystals is crystallization by vapour diffusion method, conventionally named the hanging-drop method. This technology ensures temperature and supersaturation control. Temperature control is very important in the growth of organic crystals. Most frequently, the growth proceeds under constant temperature.

A droplet containing the biological macromolecule (in our case, lyzosyme) to crystallize with buffer, crystallizing agent, and additives, is equilibrated against a reservoir containing a solution of crystallizing agent at higher concentration than that of the droplet (Figure 1).[15] Equilibration proceeds by diffusion of the volatile species (water or organic solvent) until vapor pressure in the droplet equals the one of the reservoir. If equilibration occurs by water exchange (from the droplet to reservoir), it leads to a droplet volume change. Consequently, the concentration of all constituents in the drop will change. For species with a vapour pressure higher than water, the exchange occurs from reservoir to drop.

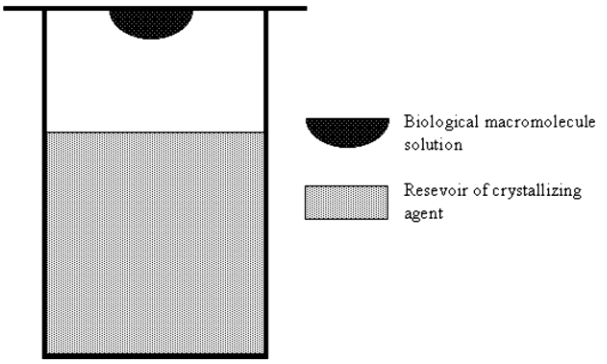

Figure 1. Exemplified scheme of the experimental setup for the growth of lyzosyme crystals (hanging drop).

The most popular crystallization protocol proceeds as follow:[15]

(i) prepare stock solution of 3 M NaCl and 50 mg ml^{-1} ($3.43 \cdot 10^{-3}$ M) lyzosyme in 50 mM acetate pH 4.5 and buffer stock solution (50 mM sodium acetate at pH 4.5),

(ii) fill up reservoir of vessel with solution of NaCl,

(iii) on a coverslip, mix 4 μl of protein stock solution with 4 μl of reservoir, flip it and set it on the greased rim,

(iv) maintain the experiment at 18 °C. We have to note that addition of NaCl to water causes association of the acid and thereby appearance of Na^+ and Cl^- ions ($NaCl \Leftarrow H_2O \Rightarrow Na^+ + Cl^-$). This phenomenon influences very strongly the growing process. (The idea of using NaCl or KCl precipitants seems quite old and appears to be efficient even for crystal growth from metallic melts, *e.g.* those based on Zn, Pb, Sn, as elaborated by J. Czochralski, who added KCl to Zn melt at 232 °C, and NaCl to Pb and Zn melts at 320 °C and 416 °C, respectively.[16] This also applies to crystals grown from a solution, *i.e.* in the case studied here.)

Why does this method suit to our model? In the first step of the growing process as well as in its later stages, the growing crystal has spherical symmetry (our model is based on spherical symmetry of the growing object). Moreover, there is quasiequilibrium between the crystal and its vapor, which makes the hanging-drop method suitable for our modelling.

CHARGED-MASS CONSERVATION LAW

Let us consider two subsequent time instants, t and t_1, where $t >> t_1$ (see Figure 2). Let us assume that the mass of the growing object is equal to:[17]

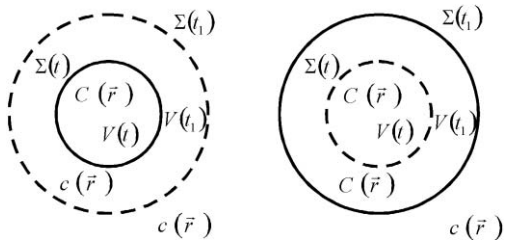

Figure 2. Two consecutive snapshots showing the mass conservation effect.

$$m(t_1) = \int_{V(t_1)} C(\vec{r})\mathrm{d}V \tag{3}$$

but for time t one has:

$$m(t) = \int_{V(t)} C(\vec{r})\mathrm{d}V + \int_{V(t_1)-V(t)} c(\vec{r})\mathrm{d}V \tag{4}$$

where the second term of the right-hand side follows from taking into account the concentration field $c(\vec{r})$ surrounding the object; here, $C(\vec{r})$ is the object (nucleus) density, $\vec{r}$ represents a particle position.

