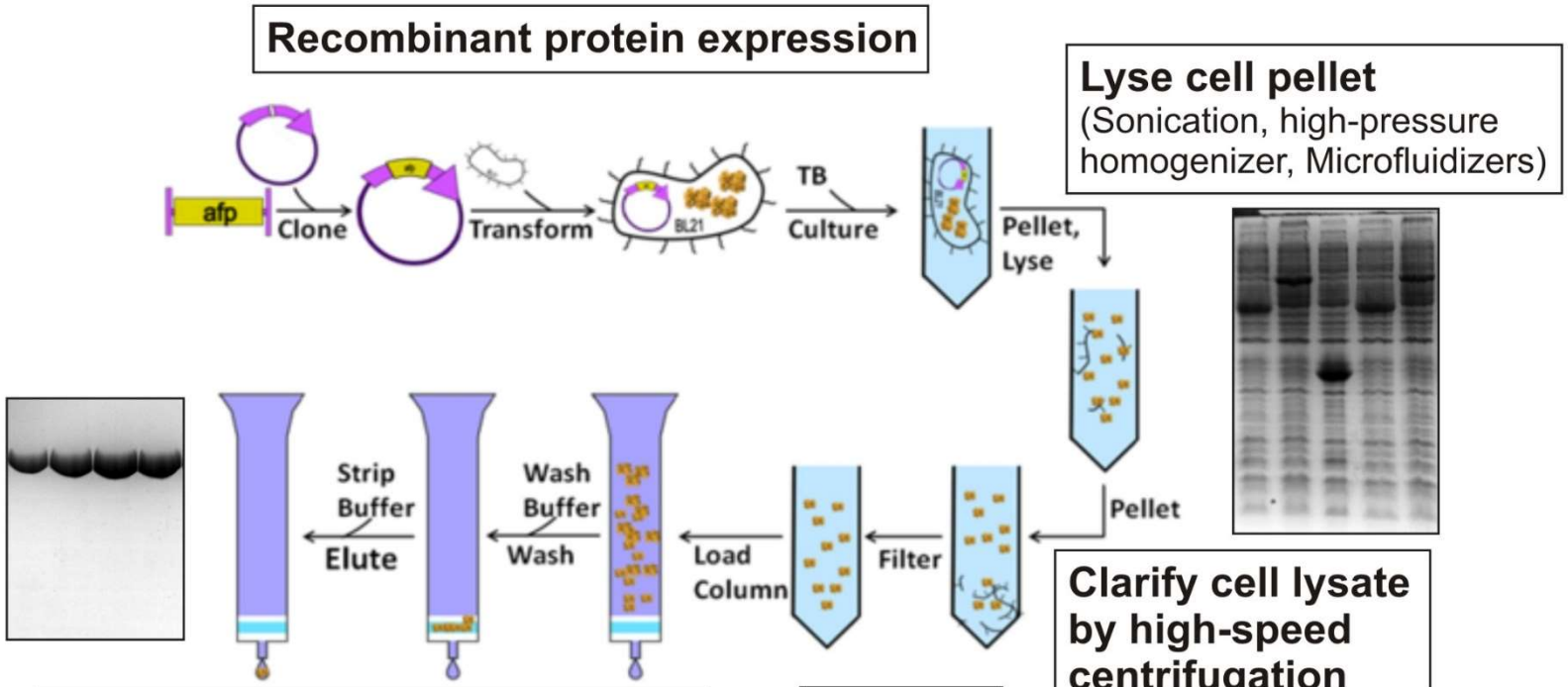
Protein purification methods

Concepts
Methods
Applications

Recombinant protein purification: step by step

The aim of a purification procedure is to obtain a highly pure and stable protein at an appropriate concentration in a buffer compatible with the intended application.



Chromatography columns in protein purification

Chromatography is the most powerful and commonly used means of purifying recombinant proteins. Each technique separates proteins based on different properties, so it is often advantageous to combine several types to maximise separation of the recombinant protein from host cell proteins.

