Simulation of twinned crystal growth and its twinning ratio distribution

bR crystal growth was modeled by a one-dimensional model. The growth begins from a single layer and proceeds by consecutive addition of new layers to each surface of the crystal alternatively (Figure 1). A new layer is added to the CP surface either by forming CP-EC or CP-CP contact, and to the EC surface by forming EC-CP or CP-CP contacts with fixed relative probabilities P_1 and P_2 (Figure 1). It was assumed that the probabilities remain unchanged during crystal growth. The total number of 4000 layers, corresponding to the experimentally observed average crystal thickness of ~ 20 μm was used in the simulation.

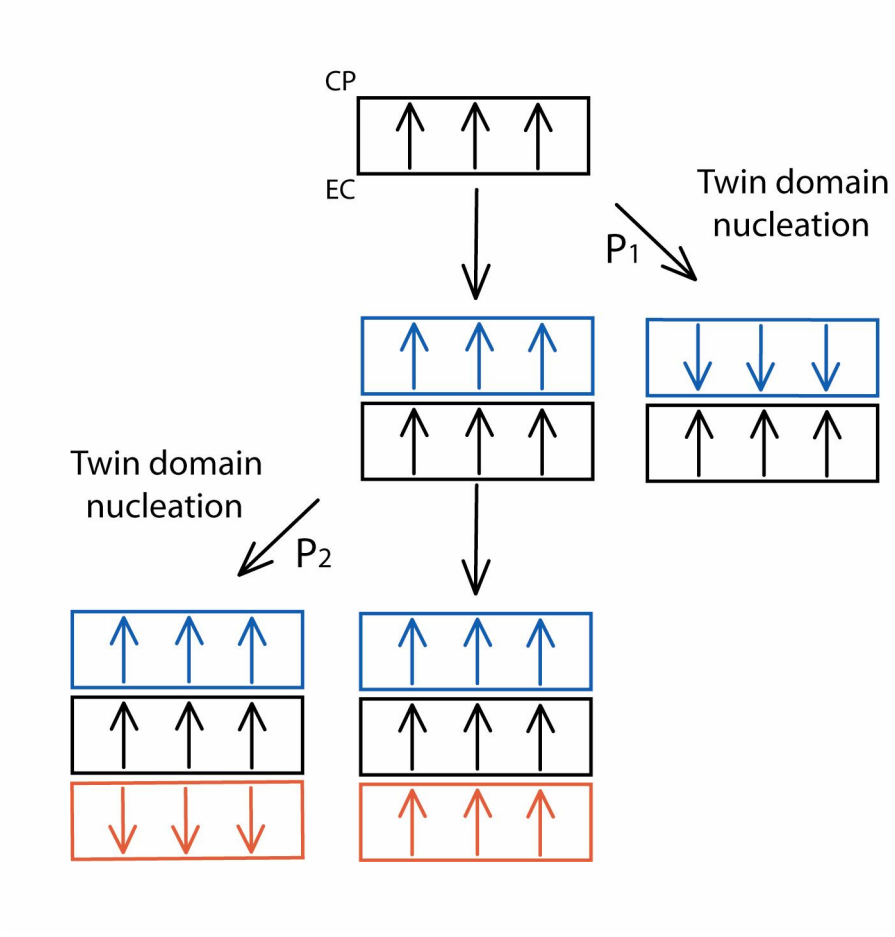

Figure 1. The figure shows sequential step of the model of crystal growth. Vertical sequence of steps corresponds to normal crystal growth, which proceeds by addition to the single layer (back) a layer from the cytoplasmic surface (blue) and than a layer from extracellular surface (red). P_1 and P_2 - relative probabilities of formation of CP-CP and EC-EC contacts, correspondingly. The layers in reverse orientation are added to the crystal with probabilities P_1 and P_2. It corresponds to nucleation of the twin domain (shown as side steps in the figure).

The model is symmetrical relative to the choice of P_1 and P_2, which are the only variables of the model. Therefore twinning ratio distributions were generated for $P_1 \geq P_2$.