Let us further evaluate the net account of mass $\Delta m = m(t_1) - m(t)$ during the time step $\Delta t = t_1 - t$, namely:

$$\frac{\Delta m}{\Delta t} = \frac{1}{t_1 - t} \int_{V(t_1)-V(t)} [C(\vec{r}) - c(\vec{r})]\mathrm{d}V \tag{5}$$

which after noting that

$$\frac{\Delta m}{\Delta t} = \int_{\Sigma(t_1)} j \bullet \mathrm{d}S \tag{6}$$

yields:

$$\frac{\mathrm{d}}{\mathrm{d}t}\int_{V(t)} [C(\vec{r}) - c(\vec{r})]\mathrm{d}V = \int_{\Sigma(t_1)} j[c(\vec{r})] \bullet \mathrm{d}S \tag{7}$$

where the limit $t_1 \to t$ is valid and »•« denotes the scalar product; here $\vec{j} \equiv \vec{j}[c(\vec{r})]$ stands for the incoming matter flux.

Because we assume a spherical symmetry of the growing object, we have to specify all the necessary details[18] of using it while performing integration (5).

Then, in a possible simple choice, it leads to an evolution equation in a full spherical coordinate system (r, θ, φ); note that now $\vec{r}$ becomes a position vector in the spherical coordinate system.

If we further assume that the nucleus is homogeneous and its density $C(\vec{r}) = C = \mathrm{const}$, and when we take, for simplicity, an ideal spherical symmetry, we are able to write down:

$$[C - c_\mathrm{S}(R)]\frac{\mathrm{d}R}{\mathrm{d}t} = c_\mathrm{S}(R)\, v(R)\,, \tag{8}$$

but under observation that[19]

$$j[c(\vec{r})] = c(\vec{r})\, v(R)\,, \tag{9}$$

on the surface Σ (the so-called overdamped regime approximation); here $c(\vec{r}) = c_\mathrm{S}(R)$.

We know that the equilibrium Gibbs-Thomson boundary condition is fulfilled

$$c(\vec{r})\big|_\Sigma = c_0\,(1 + \Gamma\, K) \tag{10}$$

provided Γ is a constant (independent of φ and θ).

$$[C - c_\mathrm{S}(R)]\frac{\mathrm{d}R}{\mathrm{d}t} = c_\mathrm{S}(R)\frac{F}{\gamma} = c_\mathrm{S}(R)\, v_\mathrm{mi}\,, \tag{11}$$

where γ is damping constant (for the overdamping region $\gamma >> 1$); note that now $v(R)$ is substituted by v_mi.

After some algebra from (8)–(11), it follows immediately that

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \sigma\, v_\mathrm{mi}\frac{R + 2\Gamma}{R - R_\mathrm{c}} \tag{12}$$

where $\sigma = c_0 / (C - c_0)$ but measured in the double layer; it is in fact an equivalent of the bulk supersaturation characteristic of the crystal growth, where the crystal is fed by diffusion over long distances from the crystallization centre.

Moreover, note that $v_\mathrm{mi} = F/\gamma$, where F is the electrostatic force, also holds (see next section). Eq. (12) will be our starting evolution equation, the solution of which reads:

$$R(t) - R(t=0) - (R_\mathrm{c} + 2\Gamma)\ln\left[\frac{R(t) + 2\Gamma}{R(t=0) + 2\Gamma}\right] = \sigma\, v_\mathrm{mi}\, t \tag{13}$$

and its large time asymptotic becomes:

$$R \sim t\,, \tag{14}$$

which means that the crystal growth rate, defined as $V_\mathrm{gr} = \mathrm{d}R / \mathrm{d}t$, approaches a constant.

Radius *vs* time dependence for three different values of parameter σ is shown in Figure 3.

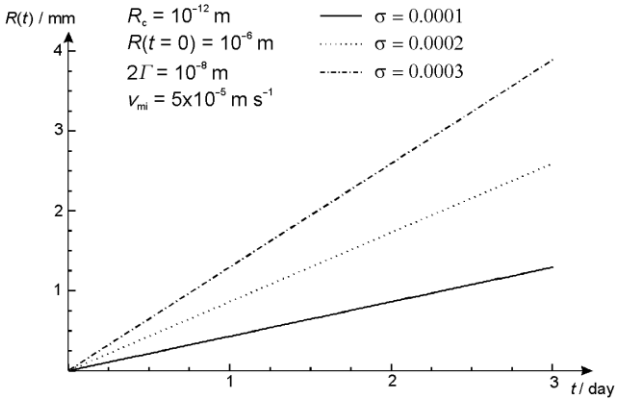

Figure 3. Radius *vs.* time dependence for three different values of parameter σ.

