

Overcoming the difficulties in obtaining pure recombinant protein



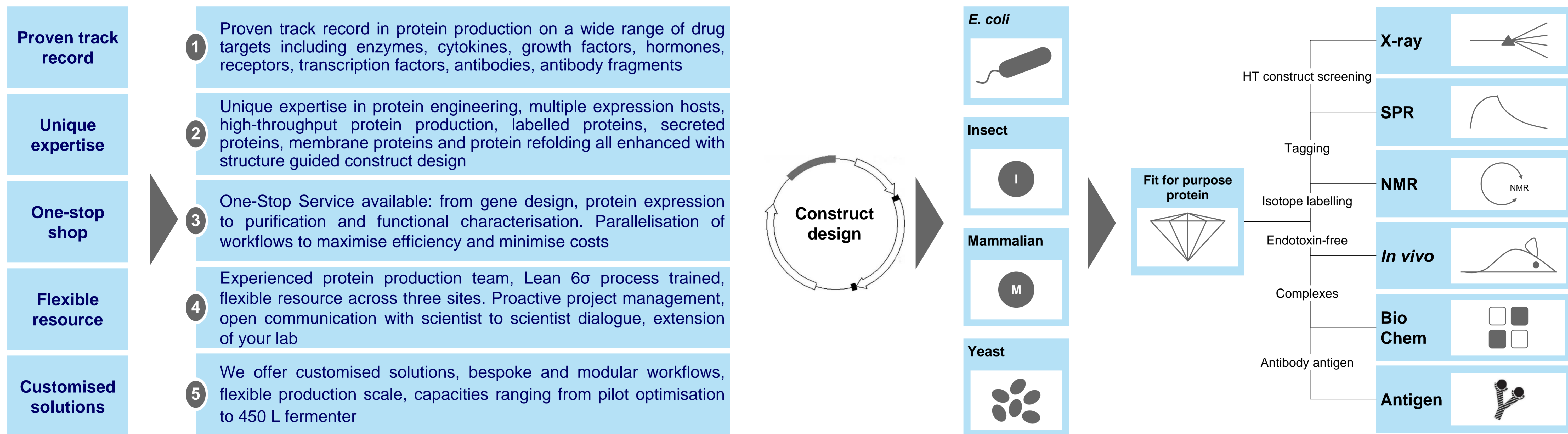
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Summary

Evotec is a recognised leader in pharmaceutical research and offers a comprehensive range of capabilities to support drug design, discovery and development. High quality protein for biochemical assay and structural biology studies is essential for any drug discovery project. A challenging target may require multiple optimisation rounds to obtain soluble, pure and active protein. Here we present diverse methodologies to overcome those challenges through construct design, vector and host selection. We also present the rigorous control methods necessary to ensure the high quality of protein produced for a variety of applications.

