Electronic Supplementary Material (ESI) for CrystEngComm.


# Supplementary Information

## Illuminating Protein Crystal Growth using Fluorophore-Labelled Proteins

**Alaa Adawy[1], Willem J. P. van Enckevort[1], Elisabeth S. Pierson[2], Willem J. de Grip[3], Elias Vlieg[1]**

[1] Radboud University Nijmegen, Institute for Molecules and Materials, 6525 AJ Nijmegen, The Netherlands
[2]Department of General Instrumentation, Faculty of Science, Radboud University Nijmegen, 6525 AJ Nijmegen, The Netherlands.
[3] Department of Biochemistry, Nijmegen Centre for Molecular Life Sciences, Radboud University Medical Centre, 6525 GA Nijmegen, The Netherlands

**Contents:**

**Photo-bleaching of monoclinic HEWL crystals**

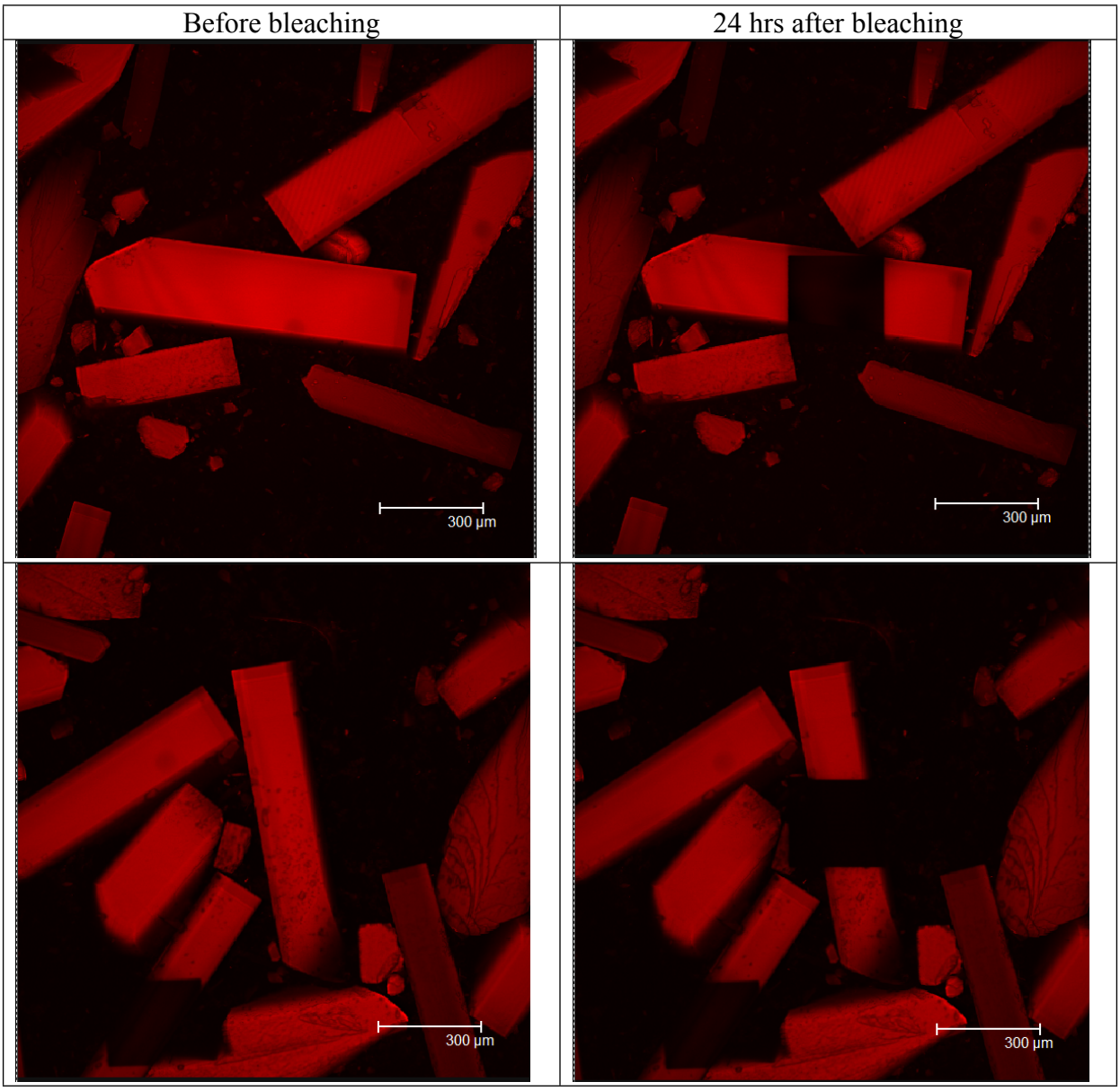


***Fig S1 A series of CLSM micrographs of the central slice of monoclinic HEWL crystals (left panel). At 24 hrs after photo-bleaching a section of the crystal, no diffusion of fluorophores was detected (right panel), confirming that the dye is only bound to protein molecules and not freely moving within the crystal.***

## The derivation of Equation 1

*Orientation of the (1-10) and (101) growth sector boundary in tetragonal Hen Egg White Lysozyme viewed in a (110) cross-section plane as a function of the relative growth rates of the adjacent (1-10) and (101) growth faces*

