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The use of electric and magnetic fields to improve the crystallization of protein crystals is not new. Different techniques have been discussed in the literature [1], [2], [3]. We will review these techniques and present new results obtained through the application of electrical fields using a novel device easily reproduced in the laboratory environment. The new device uses transparent conductive electrodes made by the deposition of ITO onto glass slides, and can easily be used in AFM scanning and topographic investigations. X-ray diffraction results will be compared to low magnetic fields attainable in the laboratory. In the end the question is if low electric or magnetic fields have an effect on crystal quality.

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Keywords: electric field, magnetic field, protein crystal quality

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Random microseeding: a theoretical and practical exploration of the Microseed Matrix-Screening (MMS) method, with new recommendations for achieving crystallization success.

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Douglas Instruments has completed theoretical and practical studies of Microseed Matrix-Screening (MMS), a powerful approach to protein crystallization, introduced by Ireton and Stoddard in 2004 [1]. The method was automated by D'Arcy et al. [2], who first used seeding with random screening kits. Experience shows that MMS with random screens generates more hits or better-diffracting crystals in about 75% of cases where at least one crystal can be obtained.

Our studies included analysis of the stability of seed crystals suspended in unconventional stock solutions and the effectiveness of seed crystals from novel sources. This has given rise to new recommendations for maximizing the number of hits, reducing the prevalence of salt crystals, increasing the diversity of crystal forms, and harvesting seed crystals from microfluidic devices. We also investigated nucleation with microporous glass, zeolites, precipitates and mixtures of crushed crystals of proteins that were unrelated to the target protein. These were less effective than conventional MMS, but may still be useful - they can of course be used before the first hits have been found. Throughout the project, the statistical significance of experiments was increased by focusing on "pregnant" conditions - defined as conditions that reliably gave crystals when seeds were present, but which otherwise gave no crystallization.

References: [1] Ireton and Stoddard*, Acta Crystallographica* section D60 (**2004**)*, 601–605*; [2] D'Arcy et al. *Acta Cryst* D63 ***(2007)***.

Keywords: crystallization-1, microseeding-2, random-3

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Fluorescence anisotropy-based macromolecule crystallization screening

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Screening for protein crystallization is typically a highly digital process, with the screening results being interpreted as yes (a crystal) or no (no crystal). This approach leads to the routine setting up of hundreds or thousands of screening experiments to obtain the ever elusive crystal. However, there may be many screening outcomes that are close to crystallization conditions but which are discarded from subsequent consideration as they gave a clear or an amorphous precipitate result. A potentially better approach would be to first determine those conditions which are proximal to those necessary for crystallization, then concentrate on them for subsequent optimization rounds.

We are developing a fluorescence anisotropy-based screening method for just this purpose. The method measures the concentration-dependent changes in the rotational rate of the macromolecule in response to the test screening conditions. Difficulties in reproducibly pipetting sub-µL volumes led to inclusion of the intensity data as a quality check, and it rapidly became apparent that intensity also increased with likely, and found, lead conditions. The method is currently implemented using 1536 well plates, assay solutions consisting of 3 µof a solution composed of 0.6 µof protein solution and 2.4 µof precipitant solution. Eight solutions are used for each precipitant concentration; 1 buffer bland and 7 protein concentrations, typically ranging from 0.2 to 4.0 mg/mL. We are at present working on speeding up the data acquisition process (it currently takes ~1.5 hrs to acquire a data set) and reducing the assay solution volume, with our target volume being in the 10-20 nL range.

Keywords: screening, rapid, fluorescence

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Structural and biochemical characterization of HP0315 as a VapD protein with an endoribonuclease activity from *Helicobacter pylori*

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Virulence associated protein D (VapD) found from various organisms, but not much information about VapD is known. Recently, the paper about novel ribonuclease family of Cas2 was reported and mentioned the relationship with VapD (1). Here, we show the first structure of a member of VapD and raise a theory about relationship among VapD, Cas2 family and toxin-antitoxin (TA) systems. The crystal structure of HP0315 from *Helicobacter pylori* was solved at 2.8 Å resolution with a modified ferredoxin-like fold. The structure of HP0315 is very similar to that of Cas2 family. Like Cas2 proteins, HP0315 has an endoribonuclease activity. HP0315 cleaved mRNA just before A and G nucleotides preferentially, which means that HP0315 has a purine specific endoribonuclease activity. Mutagenesis studies of HP0315 revealed several residues are important for RNase activity. HP0315 is arranged as an operon with HP0316 which recently was turned out to be antitoxin-related protein. However, HP0315 is not a component of TA system. HP0315 could be explained as an evolutionary

intermediate which does not belong to any of Cas2 family and toxin-antitoxin system.