Technique	Stage	Description
Affinity Chromatography (AC)	Capture or Intermediate	Based on a reversible interaction between the protein/affinity tag and a specific ligand
Ion Exchange Chromatography (IEX)	Capture or Intermediate	Separates proteins based on their net surface charge
Hydrophobic Interaction Chromatography (HIC)	Intermediate	Binding under high salt conditions, generally performed following an ammonium sulphate precipitation step
Size Exclusion Chromatography (SEC)	Polishing	Separates proteins based on their hydrodynamic volume (size)
Reverse Phase Chromatography (RPC)	-	High-resolution chromatography based on weak hydrophobic interactions. Harsh conditions generally only suitable for purification of peptides

Affinity chromatography: fusion tags

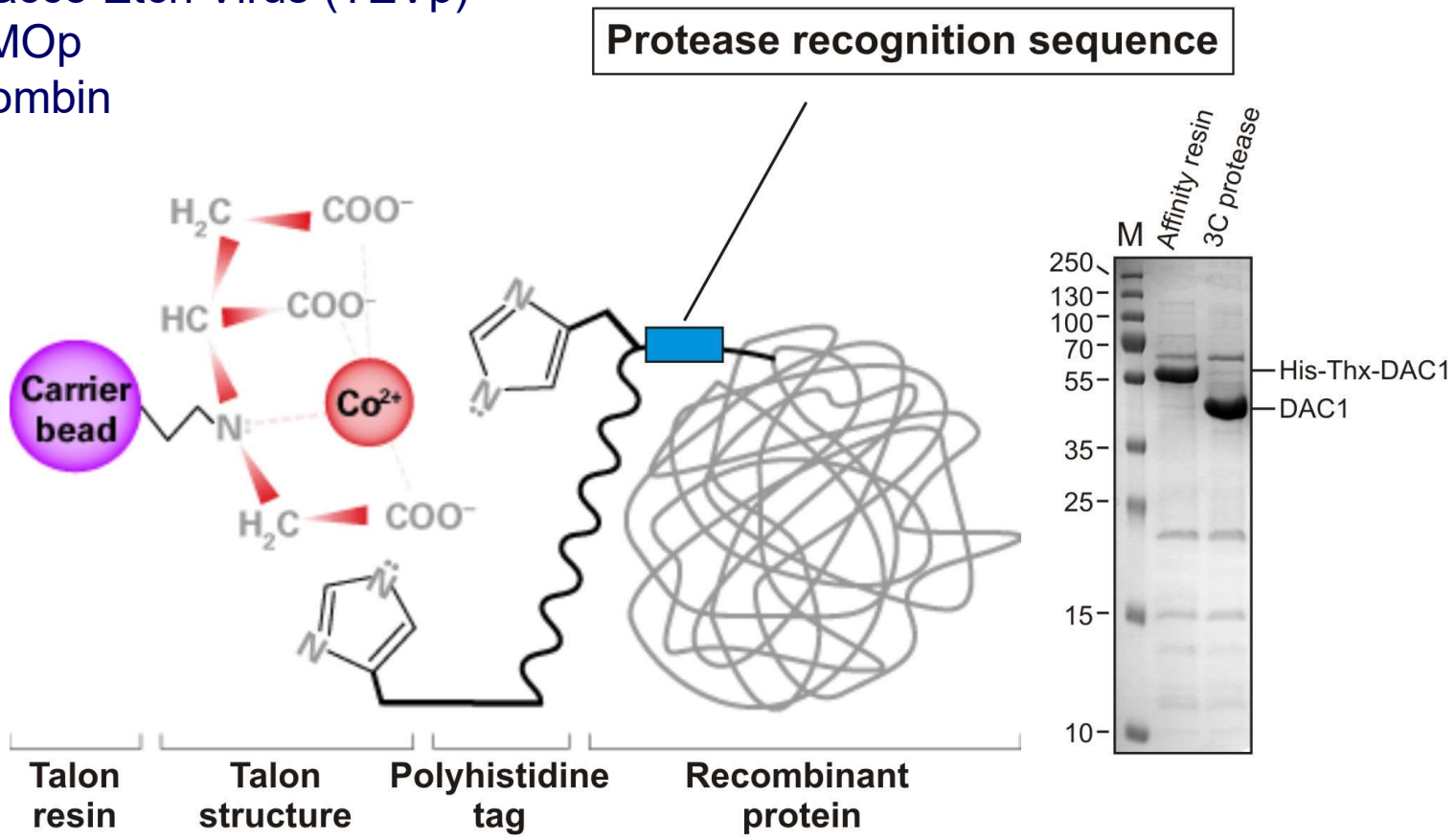
Fusion tags can improve protein expression, stability, resistance to proteolytic degradation and solubility.

