

of nucleation and control it, and in situ X-ray diffraction of crystals will be examined in this overview lecture. Conclusion: There is no universal solution in the search for good crystals. If anything, the search has become more difficult as more challenging targets are attempted. A multi-faceted approach is therefore required.

Keywords: protein crystallization strategy, automation, screening

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Photochemically induced nucleation of protein

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An important area of post-genomic research is the determination of 3-D protein structures. The main technique used for this purpose is X-ray crystallography. Protein crystallization experiments are carried out in the presence of crystallization agents, e.g., inorganic salts, non-adsorbing polymers, and alcohols, which reduce protein solubility and increase intermolecular interactions to form cluster, nucleus and crystal. In these experiments, account needs to be taken of the protein concentration, nature and concentration of the crystallization agent, pH, buffer constitution, and temperature. Here, we demonstrate photochemically-induced crystallization of metastable protein solutions by weak UV irradiation for several ten seconds. Intermediates, neutral radicals at tryptophan or tyrosine residual produced by one photon absorption, enhance nucleation. The radical forms protein dimer that is detected by an SDS-PAGE electrophoresis experiment. An addition of polyethylene glycol (PEG) greatly enhances light-induced nucleation. PEG affects to shorten the intermediate radical lifetime, which suggests that PEG assists to form dimer. We consider that the photochemical dimer behaves as smallest cluster to grow critical nucleus. The smallest cluster formation is the rate determining step in classical nucleation theory due to surface energy disadvantage. The photochemical dimer is formed by a covalent bond, and the nucleation is initiated from stable dimer. The nucleation enhancement is reasonably explained. The present researches results point out the development of a new method for controlling nucleation and growth that could be applied structural genomics and pharmaceutical industry for instance.

Keywords: photochemistry, protein, crystal growth

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Applications of designed ankyrin repeat proteins as chaperones in structural biology

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Repeat proteins are ubiquitous protein-protein interaction molecules in biology [1]. We made use of this feature in vitro and designed ankyrin repeat proteins (DARPin) which consist of repeat modules with fixed framework residues and randomized surface residues suitable for target binding. The random assembly of such modules yields combinatorial libraries of DARPins of varying length and large

diversities. DARPins are very stable, soluble and produced in large amounts by bacterial expression. By using ribosome display highly specific binders against different protein targets with low nanomolar affinity can be selected. This opens the possibility to crystallize a target protein in complex with a variety of DARPins and therefore enhances the chance of obtaining structures of target proteins that are difficult to crystallize. We selected DARPins having high affinity and specificity for proteases, kinases and membrane proteins and used them for cocrystallization of the target protein. The methodology and the X-ray structures of a DARPin-MBP- [2], a DARPin-kinase- [3], a DARPin-caspase-2- [4] and a DARPin-membrane protein-complex [5] have been published. This illustrates the usefulness of this novel technology in structural biology. It opens a new avenue in macromolecular crystallization and is an attractive alternative to antibodies for the crystallization of membrane proteins. In addition, highly specific DARPin binders and/or inhibitors of target proteins allow the analysis of the role of this protein in signalling pathways.

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Keywords: crystallization, designed proteins, membrane proteins

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A simple method to introduce anomalous scatterers in a wide number of proteins

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A recent Protein Data Bank survey indicated that more than 5500 X-ray crystal structures contain at least one Sulfate ion and the total number of X-ray crystal structures is approximately 43000 therefore approximately 13 % of the total structures contain Sulfate. Since Sulfate ions are predominantly introduced during the crystallization step, we have taken advantage of a simple substitution in the crystallization reagent. Sulfate (SO₄) was substituted with Selenate (SeO₄) during the crystallization of two model proteins known to crystallize in SO₄. Crystals were obtained in similar conditions and diffracted to similar resolution. Their SAD structure were determined solely relying on anomalous scattering from SeO₄. One structure was determined from the peak energy and the second from the high energy remote.

Keywords: protein, phasing, selenium

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Dynamic light scattering in protein crystallization: Analysis and optimization

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