PHYSICAL LENGTH SCALES

Dynamic processes occurring in complex fluids, *e.g.* protein crystallization from solution, include many disparate length scales.

We can distinguish two groups of lengths. The first is directly connected with the kinetic-thermodynamical properties of the growing object. Characteristic lengths belonging to this group are:

Γ – capillary length, which aims at smoothening out the surface of the growing object and is proportional to the surface tension; $R_c = 2\sigma\Gamma$ – critical radius of the nucleus; $R(t = 0)$ – initial radius; R_∞ – radius of the object in the mature stage of the process, *i.e.* at some t being large enough. For $t >> 1$, $R(t) \to R_\infty$; R_v – radius of the crystallization vessel, that can take on either a finite value ($R_v \to \overline{R}_v$: for real vessel crystallization) or an »infinite« value ($R_v \to \overline{R}_\infty$: no external boundary influence). Among these lengths, the following relations appear to be true:

$$0 < R_c < 2\Gamma < R(t = 0) < R_\infty < R_v \, .$$

Since we know that 2Γ is of the order 10^{-8} m, the critical radius from which the process starts to nucleate must be less then 10^{-8} m, but the growth readily occurs at some higher values, starting somewhere about $R(t = 0)$.

The second group includes lengths, that characterize both the growing system and diffusion along the surface. These are: κ^{-1} – Debye length; $L_B = Q_n Q_p / \varepsilon_0 \varepsilon_{H_2O} k_B T$ – Bjerrum length, where Q_n and Q_p are the surface charge of the nucleus and protein molecule, respectively; screening electrostatic length, $r_{min} \sim 2r_p$ (a minimalistic assumption) or higher, where r_p is the radius of the protein molecule; $L_D \approx 2\pi R_\infty$ – approximate path length of the surface diffusion of a single biomolecule on the surface of the growing object.

As we remember, our solution is an electrolytic one. When ions, which can easily diffuse, are present in the solution they will tend to concentrate in the vicinity of the oppositely charged ions. For Cl^- ions the lyzosyme molecule is an oppositely charged macroion, because under the pH of interest a lyzosyme has quite a big positive charge on its surface. This will increase the apparent dielectric constant of the solution, and will depend on the concentration of the ions. This is called the Debye-Hückel screening, and the effect is a distance dependent dielectric constant: $\varepsilon_{eff} = \varepsilon_{H_2O} \exp(+\kappa r)$, where κ is a parameter of the solution, and r is the interaction distance between two ionic forms. (In general, one can feel free to use the Kirkwood approximation for ε as well; in this way, there is a possibility of taking into account the hydrophobic effect clearly present in the electrolyte.)

Using the linearized Boltzmann equation, which relates distribution of ions around the central ion, and the Poisson equation from electrostatics, which in turn, describes the charge density distribution around the central ion, we obtain the linearized Poisson-Boltzmann equation[20] $\Delta\psi_r = (\varepsilon_0 \varepsilon_{H_2O} k_B T)^{-1} \sum n_i^0 z_i^2 e_0^2 \psi_r = \kappa^2 \psi_r$, where n_i^0, z_i^2, e_0^2 have their usual meanings, and the solution of this equation is $\psi_r = A \exp(-\kappa r) / r$, where $A = z_i \varepsilon_0 / 4\pi \varepsilon_0 \varepsilon_{H_2O}$. After expanding the exponential term, $\exp(-\kappa r) \cong 1 - \kappa r$ in a Taylor series up to the second term, we can write down the resulting equation of the electrostatic potential that would be found at a distance r from the ion:[20]

$$\psi_r = \psi_{ion} + \psi_{cloud} = \frac{z_i e_0}{4\pi\varepsilon_0\varepsilon_{H_2O} r} - \frac{z_i e_0 \kappa}{4\pi\varepsilon_0\varepsilon_{H_2O}} = \frac{z_i e_0}{4\pi\varepsilon_0\varepsilon_{H_2O}} \left[\frac{1}{r} - \frac{1}{\kappa^{-1}}\right] \quad (15)$$

Note that for relatively large r's the potential approaches a constant, $\overline{\psi}_r$, and the electric field intensity is expected to be approximately $\overline{\psi}_r$ times the distance from the crystal surface, which at some constant distances from the surface will roughly be constant, too. We see that the first term is the potential that would be found at distance r from the ion under consideration if there were no screening. This is reduced by the effect of the other ions, which screen the interactions of this ion from the others – and from the solvent – lowering the free energy. The screening is controlled by the κ^{-1}, and the screening operates exponentially with the distance. This screening has the effect of lowering the potential surrounding any ion, and the growing crystal too, which will lower the total free energy of the ionic solution, and cut back interactions between the ions.

