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)

Proven track record

1. Proven track record in protein production on a wide range of drug targets including enzymes, cytokines, growth factors, hormones, receptors, transcription factors, antibodies, antibody fragments

Unique expertise

2. Unique expertise in protein engineering, multiple expression hosts, high-throughput protein production, labelled proteins, secreted proteins, membrane proteins and protein refolding all enhanced with structure guided construct design

One-stop shop

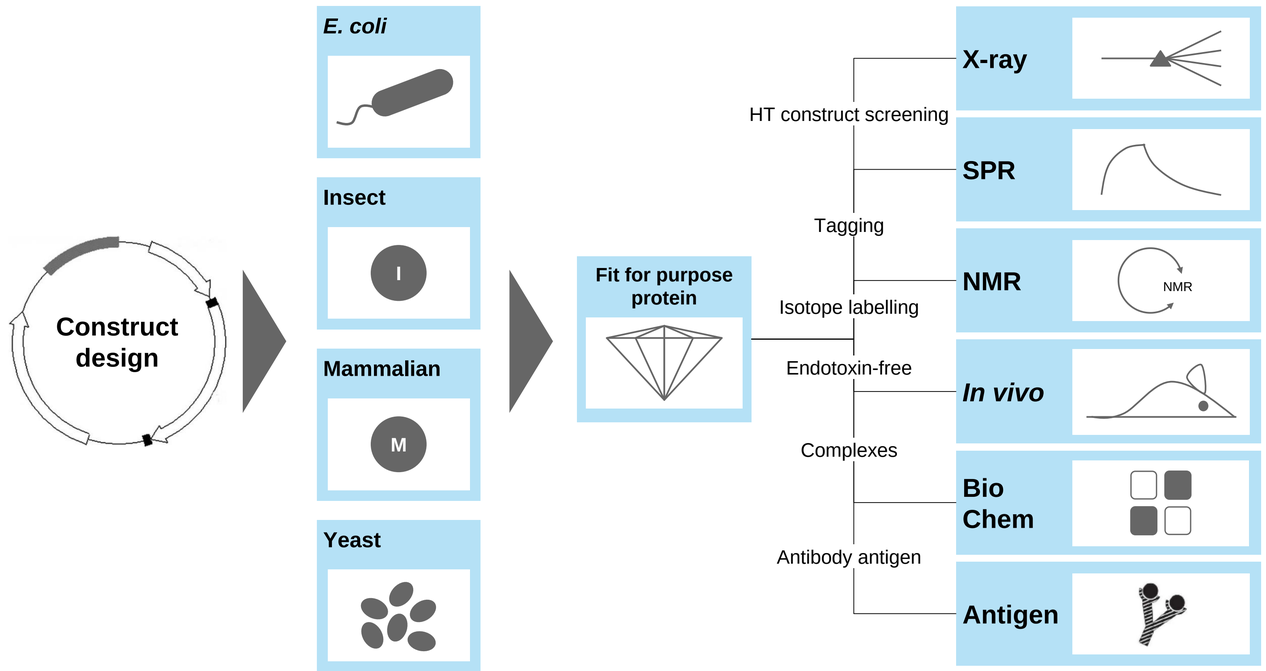
3. One-Stop Service available: from gene design, protein expression to purification and functional characterisation. Parallelisation of workflows to maximise efficiency and minimise costs

Flexible resource

4. Experienced protein production team, Lean 6σ process trained, flexible resource across three sites. Proactive project management, open communication with scientist to scientist dialogue, extension of your lab

Customised solutions

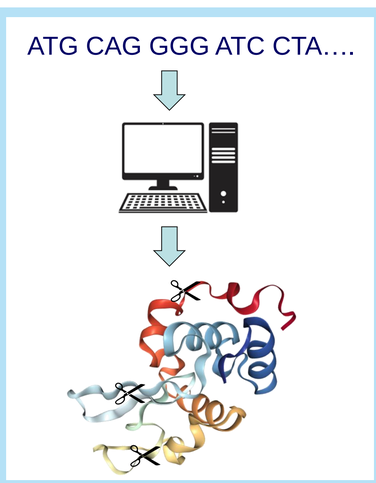
5. We offer customised solutions, bespoke and modular workflows, flexible production scale, capacities ranging from pilot optimisation to 450 L fermenter



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

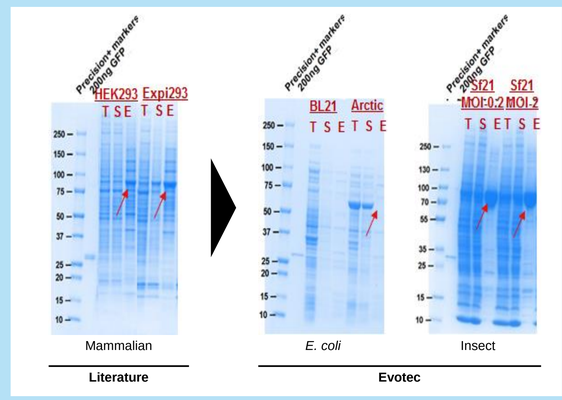
- Minimum input, maximum information output approach.
- Multiple vector selection for various expression strategies/host
- Established high-throughput downstream workflows for construct characterisation
- 1 FTE/month for analysis and design of up to 300 constructs through the above workflow



