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Computer model of a lysozyme crystal growth with/without nanotemplate – a comparison

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The results of a computer simulation of the lysozyme crystal growth influenced by monomer and tetramer (aggregate) units are discussed. A very recently introduced computer model of biopolymer crystal growth and aggregation is based on the 2D lattice Monte Carlo technique and the coarse-grained HP approximation of the lysozyme monomeric unit. Acceleration of the lysozyme crystal growth by a factor of 4/3, based on the 2AUB (PDB ID) lysozyme unit, obtained from the Langmuir-Blodgett nanotemplate method, has clearly been confirmed by means of the proposed computer simulation. It is concluded that the aggregates (tetramers) involving 2AUB lysozyme crystal growth can be expected to be slightly accelerated when compared to its monomer-based (PDB ID: 193L) counterpart, which is in excellent accord with very recent experimental findings of the emerging applied science called protein nanocrystallography.

Keywords: protein crystallization and aggregation; lysozyme; MC simulation; Langmuir-Blodgett protein thin film; nanotemplate stimulation; spiral growth; rate of crystal growth.

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1. Introduction

Nucleation-growth (N-G) transformations at a nanoscale are still becoming a subject of vital theoretical and practical interest^{1,2}, although a continuing attraction to it lasts almost over a century since C.T.R. Wilson, the 1948 Nobel prize winner, built in 1911 his particle-track-revealing cloud chamber based on the phenomenon of homogeneous (drop-wise) water nucleation. In particular, a real challenge of using the as-yet available knowledge on N-G concerns with a task of acceleration of the emerging N-G outputs, such as biomolecular crystals or aggregates, at possibly no,

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or very minor, expense of the quality of obtained microstructures, e.g. non-Kossel crystals³, or sometimes with a certain visible improvement of it².

A modern experimental technique, such as protein nanocrystallography, has recently been introduced as a unique nanotechnology-based method of stable protein crystals formation and their characterization down to atomic scale. Specifically, a protein nanostructured template appears to be able to stimulate N-G transformation of so far unsolved proteins^{4,5}. In case of lysozyme crystal growth the lysozymes appear to transfer directly from the nanostructured film into the drop, triggering the formation of the crystal⁵.

In a letter [6] it was shown that a new experimental nanocrystallographic approach allowed someone to accomplish a visible increase of the hen egg white lysozyme (HEWL) N-G rate in comparison with such a classical vapor diffusion method as hanging drop⁷.

The approach relied on a modification of the classical vapor diffusion method with the aim of crystal growth acceleration: a HEWL Langmuir-Blodgett (LB) thin film, prepared by Langmuir-Schaeffer (LS) technique variation, was used as the template for the stimulation and rate increasing of lysozyme crystal growth². Monolayers of lysozyme were formed in a Langmuir Teflon trough by spreading 500ml phosphate buffer (pH 6.5) solution with a HEWL concentration 4mg/ml; 10 μ M NaOH solution (pH 11) was used as a subphase. The subphase temperature was 22°C. The formed film was compressed with a barrier speed of about 0.1mm/s up to surface-pressure of 18mN/m and deposited by LS parallel shift technique onto the siliconized cover glass slide. Obtained nanofilm was characterized by circular dichroism, atomic force microscopy and nanogravimetric methods² and utilized as a template for crystal growth in a common crystallization apparatus, placed in a contact with a protein solution drop. For other details, see [4, 5, 6, 7].

A computer modeling is a valuable method serving for cheaper-than-by-experiment (though not necessarily more reliable) examination of any N-G transformation of interest⁸. It has been established as a standard tool in soft-condensed-matter physics to mention but one⁹. In a very recent study [10] (the present study can be dealt with as a more experimentally supported continuation thereof), we presented a computer model in which a way of preparation of the growth-unit of the HP model biomolecule for a lysozyme, and that of nucleus' basis of the structure with minimum energy of the unit cell of the non-Kossel crystal have been thoroughly described. The growth procedure, based on an epitaxial growth type, with the spiral mode being readily involved at the three-dimensional level of the description, has also been outlined. A method of calculation of the growth rate in a direction normal to the surface of the growing structure has been introduced and applied to two types of the lysozyme¹⁰.

