Open Sesame: Can We Make a Protein Cage Open at the Right Time?

Engineering a pH-Responsive Protein Nanocage for Controlled Cargo Release

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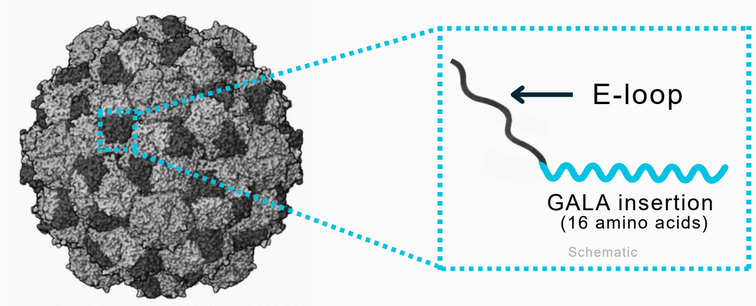
1. Background

- Encapsulins are **self-assembling bacterial protein nanocages** that can protect molecular cargo, but their **high stability can limit controlled release** [1].
- The **mildly acidic tumour microenvironment** provides a **potential trigger** for pH-responsive cargo release [7].
- Here, we engineered the **pH-sensitive peptide GALA** into the **E-loop of T=4 *Quasibacillus thermotolerans*** encapsulin (QtEnc).
- Under **acidic conditions**, GALA **changes conformation** and is hypothesised to disrupt subunit interactions, promoting cage disassembly.

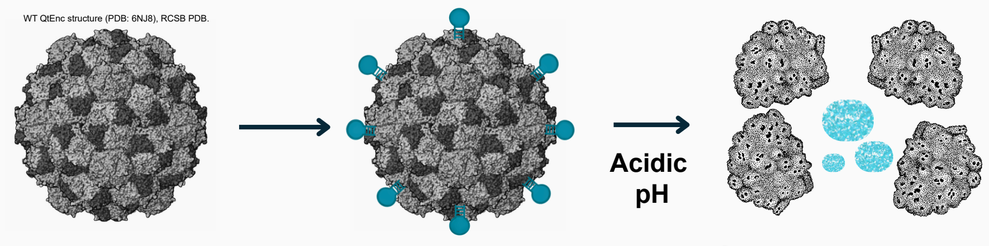


Wild Type QtEnc structure (PDB: 6NJ8), RCSB PDB.

2. Aims

- Express and purify wild-type EncStrep and T4GALA EncStrep in *E. coli*
- Characterise the pH-dependent assembly behaviour of T4GALA
- Generate T4GALA EncStrep co-expressing GFP-CLP as a model cargo system
- Assess GFP cargo loading and investigate evidence of pH-triggered release

3. Engineering the pH responsive system



WT EncStrep
(Stable at neutral pH)

T4GALA EncStrep
(pH-sensitive)

Cage Disassembly
(With Potential Cargo Release)