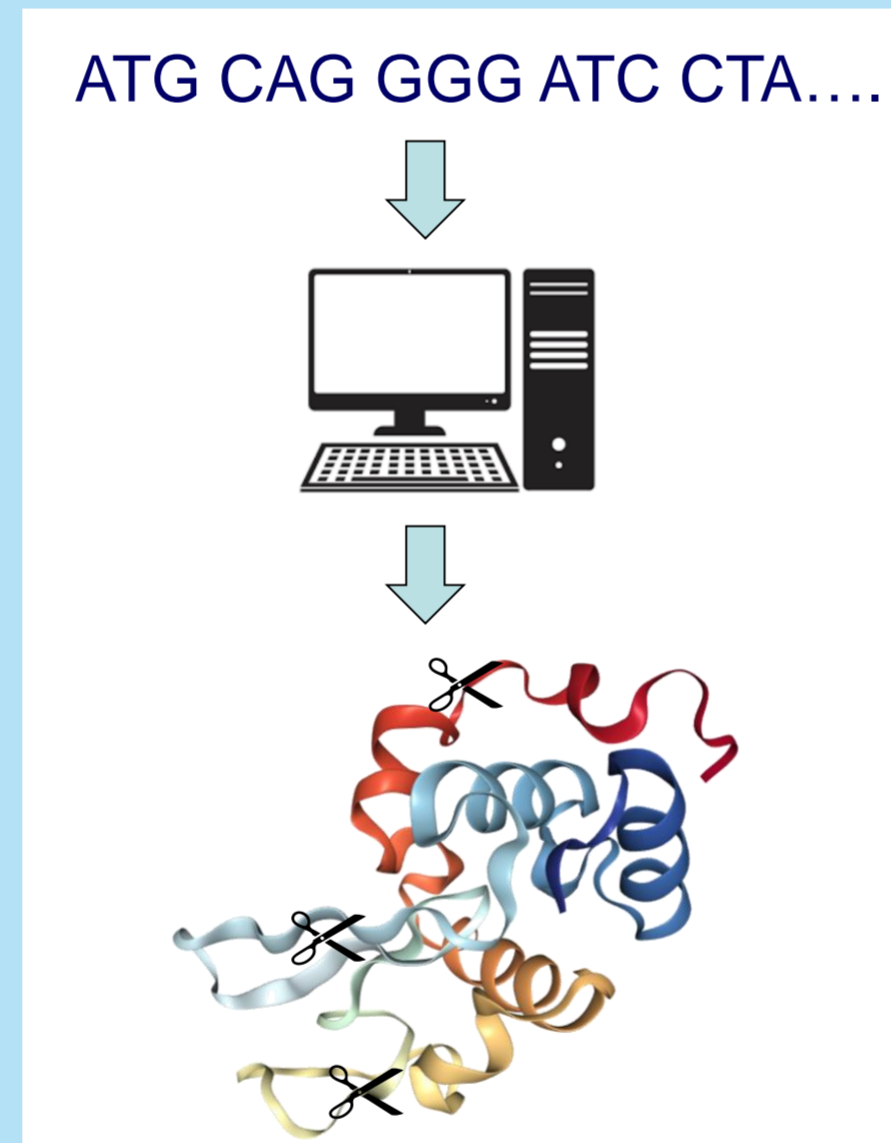
From gene to protein (flexible entry points)



Construct design for improved solubility

Secondary structure	Homology model	Sequence analysis	Panel of new constructs
Domain identification, flexible loops, disordered area etc. using bioinformatics tools	Built using MOE based on known structures pinpoints active site architecture, hydrophobic patches, disulphide bridges etc.	Modifications identified for increased expression, solubility and/or crystallisability	New sequences with new boundaries, tags and mutations

