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Precipitant-less crystallization of protein molecules induced by substrate with heterogeneous topography and surface potential gradient

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Surfaces having heterogeneous topography are known to facilitate protein crystallization by diminishing the energy barrier for nucleation. A precipitant is, nevertheless, required for shielding the charges on protein molecules, so that they can overcome electrostatic repulsion and self-assemble into crystalline structure. For yet-to-be crystallized proteins, zeroing on the right precipitant is a non-trivial problem and therefore, despite existence of several heterogeneous nucleant surfaces, number of proteins that are yet to be crystallized continues to be large. In this talk, we will present a novel surface which is decorated with nanoscopic patterns and also surface charges, the combined effect of which show the remarkable ability to induce crystallization without use of any precipitant. This effect is demonstrated by crystallizing several protein molecules with molecular weight ranging from 14-450 kDa. These surfaces are shown to induce also directed crystallization of a specific protein from a mixture of two or more protein species and even simultaneous crystallization from a mixture of proteins. In essence, these surfaces consist of nanoscopic wrinkles with spatially varying curvature and surface charges. Kelvin probe force microscopy (KPFM) measurements show that surface potential gradient as high as 140 V/μm are generated on these surfaces which can drive largescale molecular ordering in the liquid at the vicinity of the surface. As a result, the surface itself acts both as a precipitant also as a nucleant. Heterogeneity allows it to crystallize protein molecules having large range of radius of gyration, that too at low to moderate concentration of the protein in respective solutions. The prospect of precipitant-less crystallization of protein is expected to open up several possibilities in the areas of disease diagnosis, drug discovery, drug delivery and protein engineering.

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