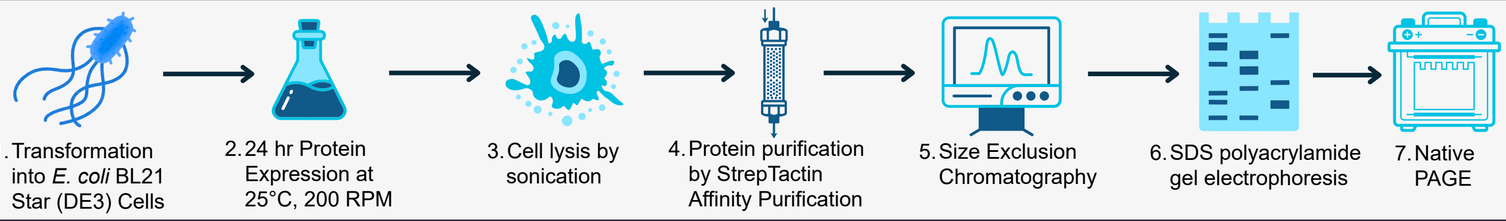
Unstructured GALA Peptide

GALA exists as a random coil at **neutral pH**

Amphipathic α helix

GALA adopts a helical configuration under **acidic conditions**

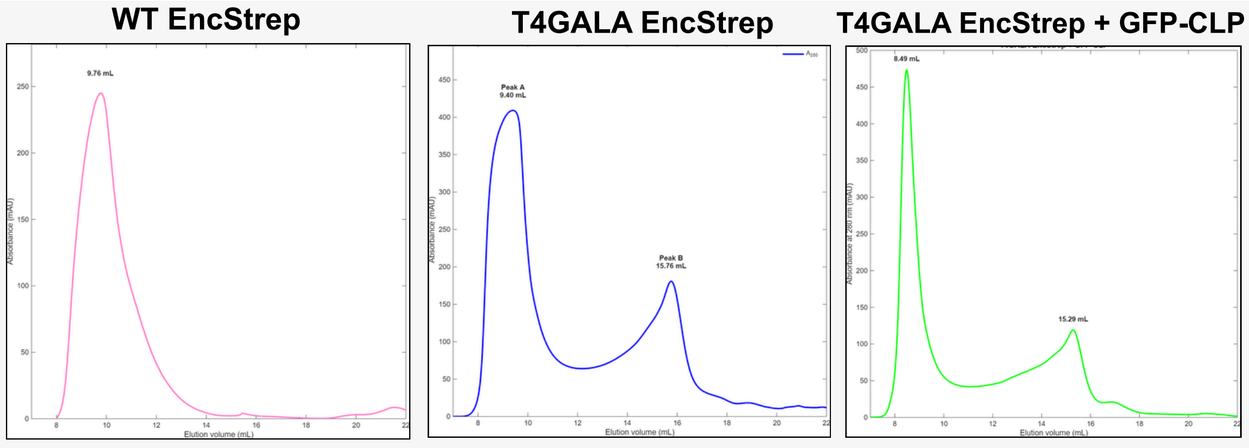
4. Overview of Methods



5. Results

5.1 Size Exclusion Chromatography (SEC)

WT EncStrep | **T4GALA EncStrep** | **T4GALA EncStrep + GFP-CLP**

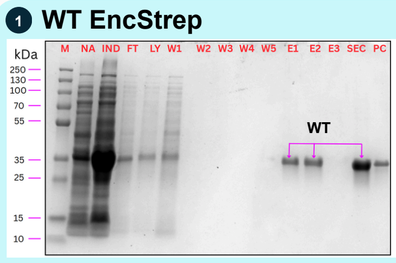


WT QtEncStrep produced a dominant early-eluting high-molecular-weight species, while T4GALA and T4GALA+GFP showed additional later-eluting species. This may reflect increased susceptibility of the GALA-modified cages to the Tris-based SEC buffer, potentially promoting partial cage destabilization[3].

A_{280} monitors protein elution

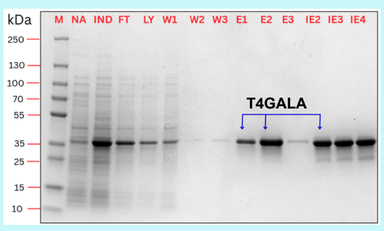
5.2 SDS-PAGE Analysis

1 WT EncStrep



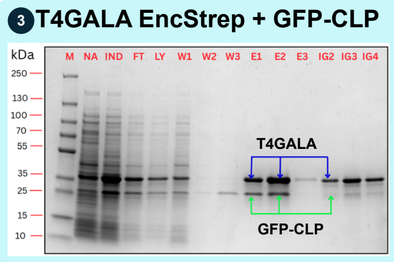
A dominant **~36 kDa** band after purification is consistent with the expected WT encapsulin subunit.

2 T4GALA EncStrep - Fresh



Fresh processing produced a dominant **~36–39** kDa T4GALA band with reduced lower-molecular-weight species.

3 T4GALA EncStrep + GFP-CLP

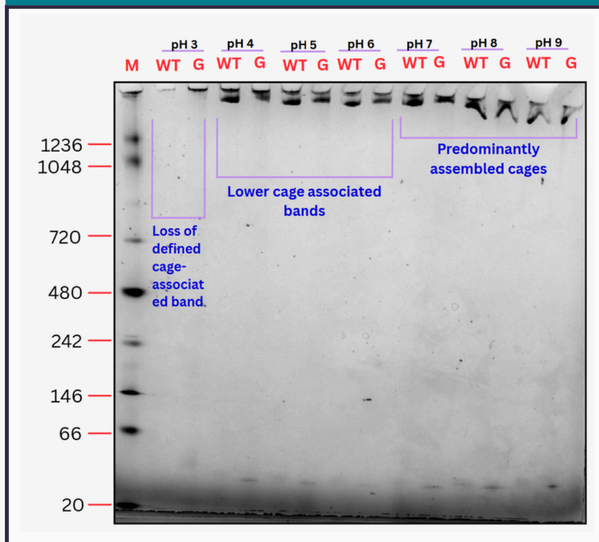


Bands consistent with T4GALA and GFP-CLP co-purified, supporting association of GFP cargo with the encapsulin system.

