Cell-derived Materials as Hydrogel Components

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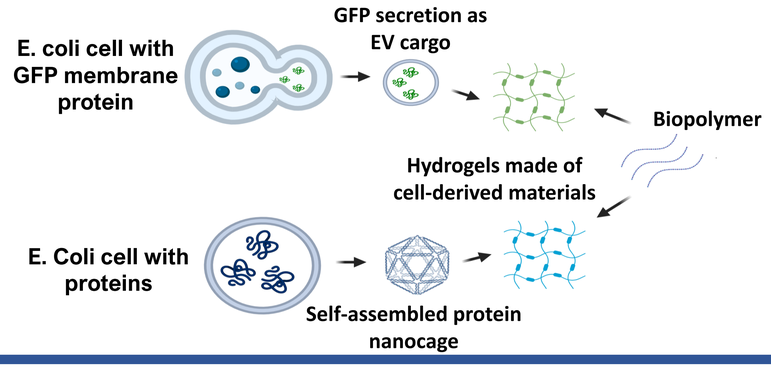
Background

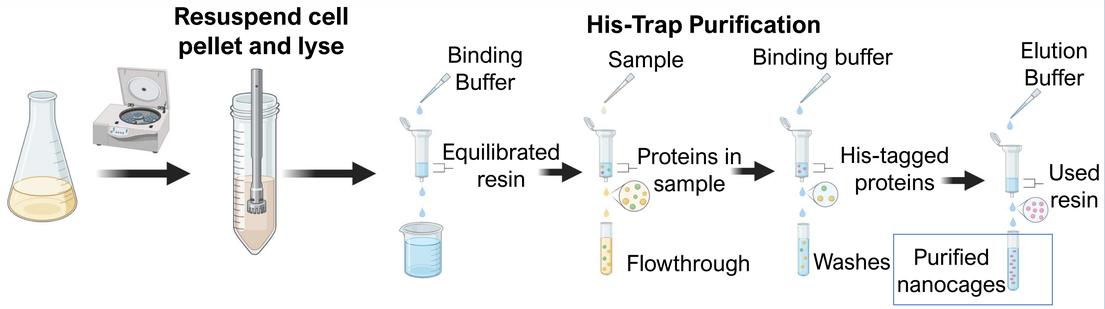
- Encapsulation of antigens and biologics into nanocarriers without compromising their activity and integrity is a challenge that requires additional engineering for successful drug delivery. (1)

Approach:

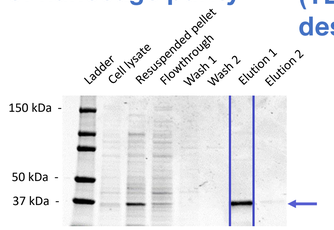
- We produced membrane-targeted NeonGreen proteins in *E. coli, which are packaged into cell-secreted* vesicles. [2]
- In parallel, we expressed self-assembling protein nanocages. [3]
- Our goal is to load these nanocarriers into injectable hydrogels, enabling their use for local, sustained cargo delivery.



Protein Nanocage Isolation and Characterization

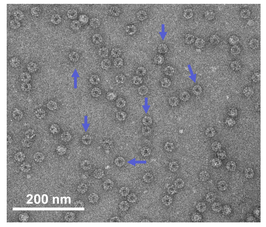


SDS PAGE analysis of nanocage purity



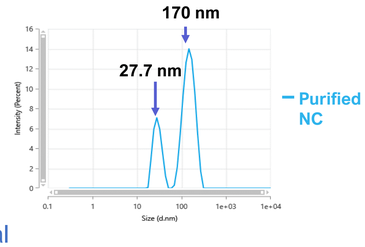
Purified nanocages (MW = 37 kDA) were isolated from Elution 1 fraction.

Transmission Electron Microscopy (TEM) shows intact nanocages with desired morphology



Nanocage-like features: icosahedral shape and hollow interior.

Dynamic Light Scattering (DLS) shows bimodal particle size



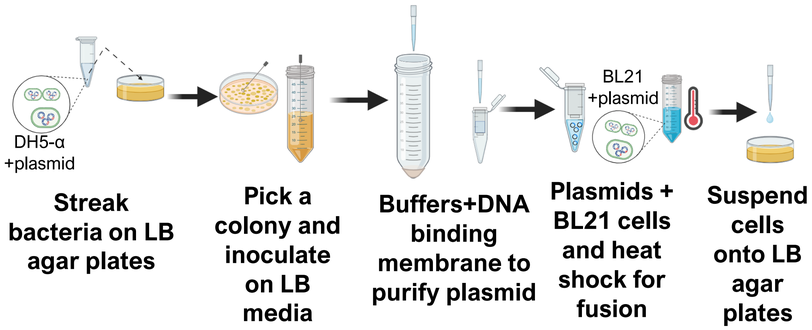
Discussion and Next Steps

- We successfully expressed and purified protein nanocages and simultaneously optimized the expression and isolation of NeonGreen-encapsulated vesicles.
- We used DLS and TEM to characterize the size, dispersity, and integrity of particles