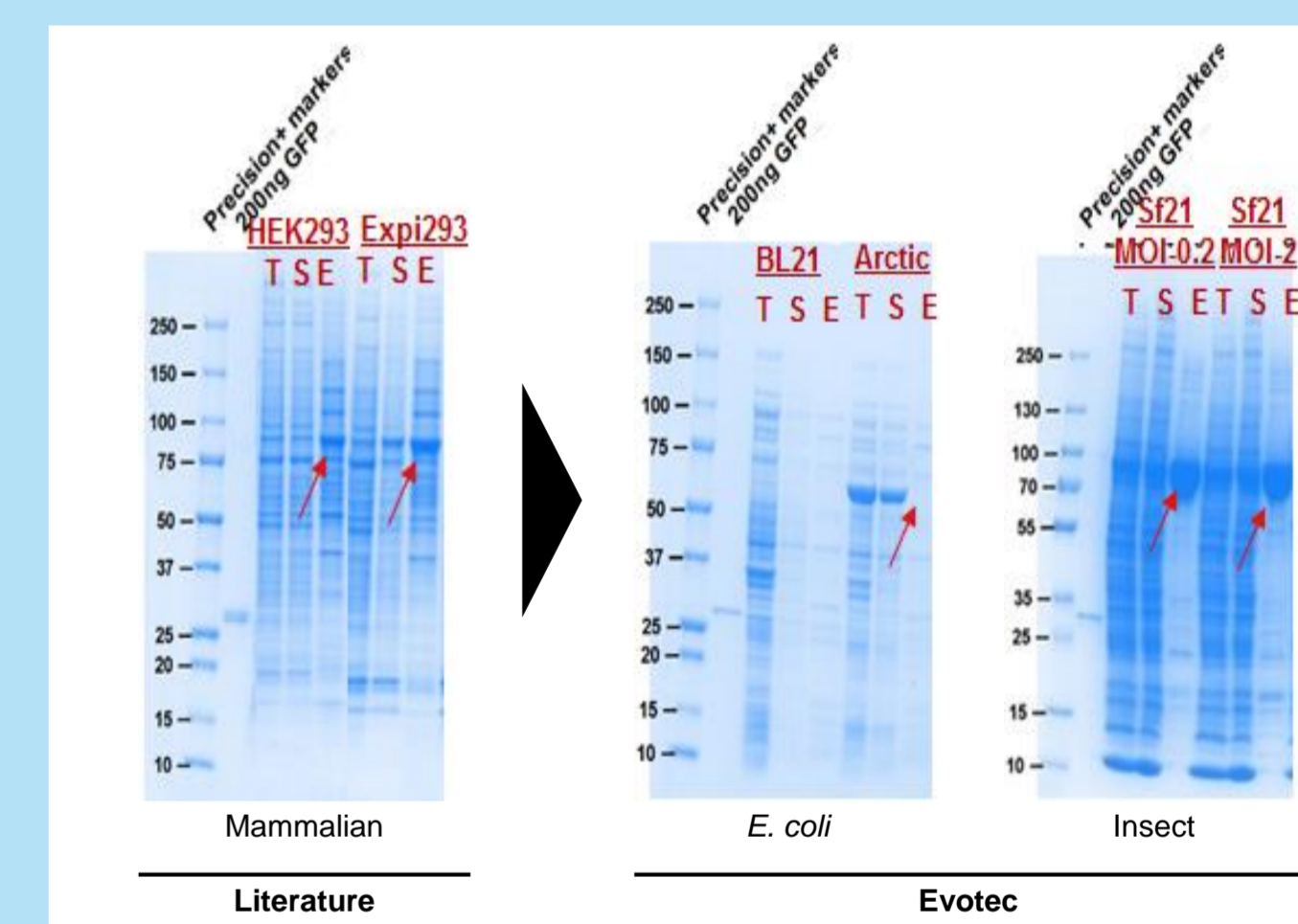
- Minimum input, maximum information output approach.
- Multiple vector selection for various expression strategies/host
- Established high-throughput downstream workflows for construct characterisation
- 1 FTE/month for analysis and design of up to 300 constructs through the above workflow



Expression scouting across multiple hosts

End Use	Target	Starting Point	Outcome	Resource	Timelines
Assays and crystallography	Cytosolic	Construct design	3 mg/L, >90% purity	1 FTE	2 months