In this note, motivated by the above mentioned experimental methods toward nanocrystallography, and starting our type of rationale with a statement, that the computer model we are going to apply points fully to an interface-controlled (towards thin films) process, we propose our computer-model based confirmation of

the experimental fact that the crystal growth based on 2AUB (PDB ID) is by a factor 4/3 faster when compared to the same type of N-G process (under the same basic thermodynamic-kinetic conditions^{10,6,4,5}) but performed on a 193L (PDB ID) lysozyme. In what follows we will develop our type of rationale that slightly modifies the before applied algorithm of simulation¹⁰ in order to achieve a result being in excellent accord with the performed experiment. Our main concern is that the resulting difference in the rates of crystal growth in both cases mentioned appears due to incorporation of tetramers, see Figure 1, by the crystal body in the case of crystals based on 2AUB lysozyme; for its full definition, unquestionably pointing to the fact that its structure is derived from thin-film-based crystals, consult [4], available by the 2AUB ID at the Protein Data Bank¹⁰. A clear origin of emergence of such ordered aggregates could very much be the ordered nanotemplate of LB type from which such entities may easily desorb², thus being dispersed in the nearby crystal vapor surrounding phase, and being therefore available for the on-nanotemplate-grown crystal drop for a final absorption.

2. Computer model and the results

Computer model of the biopolymer crystal growth and aggregation is based on the 2D lattice Monte Carlo technique and the HP approximation of the biopolymers. The most essential characteristic, properly defining this approximation, enables to use HP model with its onto-cube-walls projected (excess) HP-properties, with a special emphasis put on the outer region of the protein, which is the key feature of the proposed approximation to be applied in the present work for both types of lysozyme biomolecules, cf. Figure 1. Moreover, in the very recently invented model¹⁰, the site-dependent attachment, detachment and migration processes are involved. The probability of growth unit motion, attachment and detachment to/from the crystal surface are assumed to be proportional to the orientational factor representing the anisotropy of the molecule, for more details see [10] and refs. therein.

The tetrameric growth units, present in performed simulation of lysozyme crystal growth look like the one drawn schematically in Figure 1. They are now established as both the units dispersed in the vicinity of crystal surface as well as the units finally incorporated by the crystal surface, cf. [10] for details. When compared to the monomers, they are allowed to perform their along-surface directional Random Walk (RW) at zero-energy cost, so that no energetic penalty is ascribed to such a rolling-over effect - this makes a basic difference between monomer- and tetramer-based crystal formation that we have performed.

Clearly, a contribution of aggregates in the crystal formation modifies their final outcomes, see [11, 12]. It mainly causes a "renormalization" of the diffusion coefficient since the aggregates in the bulk diffuse slower than the monomers, their diffusion is simply mass-dependent¹². When, however, diffusing along the crystal surface, or within the interface crystal-ambient phase, the aggregates may take on a faster diffusion mode since less degrees of freedom are available in order to

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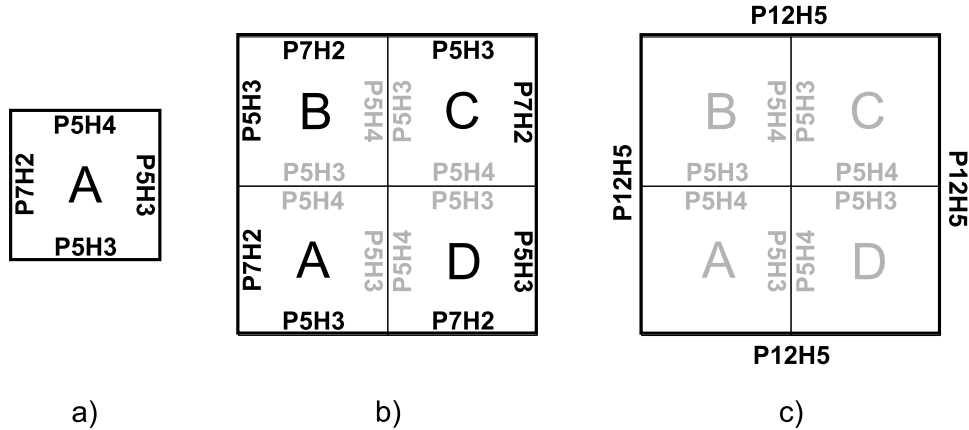