Fusion tag	Function	Size (kDa)	Description
Polyhistidine (e.g. 6xHis, 10xHis)	Affinity	1-2	The most commonly used affinity tag, binds to metal ions
Strep-tag II	Affinity	1	High affinity for engineered streptavidin
Thioredoxin (Trx)	Solubility	12	Aids in refolding proteins that require a reducing environment
Small Ubiquitin-like Modifier (SUMO)	Solubility	12	Contains a native cleavage sequence enabling tag removal with SUMO protease
Glutathione S-transferase (GST)	Solubility, affinity	26	High affinity for glutathione, often needs to be removed due to large size
Maltose Binding Protein (MBP)	Solubility, affinity	41	Binds to maltose, often needs to be removed due to large size

- Fusion tag orientation (N- or C-terminus)
- Combinatorial fusion tags (Trx/GST/MBP with an affinity tag, e.g. 6xHis)

Variety of proteases for fusion tag removal

- Human Rhinovirus (HRV 3C)
- PreScission protease
- Tobacco Etch Virus (TEVp)
- SUMOp
- Thrombin

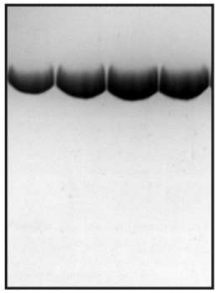


Column chromatography instrumentation

Automated chromatography systems

Chromatography steps

Affinity chromatography
Ion-exchange chromatography
Size-exclusion chromatography



Protein characterization: an aggregation problem

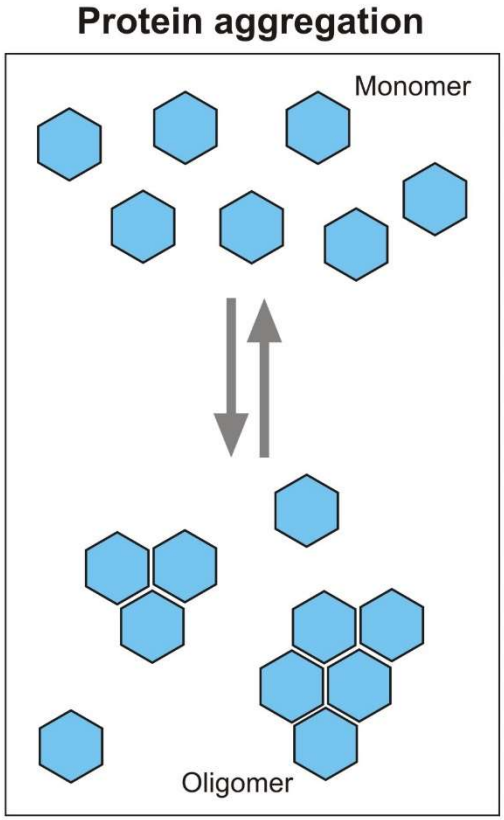
A key challenge in recombinant protein production is to maintain and store the target protein in a soluble and stable form. Protein aggregation can compromise protein function and thus it is necessary to overcome this challenge to generate functionally active protein.