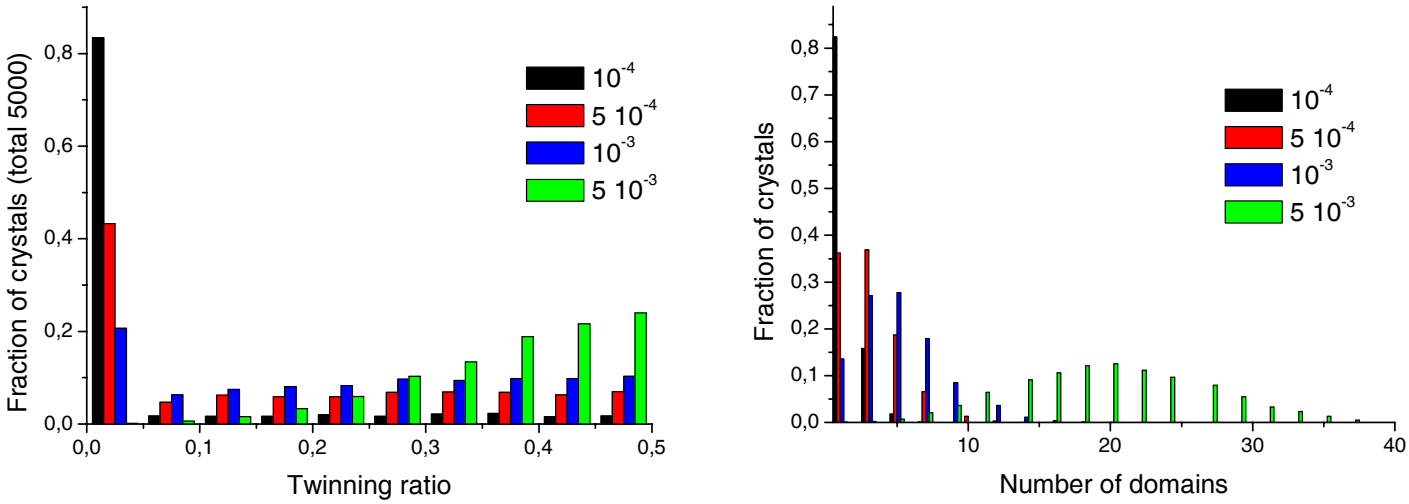

Figure 2. Distribution of twinning ratio and the number of twin domains calculated for 5000 crystals under conditions of symmetric domain nucleation (P_1=P_2) and for probabilities in the range 10^{-4} - 5×10^{-3}.

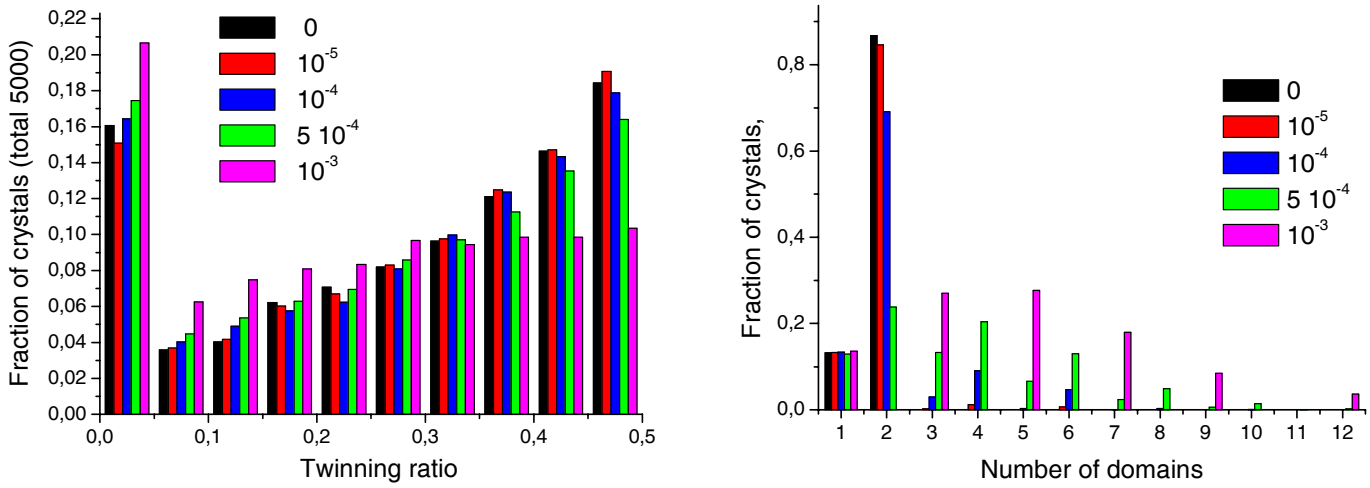

Figure 3. Distribution of twinning ratio and the number of twin domains calculated for 5000 crystals under conditions of asymmetric domain nucleation P_1=10^{-3}, P_2 in the range between 0 and 10^{-3}.