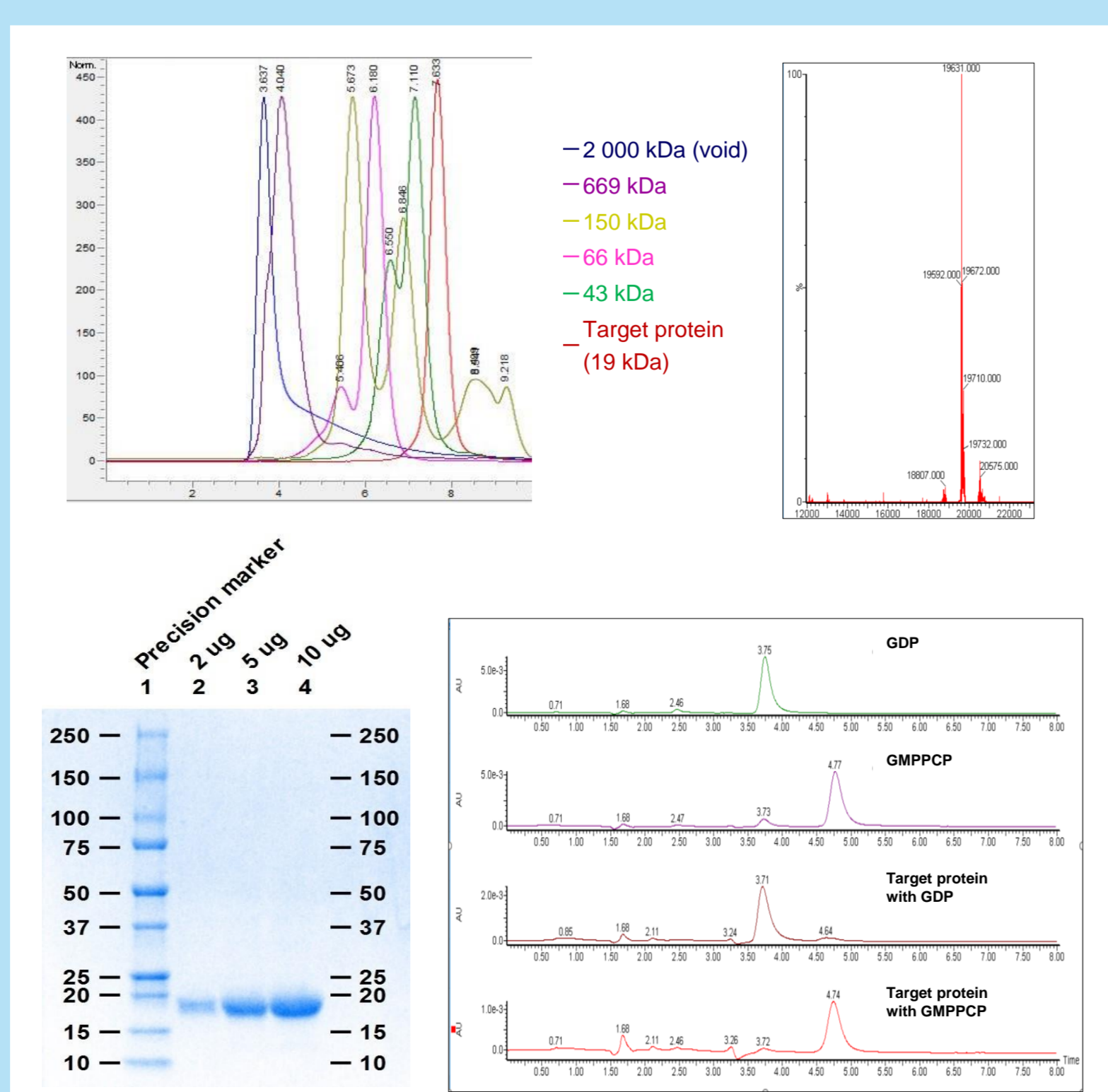
- Aim: Identify expression conditions across 3 hosts
- Expression optimisation carried out with different constructs design, Host, multiplicities of infection and timepoints
- Target showed low expression in mammalian cells and no expression in *E. coli*
- Baculovirus expression system gave high expression
- Protein expression led to successful purification with high yields
- High quality protein enabled multiple assays and shortened project timelines



