s1.m1.o2 **Generation of Phosphorylated Proteins for Structural Studies.** Tim Johnson, *Laboratory of Molecular Biophysics, Dept. of Biochemistry, University of Oxford. E-mail: tjohnson@biop.ox.ac.uk*

Keywords: Kinase; Phosphorylation; Protein

Reversible protein phosphorylation constitutes a major regulatory mechanism of signalling in eukaryotic cells. The covalent attachment of phosphate to Ser/Thr or Tyr residues of proteins may lead to local or global conformational change, with resultant modulation of enzymatic catalysis and/or the production or masking of protein-protein interaction sites. Such sites may regulate the formation of protein complexes of importance for the transduction of signal or modulate cellular localisation. Given the central role of phosphorylation in eukaryotic cellular signalling, the determination of the structural basis for phosphorylation-mediated conformational change, catalytic regulation and protein complex formation is of significant interest. To this end, methods for the specific, homogeneous phosphorylation of proteins for structural analysis are required.

In vitro methods for protein phosphorylation add an extra step of manipulation, which may lead to increased sample heterogeneity and contribute to it proving refractory to crystallisation. Furthermore, the parallel purification of the required kinase(s) is also required which may lose activity and lead to sub-stoichiometric phosphorylation, contributing further to sample heterogeneity. Here we outline methods used for the specific, stoichiometric phosphorylation of proteins for structural analysis through the use of single and multiple vector co expression systems in E. coli. The model system used focuses on the *in vivo* phosphorylation of the cell cycle regulatory kinase Cyclin-dependent kinase 2 through co expression with its respective upstream regulatory kinases.

s1.m1.o3 **Differences in the Folding Robustness of Two Variants of Green Fluorescent Protein.** Jean-Denis Pédelacq, *Los Alamos National Laboratory, Los Alamos, NM 87545 - USA. E-mail: jpdlcq@lanl.gov*

Keywords: Crystallography; Folding; Green Fluorescent Protein

Green fluorescent protein (GFP) is widely used as a tool for studying protein trafficking, protein localization, and gene expression. The wild-type GFP folds poorly when expressed in *E. coli* (1) and even enhanced versions of GFP still exhibit folding defects. For example, the F64L+S65T variant of the commonly used cycle-3 GFP, termed "folding reporter GFP", misfolds and is only weakly fluorescent when expressed as a fusion with poorly folded proteins (2).

We have engineered a more robust version of GFP, termed “superfolder GFP”, which contains six mutations. This specific variant is useful *in vivo* for high-throughput screening of protein expression levels. Thirty-six proteins from *Mycobacterium tuberculosis** were expressed in *Escherichia coli* as fusions with either the folding reporter or superfolder GFP variants. The fluorescence of the GFP folding reporter fusions was correlated with the non-fusion solubility of the proteins expressed alone, as previously reported (2, 3). In contrast, the fluorescence of GFP superfolder was well correlated with the total whole cell expression.

Using 1.07 Å synchrotron radiation, complete, highly redundant data sets were collected for the folding reporter and superfolder GFP variants, to a resolution of 2.5 Å and 1.45 Å, respectively. Structural comparison between the two variants revealed some structural changes in the vicinity of two mutations that certainly benefit the overall stability of the β-barrel structure. Amazingly, the same mutations were found to have the most profound impact towards the increased folding robustness of GFP, according to refolding kinetics experiments.

* *Mycobacterium tuberculosis* Structural Genomics Consortium (http://www.doe-mbi.ucla.edu/TB/DB/)

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