# Dynamic theory of protein crystallization

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New *"dynamic theory of protein crystallization (DTPC)"* considers protein crystallization as a competitive process between different *"protein-protein adhesion modes (PPAM)"* mutually incompatible in molecular stacking into the crystal lattice. Large surface of protein molecules offers variety of different adhesion modes and only some of them are compatible in a single crystal form. The DTPC searches for the crystallization conditions supporting spontaneous preference of a dominant protein-protein adhesion mode and suppression of all adverse adhesion modes in the newly formed solid phase. The DTPC is based on the concept of PPAM giving us tools for manipulation with protein adhesion and allowing the intuitive design of experiment to increase the number of crystallizable proteins, to choose rationally the desired polymorph form and to increase the resolution of protein structures. The necessary condition for formation of single crystal is its growth according to the *„principle of the dominant adhesion mode (PDAM)"*. It provides non-conflicting explanation for all available experimental observations regarding the protein crystallization and also for catalysis of crystal growth on heterogeneous substrates [1]. In a very simplified form, the necessary condition for the successful crystal growth can be written as a high difference of free energies of the competitive processes $\Delta F_{cryst} \sim F_{dominant\ AM} - \Sigma F_{incompatible\ AM}$

This offers the experimenter a rational way to grow diffraction quality crystals and also to select the required crystalline form. The new approach changes the situation significantly and leads to enhancement of efficiency and accuracy of all standing crystallization methods. DTPC with PDAM are general and should be strictly respected by any method of protein crystallization.

*Heterogeneous crystallization.* Historically, there were different explanations why some heterogeneous materials initiate protein crystallization. Here, we propose the universal explanation why some materials are suitable for crystal initiation and other not. The DTPC explains efficiency of crystallization catalyzers (e.g. bioglass, coarsely wrinkled foils, nano-carbon materials, imprinted polymers, porous Si, hoarse hairs, properly coated nanotubes and nanostructured carbon black [1]) as follows. The specific adhesion in depressions in the substrate surface leads to identical orientation of protein molecules. Thus, it restricts an access to their adhesive surfaces responsible for incompatible PPAM. It enforces a unique PPAM in the growing crystal nuclei. They are more stable because of lower number of stacking faults. Therefore, they do not dissolve and can continue to grow even after being released into the seemingly under-saturated bulk solution. Contrary to a number former mutually contradicting explanation, this explains all available experiments on a unique common basis. By active control of crystallization process in slightly under-saturated conditions, one can rationally limit crystal formation to cavities only. The new insight promises better design of natural and artificially prepared crystallization catalyzers promising an increase in a number of crystallizable proteins and higher resolution in structure determination.

*Homogeneous crystallization.* Also here, one should look for the systems providing the highest difference between free energies of mutually exclusive protein-protein adhesion modes. The temporary molecular clusters formed in the overcrowded crystallization solution play here an important role. The adhesion properties of the protein molecule in these complexes may differ radically from the adhesion properties of the original molecule. If the crystallographer knows the rules for the formation of these temporary complexes, he can control preferences of the adhesion modes active in the emerging crystal. He can decide which of the mutually exclusive protein-protein adhesion modes succeeds and becomes dominant by using his knowledge of the adhesion modes between the target protein and the *„protein-surface-active molecules (PSAM)"*. Very reach and natural source of the adhesion mode examples is the PDB offering a deep inshight how the *„protein surface shielding agents"* work in practice and how the *„crystal structure forming elements"* help in finding the best crystal architecture [2]. If the crystallographer fails in suppressing the mutually incompatible adhesion modes, the result cannot be quality crystal. Thus even very good precipitation agent can be a bad crystallization agent, if it does not differentiate among adhesion modes of the target protein.

*Classical theories of protein crystallization* and majority of papers on protein crystallography concentrated in last decades to an efficient and rapid precipitation. Low attention was given to reasons why the growing solid phase is regular and to the fact that protein crystallization is a competitive process between different adhesion modes. The abstract notion "*adhesion mode"* introduced by the DTPC gives the experimenter new tools controlling the crystallization and increasing predictability in protein crystallization methods.

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