CHAPTER 5
MODEL LYSOZYME CRYSTAL VERSUS AGGREGATE
(UN)CONFINED FORMATION AS VIEWED BY RECENT
THEORY AIDED BY COMPUTER EXPERIMENT

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The (dis)orderly aggregation of lysozyme is an intense field of research nowadays since such a model system contains all necessary paradigms to solve, preventing this way from the as-yet applied trial-and-error method for obtaining the desired crystalline and other forms of lysozyme aggregates. The notions of nucleation–growth phase change, combined properly with the so-called viscoelastic phase separation, and opened toward its thermodynamic-kinetic, generally non-Markovian character, may improve considerably the overall description of the process against the impractical trial-and-error method. Both, seemingly simplified stochastic-in-nature analyses, carried out at a mesoscopic level, supported suitably by submesoscopic-level computer simulations of this interface–controlled crystal–formation process, may remedy and foster further actions toward more sophisticated and efficient solution to this long-standing, truly complex phase-change and/or phase-separation involving puzzle, both of them dealt with inseparably, what stands for a certain appreciable novelty of the present study. A final message that comes from the offered study so far states that a type of hybrid model combining sensitively some parts of analytical analyses with some well–designed computer–simulation complementary co-analysis, can assure a distinctive progress toward solving practically many of systems of interest.
5.1. Introduction

Protein molecules are made up of aminoacids and possess amphiphilic nature when subjected to solvent, especially to water. Lysozyme is one protein molecule commonly met in nature and very prone to crystallize. The obtained crystals, depending on a broad spectrum of crystal–formation conditions, have, in general, polymorphic character, expressed in terms of its unit–cell, and its alterations: they are often termed non-Kossel crystals what makes them distinguishable from standard NaCl type crystal forms.

Biologically-motivated modeling becomes recently a necessary activity taken up by physicists toward obtaining first quantitative information about complex biosystems to be dealt with. Protein systems are those that are presently of central interest from the statistical–mechanical and non-equilibrium–thermodynamics points of view since they conform to basic research tasks underwent by both subdisciplines of statistical condensed-matter physics (Basu and Chowdhury, 2007).

To be more specific, let us focus our attention on, in principle, isothermal binary mixtures of lysozyme and water molecules, having eventually some other minority ingredients involved, such as ion precipitants or impurities, thus a system as a whole very prone to (dis)ordered aggregation, and in particular, to non-Kossel crystal formation (Chernov, 1997).

When looking from the statistical-mechanical point of view at such a binary lysozyme-water mixture, poured out in a three-dimensional laboratory vessel of big enough diameter, one is allowed mainly to look at interaction dynamics among: lysozyme molecules alone, lysozyme and water molecules, and finally, water molecules alone, postponing, however, other more specific and quite rarely occurring interactions, e.g. those between minority ingredients and the basic mixture’s components, called thereafter the majority ingredients (components) of the mixture (Chernov, 2003).

In the system described above, one would reasonably expect to have both majority components in the amount of ca. $6.02 \times 10^{23}$ per mole, thus a statistically big (Avogadro) number. As one may know, it is impossible to quantify readily all interactions in such immense system — therefore
one is forced to resort to statistical-mechanical description, with a suitably simplified version of the total system’s energy (Hamiltonian) on which the Newton–Langevin type complicated dynamics can be build up. Then, the central limit theorem, as well as the equilibrium formalism of statistical Gibbs ensembles would allow to get some ensemble- (or, really rarely “equivalent” time-) averages for describing the main system’s, also dynamic, properties (Reif, 1967). To be more specific, however, let us note that there is quite a big difference in physical properties of both majority ingredients of the binary mixture addressed.

A lysozyme 14.7 kDa in-molecular-weight biomolecule, in typical (dis)ordered aggregation conditions (temperature; \(pH\)), should be represented in its globular form by a positively charged sphere of radius of ca. 2 nm. Thus, its surface is somewhat like \(0.25 \times 10^{-16}\) nm\(^2\).

From dimensional viewpoint a water molecule is a dipole; its typical linear size is, however, ten times smaller that that of lysozyme, being ca. 0.2 nm, \(i.e.,\) as far as its hydrodynamic-radius approximation is concerned. Thus, assuming crudely that this dipole is also a microsphere (and its projection onto the sphere’s surface, a circle), and neglecting for simplicity the close-packing arguments on how to distribute most efficiently the two-dimensional microspheres (water molecules) over the sphere (lysozyme) surface (Shen \textit{et al.}, 2005), one may say that we can have even one hundred water dipoles occluding one lysozyme molecule.

The hydrophilic interactions between water molecules and the hydrophilic aminoacids of the given protein assure spatial stabilization of the biomolecule and the conservation of its characteristic 3D (tertiary) structure.

It must be mentioned that, the electrostatic interactions between charged biomolecules and dissolved additives, supporting the crystallization process, must be also taken into account. In the biopolimeric solutions, the most electrostatically active particles are: polar aminoacids and dissociated salts: NaCl (laboratory) and/or KCl (physiological environment) (Ducruix and Giegé, 1992). Electrostatic interactions are present between macromolecules and countercharged salt’s particles and precipitants. This way, the net positive charge of lysozyme molecule is screened electrostatically by the surrounding crowd of counterions, leading eventually to a plausible model that
involves the notion of electrostatic double layer, typically of Stern type. And, this model can be extended over the formation of aggregates of lysozyme molecules, irrespective whether they are formed as orderly or not (Gadomski and Siódmiak, 2002). It shall then be anticipated that the aggregates formed are typically full of water content, thus quite soft and semi-fragile (Booth et al., 2002) to some extent; certain exceptions, for example, in the group of non-Kossel lysozyme crystals can also be mentioned (Pechkova et al., 2005a).

The above statistical–mechanical picture can be deteriorated markedly when the laboratory vessel is not big enough, so that the interaction of the binary solution cannot be neglected any longer, as could be a case of Petri dish of small enough height. It could also be an important case of introducing purposely some geometrical confinement into the system, such as the one coming from a nanotemplate, irrespective whether it is of Langmuir–Blodgett type, thus fluid (Pechkova and Nicolini, 2001), or of nanoporous solid nature (Dobrev et al., 2005). It has been shown experimentally that the introduction of a geometrical confinement accelerates the (orderly) aggregation pace, in terms of both statistical mechanics and thermodynamics because of: (i) reduction of the degrees of freedom involved in the system’s evolution; (ii) decrease of the entropy production within the system. While point (i) looks obvious, though having, in addition, some aggregates’ desorption involving hints at work (Siódmiak et al., 2006), point (ii) requires, in general, a suitable non-equilibrium–thermodynamic knowledge, anchored tightly to the 2nd law of thermodynamics, and in addition, to the Gibbs (non-negative) entropy–production principle.

Thus, when looking from the nonequilibrium-thermodynamic point of view one is directed to use extensively the notion of entropy, applied to the aqueous protein solution, thus to explore in depth the Gibbs principle in order to get the (asymptotic) growth rule, representative of such complex stochastic binary mixture, as well as to extract in a reasonable way the corresponding cessation-to-growth conditions, pointing to the final (dis)ordered structure formation (Gadomski, 2007).

In what follows, we would like to present in this review-type study, with some other related theoretical and experimental works in a background, our argumentation line, advocating for inventing and
applying our non-equilibrium kinetic–thermodynamic analytical (stochastic) model, working at a mesoscopic level of description (next part), complemented by an in-parallel-designed computer sub-mesoscopic, coarse–grained model (following part), approaching readily some of those aspects of the former that are naturally hardly seen when being applied (Gadomski et al., 2005). In general, our presentation and argumentation line concerns the formation of protein (lysozyme) (dis)orderly aggregates in terms of interface-controlled process. An auxiliary but relevant co-argumentation is thought to view the formation as a nucleation-growth phase transformation (Gadomski, 2000), see Figure 5.4, seen from thermodynamic–kinetic point of view, intermingled, whenever appropriate, by phase–separation, often confinement-induced, tendencies toward viscoelasticity of the emerging phase as a whole (Tanaka and Nishikawa, 2005). Concluding address and remarks will summarize the presented study.

5.2. Lysozyme Crystal Versus Aggregate Formation –
Thermodynamic-Kinetic Approach at Mesoscale

5.2.1. Nucleation

The important role of nucleation step in the overall lysozyme crystal formation has been emphasized in many, mostly experimental studies, see for example (Haas and Drenth). But it has not yet been addressed comprehensively due to, in general, extremely complicated, mainly kinetic, character of the process, cf. a discussion of internal degrees of freedom, and their influence on the (homogeneous) nucleation, in Reguera et al. (1998).

First, it has since long been realized that this process appears to be of thermodynamic–kinetic, thus truly complex character. For the case of homogeneous nucleation, to be clearly distinguishable from its heterogeneous counterpart, with a pivotal role of precipitants, co- and counter-ions, and impurities, geometrical confinements, etc., the thermodynamics is mainly subjected to creation of the nucleus’ surface — such a creation comes from the equilibrium condition imposed on the
free energy of the system (Huang, 1963), written commonly as \( \delta \Phi(R) = 0 \), where \( \Phi(R) \) denotes the Gibbs’ (free) energy of a spherical nucleus of radius \( R \), and \( \delta \) - the respective variation. Thus, the thermodynamic factor as a whole, can contribute substantially to the morphology of any ripe nucleus being created by the system, as well as can influence the presence of morphological Mullins–Sekerka type, and also other, (non)linear interfacial instabilities, unavoidably present in the crystal formation, especially the one controlled by diffusion (Pimpinelli and Villain, 1998).

Second, let us note that the complications of appropriate treatment of the nucleation step of lysozyme crystal formation may likely arise not only due to the heterogeneities (by the way, even small temperature variations can also make the nucleation heterogeneous) but also due to certain internal and interfacial cross-effects prevailing within the system in the course of time (Demei and Shizgal, 1993). The cross–effects mentioned, are being specially effective right in the (bio)polymer nucleation stage, due to, in general, quite puzzling obedience, or not, of the system to the Laplace–Kelvin law (Dell’Isola et al., 1995).

The cross–effects may point to the fact that both surface tension and nucleus’ radius may depend on the subtleties of the local system’s density profile, which, in turn, would prefer to depend on the interfacial concentration of the solution, thus the biopolymer-poor phase, in contrast to the biopolymer-rich phase that is simply the crystal phase. Such a natural quasi-separation of both phases, not necessarily under a deep temperature, but rather, here, a concentration “quench” (descent) at the interface, is able to induce certain additional viscoelastic effect, e.g., a (second) gradient of the accumulated stress field, or a gradient related to textural differences at the freshly formed surface and its internal (crystal–interior) counterpart (Tanaka and Nishikawa, 2005). It may, in turn, influence the magnitude of the thermodynamic factor, termed the capillary (Gibbs–Thomson) length, which is involved in the Laplace–Kelvin law (Huang, 1963). Since the protein nucleation goes within the liquid-liquid immiscibility region one can take such a basic type of the capillary length as a first approximation, valid for correctly arranged lysozyme molecules, and then extend it to some additional curvature
correction(s) (of Tolman type, see further) if they are a bit disorganized within the nucleus’ volume.