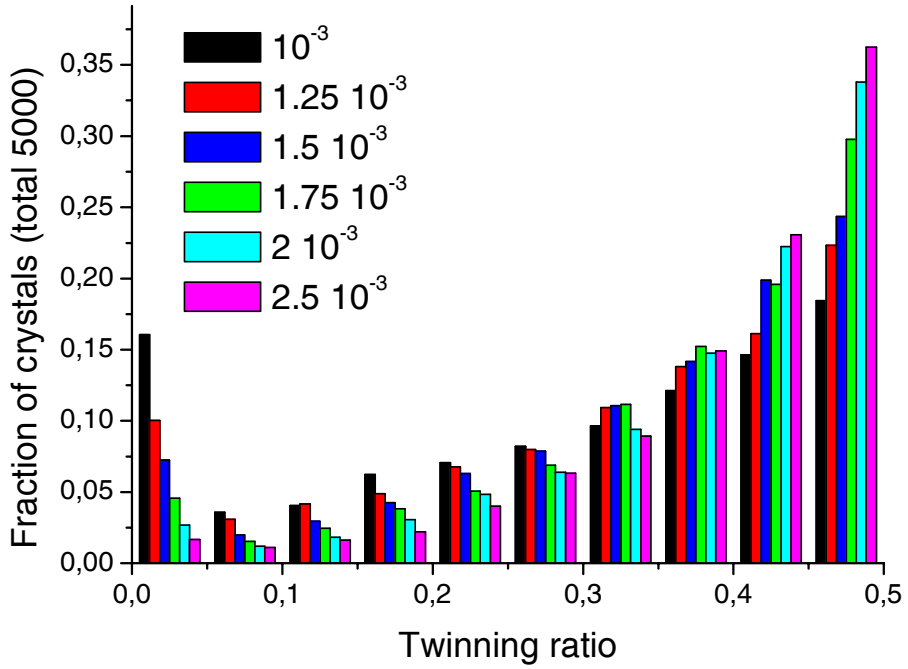

Figure 4. Distribution of twinning ratio and the number of twin domains calculated for 5000 crystals. P_1 varies between 10^{-3} and 2.5×10^{-3} and P_2=0.