An **additional lower-molecular-weight species** was observed during initial T4GALA purification. Fresh processing reduced this band, suggesting that T4GALA may be more susceptible to degradation following cell lysis.

Sample abbreviations: **M**, protein marker; **NA**, non-induced; **IND**, induced; **FT**, flow-through; **LY**, lysate; **W**, wash fractions; **E**, elution fractions; **SEC**, SEC-purified sample; **PC**, Positive Control. **IE** and **IG** indicate SEC fractions collected for native PAGE analysis of T4GALA EncStrep and T4GALA EncStrep + GFP-CLP, respectively.

5.3 Native PAGE: pH Dependent Assembly

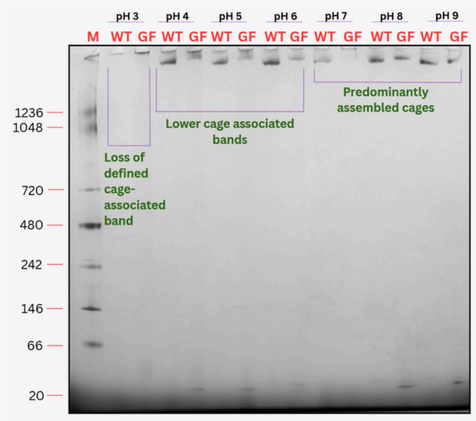


WT and T4GALA (G) were incubated in citrate (pH 3–5), MES (pH 6) or Tris (pH 7–9) buffers before native PAGE.

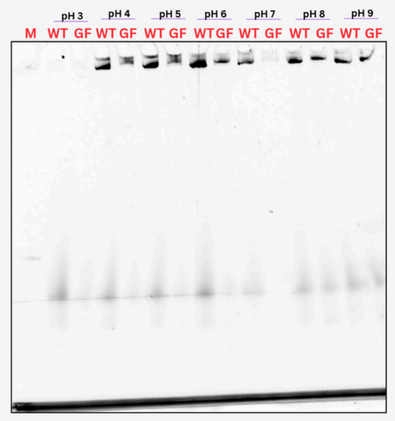
- At **pH 7-9**, both WT and T4GALA showed strong cage-associated bands, consistent with predominantly assembled encapsulins; T4GALA showed reduced assembly at pH 7.
- At **pH 4-6**, weaker bands (G-Lane) indicated a reduced assembled-cage population under increasingly acidic conditions.
- At **pH 3**, no defined cage-associated band was detected in both lanes, suggesting substantial loss of the assembled-cage population.
- Overall, these results show that encapsulin assembly changed across the conditions tested, with less cage-associated signal seen under more acidic conditions.

5.4 T4GALA EncStrep + GFP-CLP: Cargo Loading

Native PAGE comparing WT EncStrep + GFP-CLP (WT) and T4GALA EncStrep +GFP (GF)



Fluorescence image acquired before Coomassie staining to visualise GFP



GFP-loaded vs non-GFP samples under UV

GFP-CLP co-purified with T4GALA EncStrep, while fluorescence associated with slowly migrating cage species further supported GFP association with the encapsulin system. Additional lower-migrating fluorescence was observed, but its origin and relationship to cargo release could not be determined from the native PAGE results alone.

6. Conclusions

- T4GALA retained assembly under neutral and basic conditions, with reduced cage-associated signal under more acidic conditions.
- T4GALA EncStrep + GFP-CLP was successfully generated, and GFP-CLP co-purified with the encapsulin system.
- GFP fluorescence was associated with cage species, supporting GFP cargo association.
- However, clear pH-triggered release of GFP from the cage was not observed and could not be conclusively demonstrated.
- An unexpected lower-molecular-weight species was observed with T4GALA and requires further characterisation.

7. Future Work

- Identify the lower-MW species (e.g. mass spectrometry)
- Quantify encapsulated vs released GFP (e.g. fluorescence spectroscopy)
- Test Tumour-relevant pH range (6.5-7.4) using a controlled buffer system
- Optimise expression and purification conditions to reduce subunit heterogeneity

Scan to access the full report and references.

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