According to our type of modeling of the nucleation, which emphasizes the creation of the electrical double layer, circumventing the nucleus of radius $R_c$, and causing the interfacial tension to change (Sear and Warren, 2002), the critical nucleus’ radius reads (Gadomski and Siódmiak, 2002):

$$R_c = 2\Gamma_1 c_\theta / (C - c_\theta)$$  \hspace{1cm} (5.1)

cf., Figure 5.1.

The above formula assumes that the nucleus can be considered as a drop of radius $R_c$ (Huang, 1963), consisting of a certain very small number of lysozyme molecules, typically about four (Haas and Drenth, 2000) — it can, however, be treated according to the Laplace–Kelvin law when the capillary length $\Gamma_1$ fulfills the above stated obedience requirements. At this moment, let us note that a lysozyme crystal formation by hanging drop method, enhanced purposely by a fluid nanotemplate, assumes that the growing crystal tends to absorb mainly
tetramers, just small but ripe nuclei (Siódmiak et al., 2006; Pechkova et al., 2005), what has also been confirmed by another computer simulation, (Ke et al., 1998). As concerns the drop-shaped nucleus, it is reasonable to expect it, when the equilibrium surface concentration \( c_0 \), being typically small compared to the crystal density \( C \) (both quantities being proportional, according to the Raoult’s law, to their corresponding osmotic pressures (Huang, 1963), are, for our biopolymer system, not largely distinguishable, so that \( C \approx c_0 \) may hold, though \( C > c_0 \) shall always be assumed. This, because of \( c_0 \) being typically small (Gadomski and Siódmiak, 2003a) leads clearly to very small supersaturation values of \( c_0/(C-c_0) \), being effective during the diluted-solution (random) nucleation events. Thus, in order to create a ripe enough viz thermodynamically stable nucleus one had to compensate this big-dilution, or small-supersaturation effect by the corresponding increase of the capillary length \( \Gamma_1 \), which, in turn, becomes feasible when the above mentioned cross-effects, due to the second-gradient theory, will be at work, also leading to the viscoelastic phase–separation condition to be, at least partly, well approached, especially when a polynuclear system would emerge (Tanaka and Nishikawa, 2005), (Figure 5.2).

Figure 5.2. A mono- (left; denoted by a) and poly-nuclear (right; denoted by b) pathways of the single nucleus (a) and/or many nuclei (b) formation(s); the small colorful dots represent ions or dipoles; the dark bigger circles correspond to a picturesque representation of the lysozyme macroions, being sometimes “glued” together by some “entropic connectors” (thin lines), the latter being certain reactive pieces (aminoacid residues) of the lysozyme molecule. The “white hallos” around each molecule, or molecular cluster, can be considered as some drawn prerequisites of the corresponding double layers. (Consult also a review by R. Mezzenga et al. (2005) especially some electron micrographs of liquid crystalline mesophases encountered in foods, namely Figure 6 therein.)
Thus, the nucleus’ existence, or system’s tendency to avoid nucleus’ breakage, will become surely decisive factors when the nucleus’ surface tension $\sigma_c$, being proportional to $\Gamma_1$, will, for a given temperature and volume conditions, depend on the system’s density profile which, in turn, depends closely upon the concentration of the nearby external phase surrounding the nucleus. This way the Laplace–Kelvin law will very likely be satisfied in a properly modified form (Dell’Isola et al., 1995), involving the gradient trace, thus a necessary step toward growing the so-obtained nucleus will be made firmly. Then, a certain further exploration of the kinetic part of the nucleation process can be done by looking for an appropriate growth rule for the ripe, also mechanically (due to corresponding hydrophobic-type interactions between aminoacid residues) stable (Chernov, 1997) nucleus’ development. It implies that we have to turn our attention to the growth stage of the biopolymer crystal formation, the description of which, having done by means of analyzing a large number of experimental data, can be found elsewhere, especially in terms of the dynamics of layer growth in protein, mostly lysozyme, crystallization (Vekilov and Alexander, 2000). Thus, we are able to propose to consider the kinetics of the possibly time–dependent nucleation stage in terms of examining the ripe-nucleus growing effect, also with its viscoelastic peculiarities being addressed (Gadomski and Siódmiak, 2005).

It is worth mentioning that in Nanev and Tsekova (2000) a heterogeneous nucleation of lysozyme has been addressed experimentally by a method named the double-(thermal)-pulse technique, enabling to deal with nucleation and growth in a mutually independent way. This study confirmed that when nucleating lysozyme on a glass substrate, i.e., when subjected the nucleation to a two-dimensional confinement, the resulting nuclei consist of 3–4 molecules, thus comparably to what has been estimated in Haas and Drenth (2000), and utilized in Ke et al. (1998) and Siódmiak et al. (2006). Especially the latter study welcomes such an experimental result since in its computer model it has been assumed, similarly to the argumentation supporting another computational–physical study (Ke et al. 1998), that the growing pace of the protein crystal can be increased when mainly the (ordered) tetramers desorb from a nanotemplate introduced to the system.
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(Pechkova and Nicolini, 2001) — they are then absorbed by the growing crystal until a final structure is being formed. In addition, it can be stressed that the coming-from-thermodynamics driving force, namely the supersaturation, proposed to apply in Nanev and Tsekova (2000), and its influence, in the steady-state condition, on the nucleation rate, being determinable by the (small) number of molecules, constituting the nucleus, can be derived based on the so-called atomistic theory, initially developed to reveal the nucleation aspects in small molecule systems (Kashchiev, 2000). It is also worth pointing out that, as in our modeling, the necessity of two-field effect, or two-order parameter involvement, associated with the interface concentration as well as with the crystal’s (aggregate) density, and the effects they both exert on the nucleation process, has also been considered by Oxtoby (2003).

Concluding this part, it should, however, be mentioned that maintaining, while based on the mechanistic-thermodynamic grounds described above, the nucleus maximally symmetrical or, idealistically speaking, sphere-shaped within the continuum-description approach at work, does not fully guarantee any emergence of “solid” non-Kossel crystals (Chernov, 1997) but some other more disorderly biomolecular aggregates are certainly allowed to occur (Gadomski, 2007; Siódmiak and Gadomski, 2006). It is simply due to the fact that the nucleation is a random (also, living) process, which means, that the above offered argumentation line must always contain a stochastic character. This type of character can also be extended to the growth stage in which, however, the kinetic effects are allowed to prevail somehow over their thermodynamic counterpart, rendering inseparably the overall process kinetic-thermodynamic, also quite time-dependent viz non-Markovian in its basic character (Gadomski, 2007).

5.2.2. Growth

The growth step appears to be equally decisive in obtaining final output in a form of crystal or aggregate. While the nucleation step is more morphologically oriented, because it sets in the initial structure of the (dis)orderly aggregate, the growth step looks more kinetically oriented.
The most robust models proposed so far to reflect properly the kinetics of lysozyme (dis)orderly aggregation are, quite unexpectedly, very simple phenomenological models that unravel the kinetics in terms of phenomenological kinetic rules, invented to uncover most important experimental results (Chernov, 1997, 2003; Vekilov and Alexander, 2000; Chernov, 2001; Pusey et al., 1986).

The phenomenological kinetic rules are always proposed in a form of the growth rule, stated in terms of the rate of change in time of the linear nucleus' characteristic, for example, the radius or a distance to the most representative growing surface of the crystal or aggregate, measured from its mass center (Honigmann, 1958), designated by \( R_{ch} \). Such a (multiplicative) rule looks like (Vekilov and Alexander, 2000):

\[
\frac{dR_{ch}}{dt} = \sigma_T v_{lin}(R_{ch})
\]  

where \( \sigma_T \) denotes a main thermodynamic factor of the phase change, whereas \( v_{lin}(R_{ch}) \) is primarily responsible for the kinetic peculiarities of the phase change.

From all most known phenomenological models applied (Pusey et al., 1986; Chernov, 1997, 2001) it is seen that \( \sigma_T \) is related to the main driving force of the phase change, namely the supersaturation (Nanev and Tsekova, 2000), while the structure of \( v_{lin}(R_{ch}) \) is put differently, depending on whether the model imposes a diffusional control on the system’s behavior or it goes, based on experimental motivation, out of this type of control.

The diffusion-type control invokes more of less a Mullins–Sekerka (MS mode) morphological–instability involving type of the modeling (Pimpinelli and Villain, 1998) whereas non-diffusional control is mainly the interface control — it is attributed to the screw-dislocation driven growth of the terraces appearing on the crystal’s surface — the so-called Burton–Cabrera–Frank (BCF mode) growing mode (Siódmiak and Gadomski, 2006); in case of non-crystalline aggregates such a picture is markedly deteriorated, leading to serious kinetic obstacles, such as bunch cascades at the surface, and the likes (Vekilov and Alexander, 2000).

While the thermodynamic factor \( \sigma_T \) will also be recovered by our interface-controlled type of modeling (Gadomski and Siódmiak, 2002),
its kinetic counterpart, $v_{\text{kin}}(R_{ch})$, depending on whether the control is of
diffusional or non-diffusional (nearby) external-field nature, looks also
differently (Pimpinelli and Villain, 1998).

Thus, in the former case it goes like $v_{\text{kin}}(R_{ch}) \approx 1/R_{ch}$, wherein the
r.h.s. comes from counting explicitly the diffusional flux crossing the
internal (interface) boundary. In the latter case, in turn, an explicit
dependence on $R_{ch}$ cannot be seen, presumably due to either non-
standard, let us say, anomalous diffusional effects, such as those
introduced by the nearby electrostatic field (Gadomski and Siódmiak,
2003a), or when the interfacial control prevails substantially, as in the
case of BCF mode (Chernov, 1997).

When such a nonstandard confined-diffusion or purely interface-
involving control happens to the system, which might also be due to
aggregation acts, occurring at the nucleus’ surface, one had to specify
$v_{\text{kin}}(R_{ch}) \rightarrow \text{const}$ over the late-time course of the formation (Vekilov and
Alexander, 2000).

From the above it follows that in both MS and BCF modes, one
would expect $R_{ch} \approx t^\zeta$ to hold asymptotically in the course of time $t$. A
significant differentiation between these two modes manifests, however,
when looking at the growth exponents $\zeta$.

In the MS mode one gets most typically (Pusey, et al., 1986) $\zeta \approx 1/2$
while in the case of BCF mode one provides firmly (Vekilov and
Alexander, 2000) $\zeta \approx 1$.

