## MS04-O5

### Prototyping protein expression constructs with PCR, cell-free expression and fluorescence detection size exclusion chromatography

Martin Bommer[1], Udo Heinemann[1]

1. Max-Delbrueck-Centrum, Berlin, Germany

**email: martin.bommer@mdc-berlin.de**

Scouting of soluble expression constructs containing a predicted functional domain is time-consuming and appears a gamble. Further, the only direct product is often a size exclusion chromatography trace or the result of a pull-down interaction experiment. The choice of domain boundary, possibly expression tag and host cell grows into a labyrinth of pathways to be navigated by repeated manual cloning, expression and purification trials.

Yet, to allow advances in the most challenging and worthwhile targets, such as integral membrane protein receptors, ingenious methods have been developed to reduce complexity and pre-screen protein production: Cell-free expression [1] circumvents some difficulties encountered during in vivo protein expression and is easily carried out in parallel and at microliter scale. Fluorescence detection size exclusion chromatography (FSEC) [2] of proteins side-steps large-scale cultivation and purification. The green fluorescent protein (GFP)-tagged protein of interest is specifically detected within in a complex mixture.

We sought to combine these methods for the screening of soluble, interaction-competent expression constructs: Candidate gene fragments were amplified and joined to an upstream T7 promoter + solubility tag and a downstream GFP + T7 terminator by overlap-extension PCR (as is used in gene synthesis). The linear PCR products were templates for a commercial cell-free expression kit, which may simply be set up as a hanging drop experiment. Approx. one microliter protein solution was used for each quantification, FSEC or an initial microscale thermophoresis experiment. FSEC traces in the absence or presence of excess unlabelled binding partner provided convincing qualitative results.

We describe a simple setup using off-the-shelf reagents. A wealth of recent literature describes improvements, scale up and add-on experiments.

References:

[1] Haberstock et al. (2012) Protein Expr. Purif 82, 308-316

[2] Kawate, T. & Gouaux, E. (2006) Structure 14, 673-681

**Keywords: protein expression, fluorescence, in vitro**

# MS05 Structural information in drug design

**Chairs: Dr. Rob van Montford, Prof. Andreas Heine**

## MS05-O1

### X-ray free electron laser: opportunities for drug discovery

Michael Hennig[1], Robert Cheng[1], Rafael Abela[1]

1.LeadXpro AG, Villigen, Switzerland

**email: michael.hennig@leadxpro.com**

Past decades have shown the impact of structural information derived from complexes of drug candidates with their protein targets to facilitate the discovery of safe and effective medicines. Despite recent developments in single particle cryo-electron microscopy, X-ray crystallography has been the main method to derive structural information. Recently, serial crystallography has added new opportunities for X-ray data collection of challenging systems like membrane proteins. Advances in serial crystallography are a pre-requisite to use the unique properties of X-ray Free Electron Laser (XFEL). Unmet peak brilliance and beam focus allow X-ray diffraction data recording and successful structure determination from smaller and weaker diffracting crystals shortening timelines in crystal optimization. To further capitalize on the XFEL advantage, innovations in crystal sample delivery for the X-ray experiment, data collection and processing methods are required. This development was a key contributor to serial crystallography allowing structure determination at room temperature yielding physiologically more relevant structures. Adding the time resolution provided by the femtosecond X-ray pulse will enable monitoring and capturing of dynamic processes of drug molecule binding and associated conformational changes with great impact to the design of candidate drug compounds.

References:

[1] Weinert, T., et al. Serial millisecond crystallography for routine room-temperature structure determination at synchrotrons. Nature Communication, 8:542 (2017).

[2] Cheng, K.Y.R., Abela, R., Hennig, M. X-ray Free Electron Laser: opportunities for drug discovery. Essays in Biochemistry (2017) 61, 529-542.

**Keywords: Drug design, X-FEL, serial crystallography**