Advice on **Protein Purification**

- Q: How much protein is needed for crystallisations?

A: With 0.5 mg total protein and a concentration of 10 mg/ml, the volume of the protein solution (0.05 ml) allows the screening of at least 192 crystallisation conditions using a minimum volume of 0.2 microlitres protein solution per condition. If less protein is available, then the number of conditions is reduced accordingly. Nevertheless, a 48-condition screen can sometimes give valuable information for potential crystallisation conditions or even crystals – if lucky enough. In extremely difficult cases where protein is scarce, different crystallisation techniques such microfluidics can be considered.

- Q: What about purity?

A: The protein should be as pure as possible but, in general, purity >95% in an SDS PAGE is a great starting point. 90-95% purity is acceptable but it may reduce the chances of crystallisation. Other criteria such as monodispersed, stability (auto-proteolysis, denaturation), electric charges, post-translational modifications are also important in judging the quality of the protein.

- Q: What buffers are preferable to keep the purified protein?

A: In general, we avoid phosphate buffers as phosphate can easily form salts with metals used in crystallisations. This can create high hopes in the beginning if you discover crystals, but you will feel very disappointed later on when you find out that you have crystallised a salt (for example magnesium or calcium phosphate). Highly diluted buffers at neutral pHs are OK, providing that the protein is stable. If the protein requires ligands or metals, then inclusion of them in the storage buffer should be considered.

- Q: My protein contains a tag. Is that good or bad for crystallisations?

A: Tags such as 6XHis can interfere with crystallisations because of their flexibility. However, proteins may still be able to crystallise. In general, a tag-containing protein which produces no crystals should have the tag removed. Thus, expression systems that produce tag-fusion proteins with cleavable sites are highly recommended. If the tag is another large protein (e.g. glutathione transferase or maltose-binding protein), then it should be removed before any crystallisations start. In some difficult cases, though, the presence of the fusion partner has been reported to improve the changes of crystallisation.

- Q: My protein is insoluble and forms inclusion bodies. What shall I do?

A: The positive side is that the inclusion bodies are rich in protein and therefore they themselves constitute a very effective purification step. However, your protein is in an aggregated and not properly folded form and thus not suitable for further studies. It should be treated with denaturating agents such as urea or guanidine hydrochloride and then left to refold properly. Several protocols are available in our Unit for denaturation and refolding of the expressed proteins.