

s8b.m5.o1 **Protein Crystals: Growth Dynamics, Impurity Incorporation and Imperfections.** A.A. Chernov and B.R. Thomas, *Universities Space Research Association, 4950 Corporate Drive, Ste. 100, Huntsville, AL 35806, USA; alex.chernov@msfc.nasa.gov; bill.thomas@msfc.nasa.gov*

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Similarities and differences between protein and small molecule crystals in intermolecular binding, basic phenomena and parameters determining growth dynamics (growth modes, step sources, step and surface energies, kinetic coefficients, steric factor, dehydration barrier), and thermodynamics (solubility) will be discussed quantitatively on the basis of new atomic force microscopy data¹ and other experiments extending earlier findings².

New approach to the growth step propagation for the lattices including several molecules in the not equivalent positions in the unit cell shows existence of potential barrier for one-dimensional nucleation on a straight step. Classical kink concept should be adjusted to these widely spread types of crystal lattices.

Factors deteriorating crystal quality - stress and strain, mosaicity, molecular disorder - will be reviewed with emphasis on impurities³. It will be discussed how impurity deteriorate quality of inorganic and protein crystals. From our experimental findings, the not cross-linked lysozyme and, probably other protein crystals, do not show any signs of plasticity. Therefore the impurity - induced stress should be resolved during the growth process. Impurities induce mosaic block rotations around axes within the growing crystal face. Dimeric ferritin, lysozyme and acetylated lysozyme molecules, are microheterogeneous, i.e. nearly isomorphic, impurities that are shown to be preferentially trapped by ferritin and tetragonal lysozyme crystals, respectively. The distribution coefficient, K , defined as a ratio of the (impurity/protein) ratios in crystal and in solution is a measure of the trapping⁴. For acetylated lysozyme, $K = 2.15$ or 3.42 for differently acetylated lysozyme molecules, and is found to be independent of both the impurity and the crystallizing protein concentrations. The reason is that impurity flux to the surface is constant while the growth rate rises with supersaturation⁵. About 3 times lower dimer concentrations in space grown ferritin and lysozyme crystals were found⁴. Depletion of solution with respect to isomorphic impurities around a growing crystal may be K times deeper than that with respect to the crystallizing protein. This impurity depletion zone concept may partially explain better quality of some crystals grown in space (~20% as compared to their terrestrial counterparts).

s8b.m5.o2 **Nucleation of protein crystals: structures, dynamics, and control pathways.** P.G. Vekilov^{*,†}, O. Galkin[†], S.-T. Yau[†], ^{*}*Department of Chemistry, and* [†]*Center for Microgravity and Materials Research University of Alabama in Huntsville, Huntsville, Alabama 35899, USA*

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We have used atomic force microscopy (AFM) *in situ* during the crystallisation of the protein apoferritin from its solution to image, for the first time, the arrangement of the molecules in near-critical clusters that are larger or smaller than the crystal nucleus and are representative of its structure. In the supersaturation $\Delta\mu/k_B T$ range of 1.1 - 1.6, the nuclei contain 30-50 molecules. The molecular arrangement within the nuclei is similar to that in the crystal bulk. Contrary to the general belief, the observed nuclei are not compact molecular clusters, but are planar arrays of about 10-20 rods of 5-7 molecules set in one or two monomolecular layers.

We have studied the dynamics of nucleation of crystals of the model protein lysozyme using a novel technique that allows direct determinations of homogeneous nucleation rates. At constant temperature of 12.6 °C we varied the thermodynamic supersaturation by changing the concentrations of protein and precipitant. We found a broken dependence of the homogeneous nucleation rate on supersaturation that is beyond the predictions of the classical nucleation theory. The nucleation theorem allows us to relate this to discrete changes of the size of the crystal nuclei with increasing supersaturation as (10 or 11) \rightarrow (4 or 5) \rightarrow (1 or 2). This selection of the critical nuclei sizes is not compatible with existing models assuming compact structure of the nuclei.

Furthermore, we found that the existence of a second liquid phase at high protein concentrations strongly affects crystal nucleation kinetics. We show that the rate of homogeneous nucleation of lysozyme crystals passes through a maximum in the vicinity of the liquid-liquid phase boundary hidden below the liquidus (solubility) line in the phase diagram of the protein solution.

We found that glycerol and polyethylene glycol (PEG) (that do not specifically bind to proteins) shift this phase boundary and significantly suppress or enhance the crystal nucleation rates, although no simple correlation exists between the action of PEG on the phase diagram and the nucleation kinetics. This provides for a control mechanism which does not require changes in the protein concentration, acidity and ionicity of the solution. The effects of the two additives on the phase diagram strongly depend on their concentration and this provides opportunities for further tuning of nucleation rates

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