Fig. 1. Growth units: a) monomeric growth unit of A-type, b) energetically favorable (the lowest energy) configuration of four monomeric growth units, c) generalized tetrameric growth unit. For details see [10]. It can be seen that in the case of tetrameric growth unit (c) in comparison to monomeric growth unit (a) every sides look the same what plays significant role in calculation of the movement probability (practically the movement is made without crossing of any energetic barrier because the geometrically smooth surface is also energetically smooth/flat).

dissipate their kinetic energies, not mentioning, however, the electrostatics very possibly involved therein⁷. At this point, a role of a confining template has to be anticipated again, cf. [2], and examples therein.

According to our previous study¹⁰ we took for monomers an empirical estimate (see refs. of Table 1 therein) of the surface-diffusion coefficient, $D_S^{n=1} = D_B^{n=1}/100$, where $D_B^{n=1}$ stands for the diffusion coefficient of the monomer in the bulk. Because by its nature our model does not properly see the (distant) bulk diffusional effects, we may also take this estimate for granted for the tetramers that are the only (ordered) aggregates we take into account in the refined version of the model. Thus, now the surface-diffusion coefficient reads $D_S^{n=4} = D_B^{n=4}/100$ (notice a change in the index from $n = 1$ for monomers to $n = 4$ for tetramers), and will further be considered; realize that $D_B^{n=4}$ stands now for the diffusion coefficient of the tetramer in the bulk¹¹. The following rationale for setting up appropriate grounds of the simulation can be developed below.

Two important effects must be taken into account in order to get a (comparative) realistic estimate of $D_S^{n=4}$ for the aggregate/tetramer controlled lysozyme crystallization. These we can call further compensation effects, quantified by a dimensionless kinetic compensation factor ν_{comp} . As a consequence, $D_S^{n=4} = (D_B^{n=1}/100) \times \nu_{comp}$ is proposed to be applied further instead of the above. The quantification of ν_{comp} will then rely on taking thoroughly into consideration the effects of aggregate (tetramer) involvement in the N-G process under study. It can be viewed twofold: (i) By involving a dimensionless (say, superdiffusive) compensating mass-factor, $\nu_{mass} = (M_{n=4} - M_{n=1})/M_{n=4}$ (M -s being the masses of the mono- and

tetramers, respectively, $i = 1, 4$), its role being envisioned toward that heavier units are more prone to effective directional motion along the interface, e.g. by rolling over the unit¹⁰, and with nearly zero energy cost; (ii) By engaging another dimensionless compensating subdiffusion-type factor, $\nu_{diff} = [D(M_{n=1}) - D(M_{n=4})]/D(M_{n=1})$ (D -s are the corresponding bulk-diffusion coefficients), its role being attributed to the fact that heavier units realize a mass-dependent slow diffusion, such that $D(M) \propto M^{-1/2}$ here^a, cf. [12]. Moreover, according to the Einstein-Smoluchowski (ES) relation $D_B = k_B T / 6\pi\eta R_H$, where k_B - Boltzmann constant, T - the temperature, η - viscosity of the solution, R_H - hydrodynamic radius of macromolecule, cf. Table 1 in [10], one has $D_B^{n=1} = 2D_B^{n=4}$ since the hydrodynamic radius of the tetramer is twice so big than that of the monomer. Therefore $D_B^{n=4} = D_B^{n=1}/2$ holds, when compared to that monomer-oriented case.