Moreover, it may generate the possibility of bringing together lyzosyme molecule to the surface of the growing object, so close that the Van der Waals attracting force will dominate repulsive electrostatic force and hydrogen bonds between these two objects would be created.

The Bjerrum length L_B represents the approximate distance within which electrostatic interactions dominate thermal motion for two elementary charges. (We propose that this is true of interactions between two molecules or between a molecule and the growing object.) Beyond this distance, the thermal energy is bigger than electrostatic energy, and hydrogen bonds may not be created. In that case, thermal diffusion on the surface is possible as long as the molecule does not find a place where electrostatic energy is bigger than thermal energy. In such a place, an association can occur. The maximum thermal diffusion length is equal to L_D.

A careful reader has immediately noticed that ν_{mi} from Eq. (12) has not been determined yet. We will do it now.

From elementary electrostatics,[21] *i.e.* applying the Maxwell-Gauss law to a charged conductor, we know the fact that the attractive force F (see previous section) equals:

$$F = \frac{\sigma_n Q_p}{\varepsilon_0 \varepsilon_{H_2O}}, \tag{16}$$

(ε_0 has its well-known meaning), but the speed v_{mi} is, within the realm of overdamped regime approximation, just a ratio:

$$v_{mi} = \frac{F}{\gamma}, \tag{17}$$

where γ (here: $\gamma >> 1$) denotes the damping constant closely related to the viscosity (η) of the solution in the monolayer adjacent to the crystal surface (presumably half of the double-layer). The relation is:

$$\gamma = 2 r_p \eta, \tag{18}$$

where it is presumed that the adjacent monolayer is of the order of a protein molecule diameter (again, an implicit notification of the double-layer arises; the lyzosyme diameter is of the order of 3 nm). Then, after utilizing the above, applying the Einstein-Smoluchowski relation,[22] using a standard definition of the surface charge density $\sigma_n = Q_n / S_n$, where S_n stands for the crystal area, and finally replacing S_n by some L_D^2 (for simplicity, we wish to compare them directly, like $S_n = L_D^2$, but in a thorough replacement one should expect a geometrical pre-factor to appear) as well as reasonably postulating a (surface) diffusional natural scaling, like

$$\tau_D = d_0 L_D^2 \tag{19}$$

with a characteristic surface diffusion time, denoted by τ_D, one may easily arrive at the final result, namely:

$$v_{mi} = \frac{l_B}{\tau_A} \tag{20}$$

where $l_B = L_B \exp(-2 \kappa r_p)$.

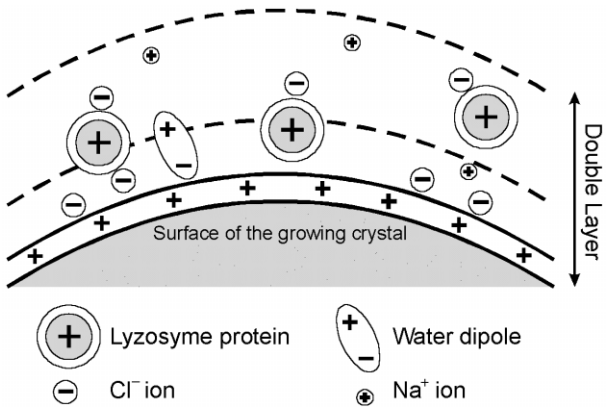

Figure 4. Naive picture of protein crystallization controlled by the double-layer effect.

Formula (20) was derived under a weak geometrical restriction, $d_0 D_0 = (3\pi)^{-1}$, because the Einstein-Smoluchowski formula $D = k_B T / 6 \pi \eta r_p$ is valid for spherically symmetric objects for any diffusion coefficient D, which in turn can be expressed by $D = D_0 \exp(-E_A \beta)$, where $\beta = 1 / k_B T$ is the inverse thermal energy, whereas E_A represents the activation energy for the surface diffusion process; as expected, k_B stands for the Boltzmann constant; the argument $E_A \beta$ is thus dimensionless.

