# Protein purification

![AKTA Explorer FPLC system; Example elution profile using a multi-step gradient.](image)

**Simple Description**
Molecules of interest are separated by differential interaction with a chromatography matrix via an affinity tag or based on the charge, hydrophobicity or the size of the protein.

Use of the AKTA system allows elution with a gradient (of ligand, salt or pH) to give better purification than step elution. Monitoring the eluate by UV absorbance shows when all unbound material has been washed off, before starting the elution. Using two (or more) columns exploiting different properties of the protein gives a product with higher purity and better stability by more thoroughly removing any proteases. Often an affinity step will be followed by ion-exchange or hydrophobic interaction chromatography. A final gel filtration step “polishes” the prep to remove any aggregated protein.

**Use this technique when…**
Use the AKTA for further purification if you have an affinity-tagged protein, such as His6, GST or MBP which you may have purified by step elution, perhaps by hand using syringes. Pure proteins are required for structural analysis by crystallography and NMR spectroscopy as well as assays such as SPR, AUC and many others.

**Related techniques**
Our HPLC equipment gives a complementary set of purification options.

**Practical Considerations**
The most common starting material will be cells over-expressing a recombinant protein, but any mixture can be further purified. Lysing cells by sonication or using a pressure cell followed by centrifugation gives an extract which can be loaded onto a chromatography column. Collected fractions can be analysed or loaded onto another column for further purification if necessary.

We have some columns available – please ask for details. Alternatively, using your own chromatography columns of suitable size and with appropriate functional groups ensures there is no contamination of your sample by material from a previous user.

**Who to talk to**
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