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

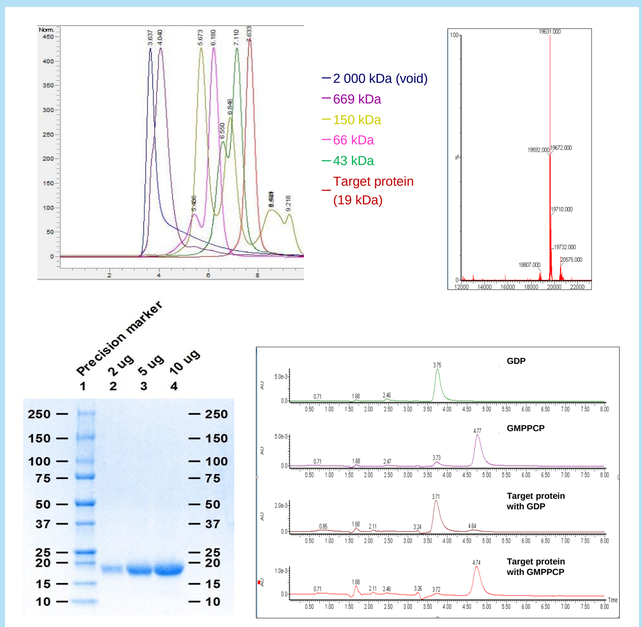
- Aim: Identify expression conditions across 3 hosts
- Expression optimisation carried out with different constructs design, Host, multiplicities of infection and timepoints
- Target showed low expression in mammalian cells and no expression in *E. coli*
- Baculovirus expression system gave high expression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



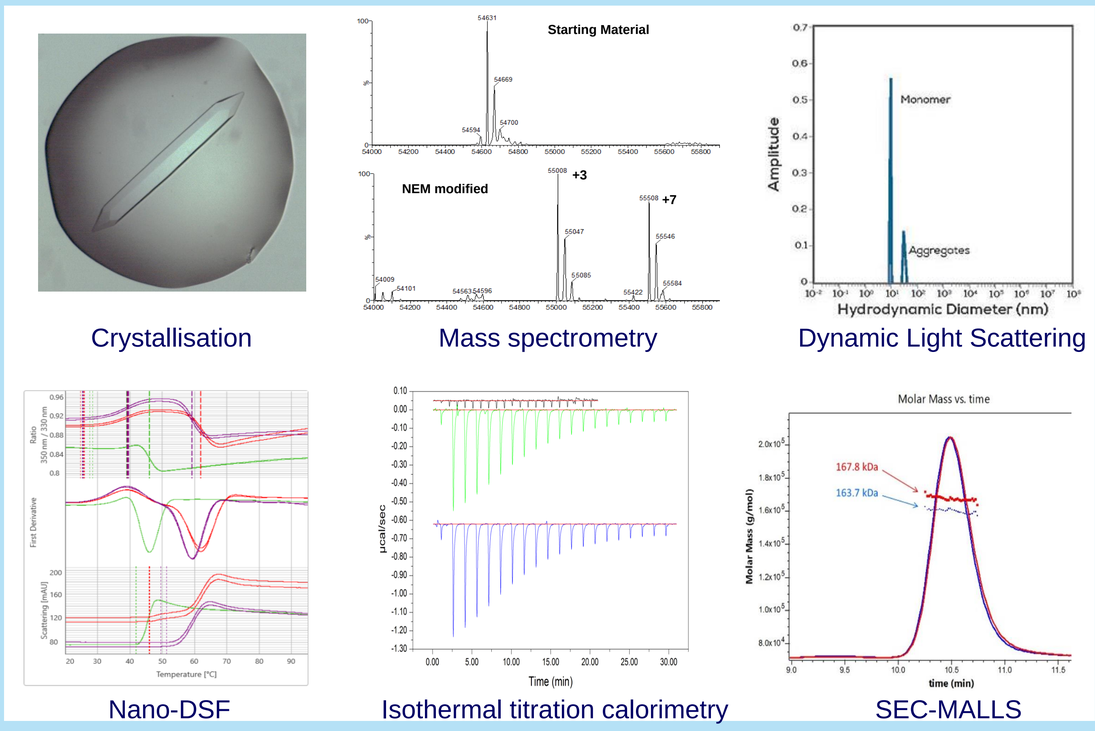
Protein purification development

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



Crystallisation | Mass spectrometry | Dynamic Light Scattering

Nano-DSF | Isothermal titration calorimetry | SEC-MALLS

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

QC extendable to comprehensive biophysics platform and complex assays

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