Keywords: HP0315, VapD, endoribonuclease

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Crystal Structure of the dimerization domain of human filamin A

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By crosslinking actin filaments, filamins play important roles in regulating the dynamics of the actin cytoskeleton which plays a central role in many cell functions such as the maintenance of cell shape, cell division, adhesion, motility, signal transduction and protein sorting. Consistent with this, mutations in human filamin genes are associated with a wide range of developmental abnormalities and defective neuronal migration. And filamins as integrators of cell mechanics and signalling by interacting with transmembrane receptors and cytosolic signaling proteins.

In humans, three filamin isoforms have been identified: filamin A, filamin B, filamin C. Of these, filamin A (FLNa) is the most abundant and widely expressed. Heterozygous null FLNa alleles result in defective neuronal migration causing periventricular heterotopia, while certain FLNa missense mutations cause familial cardiac valvular dystrophy and putative gain-of-function mutations result in a spectrum of congenital malformations generally characterized by skeletal dysplasias.

Human vertebrate filamins are homodimers of two 280kDa subunits, and each subunit contains an N-terminal actin binding domain consisted of two calponin homology domains followed by 24 tandem repeat domains (FLNa1-24) that are interrupted by flexible hinge regions between FLNa15 and FLNa16 and FLNa23 and FLNa24. Dimerization through FLNa24 is crucial for the actin-crosslinking function of filamins.

We report the structure of FLNa domain 24 (FLNa24), and compare the structure with FLNc24 and discuss how dimerization is formed in FLNa24.

Keywords: filamin A

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Protein crystallization with zeolite

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X-ray crystallography is one of the most powerful techniques for the three-dimensional structural determination of proteins. The high-resolution crystal structures allow us to study in great detail the relationship between structure and function of proteins. However, production of high-quality protein crystals is still a major bottleneck in structural biology [1]. Although the sparse matrix crystallization screening is widely used in protein X-ray crystallography, coarse and unadjustable samplings of this screening often fail in obtaining high quality of crystals especially in the case of proteins with poor crystallizability. In order to overcome this difficulty, a new effective methodology for improving crystal quality is needed.

Recently, we have reported an advanced crystallization method using synthetic zeolite molecular sieves (MS) as hetero-epitaxic nucleants by which a directed crystal nucleation on the material surface occurred in a variety of proteins, thereby providing new crystal forms with a substantial improvement of diffraction quality in some cases [2], [3].

In this study, the hetero-epitaxic nucleant method using MS was applied to the sparse-matrix crystallization screening of 20 protein samples, which provided formation of larger single crystals with better diffraction quality as compared with the conventional screening. In current protein crystallography, available sample amount for crystallization trials is limited especially when the expression level of the target protein is low. In such cases, a minimum set of sparse matrix crystallization screening in the presence of MS may be the most effective way to achieve the diffraction quality of protein crystals.

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Keywords: protein, crystallization, zeolite

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Crystallization of macromolecules in selenate to solve the phase

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Previously, the crystal structures of two model proteins were determined by the single anomalous diffraction method using scattering from selenium. Selenium was incorporated to the protein during crystallization substituting sulfate (ammonium- or sodium-), one of the most used precipitants in protein crystallization with selenate (sodium-).

The crystal structures of porcine pancreatic elastase (PPE) and proteinase K (ProtK) were solved at 1.6 Å and 0.94 Å respectively taking advantage of anomalous scattering from one SeO_4 site. A single data set was recorded slightly above the selenium absorption K edge at 12.67 keV for PPE while atomic resolution data were recorded at higher energy, 14.50 keV in the case of ProtK. In both examples, high quality experimental electron density maps were computed and the structures were solved solely relying on SAD phases from the selenate ion. In these two samples, the selenate ion occupies the exact same site as the sulfates in the reported native structures.

Additionally, a soaking experiment indicates that proteinase K crystallized in sulfate-free solution can be soaked in a cryo-solution containing SeO_4 and that the structure can be solved using Se-SAD phasing. Crystallographic data were recorded on a sulfate-free and a sulfate sample at low energy (7.1 keV and below) to confirm the absence and presence of SO_4 from S-SAD experiments. Those low energy experiments were also used to better estimate absorbed dose per sample at different energies.

Out of 63000 protein crystal structures currently available, 8500 contain at least one sulfate ion which represents more than 13 % of the total X-ray crystal structures. This quick, easy and cost-effective substitution method offers an adequate alternative to heavy atom search or seleno-methionine labeled protein production in structural biology and structural genomic. It requires minimum additional work in the work flow, exactly a single complementary step during the production of crystals.

Finally, this method was successfully applied to solve the structure of a “quasi” new structure.

Comparisons low/high energies experiments relying on S/Se atoms will also be discussed.

Keywords: sulfate, selenate, phasing