It implies that the BCF mode is, when plotting onto log-log scale,
roughly two times faster than the MS biomolecular-matter involving
mode. Certainly, depending on details of the growing conditions,
whether they are subjected to a confinement or not, (Pechkova and
Nicolini, 2001; Dobrev et al., 2005), which is the ionic strength of the
solution (Sear and Warren, 2002), or finally, which are the viscoelastic
factors governing the growth (Chernov, 1997; Haas and Drenth, 2000;
Gadomski and Siódmiak, 2005), then the growth exponent $\zeta$ ranges
typically between this two basic growing modes, thus $\zeta \in [1/2;1]$ mostly
holds, though some exceptions can also be foreseen, mainly due to
highly fluctuational growing conditions (Vekilov and Alexander, 2000;
Gadomski and Siódmiak, 2002; Chernov, 2003).
A purely phenomenological extension of the kinetic (growth) rule can also be applied, such as the one of Pusey et al. (1986), namely:

$$\frac{dR_{ch}}{dt} = \sigma_T \left[ v_{kin}(R_{ch}) \right]^{\nu}$$

(5.3)

wherein $\nu$ points to some nonlinear hydrodynamic modes, arising in the course of growth duration, i.e., if such modes would truly manifest one had to assume $\nu \neq 1$, thus having this way another fitting parameter at her/his disposal (Pusey et al., 1986). Rising $\sigma_T$ to some power in the above kinetic growth formula is rather not explicitly proposed; it could likely make sense for special-type aggregation processes such as four-nanometer thick and $R_{ch}$-long fibril formation but within a certain range of temperature or pH values applied (Arnaudov and de Vries, 2005).

In what follows we wish to propose, in a concise form, a protein-crystal growth model, motivated also by some experimental data on lysozyme (Siódmiak and Gadomski, 2006; Siódmiak et al., 2006; Gadomski, 2007, Gadomski et al., 2005), the foundations of which have deep non-equilibrium-thermodynamic origin, and the kinetics follow certain landmarks of non-Markovianity, thus being, in general, also growth-history dependent (Santamaría-Holek et al., 2007), while pointing indirectly this way to the aging effect from which many biomolecular aggregations suffer substantially (Gsponer and Vendruscolo, 2006).

In this model, which one may call readily a kinetic–thermodynamic approach to the crystal versus aggregate formation, both parts are taken inseparably — they appear in the model description together, and are also subjected to concrete specific conditions of the aggregation (Siódmiak et al., 2006; Pechkova et al., 2005b). The thermodynamic part is dedicated directly to the boundary conditions, and may be termed the boundary–condition proposal (B-CP). The kinetic part, in turn, is unambiguously dedicated to the macroions’ (lysozyme molecules) velocity-correlation proposal, to be effective in the interfacial (active) zone (Vekilov and Alexander, 2000) — this is abbreviated thereafter as V-CP. A “synergistic” mode of both growing kinetic-thermodynamic super-modes emphasized, namely, when BC-P coincides most optimally with VC-P, will indicate which growth rule, and why, is followed by the
system. Thus, by manipulating with BC-P and VC-P just “in parallel” and within the entropy–production formalism proposed, we are able to derive certain either cooperation or confliction growth rules, or while put it differently, when an easy (facilitated) growing mode emerges, or when it is hampered by the encountered kinetic–thermodynamic circumstances. Such an offer we would like to see as an original as well as useful proposal toward complementing as-yet applied, purely phenomenological kinetic laws (Chernov, 2003; Pusey et al., 1986), serving to elucidate quite a large body of experimental data (Drenth, 2002), but without explaining in detail the kinetic–thermodynamic origin of the formations under study.

5.2.3. The boundary–condition proposal (B-CP) seen as an Extension of Gibbs–Thomson boundary condition

The boundary condition \( c(R) = c_0[1 + 2\Gamma_1 / R] \) due to Gibbs and Thomson (Huang, 1963) comes from a Taylor-series based linearization of \( \exp[2\Gamma_1 / R] \) with respect to the argument \( 2\Gamma_1 / R \), with the capillary length \( \Gamma_1 = \gamma_R v_{m-c} / k_B T \), the argument due to small value of \( \Gamma_1 \) being typically distinctly less than one; notice that \( k_B T \) represents the thermal energy, whereas \( \gamma_s \) — the surface energy, while \( v_{m-c} \) denotes a volume subjected to one surface molecule, so that the capillary length brings additionally a molecular character; \( c(R), c_0 \) mean the concentrations at the curved, thus non-equilibrium, and flat (at equilibrium) interface, respectively. In fact, this standard equilibrium-thermodynamic condition, prescribed customary at the curved interface, the one represented by twice-the-mean curvature \( 2/R \) (Gadomski and Siódmiak, 2002), comes from the Wulff’s theory of equilibrium crystal’s shapes, developed under a confinement of the total crystal’s volume approaching a constant value, namely \( V_{\text{crystal}} = \text{const.} \), and under an equilibrium condition that \( \sum \gamma_i A_i = \text{min.} \), i.e., when the products of crystal–wall surface energies by the surface areas, when summed up, tend to a minimum. An exact result but for \( n \)-wall symmetrical object, thus nearly for a sphere (droplet) of radius \( R \).
certainly in the limit of large $n$, is then as follows $\ln[c(R)/c_0] = 2\Gamma_1/R$
(Honigmann, 1958).

This Gibbs–Thomson boundary condition is satisfied by a drop in an equilibrium with its supersaturated vapor (Huang, 1963). It is also applicable to small-molecule (or, atoms/ions involving) precipitation and/or crystallization from an under-cooled melt or supersaturated solution (Pimpinelli and Villain, 1998), also the one realized by the hanging-drop method (Pechkova and Nicolini, 2001). It is, however, by no means applicable without appropriate extension(s) to lysozyme crystal versus aggregate formation. The reason for being inapplicable in such a standard form may be at least twofold. First, lysozyme (dis)orderly aggregates hardly attain a global minimum on the energy axis — they may better reside in a quasi-equilibrium state, if it happens to be the case, but quite often they are out of equilibrium, though in their mature stages, close to it. This is often due to a generic competition between folding and aggregation of proteins, and it may markedly, though slowly, change over time due to mostly environmental conditions (Gsponer and Vendruscolo, 2006). Second, according to the second-gradient theory presented while describing the nucleation stage (Dell’Isola et al., 1995), a biomacromolecular system, such as that composed of lysozyme molecules of a few nanometers each, should manifest when passing between nano- and micrometer scales, a finite-size effect, pointing to a graininess of the crystal/aggregate surface, which invokes also a nontrivial contribution to the elasticity of the object’s surface. This effect is detectable when a very natural extension of Gibbs–Thomson type droplet’s equilibrium thermodynamics is allowed to be applied, namely (Gadomski et al., 2005):

$$c(R) = c_0 \left(1 + \frac{2\Gamma_1}{R} + \frac{\Gamma_2}{\bar{r}_{PMR} R}\right)$$

(5.4)

The additional r.h.s. term in the parenthesis, namely $\Gamma_2/\bar{r}_{PMR} R$, is composed as follows. In the numerator the square of the Tolman length, $\Gamma_2$ (being roughly of the size comparable to $\Gamma_1$), appears — the Tolman length is responsible for accounting for certain biomolecule stiffness-to-elasticity effects (Gadomski et al., 2005), thus to introduce readily the
finite-size macromolecular effect, which explicitly manifests in the
denominator by the molecule’s size $\tilde{r}_{PM}$ of about 2–3 nm, thus, the
signature of the graininess; presumably, it can implicitly account for a
formation of hydrogen bonds between water and lysozyme molecule,
thus, it can also be quantified by the hydrogen–bond average strength (by
the way, the hydrogen bonding is not a property of small-molecule
crystals or aggregates emerging from a solution — it is a distinguished
property of protein formations grown within the aqueous solution limit).
However, the proposed extension is still an extension of equilibrium
type, though it makes a difference, mainly when applied at the
nanometer-size scale (Gadomski et al., 2005).

Another type of the proposed boundary condition, working
effectively within quite a narrow realm of the parametric zone, appears to
be a more toward out-of-equilibrium “disputable” extension of the form

$$
c(R) = c_0 \left( 1 + \frac{2\Gamma}{R} + \sum \alpha_i \frac{R_i - R_i^0}{R_i^0} \right)
$$

(5.5)

First, it is thought of as a direct extension of the standard Gibbs-
Thomson condition, pointing to quite a drastic “environmental”
conditions (Gsponer and Vendruscolo, 2006) that may lead either to
promote the natural course of the free-energy increase or they may
contradict such a promotion by pronouncing some pinning effects, or by
hampering the growth pace due to abnormal (confined) elastic behavior
of some molecules, or certain groups of them, the latter belonging to the
surface of the growing object (Gadomski and Siódmiak, 2005). This
toward-non-equilibrium extension term reads

$$
\sum \alpha_i \frac{(R_i - R_i^0)/R_i^0}
$$

(5.5)

with the $\alpha_i$-s, the coefficients pointing to some characteristic mechanism
of elasticity, appropriate for three basic crystal-surface epitaxial
nucleation mechanisms (Pimpinelli and Villain, 1998), wherein $i = 1,2,3$
indicate the linear (by molecular rows), surface (typical epitaxy) and
volume (thick-film limit) nucleation mechanisms (Gadomski and
Siódmiak, 2005; Siódmiak and Gadomski, 2006).
Our proposed model, due to incorporation of elastic surface-nucleation effects of auxetic/non-auxetic nature (Gadomski and Siódmiaiak, 2005), allowing for appearance of non-positive Poisson coefficients at the interface (dis)orderly aggregate vs. surroundings, also includes a chance of examining certain “spurious” kinetic effects, such as formations of gels or other arrested kinetic states. It requires, however, an extension of the proposed modeling into nonlinear viscoelastic domain, (Santamaria-Holek et al., 2007 and references therein). Thus, in principle, such a procedure can be envisaged while entering the stochastic description of this modes-coupling process, wherein the modes concern both viscoelasticity as well as late-time growing conditions of the process under consideration, as has been demonstrated in Santamaria-Holek et al. (2007).

Next, from the above formula it is seen that if one wished to arrive at a constant growth pace, thus when applying the kinetic criterion, one would be enforced to work within a narrow range of $R$-domain, which implies also rather more nanometer than micrometer size scale of the obtained crystals; otherwise, only the aggregates are plausible to obtain (Gadomski, 2007 and references therein). The kinetic criterion, delineated above, could roughly be equivalent to a very narrow crystallization window (Chernov, 1997, 2003) for the protein, and also, lysozyme non-Kossel crystals. Outside this window aggregates are exclusively expected to occur (Siódmiaiak and Gadomski, 2006). Before going into last extension proposed, let us remind that in the present extension the Tolman-type term, namely $\frac{\Gamma_2^2}{\Gamma_{PMP}^2 R}$, has been consequently omitted as being inapplicable when too strong viscoelastic effects of mutual interactions between crystal surface and nearby surroundings apply, i.e., if one is set out of the linear stability regime known for epitaxial growth as Grinfeld instability (Pimpinelli and Villain, 1998).

