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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

MS92.P04

Acta Cryst. (2011) A**67,** C766

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

MS92.P05

Acta Cryst. (2011) A**67,** C766

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

MS92.P06

Acta Cryst. (2011) A**67,** C766-C767

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