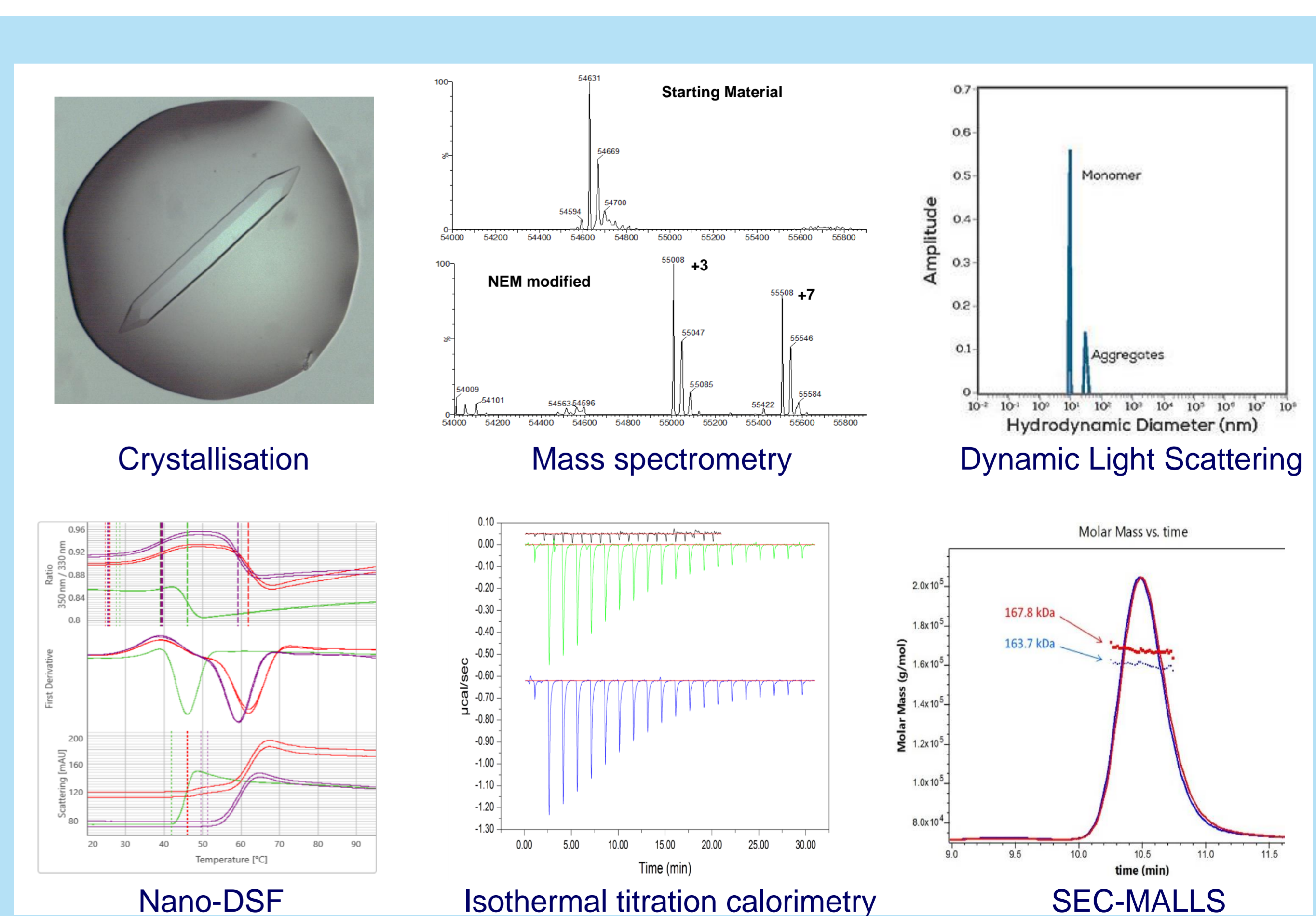
Protein purification development

End Use	Target	Starting Point	Outcome	Resource	Timelines
NMR	Cytosolic	Construct design	>100 mg, >95% purity	1.2 FTE	3 months

- Aim: Produce homogeneous labelled and nucleotide loaded protein for NMR
- Expression optimisation carried out with different tagging strategy and *E. coli* conditions
- Large-scale at 20 L scale using optimum conditions
- Purification refinement carried out with diverse affinity chromatography and size exclusion
- Nucleotide loading boosted to >95%
- QC by Mass Spec, HPLC and aSEC confirmed protein homogeneity and quality



Protein quality control....on top of the traditional



And others: biochemical assays, aSEC, fSEC, CD...

QC extendable to comprehensive biophysics platform and complex assays