Recalling all the data and experimental studies known so far, we would like to opt for a double-layer scenario of Stern type. In Stern's model, the first layer is »pinned« electrostaticly to the crystal (Figure 4). This is an immovable layer and consists of immovable ions, whereas the second layer is diffusive. We can see that a diffusion controlled adsorption mechanism can be realized also in such a case.

ASYMPTOTIC KINETIC LIMIT OF THE CRYSTAL GROWTH RATE

Typically $v_{mi} >> dR / dt$, which seems to be very natural, at least in the late time zone, because the crystal grows much slower than an individual protein molecule (»macroion«) can move. The solution of (12) is, in general, a nonlinear solution, but for the mature stage of the growing process and under the given set of growing conditions, a simple asymptotic (late time) solution can be typically recast, namely $R \sim t$, which in turn leads to the conclusion that the growth rate, V_{gr}, should, roughly speaking, be constant, *i.e.* $V_{gr} = dR / dt \rightarrow$ for $t >> 1$.

As seen from (13), the main rate-limiting factor appears to be the product of σv_{mi}, where σ and v_{mi} have been thoroughly determined above.

Let us assume that the double-layer experiences fluctuations of the velocity field within it. Fluctuating part of the velocity field is a Gaussian white noise (non-correlated fluctuation) of the strength $1 >> D > 0$:

$$v(r,t) = v_{mi} + V(t), \tag{21}$$

where $v_{mi} = \langle v(r,t) \rangle$ is a positive constant and $V(t)$ is the fluctuating part

$$\langle V(t) \rangle = 0, \quad \langle V(t) V(s) \rangle = 2D \delta(t-s) \tag{22}$$

where $\delta(t-s)$ is the Dirac distribution.

In this case, our starting Eq. (12) is:

$$\frac{dR}{dt} = \sigma (v_{mi} + V(t)) \frac{R + 2\Gamma}{R - R_c} \tag{23}$$

Relation $\langle R(t) \rangle$ *versus* t for several values of the fluctuation strength D is shown in Figure 2 in Ref. 23. We can see that when the field fluctuations grow, then the growth velocity increases. At the early stage of evo-

lution, field fluctuations cause a very fast growth of the sphere in a nonlinear fashion. For long times, in turn, the growth is observed to be linear in time.

COMPARISON WITH SEMINAL LITERATURE DATA (on lyzosyme crystal growth)

The comparison, we would like to sketch briefly, is due to the kinetics of the crystal growth presented in the Chernov's model.[1]

In the Chernov's model, the kinetic coefficient of a crystal face taken as a distinguished unit is $\beta = \beta_{st}\, p$, where the vicinal slope, p, is typically equal to 10^{-2} (p is the average step density normalized by the step height). Therefore $\beta \approx 10^{-6} - 10^{-5}$ cm s^{-1}.

In our model we have $V_{gr} = \sigma\, \nu_{mi}$, where V_{gr} – growth rate and ν_{mi} – single molecule speed, and $\nu_{mi} = (L_B / \tau_D) \exp(-2\kappa r_p - E_A / k_B T)$, where τ_D is the elementary diffusion jump time from one of the minima to another: the deepest minimum is the best; E_A is the energetic barrier (activation energy of the jump).

In the Chernov's model, the lyzosyme diffusivity is $D \approx 10^{-6}$ cm^2 s^{-1}. Therefore, the growth length of a crystal less than $D / \beta \approx 0.1$ cm in size should be controlled mainly by incorporation of species at the interface rather than by bulk diffusion, though in generally coupling is possible between the interface kinetics, diffusion and liquid flow to produce instabilities.

Our characteristic length for the growing crystal reads $l_{char} = \sigma^{-1}(L_D^2/L_B)e^{y_{min}}$, where $y_{min} = 2\kappa r_p + E_A \beta$, and where $0 < y_{min} \leq 1$.