Detection of protein aggregation

- Analytical size-exclusion chromatography (SEC)
- Dynamic light scattering (DLS)
- Analytical ultracentrifugation (AUC)

Troubleshooting

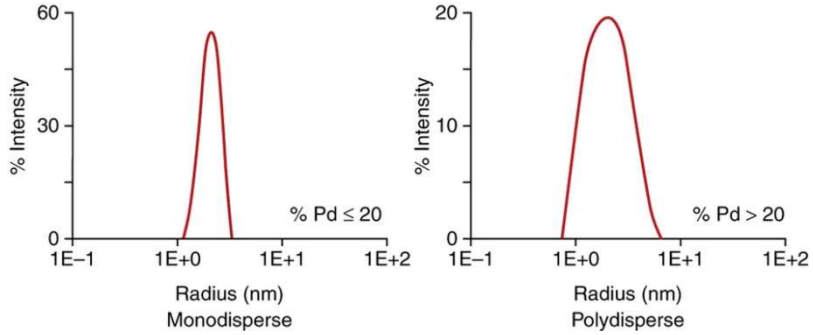
- Culture conditions (e.g. reducing temperature)
- Buffer composition (ionic strenght, pH, reducing agents)
- Presence co-factors (Acetyl-CoA, metal ions)
- Fusion tags (Trx, MBP, SUMO)
- Minimising sample handling
- Avoiding time delays between purification steps
- Performing purification steps at 4°C
- Store purified proteins in -80°C



Protein quality control (QC) analyses

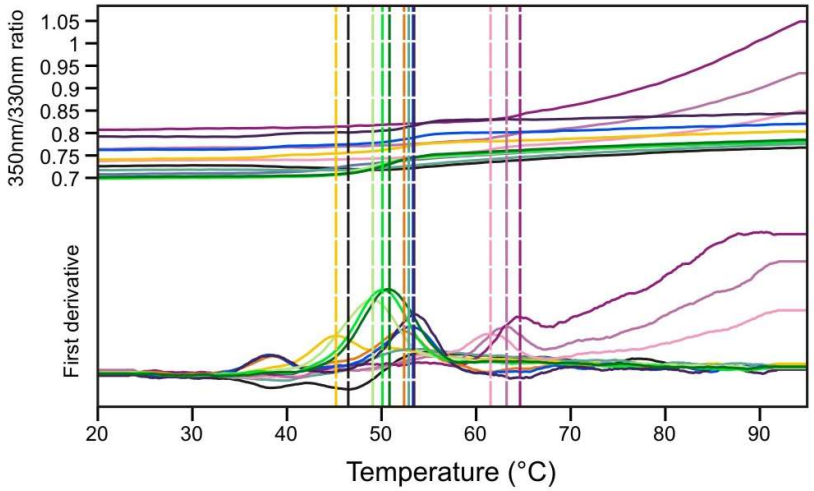
High purity and homogeneity of the protein sample are crucial for the downstream processes to be successful.

- Dynamic light scattering (DLS): To characterize the polydispersity of sample

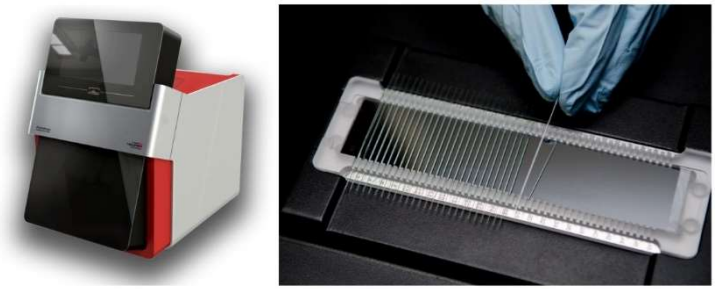


Identification of different oligomeric forms or aggregates, which are preventing crystallization

- Differential scanning fluorimetry (DSF): analysis of protein stability



To characterize the stability of the protein in different buffers and in the presence of different ligands, which stabilize the protein for crystallization



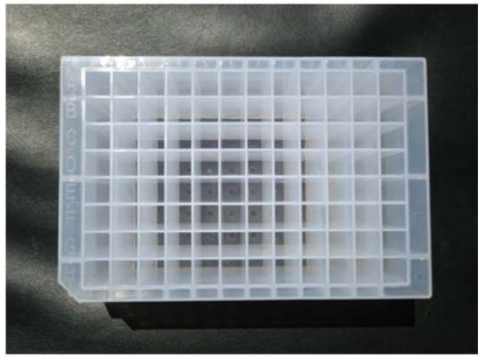
Prometheus NT.48 (nanoDSF)

