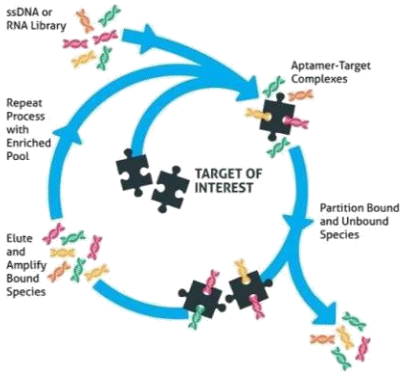
AptaBind: Enabling Improved Protein Purification with Aptamer-Mediated Affinity Chromatography (AMAC)

Aptamer Technology Platform

Aptamers (short, synthetic DNA- or RNA-based molecules) are developed at Aptamer Group under strict laboratory conditions utilising our automated high-throughput selection platform. Aptamers have flexible, sequence specific structures that can form complimentary surfaces to all or part of their target. This structural synergy facilitates strong electrostatic, hydrogen bonding or stacking interactions between the aptamer and its target; the specificity of which underpins AptaBind's AMAC technology. Aptamers are selected from an incredibly diverse library (~10^{15} molecules) by an iterative, systematic process that enriches the library until it consists only of aptamers with the required target binding characteristics.



Target specific aptamers are isolated using a cyclic process that selects only aptamers with the required characteristics.

Challenges with Protein Purification

Proteins are often expressed with fusion-tags (e.g. His tags, FLAG tags, protein fusions etc) for affinity purification and to aid solubility in commercially available plasmid vectors. Although well-characterised and widely used, this method cannot be applied universally. Potential issues include;

- Binding / elution conditions are specific for the tag and not the protein of interest
- Solubility tags can 'mask' target protein behaviour
- Column efficiency often drops with regeneration and reuse
- Non-native protein purification
- Necessitates subsequent cleavage and secondary clean-up

The purification of native proteins on an industrial scale relies on traditional ion exchange (IEX), hydrophobic interaction (HIC) or size exclusion chromatography (SEC). Although these techniques are relatively 'gentle', obtaining the desired level of purity requires many sequential and repeated purification steps.

Fusion Tag	Purification Method	Challenges
Polyhistidine (His_6)	Ni-affinity	Must be carried out above pH 7.5. EDTA or other chelators cannot be used. Limited use of reducing agents.
Glutathione-S-transferase (GST)	GST-affinity	Maximum recovery requires specific conditions. Column efficiency deteriorates.
Strep-tag	Strep-Tactin affinity	Maximum recovery requires specific conditions. Column efficiency deteriorates.
Maltose binding protein (MBP)	MBP affinity	High expression levels can mask target protein behaviour. Purification can be affected by detergents.
Small ubiquitin related modifier (SUMO / His_6-SUMO)	Ni-affinity	High expression levels can mask target protein behaviour. Must be carried out above pH 7.5. EDTA or other chelators cannot be used. Limited use of reducing agents.

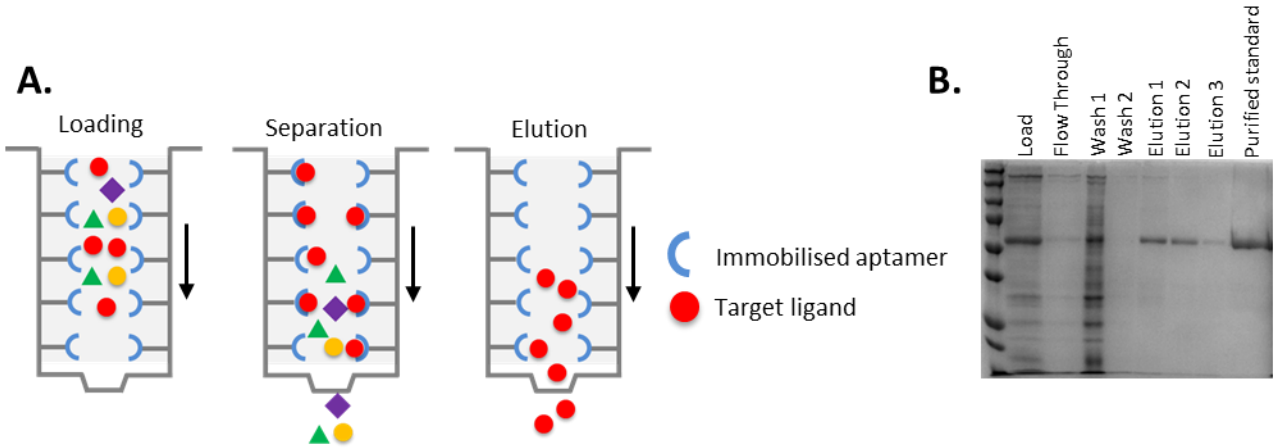
Common fusion tags, their purification strategy and associated challenges.

The Solution: Aptamer-Mediated Affinity Chromatography (AMAC)

Aptamer-mediated affinity chromatography (AMAC) enables purification of proteins in a single step. Aptamers selected to bind to a specific target protein are readily modified to allow subsequent immobilisation on a suitable resin. Protein loading, washing and elution can then be carried out under customer-specified, pre-determined, often mild conditions.

The key features of AMAC are;

- Highly specific binding to the protein of interest
- Tailored binding, washing and elution conditions to maintain protein integrity
- Increased yield through single-step purification
- Modification of aptamers to allow immobilisation on customer preferred resins
- Straightforward column regeneration for repeated use
- Rapid process; novel aptamers can be selected in 10 weeks, functional columns developed in 2 weeks



A) Schematic representation of AptaBind purification.
B) Case Study: SDS-PAGE analysis of a therapeutic protein purified from expression medium using AptaBind aptamers.

AptaBind

In addition to identifying specific aptamers for each target, the AptaBind process isolates only those that bind and release under the desired purification conditions. These AptaBind aptamers are then immobilised onto an appropriate resin for subsequent use in aptamer-mediated column purification.

The specificity of aptamers for their targets ensures that high levels of purity can be achieved from complex medium in a single AMAC step. Elution under pre-determined conditions removes the need for harsh, denaturing reagents often required with other affinity ligands such as antibodies. Mild elution conditions guarantee protein integrity following purification. In addition to these benefits, AMAC does not rely on affinity / solubility tags and therefore offers a simpler workflow for purifying 'native' proteins. Single-step AMAC purification increases the final yield of protein and dramatically reduces the purification time; important considerations for industrial purification applications as production costs can be significantly reduced.

Benefits of AptaBind

Generic purification strategies often involve passing the extracted protein through multiple types of column to achieve the desired purity. The broad spectrum of recombinant expression systems (many incorporating solubility and affinity tags), mean that these processes can be applied to many proteins with reasonable results. However, proteins are diverse in nature and properties such as stability, hydrophobicity or low expression levels represent major challenges for the purification of a large number of proteins, even within commercially available systems. In cases where highly pure protein is required, it is not uncommon for 5 or more different column purification steps to be needed. Each column pass has inherent losses that collectively reduce the yield of the final purified product and thereby increase production time and costs.

AMAC addresses these issues though the rapid development of bespoke, high affinity and specific purification reagents, and pre-determined, user-defined elution conditions.