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Accelerating protein production: High efficiency tags, fusions and automated purification

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The protein production section of the Beatson Institute's Drug Discovery Program supplies a considerable number of highly purified and active recombinant proteins for structural biology, biophysical and biochemical applications. Pressure to satisfy high demand has prompted the development of novel and innovative methods to streamline workflow maximize output and ensure rapid delivery of critical proteins. Driving up soluble yields, ensuring productive cell culture and reducing the burden on purification resources can enhance the output of a protein production facility. We have substantially improved our productivity by implementing a series of new bacterial MBP fusion vectors. These systems substantially elevate soluble expression but also co-express a protease that removes the MBP tag *in vivo*, affording the benefits of MBP fusion on soluble yield but eliminating the MBP moiety from downstream purification. As an extension of this, we have developed several systems that allow the monitoring of recombinant expression during fermentation, using an auto-cleaved fluorescent protein tracer. This allows the operator to infer adequate target expression in near real-time, ensuring that cultures allowed occupy fermentation resource lead to satisfactory product yields. We have also accelerated productivity by reducing the number of chromatography steps in common protocols and automating FPLC purification. Taking our inspiration from several emerging and newer multimodal chromatography technologies, we have developed rapid, mixed chemistry affinity strategies to purify tandem tagged proteins. This allows the isolation of high purity target in a single column, often removing the need for slow size exclusion chromatography to polish the product. Finally we have implemented routine multidimensional chromatography on our AKTA AVANT systems performing complex sequences of protein purification and conditioning steps with a minimal requirement for user intervention. The net result is more reliable and frequently higher yield preps, delivered to the downstream user in the shortest possible time.

Biography

Christopher H Gray has obtained his BSc and PhD from the University of Glasgow, examining the biochemical basis of multidrug resistance in pathogenic fungi. He has then moved to the Institute of Cancer Research in London to gain experience as an X-ray Crystallographer where he was trained in a diverse range of protein production techniques. Following this he took up a position at the CRUK Beatson Institute becoming an early Member of Staff in the Institute's Drug Discovery Program. As a Structural Biology Team Leader he has day to day responsibility for the protein production, NMR, biophysics and X-ray Crystallography in the program.

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