## MS02 O1

**The Oxford Protein Production Facility; a biomedically driven structural proteomics project**
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**Keywords: high throughput, protein production, Crystallization**

High throughput sequencing of eukaryotic, viral and bacterial genomes is providing a huge database of proteins with potential for structure-function analysis. In response to this opportunity, structural proteomics projects, including the Oxford Protein Production Facility have been established focused on high throughput (HTP) structure determination. Crucial to this effort has been the development of technologies for HTP protein production and crystallization. For all projects, there has been an emphasis on parallel processing driven by the need to accommodate relatively large numbers of potential targets at an acceptable cost. The OPPF, in common with most other groups has set up semi-automated liquid handling systems to carry out some of the protocols for protein production [1] and crystallization [2]. However, many of the methods can equally well be carried out manually with appropriate equipment e.g. multi-channel pipette dispensers. The motivation to implement automation is largely to enable processes to be scaleable and sustainable as error-free operations. Target selection in the OPPF is focused on human proteins and those of human pathogens, both viral and bacterial, selected for their direct biomedical relevance. Recent technical developments in OPPF to address some of these challenging targets will be reviewed.

[1] Berrow N, Alderton, D., Sainsbury, S., Nettleship, J., Assenberg, R., Rahman, N., Stuart, DI, Owens, RJ*, Nucleic Acids Res*. 2007 March 12, doi:10.1093/nar/gkm047.
[2] Walter TS, Diprose JM, Mayo CJ, Siebold C, Pickford MG, Carter L, Sutton GC,Berrow NS, Brown J, Berry IM, Stewart-Jones GB, Grimes JM, Stammers DK, EsnoufRM, Jones EY, Owens RJ, Stuart DI, Harlos K. (2005) Acta Crystallogr D Biol Crystallogr. 61: 651-7.

## MS02 O2

**Application of high throughput ligand screening to promote protein crystallization**
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**Keywords: Structural genomics, thermal stability, ligand screening.**

Structural genomics efforts have led to the expression of thousands of proteins, many of which have not been purified or characterized previously. Identification of small molecules that bind to and stabilize these proteins can promote their crystallization as well as provide valuable functional information. We have employed differential static light scattering (DSLS) and differential scanning fluorimetry (DSF) to investigate the thermostability and ligand binding specificity of our protein targets. Optimum buffer conditions which further stabilized the aggregating or hard to concentrate proteins often resulted in more soluble proteins which were further concentrated. Presence of identified ligands in many cases resulted in crystallizing those hard to crystallize proteins and improving crystal quality leading to structure determination. Screening different members of families of proteins against customized libraries of compounds also resulted in binding profiles for each protein and providing the opportunity to compare small molecule binding specificity of different members of each family of protein. The screening results facilitated the comparison of substrate specificities and also identified compounds which appeared to be general inhibitors for each of these protein families. Moreover, other compounds were discovered that only bind to a subset of proteins in each family of proteins and thus appear to discriminate among different members of the family.

## MS02 O3

**Split-GFP as a Tool for Finding Soluble Protein Domains**
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**Keywords: solubility reporter, domain trapping, structural genomics.**

The high-throughput detection and quantification of proteins *in vivo* and *in vitro* remains challenging. To help address these issues, we have developed a quantitative, fluorescent universal protein tagging and detection system based on self complementing fragments of the Green Fluorescent Protein (GFP) [1,2,3]. These fragments originally poorly folded have been engineered for improved solubility and complementation. Using a set of control proteins, we validated the ability of the system to quantify soluble and total protein expression *in vitro* and *in vivo*.
As part of our structural genomics initiatives on *Mycobacterium tuberculosis**, we applied this system to the identification of soluble domains of large multi-domain proteins that failed to crystallize. We have developed a new domain trapping strategy which includes several selection steps, enabling to reduce progressively the throughput and increase the fidelity of our screening process. The first step consists of selecting DNA fragments containing in frame inserts regardless of protein solubility. This is achieved using a DHFR insertion construct which confers bacteria survival in the presence of selecting agent only when “in frame” fragments are cloned in the DHFR scaffolding. We applied the split-GFP tagging system to select soluble clones from the selected pool of “in frame” variants. The most soluble clones are sorted by a first solubility screening *in vivo.* A second screening *in vitro* enables the selection of a smaller subset of domains that will be further characterized by DNA sequencing to determine precise boundaries. We demonstrate the ability of the ORF-selector combined with the split GFP solubility screen for creating repertoires of soluble protein fragments from several intractable proteins and genomes.