Therefore:

$$l_{cr} \sim \sigma^{-1} \frac{L_D^2}{L_B} \tag{24}$$

because again $e^{y_{min}} \cong 1 + y_{min}$, and for water one has $L_B \sim 10^{-9}$ m. One may state that for a sphere $L_D^2 \cong 4\pi r^2 \sim 10^{-16}$ m^2, so that for $\sigma \sim 10^{-4}$ we are able to get $l_{char} \sim 10^{-3}$ m = 10^{-1} cm, which confirms, at least qualitatively, a typical characteristic crystal length as estimated by Chernov[1] and others (see also Drenth & Haas, private communication).[24]

We see that our model is in agreement with other types of models. Other models are geometrical models, because there are terraces and spiral growth. We can crudely substitute terraces characteristic of the Burton *et. al.* model[25] just by including a perturbation of sphericity. Other models do not have any parameters characteristic of the electrolyte. Our model includes some parameters that characterize the growth from electrolyte: l_B and κ^{-1}. Our model, like that recommended in Chernov's review, includes also the quantities that can be available from experiment.

FINAL ADDRESS

First of all, we wish to underline that we have investigated a model of protein crystal growth in its, perhaps oversimplified, version, which can be named: the evolution of a spherical object in water-based electrolyte. There are, however, certain reasons for which our approximation looks promising:

(i) The apparent simplicity of our approach: to obtain an evolution equation, we use a straightforward and very natural procedure.

(ii) The above is, in fact, in full agreement with prescribing an equilibrium boundary condition for the evolving sphere, where, however, the evolution is realized in its large time limit, so that the curvature of the sphere is of negligible value, *cf.* the Gibbs-Thomson capillarity condition. This is the so-called mature stage of the growing process, being certainly of prior technological importance.

(iii) The PDH electrostatic scenario, assigned to the double-layer, fits very well such a prescription at the boundary, and appears to be a context, being in excellent agreement with the diluted regime approximation (see Introduction).

(iv) The crystal growth rate, predicted by our deterministic evolution equation, resulting from the charged-mass conservation law, takes on a constant value for the large time limit, and shows some »logarithmically-influenced« discrepancies from that linear growth, but mostly for initial stages of the evolution; it should be underlined that it is in excellent accord with the well-known Burton *et. al.* model,[25] where the growth is realized *via* deposition on surface terraces in a spiral-like manner, for which also the growth rate appears to tend to a constant.[26] Moreover, an analogous product to our $\sigma\, \nu_{mi}$ appears also in another study, *viz.* that of the surface diffusion controlled ADP crystal growth from solution[27] (see Eq. 16 therein).

(v) The physically-motivated incorporation of a stochastic perturbation into our model (in general, some well-defined escape of the system from the PDH scenario could be modelled in this way) acts at least twofold: first, it gives an opportunity to determine a characteristic crystal growth length (see above), and second, it enables to make a comparison between our modelling and some other models, accepted recently as a thorough process description.

(vi) Our final result(s) can be fully expressed by quantities that are, no doubt, measurable quantities, so that such a comparison can, in principle, be quantified.

(vii) For simplicity, it is possibile to extend our model toward applying to nanocrystals and/or to make it more specific, *e.g.* modify the equilibrium Gibbs-Thomson internal boundary condition ($c(R) = c_0(1 + \Gamma K_0)$, where $K_0 = 2/R$), by adding a nonlinear curvature term *i.e.* the

Gaussian curvature ($K_1 = 1/R^2$), specifically $c(R) = c_0(1 + \Gamma K_0 + \delta_T^2 K_1)$ where δ_T is the Tolman parameter.[10,28] The solution of our starting evolution Eq. (12), asymptotically, is linear in time, but in the entire time domain (for specific physically-motivated choice of parameters), the solution includes additional (*e.g.* logarithmic) corrections.

(viii) One ought to be aware of simplifications, enabled by our modelling, which at the first glance seem to abandon all the specifics of individual protein crystal growth processes, which is surely very important as well.

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SAŽETAK

Novi model rasta proteinskih kristala: kinetičke granice i uloga električnoga dvosloja

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Predložen je kinetički model, kojim se, uz pretpostavku očuvanja mase i naboja, mogu opisati najvažnija fizikalno-kemijska svojstva sustava pri kristalizaciji iz vodene otopine elektrolita. Iako se proučavane pojave odvijaju u režimu konvekcije masa, one su ipak kontrolirane pojavama na međupovršini, i to vjerojatno električkim dvoslojem Sternova tipa, koji okružuje rastuće čestice. Umnožak presićenja i brzine biomolekule u dvosloju pokazao se kontrolirajućim kinetičkim faktorom, a ujedno i asimptotskom granicom kinetike promatranoga procesa. Model je uspješno testiran na rastu kristala lizozima.