Finally, to properly account for the overall rate increase and/or decrease (competition) net effects expected in the modeled crystal growth, let us define $\nu_{comp} = \nu_{diff}/\nu_{mass}$. (The quantity in the numerator accounts for deceleration effect, carrying from the bulk effect on the diffusion within the interface whereas its denominator counterpart accounts for the accelerating mass effect.) It gives $\nu_{comp} = (1/2)/(3/4) = 2/3$, so that $D_S^{n=4} = (D_B^{n=1}/100) \times 2/3$, which finally reads $D_S^{n=4} = D_B^{n=1}/150$, and which above all conforms in an excellent way to the experimental results^{6,4,5}, see also Fig. 1 of [6] or another Fig. 1 of [4] for comparison. This is also indicated by Figure 2 obtained by our computer simulation. Note that all figures mentioned point to the crystal-growth acceleration rate of about $4/3$ when compared to the non-template (viz monomer-involving) case. In comparison to our previous study¹⁰ the application of tetrameric growth units modifies growth rate by multiplying it by ν_{comp} , because the simulation time t , involved in the equation which expresses the growth rate, see Eq. 4 in [10], is expressed by $D_S^{n=4} = D_S^{n=1} \times \nu_{comp}$. Ultimately, the obtained growth rate of modeled structure for the tetrameric growth units $V_{gr}^{n=4}$ is about $4/3$ times greater than that derived for the case when monomeric growth units yield the crystal $V_{gr}^{n=1}$, cf. [6].

To recap the above in a simpler way, it is now easy to conclude that all effect on the accelerated crystal formation essentially comes from the contribution of masses of the tetramers relative to the masses of monomers, namely by realizing that the inverse of ν_{mass} actually reads $\nu_{mass}^{-1} \equiv 4/3$ which is formally the experimentally confirmed factor⁶ of interest (realize that the coefficient D also depends on mass¹²).

3. Conclusions

In summary, we can draw the following conclusions:

- (i) An interface-controlled model crystal growth of two types of lysozymes, in which the crystal formation occurs with or without a nanotemplate, has been compare-

^aA stochastic attachment/detachment kinetics of the aggregation stays behind the presented formula.

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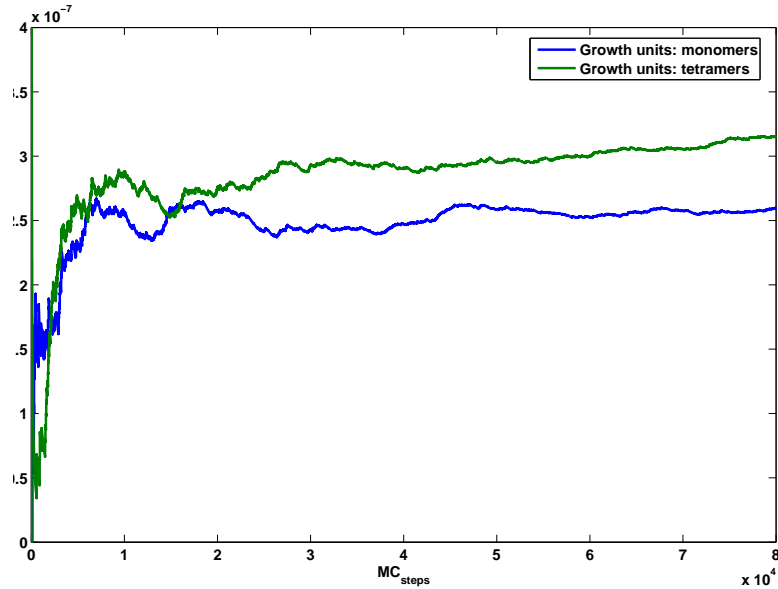