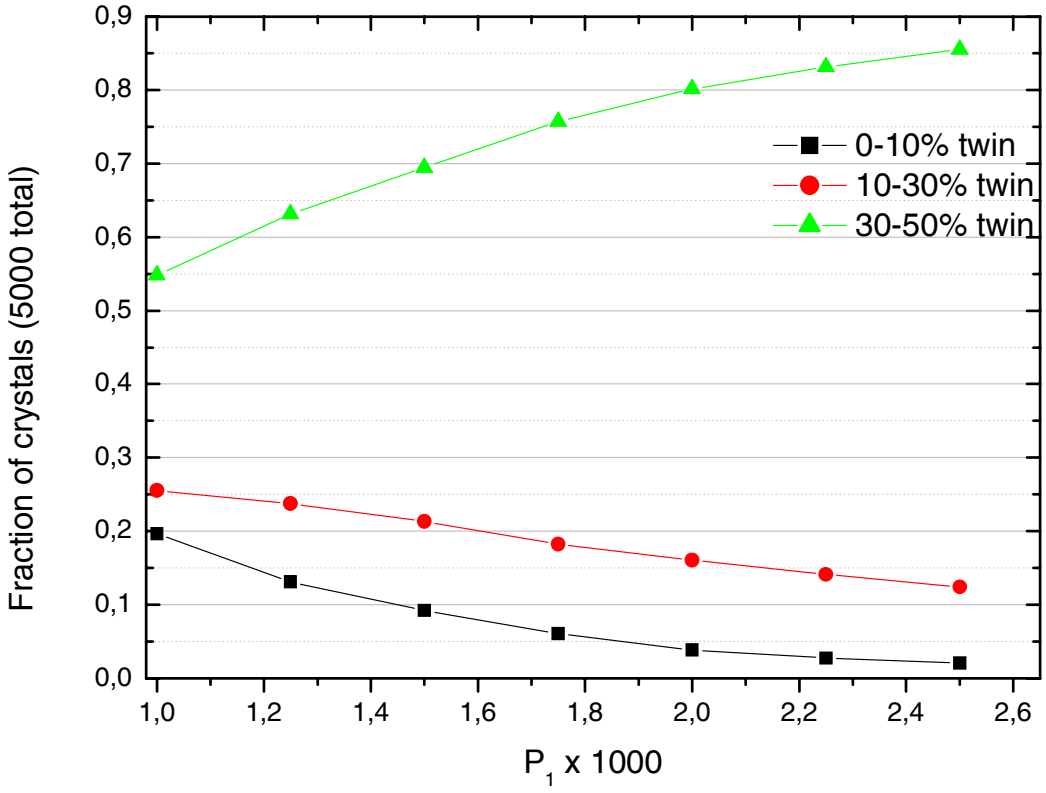

Figure 5. Dependence of fraction of crystals with twinning ratio in the range 0-10, 10-30 and 30-50 % on probability P_1, P_2=0. The fastest changes occur for twinning ratios around 0 and 50 %.

Rate of crystal growth and probability of twin formation

The model proposed for twinning formation and our numerical simulations do not explain how changes in the crystal growth rate may cause differences in relative probabilities of the addition of differently oriented new proteins layers to the crystal interfaces. The thermodynamical consideration of the crystallisation process may give the explanation to this phenomenon.

The idea that limiting step of crystal growth is 2D nucleation of a new layer on the crystal surface is central in our model of crystal growth. It is based on experimental data of AFM (Qutub *et al.*, 2004) and morphological properties of the type I crystal.

According to classical thermodynamic theory thermodynamic potential of 2D nucleus formation on a crystal surface is (I.V. Markov, Crystal growth for beginners, Page 98):

$$\Delta G=-n\,(\mu_v-\mu_c)+\Sigma_i l_i \kappa_t\ ,$$

where μ_v and μ_c are chemical potentials of protein molecule in the volume of crystallization media and in crystal respectively, n - number of protein molecules in the nucleus. The second term describes edge energy, where κ_t is a specific edge energy and l_i is the length of i's edge. Based on the above expression free energy of nucleus formation can be written in the general form as (I.V. Markov, Crystal growth for beginners, Page 99):

$$\Delta G^*=A/(\Delta\mu-B), \qquad (1)$$

where A and B are the terms independent of supersaturation $\Delta\mu=\mu_v-\mu_c$. Here A depends on the specific edge energy and is virtually the same for normal and inverse nucleation; B depends on interaction of proteins in different layers and is different for different nuclear orientations.

Now consider the rate of 2D nucleation for which general expression looks like:

$$J \sim \Gamma \exp(-\Delta G^*/kT) \qquad (2)$$

where ΔG^* is free energy of nucleus formation and Γ is a term that has slow dependence on supersaturation, compare to exponential term. During twin crystal growth nucleation of domains with normal orientation is much more probable than with twinned orientation (the condition imposed by requirement for the twin domains to be macroscopic). Thus:

$$J_o >> J_1 \text{ and } J_2 \Rightarrow \Delta G^*_o < \Delta G^*_1 \text{ and } \Delta G^*_2 \qquad (3)$$

Where J_o, J_1 and J_2 are the rates of nucleation for normal crystal growth and two possible orientations of twin domains and ΔG^*_o, ΔG^*_1 and ΔG^*_2 are corresponding free energies of 2D nucleation. The rate of crystal growth is regulated by supersaturation $\Delta\mu$ and at very high values of supersaturation the difference between J_o, J_1 and J_2 vanishes (see (1)). When

supersaturation decreases the value of ΔG^*_o decreases and crystal growth slows down. Simultaneously ΔG^*_1 and ΔG^*_2 approaches 0 faster than ΔG^*_o and at some $\Delta\mu$ become negative (nucleation of corresponding twin domain ceases), which follows from equation (1) and interrelation (3). Due to exponential term in equation (2) the gap between probability of normal and twin domain formation grows.