Success stories

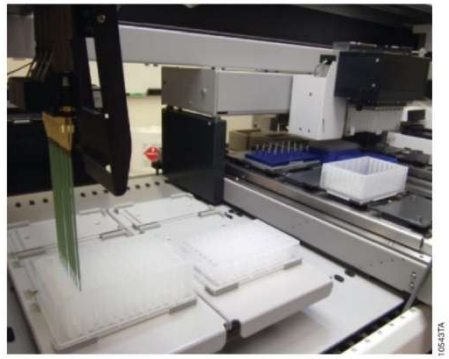
Development of expression and purification protocol for *Schistosoma mansoni* HDAC8: mini-scale tests

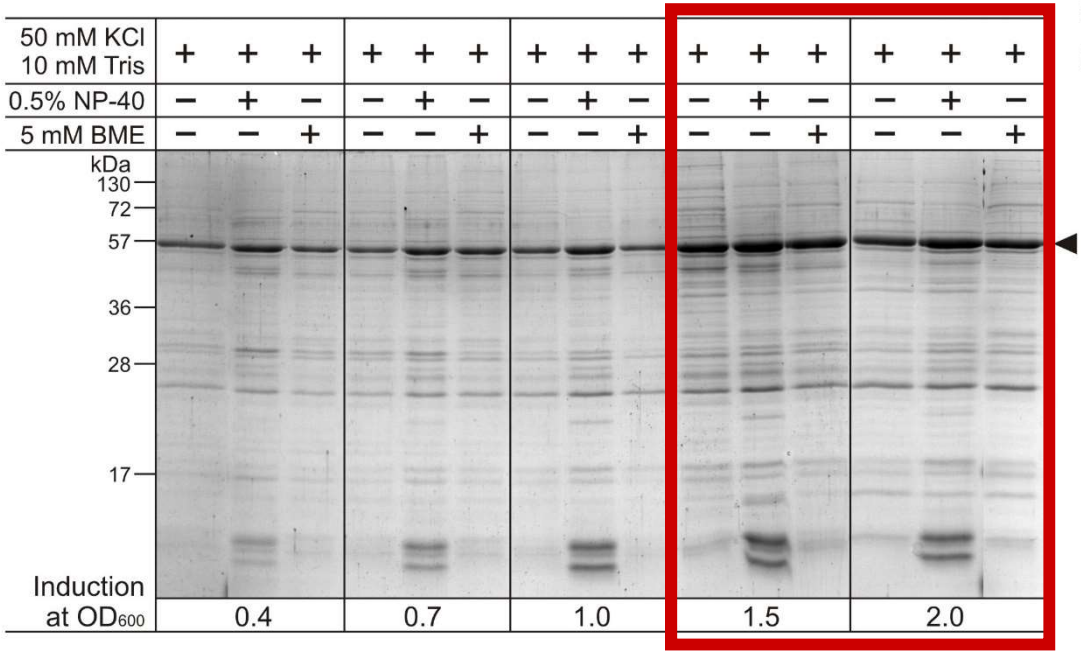
24-well deep plate for *E. coli* cultures