Fig. 2. Simulated growth rate of lysozyme crystal obtained from the Langmuir-Blodgett template motivated technique (tetrameric growth units) - green (higher) line versus classical method (monomeric growth units) - blue (lower) line. Experimental data used in simulation for monomeric growth unit¹⁰: lysozyme concentration in solution 6%, temperature $T = 310K$, viscosity of the lysozyme solution $\eta = 2.4 \cdot 10^{-3} kg/m \cdot s$, derived from Einstein-Smoluchowski relation bulk diffusion coefficient of the lysozyme molecule $D_B^{310K} = 4.977 \cdot 10^{-11} m^2/s$, surface diffusion coefficient of the lysozyme molecule $D_S \approx D_B^{310K}/100 = 4.977 \cdot 10^{-13} m^2/s$, time step $t_{step} = \langle x^2 \rangle / 4D_S = 7.2532 \cdot 10^{-6} s$, movement probability $p^{(m)} \simeq \exp(-\Delta E/k_B T)$, attachment probability of the well suited growth unit $p^{(+)} = 1$, detachment probability $p^{(-)} \simeq \exp(-\Delta E/k_B T)$, lysozyme radius $R_M (= 1/2d) = 1.61 \cdot 10^{-9} m$, hydrodynamic radius of lysozyme $R_H = 1.9 \cdot 10^{-9} m$, critical radius for lysozyme crystal $R_c = 2.73 \cdot 10^{-8} m$, minimum distance between two steps $\lambda_0 = 4R_c = 1.09 \cdot 10^{-7} m$.

tively discussed, cf. Figure 2. From this comparison it follows that incorporating a tetrameric unit, Figure 2, will result - without any special modification of the algorithm explored - in obtaining some acceleration mode of the process, cf. [6, 4, 5]; (ii) As shown in the experiment⁶, the acceleration can be quantified by a factor of 4/3 which follows in a very natural way from our type of modifying the recently introduced algorithm¹⁰; (iii) It should be clearly underlined that the obtained acceleration factor is only possible to occur when one assumed the tetramers in our model¹¹; (iv) The phase diagrams of the interface-controlled N-G process, characteristic, however, of a standard ES relation¹³ and an energetic-penalty involving RW (or, free of it when the tetramers are engaged in a decisive way¹¹), cf. Fig. 7 of the previous study [10], will result in a possible small shift^b of all regions of interest

^bTetrameric (dry) growth units are a little bit more resistant to increasing temperature and acts

concerning the (dis)ordered aggregation of proteins, and would remain for a future task; (v) It should also be noted that no direct interconnection of the present computer model with the computer simulation of LB film, recently further characterized by microGISAXS method during crystallization process has been explored but exclusively an experimentally-supported² premise was taken in the form of choosing lysozyme tetramers as suitable 'building blocks' of the crystal resulting from LB (virtual) nanotemplate¹⁴; (vi) Let us also state clearly that the presented model does not account explicitly for a contribution of the structured water, detected experimentally in [2]; a way of how to try to deal with it can perhaps be started from [12] by really incorporating the solute-solvent interaction conditions (Flory-Huggins parameter being involved) at a given temperature; (vii) a role of $2D$ template in order to optimize from the late-stage growing and mechanical-relaxation points of view the rates of complex colloid-type matter agglomerations at a mesoscopic level of matter organization has thoroughly been discussed¹⁵.

To sum up, let us note that the overall rationale presented above (see, Sect. 2, mainly) supports a recently accepted view, namely that the achieved crystal growth morphology preferentially results from an interplay of crystallographic anisotropy and growth kinetics by means of interfacial processes and long-range transport. Moreover, the crystals that grow under the control of interfacial kinetic processes tend asymptotically toward a kinetic shape, being an analogue of the Wulff shape, except that it is based on the anisotropic "nonequilibrium" interfacial kinetic coefficient¹⁶ such as the proposed ν_{comp} .

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of detachment can be observed for a little bit higher temperature in comparison to monomeric growth units.

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