MS1-P7 **Microseed it! A Theoretical and Practical Exploration of Seed Stability and Seeding Techniques for Successful Protein Crystallization** Patrick D. Shaw Stewart[a] Stefan A. Kolek[a], Richard A. Briggs[a], Naomi E. Chayen[b], and Peter F. M. Baldock[a] *[a]Douglas Instruments, Douglas House, East Garston, Hungerford, Berkshire, RG17 7HD, U.K.,[b]Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, London SW7 2AZ, U.K.*
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Random Microseed Matrix-Screening (rMMS), where seed crystals are added automatically to random crystallization screens, is a significant recent breakthrough in protein crystallization[1]. One industrial group has used the method to solve 38 out of 70 structures generated, finding particular success with antibody complexes[2] . rMMS not only produces more hits, it also generates better-diffracting crystals - because crystals are more likely to grow in the metastable zone[3].

The theory and practice of the rMMS method will be described with case studies, and studies of novel approaches to rMMS by Douglas Instruments[4] will also be presented. The use of the method with membrane proteins and complexes will be described. Finally, a simple and novel experimental design for optimizing seeding levels will be presented (this design also has applications for reshuffling the ingredients of several crystallization hits).

[1] Allan D'Arcy, Frederic Villarda, May Marsh. 'An automated microseed matrix-screening method for protein crystallization' (2007). *Acta Cryst* **D63** 550–554.
[2] Microseed Matrix Screening Crystallization of Antibody Fragments and Antibody-Antigen Complexes (2011). *RAMC, Strasbourg, France. Galina Obmolova, Biologics Research,CentocorR&D*
[3] Further information on the theory and practice of the MMS method is available at the Douglas Instruments web-site, http://www.douglas.co.uk/mms.htm
[4] Patrick Shaw Stewart, Stefan Kolek, Richard Briggs, Naomi Chayen, and Peter Baldock. Random Microseeding: A Theoretical and Practical Exploration of Seed Stability and Seeding Techniques for Successful Protein Crystallization (2011).*Crystal Growth Des*.,**11**, 3432-3441.

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MS1-P8 **Structure of UvrA nucleotide excision repair protein in complex with modified DNA.** Elżbieta Nowak, Marcin Jaciuk, Krzysztof Skowronek, Anna Tańska, Marcin Nowotny. *International Institute of Molecular and Cell Biology, Warsaw, Poland.*
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DNA damages are structural abnormalities which must be removed for the organism to survive.One of the primary mechanisms to achieve this is Nucleotide Excision Repair (NER) pathway. Its main feature is the ability to recognize and repair a wide spectrum of different DNA lesions. In bacterial NER system damage detection is carried by UvrA protein. Its role is to locate the site of the DNA lesion. The DNA is then handed over to UvrB which verifies the presence of the damage. UvrC nuclease next excises the DNA fragment containing the lesion.

In order to elucidate the mechanism of DNA damage detection, we solved a crystal structure of *T. maritima* UvrA protein in complex with a modified DNA [1]. In the structure, the DNA is bound in a cleft running across the UvrA dimer. The protein binds the DNA duplex in its terminal regions on both sides of the modification site. The DNA is deformed - bent, stretched and unwound. Only this deformed conformation is complementary with the protein surface. Since these types of deformation are often observed in various modified DNAs, we propose, that UvrA uses them for indirect readout of the presence of the damage. The protein not only senses the deformations of the DNA caused by the lesion but it may also adjust them, so that the duplex fits to the protein surface. It is facilitated by the fact that modified DNA duplexes are more flexible.

Binding of the damaged DNA activates the ATPase activity of UvrA which is thought to be the signal that the DNA lesion was found and downstream proteins can be recruited. Based on a comparison of our UvrA-DNA structure with the previously determined structures of UvrA proteins without the DNA bound we proposed the mechanism for the ATPase activation.

[1] Jaciuk, M., Nowak E., Skowronek, K., Tańska, A., Nowotny, M. (2011). *Nat Struct Mol Biol.* **18(2)**, 191-197.

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