We consider a cross-section plane (110) (the plane scanned by the confocal fluorescence microscope, shown in Fig.4 of the main text) in the tetragonal HEWL crystal. The boundary between the sectors of the two adjacent side faces (1-10) and (101) is the trajectory of the intersection point of the two intersecting lines of both faces with the basic plane (110), see Figure S1. In the following the indices 1 and 2 refer to the (101) and (1-10) planes respectively.

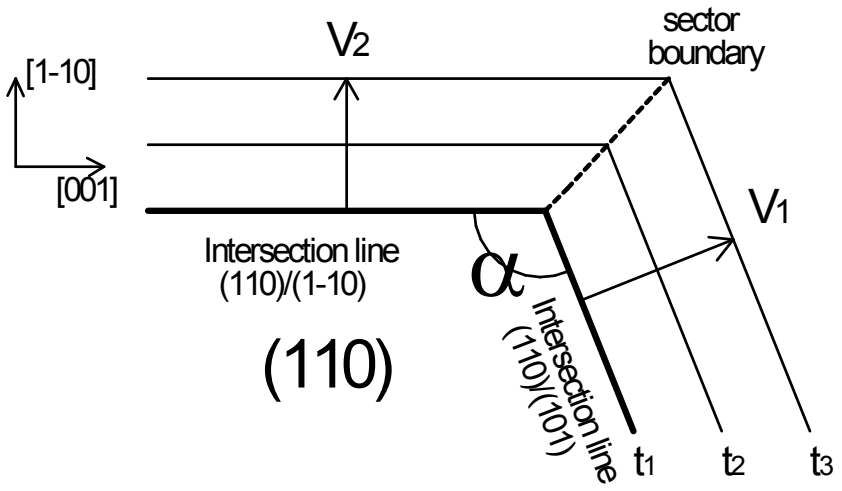


***Fig S2. The trajectory of the sector boundary between the two adjacent faces (1-10) and (101) imaged on the cross-section plane (110) is given by the intersection point of the two intersection lines of both side faces with the basal plane (110). Top view on (110).***

The advancement rate of the two intersection lines is given by (Figure S2)

$$V_i = \frac{R_i}{\sin \beta_i}, \qquad \text{S1}$$

with $R_i$ the growth velocity of side face $i$, $V_i$ the advancement rate of the intersection line and $\beta_i$ the angle between the basal (110) plane and the side face in question. For the (101) plane $\beta_1$ = 72.21°; for (1-10) $\beta_2$ = 90°.