The values of ΔG^*_1 and ΔG^*_2 depend on interaction energy of CP-CP and EC-EC surfaces of bR 2D crystals. As has been discussed in (Efremov *et al.*, 2004), these surfaces have very different electrostatic properties. This suggests that one of the nucleation rates of twin domains will vanish faster than the other as crystal growth rate decreases. This point explains why asymmetry in twin domain formation is observed when crystal growth is slow. The above considerations are generic for crystals in which twinning is a result of 'erroneous' 2D nucleation on the crystal surface, thus correlation between crystal growth rate and probability of twin formation might be a general property of such crystals.

The above explanation is general and based on thermodynamics. But the field of *in meso* crystallisation is separate from the soluble protein crystallisation. The driving force of crystallisation *in meso* and soluble proteins crystallisation probably are different (Grabe *et al.*, 2003). For this reason we provide an additional scenario of how changes in the crystal growth rate may cause differences in relative probabilities of nucleation in *in meso* crystallisation.

It is well justified that all membrane protein crystals (including those of bR) grown in lipidic meso phases are crystals of type I (i.e. the crystals represents a stack of membrane-like layers) (Michel, 1991). There is an evidence that the crystals grow layer by layer (Caffrey, 2003). The current state of view on membrane protein crystallization *in meso* is illustrated on the Fig.1 at (Cherezov & Caffrey, 2007). Protein molecules are inserted into the curved bilayer of lipidic mesophase. But according to the hypothesis, the cubic lipid phase is adapted to create a lamellar lipid phase which surrounds the growing membrane protein crystal. Still in the lipid bilayer protein molecules diffuse the from the bulk meso phase to the lamellar phase where

they are incorporated into the growing crystal. This hypothesis is strongly supported by AFM (Qutub *et al.*, 2004), EM (Paas *et al.*, 2003) and X-ray (Cherezov & Caffrey, 2007) studies. It is highly important that there is a curved intermediate part of the bilayer between the bilayer of the cubic phase and that of the lamellar phase (since the bilayer of the cubic phase is highly curved and lamellar is not). The increase of the curvature of lipid meso phase will also lead to the increase of the curvature of the intermediate bilayer.

Here we have to turn to another important aspect of *in meso* crystallization. In accordance to the theoretical work of Grabe (Grabe *et al.*, 2003), a driving force of *in meso* protein crystallization is the elastic energy of deformation of a curved bilayer due to the hydrophilic-hydrophobic mismatch of the lipid bilayer and protein interfaces. The deformation energy strongly depends on the ratio of curvature of the bilayer and the length of protein hydrophobic-hydrophilic interface. It is shown (Grabe *et al.*, 2003) that it is the change of the curvature which is the determinant of the crystal growth rate. Our experiments on the crystallization in lipidic meso phases with different curvature also support this conclusion (Gordeliy et al. To be published). The decrease of curvature slows down the rate of crystal growth. Presumably, this is the phenomenon which we observed at the current work.

The elastic energy also depends on the length of the hydrophilic-hydrophobic boundary of the protein (Grabe *et al.*, 2003). Generally speaking there are two not equal hydrophilic-hydrophobic boundaries of the protein which corresponds to extracellular and cytoplasmic interfaces of the protein. It is evident that curved bilayer is also asymmetrical relative to the perpendicular to the bilayer surface. Since both protein and bilayer are asymmetrical the elastic energy of protein will not be equal to those of upside down protein.

Here we come to the main point of the hypothesis. Since the protein moves towards a growing crystal across curved intermediate bilayer and the elastic energy for two different orientations of the protein will be different. Moreover, the elastic energies will change in different ways with the change of the curvature of the bilayer. Thus, the decrease of lipid meso phase

curvature will lead to the slowing down the crystal growth rate. But it also will cause the decrease of the curvature of the intermediate layer. In its turn it will differently change the elastic energies of different protein orientations. And thus it may lead to the change in relative probabilities of the addition of new protein layers to the crystal.

The first proposed scenario of the twinning phenomenon relies on the thermodynamics of nucleation, and the second one relies on current hypothesis of the mechanism of membrane protein crystal growth in the lipidic phase and seems to be plausible. However, we should stress that both of these hypotheses are not yet well justified.

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