Last but not the least, a very natural extension of perturbed–surface droplet’s equilibrium thermodynamics into nonequilibrium occurs when

$$c(R) = c_0 \left(1 + \frac{2\Gamma_1}{R} + \frac{\Gamma_2^2}{\Gamma_{PMP}^2 R} - \beta K \frac{dR}{dt} \right)$$

(5.6)
is presumed. This experimentally–motivated condition, which has been first proposed by Goldenfeld in 1987 for accounting for the kinetics of round polycrystalline forms, named spherulites (Gadomski et al., 2005 and references therein), contains a term that expresses directly a (local) kinetic departure from the equilibrium. This term, equipped with a minus sign, see above, looks like \(-\beta_K (dR/dt)\), and quantifies for a local growth pace due to non-uniform evolution of the interface (\(\beta_K\) stands for a phenomenological coefficient). Notice, however, that \(-\beta_K (dR/dt) \to 0\) when the typical non-Kossel crystal growth pace of roughly constant value applies. Therefore, when this term preserves over relatively long time scale, the non-Kossel crystal is not very close to its thermodynamic equilibrium, and the polycrystalline form eventually wins over its ‘purely’ single–crystal counterpart. This departure from equilibrium can also be utilized when some defects of the temporarily formed crystal structure tend to dominate at the interface; then the coefficient \(\beta_K\) could be proposed as an indirect measure of the interfacial defects’ concentration — then another model mentioned in Gadomski et al. (2005), named a UCJ (from the names of Ulman, Chalmers and Jackson, known since 1964) solidifying-front crystal–growth model ultimately applies.

When looking from a phenomenological (non-stochastic) point of view the deterministic growth rule that we propose to apply reads (Gadomski, 2007)

\[
\frac{dR}{dt} = \left[\sigma(R)\right]^{-1} \nu(R) 
\]

(5.7)

where the near-surface solubility, being just the inverse of the supersaturation (Nanev and Tsekova, 2000) reads

\[
\sigma(R) = \frac{C - c(R)}{c(R)} 
\]

(5.8)

The verification of our deterministic growth rule has first been included in Gadomski and Siódmiak (2002) for experiments with lysozyme crystals growing up to a characteristic length of \(\sim 0.1 \text{ cm}\) when it is allowed that the process is controlled mainly by incorporation of lysozyme molecules at the interface rather than by bulk diffusion. The
obtained result is of the same quantitative weight as another estimation coming from applying a phenomenological growth rule, apparently within the BCF mode (Chernov, 1997). In addition, let us note that the deterministic rule, also according to Gadomski and Siódmiak (2002) can be rewritten with an inclusion of the critical nucleus’ radius as

\[
\frac{dR}{dt} = v(R) \left[ \frac{c_s}{(C - c_s)} \right] \frac{R + R_c}{R - R_c}
\] (5.9)

Figure 5.3. A growth of the (simplified) spherical nucleus in two consecutive time instants; for explanations of symbols used, see Text, especially a sketch of the derivation of the deterministic growth rule (0→R means: the crystal’s radius range — interior of the crystal).

Let us briefly summarize here the main points of the derivation of the above deterministic growth rule (Gadomski and Siódmiak 2002, 2003a), cf. Figure 5.3. First, we take the mass of the growing object in two consecutive time steps, \( t \) and \( t_1 \), where \( t_1 > t \), expressed via the corresponding densities (concentrations) of the object and of the surrounding field, marked in Figure 5.3, \( c(\vec{r}) \) and \( c(\vec{r}) \), respectively. Second, we take the difference between the masses, \( \Delta m \), and construct the ratio \( \Delta m / \Delta t \), wherein \( \Delta t = t_1 - t \). Then we go into the limit \( \Delta t \to 0 \).

Further, we make use of the physical observation that \( J = \Delta m / \Delta t \), where \( J \) is the global flux taken at the object’s surface \( \Sigma(t) \), containing the object’s volume \( V(t) \) as a whole. The above procedure is called thereafter the global mass-conservation law. Then, we make use of some
simplifications, such as the spherical symmetry of the growing object, 
assume its density to be constant, \( C(\vec{r}) = \text{const.} \), utilize the (extended) 
Gibbs–Thomson type thermodynamic internal boundary condition(s).
Finally, we apply the formula for the mass-convective flux, appropriate 
for protein and/or colloid-type systems (Tanaka and Araki, 2007), which 
 at a local level of mass-counting reads \( j = v(\vec{r})c(\vec{r}) \), with \( v(\vec{r}) \) being a \( \vec{r} \) 
(position) dependent vector representation of \( v(R) \), involved directly in 
the growth rule above. This procedure in total enables to write down 
formally the deterministic growth formula in the form presented above, 
(Gadomski, et al., 1993).

Then, the asymptotic (late-time) solution to the above, presumed that 
the lysozyme-molecule velocity, \( v(R) \), at the interface, is a constant 
parameter (Gadomski and Siódmiak, 2002; Vekilov and Alexander, 
2000) reads

\[
R(t) = \bar{R}e^{v_g t} \tag{5.10}
\]

with a tendency of \( v_g \to 1 \) for crystalline formations (Gadomski, 2007); 
\( \bar{R} \) stands for a kinetic set-point constant radius, roughly close to the 
equilibrium nucleation radius \( R_c \) described above. By the way, an 
extension to the above proposal can be seen in terms of the two 
thermodynamic lengths, \( \Gamma_1 \) and \( \Gamma_2 \). It looks like \( R_c = 2\bar{\Gamma}_c c_0/(C-c_0) \), 
wherein \( \bar{\Gamma}_c = \Gamma_1 + (\Gamma_2^2/2\bar{\Gamma}_{\text{MP}}) \) (Gadomski et al., 2005). This could then be 
considered as the second-gradient type extension (Dell’Isola et al., 1995) 
introduced for the purpose of properly approaching the nucleation stage, 
thus to appropriately account for the above mentioned kinetic set-point 
constant radius, \( \bar{R} \).

When looking from a stochastic point of view, in turn, the growth 
rule that we propose to apply can be provided by (Gadomski, 2007; 
Gadomski and Siódmiak, 2002)

\[
\frac{dR}{dt} = [\sigma(R)]^{-1}V(t) \tag{5.11}
\]

The only difference when compared to the deterministic rule relies on 
the replacement of \( v(R) \) by \( V(t) \) — the stochastic velocity of the incoming 
macroions (the statistical properties of \( V(t) \) are determined by thermal 
fluctuations — see further), a much more realistic velocity correlational
proposal (Gadomski, 2007) to be met within the active zone of the crystal or aggregate growth (Vekilov and Alexander, 2000; Gadomski and Luczka, 2000). In what follows we would like to sketch how does it look like, and which is the motivation that stays behind it.

5.2.4. The Velocity-Correlation proposal (V-Cp) seen as a relevant extension of the Mullins–Sekerka (MS) like mode

While the MS mode expresses purely external-field diffusional growth of the evolving object as a whole, its surface-perturbation parameter decays exponentially with time, (Pimpinelli and Villain, 1998; Pusey et al., 1986; Gadomski et al., 1993; Gadomski and Luczka, 2000). In the case of our type of modeling such a decay seems quite unrealistic, therefore, we would like to propose an interface-controlled growth, wherein the width of the interface is roughly of comparable size to the size of above mentioned perturbation (Figure 5.1). The width, depending on the concrete from-solution crystallization conditions (Poon et al., 2000), may change over time, also responding to a suitable concentration–temperature phase–diagram zone (Haas and Drenth, 2000). Within the interface, however, a diffusional microscopic motion, just a random walk, in fact, of lysozyme macroions is observed (Vekilov and Alexander, 2000). Thus, the diffusion goes internally, so to say, being likely “intermittently” interrupted by the, let us say, almighty electrostatics (Poon et al., 2000; Gadomski and Siódmiak, 2003a), settling up the rules of accretion and motion, or attachment and detachment (Siódmiak and Gadomski, 2006), if a molecule, or a molecular aggregate (Pechkova and Nicolini, 2001; Siódmiak et al., 2006), is still not absorbed by the growing object. The time–dependent diffusion within the interface is given by the correlations, namely

\[ D(t) = \int_0^t K(s)ds \] (5.12)

wherein the correlation function \( K(s) \) is defined by some stochastic averages \( <V(t)\geq 0 \) and \( K(t_1-t_2) = <V(t_1)V(t_2)> = 0 \) (a stationary Gaussian noise for the stochastic velocity field with nonzero temporal and algebraic correlation) (Gadomski, 2007), and where \( s := t_1-t_2 \) has to
apply. This conditions look fairly complicated at first look, but point to the stationary \(<V(t) \geq 0\) Gaussian process. The truth is, however, more obvious: they simply establish the necessary and sufficient conditions for stating the diffusion coefficient \(D(t)\) by means of the velocities of the lysozyme molecules walking at random in the interfacial zone. It is another, not Einstein-like definition of the diffusion coefficient, coming directly from the dissipation-fluctuation theorem — the formula, in turn, can be named the Green–Kubo relation, (Gadomski, 2007 and refs. therein). It is worth to mention, that only temporal correlations are discussed here, because the growth is considered within its (narrow) interface-controlled approximation, the spatial correlations of the velocity macroions' field are consequently postponed; they may be valid for another, more diffusion-type approximation, in turn. For the non-Kossel lysozyme crystal growth to prevail, an asymptotic correlational proposal has to look as follows

\[
D(t) \propto t^{1-\nu}
\]

(5.13)

where \(\nu \in (0,1)\) holds (Gadomski and Siódmiak, 2005). The limit of \(\nu \to 0\) corresponds to some weak-interaction, very small correlation-strength parameter, describing thereby the average interactions within the binary system (Tanaka and Nishikawa, 2005), consisting of solute (lysozyme) and solvent viz water molecules (Gadomski and Siódmiak, 2003b). This stands also for the kinetic criterion, see above, of the non-Kossel crystal formation. Outside this limit a (dis)orderly aggregation occurs. The limit of \(\nu \to 1\) corresponds to time–independent diffusion coefficient, which implies the standard diffusion condition (or, a normal Einstein type random walk of a lysozyme molecule). It should be outlined that the stochastic growth rule \((dR/dt) \propto V(t)\) (where \(R\) is the sphere radius; for detailed description of the sphere-type approximation for description of the growth rule see Gadomski et al., 1993) with the proportionality thermodynamic factor \([\sigma(R)]^{-1}\) is, in a mathematical sense, completely equivalent to the following Smoluchowski type equation (Doi and Edwards, 1986)

\[
\frac{\partial}{\partial t} P(R,t) + \frac{\partial}{\partial R} J(R,t) = 0
\]

(5.14)
with the aggregating-matter flux

\[ J(R, t) = -D(R, t) \frac{\partial}{\partial R} P(R, t) - \frac{D(R, t)}{k_B T} \frac{\partial \Phi}{\partial R} P(R, t) \quad (5.15) \]

and with the (Kramers’ type) boundary conditions of the Smoluchowski equation

\[ J(R = R_\sigma, t) = J(R_\sigma, t) = 0 \quad (5.16) \]

Figure 5.4. Surmounting (in a classical way) the so-called Kramers’ barrier, (Reguera et al., 1998, and refs. therein) appears to be a necessary step in getting the crystal and/or molecular-aggregate phase; for explanations of the symbols plotted, see Text.