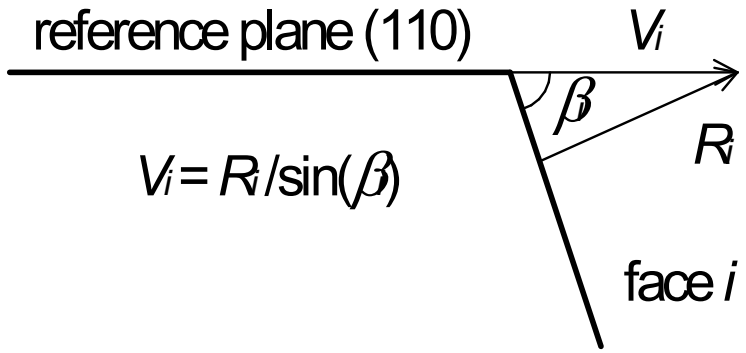


***Fig S3. The advancement velocity of the intersection line between the basal face and a side face as a function of side face growth rate velocity and its angle with the basal face. Side view.***

The trajectory angle, $\varphi$, of the intersection point of the of the two intersection lines can now be derived by using figure S3 and considering that the trajectory velocity is given by the vector sum $\mathbf{V_t'} = \mathbf{V_1'}+\mathbf{V_2'}$. Here $\mathbf{V_i'}$ prime holds for the intersection point velocity 'component' of face *i* parallel to the intersection line of the other face. From figure S3 it follows that

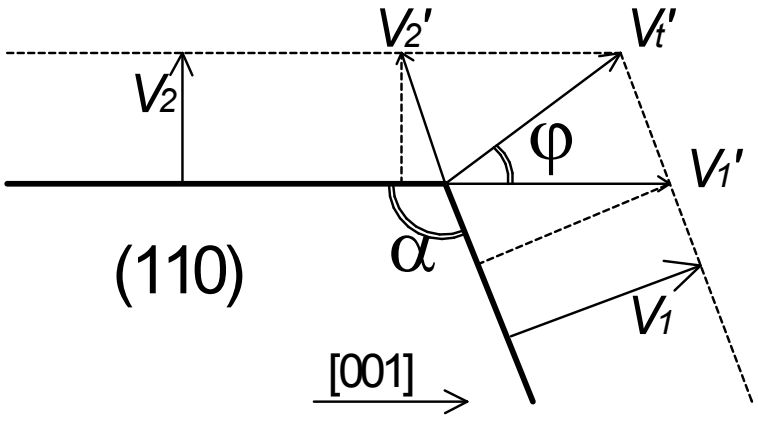


***Fig S4. The trajectory angle, φ, is determined by the angle between $V_t' = V_1' + V_2'$ and the horizontal [001] crystallographic direction. Top view on (110).***

$$V_1^{'} = \frac{V_1}{\sin\alpha} \text{ and } V_2^{'} = \frac{V_2}{\sin\alpha} \qquad \text{S2}$$

and

$$V_t^{'2} = V_1^{'2} + V_2^{'2} + V_1^{'}V_2^{'}\cos\alpha \qquad \text{S3}$$

with $\alpha$ the angle between both intersection lines. The trajectory angle, $\varphi$, (which is the angle between $\mathbf{V_t'}$ and the horizontal [001] direction) is given by

$$\sin\varphi = \frac{V_2}{V_t^{'}} \qquad \text{S4}$$

or

$$(\sin\varphi)^{-1} = \frac{V_t^{'}}{V_2} = \frac{\left(V_1^{'2} + V_2^{'2} + 2V_1^{'}V_2^{'}\cos\alpha\right)^{1/2}}{V_2}. \qquad \text{S5}$$