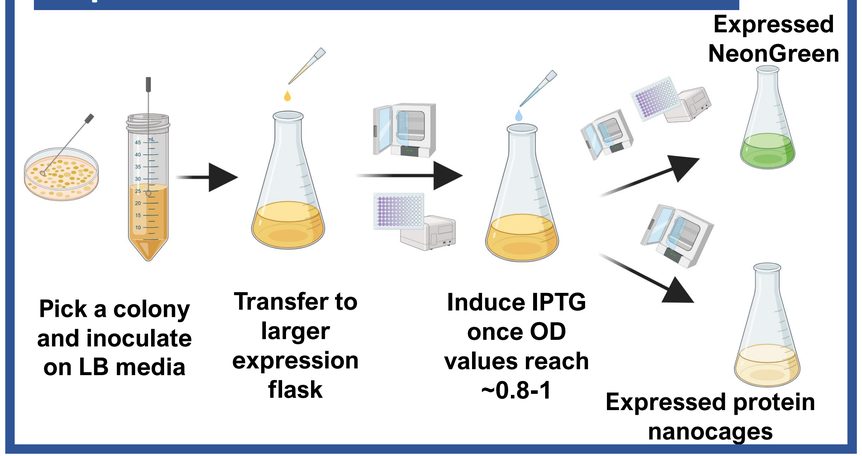
Next steps:

- Track and label vesicles with lipohilic dyes (e.g., DiI, FM-4-64).
- Optimize nanocage expression
- Incorporate antigens (e.g., OVA) or biologic cargo into vesicles or particles.
- Make and test gels using of cell derived polymers (e.g., hyaluronic acid, hydroxypropyl methyl cellulose) and nanocarriers.
- Monitor release from and decomposition of gels.

Miniprep and Transformation

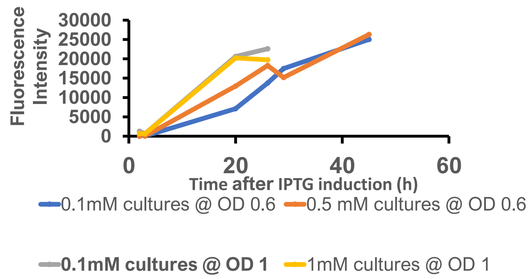


Expression of Cell-Derived Nanocarriers

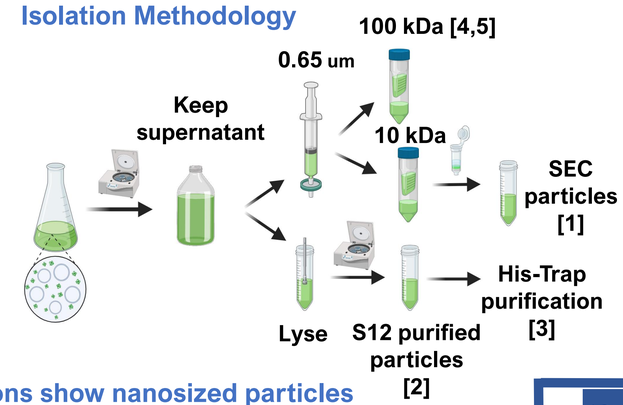


Extracellular Vesicle Isolation, and Characterization

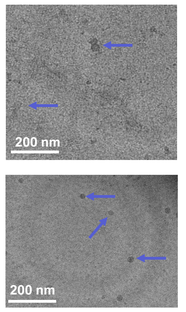
Optimizing secretion of NeonGreen over time



Isolation Methodology

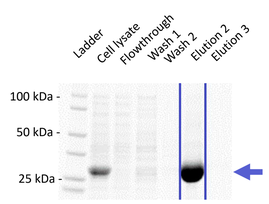


TEM



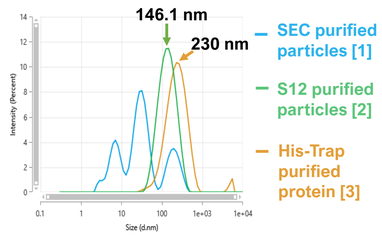
Negative staining EM shows vesicle-like structures.

His-Trap purification yields pure NeonGreen

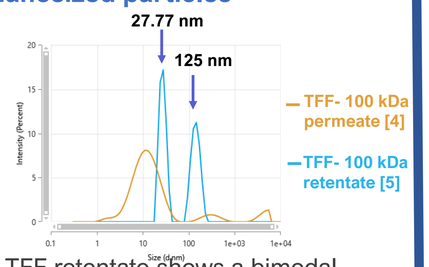


NeonGreen (MW ~30.6 kDa) was isolated from the Elution 2 fraction.

Vesicle fractions show nanosized particles



Vesicle fractions (SEC, S12) show particles with 146.1 and 230 nm.
SEC purified particles are polydisperse.



TFF retentate shows a bimodal distribution with 27.77 and 125 nm particles.

TFF permeate fraction was highly polydisperse.

References

1. Peruzzi (2024) *Nat. Commun.*
2. Eastwood (2023) *Cell Rep.*
3. Hsia (2016) *Nature.*
4. Correa (2021) *Chem. Rev.*
5. Toyofuku (2023) *Nat. Rev.*

Acknowledgements

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- SURE for funding.
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