The overall diffusion function of the formation reads (Gadomski, 2007)

\[ D(R, t) = D(t) / \sigma^2(R) \quad (5.17) \]

and represents by itself a competition effect between both proposals, namely the BC-P (“static”) and its VCP (“dynamic”) counterpart (Gadomski and Siódmiak, 2005). This diffusion function, and the analyses staying behind it, enable someone to differentiate between orderly and disorderly aggregations. For example, an orderly aggregation would manifest when \( D(R, t) \) diminished with time. This implies that the “static” condition \( \sigma^2(R) \) wins over its “dynamic” counterpart \( D(t) \). A disorder governs the system’s behavior, in turn, when a converse
reasoning applies, i.e. the velocity field is “wild” enough to overcome its “static” peaceful could-be antagonist, the latter being always in a MS type description responsible for smoothening the surface of the growing object (Pimpinelli and Villain, 1998; Gadomski and Siódmiak, 2003a). When \( \sigma^2(R) \) and \( D(t) \) are cooperating, none of them being the winner of the aggregation versus phase separation game, a certain intermediate state clearly arises, such as the one reminiscent of the emergence of the hexatic phase during an order-disorder (crystal versus molten phase) phase change, presumably resembling that of Thouless-Kosterlitz type — in the case of lysozyme aggregation it is simply the viscoelastic liquid-liquid phase separation (Wentzel and Gunton, 2007; Tanaka and Nishikawa, 2005) or immiscibility region (Haas and Drenth, 2000). The suitably reformulated stochastic depiction of the crystallization phenomena, taken as a nucleation-and-growth phase transformation change, could afford to discern the gelation processes, often occurring during lysozyme aggregation/crystallization process (Gadomski, 2007; Gadomski and Ausloos, 2006).

The last but not least quantity in the Smoluchowski equation above is the Gibbs free energy; it is provided by

\[
\Phi(R) = -k_BT \ln[\sigma(R)]
\]  

(5.18)

and expresses truly non-equilibrium character until the late-time limit, fairly equivalent to a stationary state, applies. Notice that the equilibrium conditions can also be recovered while based on the function, namely when

\[
\Phi'(R) = 0
\]  

(5.19)

then a simple evaluation shows that

\[
\ln[c(R)]x \frac{1}{1-1/(\sigma(R)+1)} = 0
\]  

(5.20)

thus

\[
\frac{1}{1-1/(\sigma(R)+1)} \xrightarrow{t \gg t_c} \text{const.}
\]  

(5.21)

and
finally results (the prime denotes respective differentiation). This gives
then exactly the equilibrium condition, the same that has been revealed
above (see, the nucleation step) within the Wulff’s approximation taken
strictly at equilibrium; moreover, one had also \( \ln[c(R)/c_o] = -2\Gamma_1/R^2 = 0 \)
which would imply that the explicit contributions of the second order
correction to the Gibbs–Thomson boundary condition should be
forbidden — this contribution is simply too small to be effectively applied
for the mature growing stage. Ultimately, let us report on recovering this
way formally the equilibrium (certainly, very late-time, \( t >> t_o \) ) state,
namely
\[
\ln[c(R)] \xrightarrow{t > t_o} \ln c_o = \text{const.} \quad (5.23)
\]
and
\[
c(R) \xrightarrow{t > t_o} c_o \quad (5.24)
\]
It is worth introducing right at this stage the so-called Kramers’ barrier,
cf. Figure 5.4, that is given by
\[
\Delta \Phi(R) := \Phi(R(t_2)) - \Phi(R(t_1)) \quad (5.25)
\]
(To understand Figure 5.4 formally, \( R_0 = R(t_1) \) and \( R_2 = R(t_2) \) have to be
taken.) When \( \Delta \Phi(R) < 0 \), the aggregating system goes toward
equilibrium; when \( \Delta \Phi(R) > 0 \), the system goes away from equilibrium,
while at \( \Delta \Phi(R) = 0 \) it is supposed to be just in it (Gadomski and

Let us point out that another thermo-kinetic model of crystal growth
of complex molecules from solution has also been proposed more than
twenty years ago (Tiller, 1986). It takes also into account the presence of
electrical double layers, this time mainly around the (protein) molecules,
but around suitably small crystallites too, and shifts the crystal–growth
comprehension from far-field diffusion controlled to interface–
controlled, i.e., somehow confined, thus exploring also the pathway that
we have actually followed by our Smoluchowski-type of modeling, with
the involvement of Kramers’ barrier (Gadomski, 2007). The formulation
by Tiller (1986) is, however, more — both phenomenological and
dynamic — using mainly a typical Lennard–Jones type attraction–
repulsion potential’s rationale for describing the dynamics in a colloid type systems of interest. In our formulation, in turn, the emphasis is put more on kinetics, having, roughly speaking, the thermodynamic aspects of the two just compared models of the same (productive) entropy-involving character (Gadomski, 2007).

5.2.5. Cessation-to-growth and final-structure creation

Stationary solution to the above aggregate size dependent Smoluchowski equation, i.e., when

$$\frac{\partial}{\partial t} P(R,t) = 0$$

(5.26)

which means practically that

$$J(R = R_\infty, t) = 0$$

(5.27)

($R_\infty$ — final, typically large enough size of the obtained aggregate or crystal, obtained when $c(\vec{r})$ becomes $c_0$, i.e., in the readily long-time limit) and implies that the corresponding probability of attaining a quasi-equilibrium form of the protein crystal/aggregate, $P_\infty$, goes as (Doi and Edwards, 1986)

$$P_\infty \approx c_i / (C - c_n) = R_\infty / 2 \Gamma;$$

(5.28)

i.e., it is purely thermodynamically controlled, or equivalently, fully set in by the nucleation stage. Note that $0 < c_0 < C$ firmly applies (Gadomski and Siódmiak, 2003a). Let us simply remind the reader a “naked truth” coming out from the so-constructed model as a whole, see above. Namely, each experimenter would expect, when the crystal, or the aggregate, ceased to grow, what typically becomes effective after an appreciably long (stationarity-invoking) time, that the concentration near the interface crystal-surrounding would take on a constant value. It is seen from the above probability-addressing formula that this is really the case. Moreover, the mentioned formula, does not include any signature of even putative changes-in-time of the involved quantities.

Last but not least, the obtained formula, cf. the beginning of the Chapter, in addition, explicitly bears the landmark of the nucleation radius — therefore one might conclude that the cessation-to-growth stage...
is somehow nucleation-predetermined. The last sentence can also be viewed as a natural observation attributed to any phase-change phenomenon in which a children phase grows at the expense of its parent counterpart. Notice that $0 < R_\infty \leq 2\Gamma_1$ must hold (Gadomski et al., 2005). Bear in mind that at this crystal–formation stage we refer to the stable, thermodynamically ripe nucleus of radius $R_\infty$ — its stability is somehow guaranteed by the so-called second-gradient theory, first invented for microscopic bubbles (Dell’Isola et al., 1995 and references therein). To sum up in part, it shall be ascertained that if $R_\infty \to 2\Gamma_1$ then the probability $P_\infty \to 1$, i.e., the more firmly one can attain the equilibrium-crystal form (Honigmann, 1958; Chernov, 1997). Note that no kinetic subtleties are explicitly contained in the final form of $P_\infty$. Thus, in other words, the crystal formation viewed by our type of modeling, and supported by helpful findings of the others (Chernov, 2003; Pechkova et al., 2005a; Haas and Drenth, 1999, 2000; Sear and Warren, 2002; Vekilov and Alexander, 2000; Nanev and Tsekova, 2000; Poon et al., 2000; Wentzel and Gunton, 2007), proclaims univocally that the nucleation stage appears to be the most important or decisive step, thus a careful preparation of the nucleus (Kashchiev, 2000) is really worth doing (Siódmiak and Gadomski, 2006; Siódmiak et al., 2006). It is so indeed since it pre-determines the last stage of the formation (cessation-to-growth) when the (pre)final structure is going to emerge ultimately.

A more careful analysis of the nucleus’ preparation, and then a realization based on coarse–grained molecular, carefully prepared building blocks, carried out mainly within the framework of BCF mode of growing a few types of lysozyme crystals, has since now on been shifted to the next part of our outlook on the lysozyme (dis)orderly aggregation seen preferentially from our theoretically-oriented but also thoroughly experimentally–motivated perspective (Chernov, 1997, 2001; Santamaría-Holek et al., 2007; Gadomski, 2007).
5.3. Lysozyme Crystal Versus Aggregate Formation — Coarse-grained Approach at Sub-Mesoscale by Monte Carlo Simulation

5.3.1. Coarse-graining procedure

Sub-mesoscopic simulations of the protein crystal growth are enormously demanding computationally. This situation is going on by virtue of very complex structure of single macromolecule, often consisted of hundreds of aminoacids. Moreover, on account of complex composition of the growth milieu (water based protein solution is a mixture of: biomolecules, dissociated salts, pH stabilizers and a number of precipitants conjuring up the growth process) and what follows very complex interactions including: (i) strong hydrophobic interactions (which is the driving force in protein crystallization process) between hydrophobic/hydrophilic groups of protein chain(s) and water molecules; (ii) electrostatic interactions between charged groups of protein chain(s) and dispersed in the solution salt’s ions (Ducruix and Giegé, 1992).

For this reason, there is a need to jettison full atom or even aminoacidal representation of the protein molecule on aid of advanced coarse-grained exposition of a single biomolecule.

Figure 5.5. From the full atom representation of the lysozyme (PDB ID 193L) to the 2D growth unit: a) Full atom representation of the lysozyme, b) HP-aminoacidic (Hydrophobic–Hydrophilic) representation –blue balls represent hydrophilic whereas red balls represent hydrophobic aminoacids, c) Exterior part of the macromolecule, d) 3D cubic representation. The net excess in a number of one of the monomer types determines the type of the wall (side) of the cubic box, e) A 2D growth unit of A-type (Reprinted with the permission from Siódmiak and Gadomski, Growing lysozyme crystals under variety of physicochemical conditions - a computer modelling. Journal of Non-Crystalline Solids, 354, pp. 4221–4226, © 2008, Elsevier).
In this picture, proteins are represented as a rigid bodies (spheres or cubes) with specific active or inactive surface elements, where the degree of activation is determined by the local structure of the molecule under investigation (Siódmiak and Gadomski, 2006; Lomakin et al., 1999). The coarse–graining procedure, which the task is emphasizing amphiphilic properties of the biomolecule’s surface both in 3D and 2D depiction, for the lysozyme protein (PDB ID 193L) is presented on Figure 5.5 (for more details see following subsections and Siódmiak and Gadomski, 2006).