96-well deep plate for purification



Tecan robot



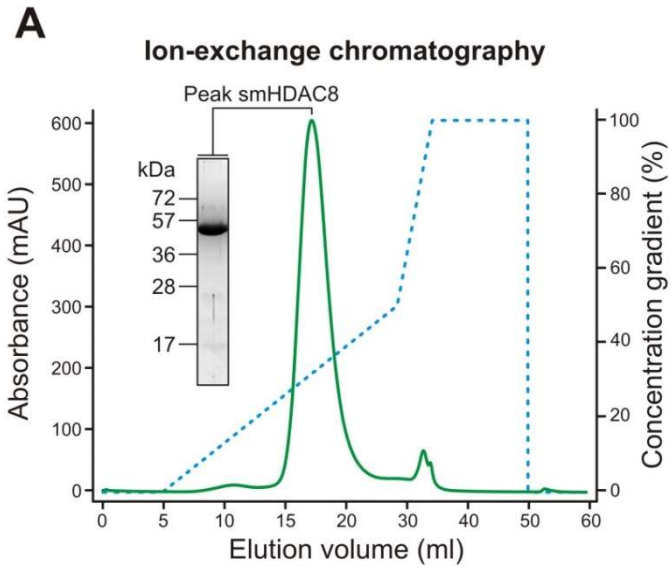


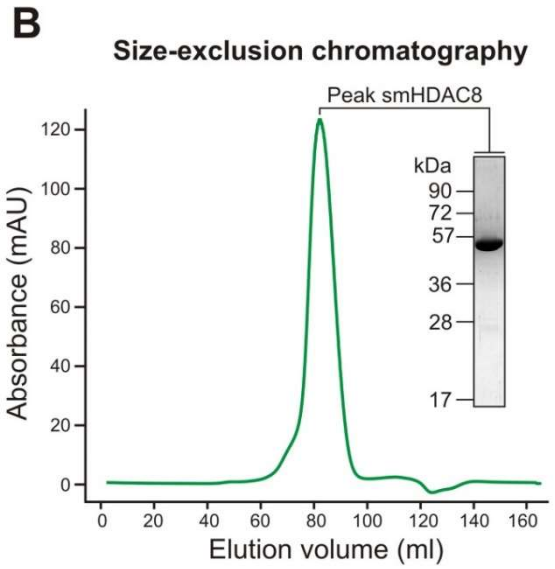
Semi-automated affinity purification

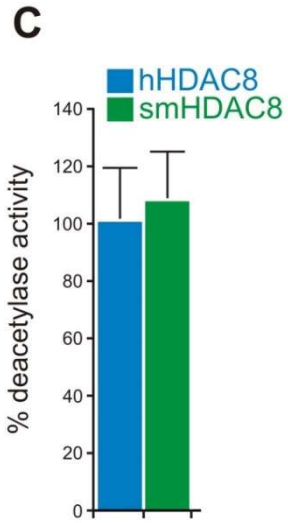
Induction at high O.D. results in higher yield

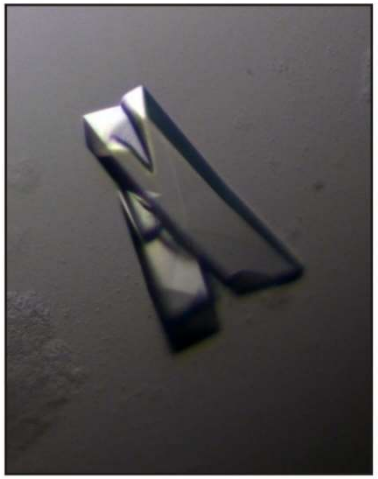
Harvest cells 1 h post induction

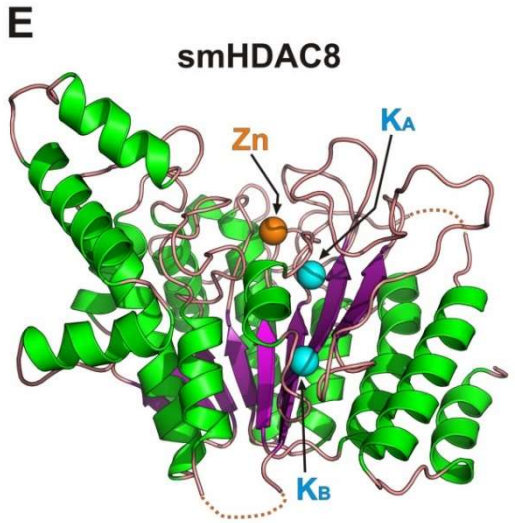
Large-scale production and crystallization of smHDAC8











Conclusions

- The project design is ultimately determined by the end-use of the recombinant protein
- The overall success of a project lies in an effective project design

Recombinant DNA Technology Market Worth $844.6 Billion by 2025: Grand View Research, Inc.

GRAND VIEW RESEARCH

Questions

Dr. Martin Marek
Loschmidt Laboratories
Faculty of Science, MUNI
Kamenice 5, bld. A13, room 332
martin.marek@recetox.muni.cz