PhD Open Days

Biomanufacturing of protein nanocages for biomedical applications

PhD Programme in Biotechnology and Biosciences (BIOTECnico)

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MOTIVATION

- **Protein nanocages** are versatile vehicles for **drug delivery** and **bioimaging**.
- The **exterior** of these nanometer scale architectures can be **conjugated with functional ligands** and the **inner core** can be **loaded with different molecules**.
- The **clinical development** of protein nanocages requires large amounts of **pure, well-folded assemblies**.

- **However, the currently used upstream and downstream processing approaches are only suitable for proof-of-concept studies at lab scale.**
- **Biomanufacturing at large scale will require more efficient bioprocess technologies to enable the use of protein nanocages for biomedical applications.**

OBJECTIVE OF THIS WORK:

Development of a scalable and cost-effective process for the biomanufacturing of non-viral protein nanocages

BACKGROUND

- **Non-viral protein nanocages** are formed by **homo- or hetero-self-assembly of monomers** into **defined symmetric structures** of different size and shape and can be classified as **natural** or **synthetic**.

- **Protein models** used in this work:
  - A 16.5 kDa natural **small heat shock protein from *Methanococcus jannaschii* (MjsHSP)**: the *in vivo* assembly of 24 units of MjsHSP originates 12 nm nanocages with octahedral symmetry and 3 nm pores.
  - A 92 kDa engineered ring-shaped **trp RNA-binding attenuation protein (TRAP)**: the assembly of 24 or 12 units of TRAP rings in the presence of gold nanoparticles originates 22 nm or 16 nm nanocages, respectively.

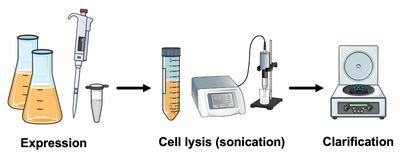
- Protein nanocages are usually **expressed in *Escherichia coli*** but can also be **produced in an alternative expressing host** such as ***Vibrio natriegens***.
- **Conventional lab scale protocols** are typically applied for the **downstream processing** of these protein nanocages.

STRATEGIES AND RESULTS

Upstream Processing

Expression in *E. coli* cells

- ***E. coli*** is one of the **most used expression systems** due to its **advantageous characteristics**.
- A **standard approach** with a **high level** of **MjsHSP nanocages expression** was established for this bacterial host.



Expression in *V. natriegens* cells

- ***V. natriegens*** is a very **interesting and promising platform** for different applications in **biotechnology**.
- Using a **standard procedure** and a **specific strain** (*Vmax™ X2*), it was possible to produce **MjsHSP nanocages** in this alternative bacteria but in a very low concentration.

→ **Optimization of the protein nanocages production (both MjsHSP nanocages and TRAP nanocages) will be performed particularly for the *V. natriegens* cells.**

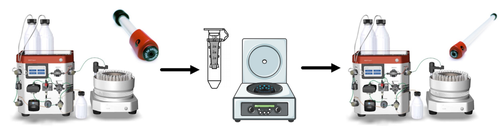
→ **Parameters to be tested include growth media composition, aeration conditions, expression temperature, concentration of expression inducer (IPTG) and induction time.**

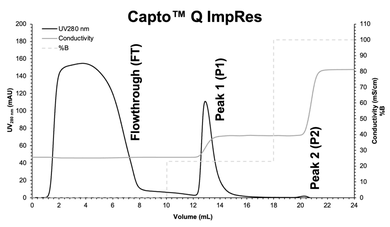
→ **The scale-up for a bioreactor of the optimized production will be considered.**

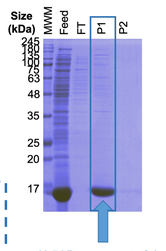
Downstream Processing

1. Anion Exchange Chromatography (AEXC)

- Four strong AEX chromatographic supports were tested: HiTrap® Q FF (Cytiva), **Capto™ Q ImpRes** (Cytiva), POROS™ HQ (Thermo Scientific) and CIMmultus™ QA (BIA Separations).



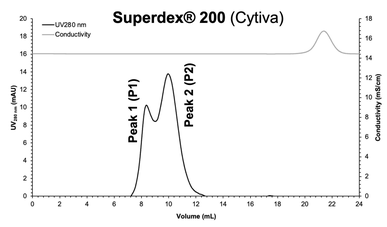




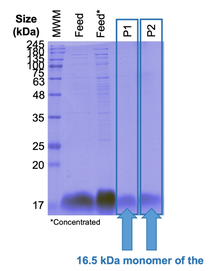
16.5 kDa monomer of the MjsHSP nanocages

→ **Versatility and robustness of the purification process will be evaluated using MjsHSP nanocages produced in *V. natriegens* and using the TRAP nanocages.**

2. Size Exclusion Chromatography (SEC)



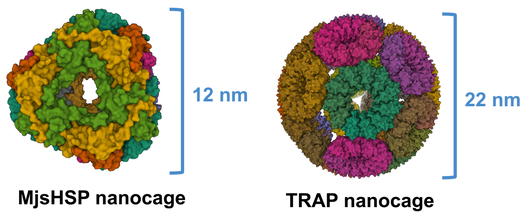
→ **Presence of two populations of MjsHSP nanocages in P1**



16.5 kDa monomer of the MjsHSP nanocages

Characterization Techniques

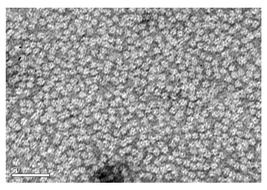
3D Structure Simulation



MjsHSP nanocage — TRAP nanocage

Negative Staining TEM

→ Estimation for the **hydrodynamic diameter** of the MjsHSP nanocages: **10.2 ± 0.9 nm**



→ A promising technique under study is **atomic force microscopy (AFM)**

Collaboration with the AFMaRT Lab – Bio-PhysNano – BioISI – FCUL

Dynamic Light Scattering (DLS)

MjsHSP Nanocages	Hydrodynamic Diameter (nm)
	Intensity Distribution
P1 (SEC)	29.0
P2 (SEC)	12.2

Fluorescence Correlation Spectroscopy (FCS)

Collaboration with Professor Pedro M. Paulo (MPPM – CQE – IST)

→ **MjsHSP nanocages labelled with Alexa Fluor 647**

MjsHSP Nanocages	Diffusion Coefficient (μm²/s)	Hydrodynamic Diameter (nm)
P1 (SEC)	33	14.8
P2 (SEC)	41	12.0

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