5.3.2. Mechanism of growth of the crystal’s surface

Most of the computer models of biomolecular crystal growth, in which the growth unit is prepared in the spirit of the coarse-graining procedure, reproduce/mimic the growth on one of the crystal’s faces, e.g. (110) or (101) (Vekilov and Alexander, 2000). The growth mechanisms, almost all of them, more or less, base on the growth rules characteristic of the epitaxial growth where adatoms are deposited on a nucleus’ surface.

Figure 5.6. (a) The growth of a terrace. The kink positions are the most (from energetic point of view – occurrence of a Ehrlich–Schwoebel barrier) favorable places at the surface. Solute units aspire to be absorbed by the crystal. (b) Ehrlich–Schwoebel barrier experienced by the system at the edge of the terrace. eDL — electrostatic double layer. (Reprinted with permission from Liu et al., Prediction of crystal-growth morphology based on structural-analysis of the solid fluid interface. Nature, 374, 342–345, ©1995 Macmillan Publishers Ltd).
When the chemical reactants are controlled and the system parameters are set correctly, the depositing adatoms arrive at the surface with sufficient energy to move around on the surface and orient themselves to the nucleus/crystal arrangement of the already crystallized molecules (e.g. proteins). Thus, an epitaxial film deposited on a (110)-oriented surface of the nucleus will take on a (110) orientation.

It must be mentioned that adatoms which arrived at the surface can be absorbed by the crystal only in specific places, such as: kinks (ledge) positions or point defects (Burton et al., 1951), see Figure 5.6a. One of the most frequent handicaps occurring on the crystal surface is a dislocation-type defect. In the case of dislocation-type defects a long ledge is formed the height of which ranges from zero to one lattice constant’s height.

Figure 5.7. A schematic of the spiral growth of a crystal surface growing from solution — the BCF-type of growth (Burton et al., 1951; Liu et al., 1995). The spiral is conducted by the screw dislocation center and becomes roughly equivalent to concentric circuit steps of height $d$ with a separation distance $\lambda_0$ (Liu et al., 1995; Żmija, 1987). (Reprinted with permission from Liu et al., Prediction of crystal-growth morphology based on structural-analysis of the solid fluid interface. Nature, 374, 342–345, ©1995 Macmillan Publishers Ltd).

Because the kink positions are the most (anticipating from energetic point of view occurrence of a Ehrlich–Schwoebel barrier (Schwoebel and Shipsey, 1966; Pimpinelli and Villain, 1998), see Figure 5.6b) favorable places on the planer surface, adatoms (or macromolecules) aspire to be
incorporated by the crystal in these places. This phenomena occurs when
the incoming material accumulate in the kink positions and so formed
terrace grows (terrace’s face propagates) to the direction perpendicular to
its limbs. The addition of growth units along a dislocation growth step
results in the formation of a hillock as shown in Figure 5.7, and
eventually the growth of the face as a whole, cf. Figures 5.7 and 5.8.

The dislocation-driven-growth mechanism was well described by
Burton, Cabrera, and Frank — BCF model (Burton et al., 1951), as
above consequently called thereafter the BCF mode of growing crystals.
In this model the incorporation of adatoms into a surface site depend on
many factors, including the: adatom density, edge atom density, kink
density, number of terraces, equilibrium vapor pressure, the impingement
flux, the diffusivity of the impinging species, surface diffusity and the
binding energies of the adatoms, to mention but most important. The
BCF mode assumes that the growth occurs on the surface with low
concentration of dislocations and exchange of the material between the
growing crystal and vapor phase is acceptable (adsorption and
desorptions phenomena proceed simultaneously).

Figure 5.8. Formation of facets: (a) Spheroid-type crystal of radius $R$. (b) Flat
crystalline surfaces formation. (c) Screw dislocation driven growth. d) Section of the
growing spiral.

5.3.3. From spheroidal to faceted crystal growth

In the deterministic as well as in the stochastic description one assumed
that the crystal has spherical symmetry. Once a symmetrical spherical
crystal grows (Libbrecht, 2005), see above, the expanding crystal
develops facets because some crystalline surfaces accumulate material
slower than others — this way an asymmetry is imposed on the system
as whole. Condensing macromolecules are especially attracted to
rounded surfaces (curvature effects) that are rough on atomic scales,
because such areas present greater available molecular binding, Figures
5.6a,b and 5.8a,b. 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 (Libbrecht, 2005).
Molecularly flat regions — the facet surfaces — have fewer dangling
chemical bonds and thus are less favorable attachment spots.

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
(Pimpinelli and Villain, 1998), the 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
Figure 5.8c, and eventually the growth of the face as a whole (Burton
et al., 1951). The growth rate of the faceted crystal is determined by the
surface diffusivity of attracted macromolecules and the geometry of the
steps, Figure 5.8d. In this case the average growth rate of the spiral in the
direction perpendicular to the surface could be identified with the growth
rate of the spherical crystal (Siódmiak and Gadomski, 2006), cf. Figures
5.7 and 5.8a,

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

(5.29)

where \(v_{\text{step}}\) is a step propagation velocity parallel to the step, \(\lambda_0\) —
average distance between two steps and \(d\) is a steps height, \(R\) stands (see
above) for the radius of the spherical nucleus.

5.3.4. Computer implementation of spiral growth

Computer model of the lysozyme crystal growth is based on the
implementation of the crystal’s surface which can grow in a spiral way
(strictly 3D structure) into a 2D lattice (Figure 5.9). The Monte Carlo
technique and the HP approximation of the biopolymers (Lau and Dill,
1989; Larson et al., 1985) are used to simulate the growth process. The
most essential characteristic, properly defining this approximation,
enables to use HP (hydrophobic-polar) model with its onto-cube-walls projected (excess) HP-properties, with a special emphasis placed on the outer “skin” region of the protein, which is the key feature of the proposed approximation to be applied in the present work for lysozyme biomolecules (Siódmiak and Gadomski, 2006).

5.3.5. **Growth unit and unit-cell preparation**

The simulation begins with an atomic-level analysis of the protein molecules under study, as taken from the RCSB Protein Data Bank (Berman et al., 2000, 2003), see Figure 5.5a (Siódmiak and Gadomski, 2006). The HP (hydrophobic–polar viz hydrophilic) representation of amino-acid is applied to reduce a number of particles used in simulation, see Figure 5.5b. Next, the exterior part of the macromolecule, Figure 5c, is projected on the walls of the virtual cubic box, surrounding the protein, Figure 5.5d. Finally, neglecting the most non-reactive sides, the 2D representation is proposed, Figure 5.5e. Symbols on the sides of the growth unit represent degrees of hydrophobicity, i.e., the numbers of the hydrophobic and hydrophilic aminoacids on each side. Interaction energies between each type of aminoacids (HH, HP and PP) can be taken from the well known models of the lattice proteins (Young-Yun et al., 2005) and amount respectively to: $E_{HH}=-2.3$, $E_{HP}=-1$ and $E_{PP}=0$. The growth unit presented in Figure 5.5e will be called A-type. B-type arises
from the clockwise rotation of A-type unit by an angle of 90°. C-type arises from the clockwise rotation of B-type unit and D-type arises from the clockwise rotation of C-type unit, all of them again by 90°-rotation. A unit cell, as a structure made up of the four 2D growth units, is a minimum energy configuration, see Figure 5.10. The ABCD-type unit cell means that growth units are placed, in a spiral-like fashion, in the following positions: A-lower left, B-upper left, C-upper right, D-lower right. It can be seen that each growth unit is differently oriented in space. Moreover, unit cell has hydrophobic core and hydrophilic surface turned to the virtual solution.

5.3.6. The growth of the lattice crystals

The growth procedure is similar to that specified by the well-known Frenkel–Kontorova (1938) and/or solid-on-solid (Abraham, 1986) (SOS) models. In these models, virtual particles (adatoms or macromolecules) are placed above lattice points.

Figure 5.10. Non-Kossel-type (Chernov, 1997) ABCD unit cell (for growth unit type see text and Figures 5.5c,e; growth units in ABCD unit cell: A-lower left, B-upper left, C-upper right, D-lower right). A hydrophobic core and also hydrophilic surface are visible (Reprinted with the permission from Siódmiak and Gadomski, Growing lysozyme crystals under variety of physicochemical conditions - a computer modelling. Journal of Non-Crystalline Solids, 354, pp. 4221–4226, © 2008, Elsevier).
Particles hop to neighboring points. The direction of hoping is random and the hopping rate depends on the height of the energetic barrier between two neighboring positions (Figure 5.11). To keep constant concentration of free particles on the surface instead of the particle which permanently enclose to the crystal new particle is deposited above random lattice point.

Figure 5.11. Energetic barrier between two neighboring points at the crystal’s surface in the Frenkel–Kontorova (SOS – solid-on-solid) models. A commensurability of two neighboring crystal layers is revealed.

Another group of protein crystal models assumes that the probability of monomer attachment to the growing crystal is proportional to the protein volume fraction and the orientational factor representing the anisotropy of protein molecules (Kierzek et al., 1999). The rate of detachment depended on the free energy of association of the given monomer in the lattice, as calculated from the buried surface area.

Also in Bratko and Blanch (2001), authors point at very important phenomenon accompanying the protein crystallization process viz refolding process which leads to increased aggregation. Their model is designed to examine the competition between intramolecular interactions leading to the native protein structure, and intermolecular association, resulting in the formation of aggregates of misfolded chains.

During the whole course of the presented simulation one observes exclusively the nucleus’ surface and the growth units which are at the surface at each simulation step. In principle, molecules in the bulk are not taken into consideration what is consistent with the above presented
mesoscopic-modeling idea of the electrostatic double layer (eDL — see Figure 5.6b) which surrounds the growing crystal and in which the most important processes seen in terms of surface formation take place (Figure 5.1). This hint saves the processor time and also speeds up the growth procedure. The number of the free growth units which are present in a given moment at the surface is always considered to be proportional to the molecule concentration in a virtual solution (“bulk”), e.g., for bulk concentration of 6%, statistically 6% of the surface area is occupied by the growth units. In the beginning, a given number of the growth units, with their random orientations (A, B, C or D), is placed by chance at the surface. There is no possibility that two movable growth units were put one over one because presented modeling is purposely confined to the one really nearest layer, only.