Rewriting S5, using equations S2 gives

$$\frac{\sin^2\alpha}{\sin^2\varphi} = \Gamma_{12}^{l\,2} + 2\Gamma_{12}^{l}\cos\alpha + 1 \qquad \text{S6}$$

with $\Gamma_{12}^{l} = \frac{V_1}{V_2}$ the ratio of the two intersection line displacement velocities. Expressed in the ratio of the growth velocities of the side planes, $\Gamma_{12}^{p} = \frac{R_1}{R_2}$ one obtains

$$\Gamma_{12}^{p\,2}\left(\frac{\sin\beta_2}{\sin\beta_1}\right)^2 + 2\Gamma_{12}^{p}\left(\frac{\sin\beta_2}{\sin\beta_1}\cos\alpha\right) - \frac{\sin^2\alpha}{\sin^2\varphi} + 1 = 0. \qquad \text{S7}$$

Now using the HEWL values $\beta_1 = 72.21°$, $\beta_2 = 90°$ and $\alpha = 108.72°$ one obtains the quadratic equation

$$1.1029\Gamma_{12}^{p\,2} - 0.6740\Gamma_{12}^{p} + \left(-\frac{0.8970}{\sin^2\varphi} + 1\right) = 0 \qquad \text{S8}$$

Solving this equation gives the expression of the relative growth rates of the adjacent faces (1-10) and (101) as a function of sector boundary slope φ (equation 1 in the main text)

$$R_{\{101\}} / R_{\{1-10\}} = \Gamma_{12}^{p} = 0.3056 + \sqrt{\frac{0.8133}{\sin^2\varphi} - 0.8133}. \qquad \text{S9}$$

**Hypothesis for the complete rejection of F-BSA from BSA crystals**

Observation: BSA labeled with a highly negatively charge fluorophore (Fig. 6) is completely segregated from growing BSA crystals.

Background: BSA has a pKa of 4.7 [S1], hence carries a net negative charge under the applied crystallization conditions (pH 6.5-7). According to spectroscopic and MALDI-TOF analysis, about one fluorophore is covalently bound in F-BSA. Thus F-BSA also carries a net negative charge in the crystallization solution, but has an additional negative lump at the site of the label.

Hypothesis: In our experiments, we used fatty acid-free BSA and therefore the 7 available fatty acid (FA) pockets are essentially FA-free in our experiments (Fig S4).

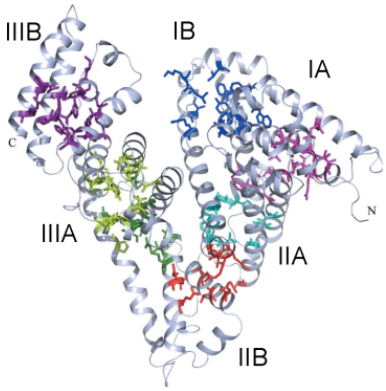


***Fig S5 (a)BSA monomer with the main domains I, II and III and their A and B subdomains. FA pockets coloured FA1, blue; FA2, pink; FA3, green; FA4, light green; FA5, violet; FA6, red; FA7, cyan. (Reproduced with permission of the International Union of Crystallography[40])***

It was shown that during BSA crystallization, PEG fragments and sulfate groups occupy fatty acid pockets on "A" subdomains in the macromolecule[40]. The FA1 which lies on the upper side of the molecule in IB is thus the only possible *major* free position for binding with the fluorophore (Lys114, Lys116, Lys127, Lys136, Lys173). The unit cell of BSA crystals was shown to have an asymmetric unit of 2 BSA monomers that lie head to head. This means that an F-BSA with a fluorophore hanging at that side not only increases the local negative charge and causes repulsion, but also would perturb the packing within the crystal lattice**.**

**Additional References**

S1 Ge, Shouren, et al. "Bovine serum albumin adsorption onto immobilized organotrichlorosilane surface: influence of the phase separation on protein adsorption patterns." *Journal of Biomaterials Science, Polymer Edition* 9.2 (1998): 131-150.