MS1-01 **Record Resolution Protein Crystals Using an Efficient Convection-free Frowth Geometry**. Elias Vlieg,[a] Alaa Adawy,[a] Etienne Rebuffet,[b] Susanna Törnroth-Horsefield,[b] Willem de Grip,[a] Willem van Enckevort,[a] *[a]Radboud University Nijmegen, The Netherlands, [b]University of Gothenburg, Sweden*
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The growth of high quality single protein crystals, yielding the highest X-ray resolution, remains a bottleneck in macromolecular crystallography. Convection-free conditions should lead to improved crystal quality, because in such a case growth is slow and the formation of defects and the incorporation of impurities minimized. Convection-free conditions have been achieved in the microgravity environment of space and in magnetic fields, but the impact on protein crystal growth has been limited due to the experimental difficulties with these methods.

Here we show that through a laboratory-based setup [1], an entirely convection-free crystallization environment is achieved, which enhances the purity and crystallinity of protein crystals. This is accomplished by using an upside-down geometry, where crystals grow at the 'ceiling' of a growth cell completely filled with the crystallization solution. The 'ceiling crystals' experience the same diffusion-limited conditions as in space microgravity environments. The new method was tested on bovine insuline and two hen egg-white lysozyme polymorphs. In all cases, ceiling crystals diffracted X-rays to resolution limits beyond their current world records, even while solutions of moderate purity were used. The structure for bovine insuline was solved to a resolution of 1.6 Å (compared with the previous record of 2.25 Å). The enhanced purification in the ceiling geometry is further demonstrated by comparing the crystal morphology and the fluorescence from impurities between normal batch-grown and ceiling crystals.

[1] Pooth, P. et al (2009). *Cryst. Growth & Design,* **9**, 885-888.

Keywords: crystal growth; diffusion-limited growth; X-ray resolution

MS1-02 **Producing stable membrane protein for structural studies.** Bernadette Byrne[a] *[a]Imperial College London, South Kensington, London SW7 2AZ, UK*
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Despite major advances over the last decade, the production of membrane proteins for structural studies remains problematic. Many membrane proteins are highly unstable in detergent solution. The protein isolation process is often complicated by significant losses through aggregation during purification. It is often difficult to obtain a fully homogeneous preparation and in addition, pure membrane protein often exhibits low conformational and thermal stability.

In my group use a number of different strategies to identify the most stable constructs and buffer compositions. In the case of the eukaryotic transporter, UapA, we have adopted a rational mutagenesis approach in combination with heated fluorescent size exclusion chromatography [1]. In contrast, in the case of $A_{2A}R$, a G-protein coupled receptor, radioligand binding analysis was used to facilitate the identification of the most stable construct and guide development of an efficient isolation protocol [2].

Other recent work in my group has focused on altering the detergent used rather than the protein. To this end we have been involved in the characterisation of a range of different detergents. The best known of these are the maltose neopentyl glycol (MNG) detergents which contain a tetra-substituted central carbon atom bonded to two hydrophilic groups and two lipophilic chains [3]. These dramatically increase the stability of a range of different membrane proteins compared to standard detergents. In addition the use of these detergents has facilitated the crystallisation of a plant transporter.

[1] Leung et al. (2012) Stabilizing the heterologously expressed uric acid-xanthine transporter UapA from the lower eukaryote *Aspergillus nidulans. Mol. Memb. Biol.* In press.
[2] Singh et al (2010) A purified C-terminally truncated human Adenosine A_{2A} receptor construct is functionally stable and degradation resistant. *Prot. Expr. Purif.* 74: 80-87.
[3] Chae et al. (2010) Accessible new amphiphiles for solubilization and stabilisation of membrane proteins. Nature Methods 7: 1003-8.

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