The movement direction of each growth unit placed at the surface is then randomly chosen: go to the left, go to the right, come off the surface or stay at the same location. The movement probability, \( p^{(m)} \), is consistent with the Metropolis Monte Carlo acceptance rule(s) (Metropolis et al., 1953). This means that downhill transitions that lower the total energy are accepted with probability one and uphill transitions with probability proportional to the Boltzmann factor:

\[
p^{(m)} = \begin{cases} 
1 & \text{for } \Delta E < 0 \\
\frac{\Delta E}{e^{\frac{\Delta E}{k_B T}}} & \text{for } \Delta E > 0
\end{cases}
\]  

(5.30)

There is a possibility that two movable growth units will come across each other. In this case, the movement is possible to occur after consideration of two components of the interaction energy: (i) interaction energy of the growth unit with the crystals surface and (ii) interaction energy of the growth unit with the neighboring movable growth unit(s). This way one can also observe some temporary aggregated forms of the growth units, e.g., 2D islands.
Figure 5.12. Consecutive snapshots of the growth of the crystal surface, from top to bottom \((t_1 < t_2)\). Grey boxes represent the units which already became a part of the crystal, whereas the white ones represent units which may still walk along the crystal's surface. \(\lambda_0\) — average distance between two steps for which the next step can start to growth, the acts of the movement and of the detachment are made with probabilities \(p^{(m)}\) and \(p^{(-)}\) respectively (Reprinted with the permission from Siódmik and Gadomski, Computer model of biopolymer crystal growth and aggregation by addition of macromolecular units. A comparative study. International Journal of Modern Physics C, 17, pp. 1037–1053, © 2006, World Scientific Publishing Company).

Every step is a combination of linear transition and rotation, i.e. the growth unit rolls over the crystal’s (or, aggregate) surface. The growth unit becomes a part of the crystal when it is situated in a kink position and its orientation suits the other elements of the crystal unit cell (non-Kossel structural rule). Moreover, there is usually a substantial energy barrier. The reason for this barrier is that an exposed edge site has a higher potential than a corner (kink) site because a molecule sitting at an edge site has the smallest number of nearest neighbors. The resulting barrier is called the Ehrlich-Schwoebel barrier (Schwoebel and Shipsey, 1966; Pimpinelli and Villain, 1998), see Figure 5.6b. Moreover, the kink site is the smallest potential site on the surface. In the case of energetically favorable move into a kink position and if the growth unit orientation does not suit the other elements of the crystal unit cell the growth unit will not become a part of the crystal. It is because in the case of lysozyme and other molecular crystals molecules occupying different positions are identical, and thus, are characterized by the same chemical potential in solution, vapor or melt. Correspondingly, the crystal should be characterized by one chemical potential averaged over the unit cell.
The growth unit that would become a part of the crystal would still have a small chance to detach but only from the kink position. Detachment probability is proportional to the difference of interaction energies between the new and the old positions, namely $p^- \equiv \exp(-\Delta E/k_B T)$. The new layer can start growing only when the bottom layer achieves a certain length equal to some $\lambda_0$. This is the minimum length for which the next twist of the spiral can arise, cf. Figure 5.8 (Żmija, 1987).

Consecutive snapshots taken from applying the PDB-based computer model of the growing crystal surface, from top to bottom ($t_1 < t_2$), in the subsequent simulation steps are shown in Figure 5.12 (Siódmiak and Gadomski, 2006). Grey boxes represent the units that already became a part of the crystal, whereas the white ones represent units being still able to walk along the crystal surface. Letters mean the growth unit type (orientation in the crystal’s structure). Because the growth unit movement is site-dependent, probabilities of the movements ($p^{(m)}$) and detachment ($p^{(-)}$) are also shown and are determined by the rules of the Metropolis Monte Carlo algorithm (Metropolis et al., 1953). Moreover, 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 Siódmiak and Gadomski (2006) and references therein.

5.3.7. Growth rate and morphological phase diagrams

Figure 5.13 presents some repeatable tendencies observed while examining the tempo of the formation of mutant (PDB ID 193L) vs. non-mutant (PDB ID 1LYY) lysozyme crystal (growth unit and unit cell preparation were made in the same way), depending on the lattice size: $30(2R_M) \times 351(2R_M)$ and $50(2R_M) \times 251(2R_M)$, where the first dimension is a lattice height, the second is a lattice width and $2R_M$ is a lattice constant which is equivalent to the size of modeled growth unit.

The growth rate can be calculated using many experimental data viz: protein radius (physical as well as hydrophobic), protein concentration, surface and bulk diffusivity, temperature, viscosity. Using these experimental data it is easy to convert MCsteps ($x$-axes on Figure 5.13) to real (clock) time, for details see Siódmiak and Gadomski (2006).
Figure 5.13. Certain most repeatable tendencies observed while examining the rate of the formation of mutant vs. non-mutant lysozyme crystal, depending on the lattice size: $30(2R_m) \times 351(2R_m)$ and $50(2R_m) \times 251(2R_m)$ and temperature $T = 310 \text{ K}$, cf. Table 5.1. Four regions are depicted on the plot: I — region of pre-nucleational effects, II — region of nucleus formation, III — region of non-stationary crystal growth, IV — region of stationary, nearly constant-tempo crystal growth. The as-yet detected differences are mostly due to a finite-lattice size effect. The curves have been obtained for the 6% protein concentration which is noticed to show optimal growing aggregation-oriented, kinetic-thermodynamic trends for both protein forms under study. The final values of the growth rates for the lattice size $30(2R_m) \times 351(2R_m)$ correspond to the appropriate values on Figure 5.14 (Reprinted with the permission from Siódmiak and Gadomski, Computer model of biopolymer crystal growth and aggregation by addition of macromolecular units. A comparative study. International Journal of Modern Physics C, 17, pp. 1037–1053, © 2006, World Scientific Publishing Company).

The differences are attributed mostly to a finite-lattice size effect. The curves have been obtained for the 6% protein concentration. Four regions are depicted on the plot: I — region of pre-nucleational effects: At this stage the first growth units try to join the crystal surface. One can see that the growth rate fluctuations are very strong and the growth rate increases very rapidly with the increasing number of new terraces. II — region of nucleus formation: At this stage the number of terraces tends to a constant value and depends on the lattice size. III — region of non-stationary viz transient crystal growth: At the end of this stage the
number of terraces is fixed and is approximately equal to \( h = \frac{\text{lattice width}}{\lambda_0} \). IV — region of stationary, close-constant-tempo crystal growth: a constant—growth rate of lysozyme crystals is consistent with often experimentally observed behavior of lysozyme crystals grown from aqueous solution (Pusey et al., 1986). Based on this result, one can conclude that the growth of lysozyme is controlled by the incorporation of lysozyme molecules to the surface of the crystal. In other words, the overall crystal formation has been mainly designed as an interface—controlled phenomenon. Investigations on several types of proteins (insulin, canavalin, and lysozyme) performed by other researchers (Monaco and Rosenberger, 1993; Forsythe and Pusey, 1994) have also suggested that the growth of such proteins is limited by interface—involved rather than by volume (in-bulk) transport.

Figure 5.14 presents a comparative plot, roughly revealing three regions of temperature behavior seen in terms of the rate of growing of the (poly)crystalline aggregates for the set of protein (non-mutant lysozyme) concentrations: 3, 6, 9, 12%. For non-mutated form of lysozyme (PDB ID 193L) several regions viz parametric windows (slots) have been found: I — Region below an optimal temperature for a given lysozyme concentration: In this range of temperature values the growth rate increases with increasing temperature. This behavior is associated with increasing mobility of the growth units but the temperature is still too small for dissolution effects to prevail. II — Crystallization slot, typically detectable by means of calculating the second virial coefficient from the state equation (Chernov, 1997): In this range of temperature values the growth rate conforms to an approximately constant value and the acts of attachment/detachment are balanced. A deflection point is only seen for the non-mutant form of the lysozyme; the mutant form, in turn, expresses a certain resistance against smooth crystallization and the deflection point is hardly visible for it (probably it could be seen above denaturation temperature). III — Region above an optimal temperature for a given lysozyme concentration: In this range of temperature the growth rate is decreasing because detachment starts dominating over the attachment. Outside the window for both variants one detects the protein aggregates that are going to be present for each of the concentration value chosen. The data points for \( T = 310 \text{ K} \) and concentration 6%, both for non-mutated
and muted lysozyme, correspond to the final values of the growth rates from Figure 5.13 for lattice size $30(2R_M) \times 351(2R_M)$ (Siódmiak and Gadomski, 2006).

Figure 5.14. A comparative plot, revealing three regions of temperature behavior seen in terms of the tempo of the growing (poly)crystalline aggregates for the set of protein (non-mutant lysozyme) concentrations: 3, 6, 9, 12%. I — region below an optimal temperature for a given lysozyme concentration, II — crystallization window, III — region above an optimal temperature for a given lysozyme concentration. The data points for $T = 310$ K and concentration 6%, both for non-mutated and muted lysozyme, correspond to the final values of the growth rates from Figure 5.13 for lattice size $30(2R_M) \times 351(2R_M)$. (Reprinted with the permission from Siódmiak and Gadomski, Computer model of biopolymer crystal growth and aggregation by addition of macromolecular units. A comparative study. International Journal of Modern Physics C, 17, pp. 1037–1053, © 2006, World Scientific Publishing Company).

A tendency of dissolving the crystal appears too — see the lowest curve (LYS 3%) with $V_{gr} < 0$ (due to enormous evaporation).

### 5.3.8. Crystal growth using aggregates as the growth unit

A gob of theoretical as well as experimental works substantiate that the growth of (100) and (101) faces of tetragonal lysozyme crystal can be
fully explained if not only monomeric growth units but also larger than monomer, e.g. tetramers, growth units are assumed (Nadarajah et al., 1997, 1999; Kierzek et al., 1997, 2000; Durbin and Feher, 1991).

Tetrameric growth units can be obtained through some modification of the classical vapor diffusion method. A HEWL (hen-egg white lysozyme) Langmuir–Blodgett (LB) thin film, prepared by a Langmuir–Schaeffer (LS) technique variation thereof (Ulman, 1991), was used as the template for the stimulation and rate increasing of lysozyme crystal growth (Pechkova and Nicolini, 2003). Monolayers of lysozyme were formed in a Langmuir Teflon trough by spreading 500 ml phosphate buffer (pH 6.5) solution with a HEWL concentration 4 mg/ml; 10 μM 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 0.1 mm/s up to surface–pressure of 18 mN/m and deposited by LS parallel shift technique onto the siliconazed cover glass slide. Obtained nanofilm was characterized by several experimental techniques such as: circular dichroism, atomic force microscopy and nanogravimetric methods (Pechkova and Nicolini, 2003) 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 Pechkova et al., (2005a,b), Pechkova and Nicolini, 2001 and Gadomski and Siódmiak, 2003a.

Because in the computer modeling presented here a face of growth is not well defined (is it (100) or (101) face?) the incorporation of non-monomeric growth units was applied as an universal method (crystal’s face independent) and is realized as follows. (The additional physical reason for the incorporation consists in some expected increase of the osmotic pressure of the solution.)

The tetrameric growth units look like the one drawn schematically in Figure 5.15 (Siódmiak et al., 2006). 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, see Siódmiak and Gadomski (2006) for details. Now, the growth unit is identical with unit cell. When compared to the monomers, they are allowed to perform their along-surface biased directionally Random Walk (RW) at practically 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 one wished to perform.

The main result obtained from the comparative (monomeric vs. tetrameric growth unit) simulation is presented on Figure 5.16. It can be seen, that for a long time interval (here MCsteps) a difference in a value of the growth rate reaches 25%. This result can be mathematically proved after taking into consideration differences in the size, mass and diffusity of monomeric as well as tetrameric growth unit, for detail see Siódmiak et al. (2006).

From this comparison it follows that incorporating a tetrameric unit, Figure 5.15, will result — without any special modification of the algorithm explored — in obtaining some acceleration mode of the under-confinement developing process (Pechkova et al., 2005a,b, Pechkova and Nicolini, 2001). As shown in the experiment (Pechkova et al., 2005b), 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
algorithm (Siódmiak and Gadomski, 2006). It should be clearly underlined that the obtained acceleration factor is only possible to occur when one assumed the non-monomeric growth units.

Let us also state clearly that the presented model does not account explicitly for a contribution of the structured water, detected experimentally in Pechkova and Nicolini (2003); a way of how to try to deal with it can perhaps be started from Gadomski and Łuczka (2000) by really incorporating the solute–solvent interaction conditions (Flory–Huggins parameter being involved) at a given temperature.

Some trials of incorporation into the model some important — for protein crystal growth processes — physicochemical parameters are presented in following subsection.

Figure 5.17. Lysozyme crystals grown out from orifices punched in kapton flat-and-porous membranes (Siwy et al., 2003): top view, a setup consisting of 24 Limbro cells; bottom view, a magnified picture of a single crystal of lysozyme grown from the kapton–membrane orifice (see, a paper “Could one effectively grow protein crystals: what comes out from theory and what from experiment?” at http://www.mischer-expo.de/achem/achema-2.htm, presented by A. Gadomski at Achema 2006, in Frankfurt on Main, and coauthored by C.B. Trame).
5.3.9. Growing lysozyme crystals under variety of physicochemical conditions

The most important variables in the search of crystallization conditions are: pH value, ionic strength and temperature of the solution (Gilliland and Ladner, 1996; Pusey et al., 2005). All parameters are strictly related to the virial coefficients, especially to the second virial coefficient (Chernov, 2003; George et al., 1997; Bonnete et al., 1999), $B_2$, which strongly influences the protein crystallization behavior (George et al., 1997; George and Wilson, 1994). The temperature is directly used in presented model, i.e. it is used to calculate the probability of movement, $p^{(m)}$, and probability of the detachment of already crystallized growth unit, $p^{(-)}$. Because the pH and electrolyte concentration influence $B_2$, which characterizes interaction energies between aminoacids, interactions energies: $E_{HII}$, $E_{HP}$ and $E_{PP}$, must be changed to control and/or mimic pH or ionic strength of the solution (Rosenberger, 1996).

Because there is not unambiguous estimation of the interactions energies for model lattice proteins there are some possibilities of choosing various combinations of energy values. The chosen interaction-energy values must be merely in agreement with a Miyazawa–Jernigan (MJ) matrix (Miyazawa and Jernigan, 1985; Rosenberger, 1996). In this matrix effective inter-residue contact energies for protein in solution are estimated from the numbers of residue-residue contacts observed in crystal structures of the globular proteins. Splitting all 20 aminoacids into two groups, H-hydrophobic and P-hydrophilic, it is possible to describe general rules of interaction energies within these two groups of residues. Effective inter-residue energies must fulfill the following inequalities (Miyazawa and Jernigan, 1985):

$$2E_{HP} > E_{PP} + E_{HII}$$
$$E_{PP} \geq E_{HP} > E_{HII}$$

(5.31)

It is easy to check that inter–residue contact energies proposed by the well known HP model, first introduced by Larson et al. (1985) for surfactant-containing systems, and then successfully applied by Lau and Dill (1989) to describe conformational behavior of lattice proteins, i.e. $E_{HII} = -2.3$, $E_{HP} = -1$ and $E_{PP} = 0$, fulfill foregoing conditions. The use of
the HP model implies automatically that the implicit solvent conditions have been assumed (Lau and Dill, 1989; Larson et al., 1985).

Some combinations of the interaction energies lead to the different values of the growth rate (for the growth rate calculation see Siódmiak and Gadomski 2006) and to the various unit cell configurations (#UC types), characterized by the same binding energy (UCe), see Table 5.1 and Figure 5.18.

<table>
<thead>
<tr>
<th>$E_{HP}$</th>
<th>$E_{PP}$</th>
<th># UC types</th>
<th>UCe</th>
<th>$V_g$ [m/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3.0</td>
<td>-1.0</td>
<td>0.0</td>
<td>1</td>
<td>-40.0</td>
</tr>
<tr>
<td>-2.3</td>
<td>-1.0</td>
<td>0.0</td>
<td>1</td>
<td>-31.6</td>
</tr>
<tr>
<td>-3.0</td>
<td>0.0</td>
<td>1.0</td>
<td>1</td>
<td>-20.0</td>
</tr>
<tr>
<td>-3.0</td>
<td>1.0</td>
<td>1.0</td>
<td>16</td>
<td>-16.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>1.5</td>
<td>1</td>
<td>-12.0</td>
</tr>
<tr>
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<td>0.0</td>
<td>1.0</td>
<td>1</td>
<td>-11.6</td>
</tr>
<tr>
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<td>1.0</td>
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<td>1.0</td>
<td>1.5</td>
<td>1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 5.1: Number of the unit cell configurations (# UC types), unit cell’s binding energies (UCe) and the growth rates for a various inter-residue energies (for the growth rate calculation see Siódmiak and Gadomski, 2006).

It can be seen that for unit cell binding energies: -7.6 and -16 two groups of velocities were obtained: “fast” and “normal” growth occurs. Analyzing these two groups characterized by different growth rates, it can be seen that in cases of $E_{HP} = E_{PP}$, 16 different types of the unit cell (different than ABCD), characterized by the same value of the binding energy, were obtained. Among them there are few for which crystals grow faster than for most types. Therefore, estimation of the mean value of the growth rate for a given set of “conditions” (when $E_{HP} = E_{PP}$) is not possible. In other cases, when $E_{HP} \neq E_{PP}$, one type of the unit cell was obtained, see Figure 5.6, and the overall growth rate could be calculated.

A detailed (morphological) phase diagram showing the growth rate dependence on the unit cell configuration is shown in Figure 5.18. In this diagram four groups of the unit cells can be distinguished. Two of them, I and IV, lead to rhomboid-like final shape of the crystal, while remaining two groups, II and III, lead to needle-like crystals. It can be
seen that if the crystal is made up of unit cells which form the horizontal sliding layer (monolayer), group II, modeled crystal grows faster in a direction perpendicular to this layer and elongated shape is observed for a long time. The same results are seen in group III, where vertical monolayer is formed and the crystal grows faster horizontally.

This phenomenon occurs because the growth unit can move faster (smaller energetic cost of the movement) on the monolayer. In the case when no sliding layer is formed, group I, or two horizontal or vertical sliding layers are formed, group IV, no elongated shape is attained. By the way, similar results can also be obtained when varying the lysozyme concentration at constant temperature (24 °C) and fixed pH ≅ 4.6 but upon adding different amounts of the NaCl precipitant. Such a procedure leads to favoring either (101) or (110) planes grow predominantly in a tetragonal lysozyme crystal (Durbin and Feher, 1991).

Figure 5.18. A (picturesque) diagram of the anisotropic growth. It can be seen that if the crystal is made up of unit cells which form the horizontal (or vertical) sliding layer (monolayer), group II (or group III), modeled crystal grows faster in a direction perpendicular to this layer and for a long-time period an elongated shape is observed. In the case when no sliding layer is formed, group I, or two horizontal or vertical sliding layers are formed, group IV, no elongated shape is admitted to the system.
This phenomena can be explained also on the probabilistic grounds. The formation of the monolayer proceeds two times faster than the bilayer. In the case of monolayer, for example made up of growth units of type B, the probability that the B-type growth unit appears in the kink position, where the neighboring already crystallized growth unit is also of the B-type, is 1/4. In the case of the bilayer, for example made up of growth units of type A and C alternately, the probability that the A-type growth unit appears in the kink position, where the neighboring already crystallized growth unit is of the C-type, is 1/8. This probability is lower because of the kinked (edged) growth unit must be exactly of the C-type (not A-type) and the probability that in the case of A/C bilayer the kinked growth unit will be C-type is 1/2. Multiplying both probabilities one obtains exactly 1/8.

Extremely pH value and electrolyte concentration (ionic strength) dependent shapes effect experimentally observed for the lysozyme crystals (Velev et al., 1998) is shown in Figure 5.19. For low values of the pH strictly three-dimensional (rhomboid-like) crystals are obtained, like in groups I and IV presented in Figure 5.18, whereas for the high values of the pH significantly elongated (needle-like) crystals are observed, like in groups II and III presented in Figure 5.18.

Figure 5.19. Extremely pH value and electrolyte concentration (ionic strength) dependent shapes effect experimentally observed for the lysozyme crystals. For low values of the pH exactly three-dimensional (rhomboid-like) crystals are obtained, whereas for the high values of the pH significantly elongated (needle-like) crystals are detected (Reprinted with the permission from Velev et al. Protein interactions in solution characterized by light and neutron scattering. Comparison of lysozyme and chymotrypsinogen. Biophysical Journal, 75, 2682–2697, © 1998, Elsevier).
5.4. Conclusions

Lysozyme crystal growth, and possibly other protein crystal growth types, such as the one of ribonuclease A, may be modeled by means of the sub-mesoscopic computer-aided approach proposed, in particular, when small aggregates, such as ordered tetramers coming from Langmuir–Schaeffer supports, are suitable for being incorporated in the crystal structure when the boundary conditions determine somehow a kinetic optimality and get ultimately the process accelerated markedly, thus playing a role of its intimate (chemical) catalysts.

Non-equilibrium thermodynamic mechanism at a mesoscale appears to be amenable to model a wide class of growing processes, taking place in entropic environments, in which memory effects as well as boundary constraints are their basic growth-promoting landmarks toward hybrid (analytical plus computer-aided) modeling. Therefore a better comprehension of experimental material, and solid cooperation with experimenters is needed for making substantial progress in this complex task, commonly named protein (lysozyme) crystal growth.

Sub-mesoscopic computer model in comparison to mesoscopic modeling gives us some opportunities of bringing out some details of complex structure of biomolecules. The knowledge of the structure of proteins is important since they often reveal anisotropic properties such as mechanical properties and surface charge distribution. A huge number of detailed information on particles, which reveal anisotropic properties, used in modeled system intrude very often on receiving some statistical information on mean values of thermodynamical parameter. Non-equilibrium thermodynamic gives us an opportunity to use an “averaged” particles in a model characterized by mean size, mean surface charge and its velocity what finally brings us closer to obtain an average size of the crystal/aggregate and the kinetics of the growth process in a long–time regime. The interchange of information between both presented types of modeling causes them to be in some (pronounced) sense complementary.

As a certain perspective, the hybrid model and some useful liaison with experimenters arise naturally for the future.
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References


Model Lysozyme Crystal versus Aggregate (Un)Confined Formation


