PhD Open Days

Biomanufacturing of non-viral protein nanocages for biotechnological and biomedical applications

PhD Programme in Biotechnology and Biosciences (BIOTECnico)

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Motivation

- **Non-viral protein nanocages (NVPNs)** are very attractive as a **biological nanomaterial** due to their intrinsic characteristics: **high surface/volume ratio**, **multi-functionality**, **ease of modifying**, **high stability and solubility**, **biocompatibility**, **biodegradability**, etc..
- **Biotechnological and biomedical applications** (e.g., drug delivery, vaccine development, bioimaging and biomineralization) have been investigated, showing that **NVPNs** can be a **promising and interesting tool**.
- The development of applications for **NVPNs** requires **large amounts** of **pure and well-folded assemblies**.

- Consequently, the **availability** of more **efficient biomanufacturing processes** is needed to transform **NVPNs** into **valuable bioproducts**.
- These processes should be supported by **analytical techniques** adequate for the **quality control and characterization** of their **biological function and structure**.

Background

- **NVPNs**
  - Defined as **highly ordered** architectures in a **nanometer scale**.
  - Produced through the **self-assembly** of **identical** or **different multiple monomers**.
  - Assembled structures are **symmetric** and **homogeneous**, presenting several shapes and sizes (10-100 nm).
  - Perform **relevant natural functions** at **cellular level** in procaryotic and eucaryotic organisms.
  - Can be classified as **natural** or **artificial** (bioinspired and designed *de novo*).

- **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 91.7 kDa engineered ring-shaped **trp RNA-binding attenuation protein (TRAP)**: the *in vitro* assembly of 24 TRAP O-rings in the presence of gold originates 22 nm nanocages with octahedral symmetry.

OBJECTIVE OF THIS WORK:

Development of scalable and cost-effective processes for the biomanufacturing of NVPNs

- **Few scientific studies** have addressed the **upstream and downstream processing of NVPNs**.
- **Standard production and purification processes** based on laboratory scale protocols need to be **improved and adapted for scale-up**.
- The **conceptual design** of **downstream processing of NVPNs** is restricted by **limited data** and **information on alternative strategies** as well as **process yields**.

REVIEW ARTICLE

Strategies and Results

Upstream Processing

Expression in *E. coli* cells

- A **standard approach** with a **high level** of both **MjsHSP and TRAP nanocages expression** was established for this bacterial host.

Expression in *V. natriegens* cells

- Using a **standard procedure** and a **specific strain,** it was possible to produce **MjsHSP nanocages** in this alternative bacteria but in a very low concentration.

- **Growth and production conditions**:
  - *E. coli* BL21(DE3) cells | *Vibrio natriegens* Vmax™ X2 cells;
  - LB medium | LB medium with V2 salts (204 mM NaCl, 4.2 mM KCl and 23.1 mM $MgCl_2$)
  - Incubation at 37 °C and 250 rpm;
  - Induction with 1 mM isopropyl-β-D-thiogalactoside (IPTG) at OD_{600nm} ≈ 0.5-0.6;
  - 4 hours of induction.

→ **Optimization of the NVPNs production is being performed particularly for the *V. natriegens* cells.**

→ **Parameters being tested include growth medium composition, aeration conditions, temperature of protein expression, concentration of IPTG and induction time.**

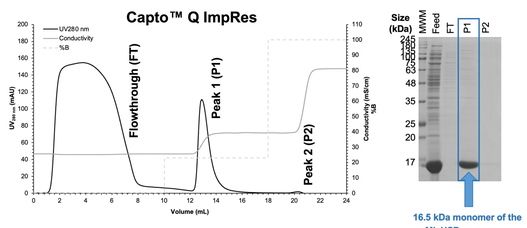
→ **Cell lysis: Mechanical by sonication vs. Thermal**

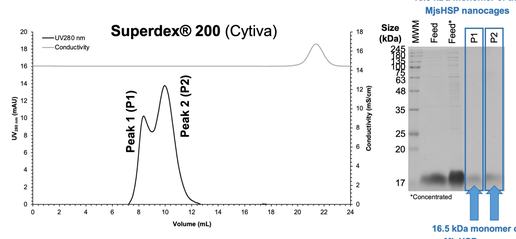
Downstream Processing

→ **MjsHSP nanocages**

1. Anion Exchange Chromatography (AEXC)

- Binding buffer: 50 mM sodium phosphate, 250 mM NaCl, pH 6.0
- Elution buffer: 50 mM sodium phosphate, 1 M NaCl, pH 6.0





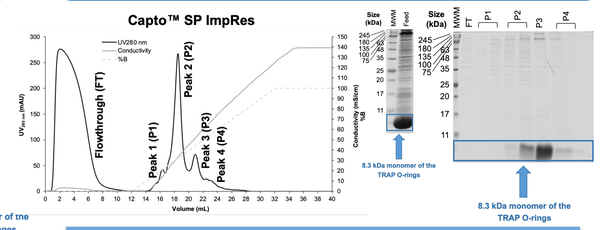
2. Size Exclusion Chromatography (SEC)

- Running buffer: 1X phosphate buffered saline (PBS)

→ **TRAP nanocages**

- Binding buffer: 50 mM MES, pH 5.5
- Elution buffer: 50 mM MES, 2 M NaCl, pH 5.5

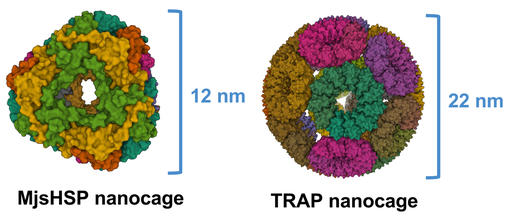
Cation Exchange Chromatography (CEXC)



→ **After the *in vitro* assembly of TRAP O-rings: SEC vs. Multimodal vs. Hydrogen bond**

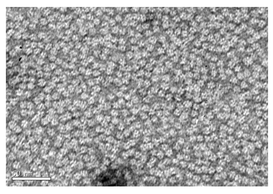
Characterization Techniques

3D Structure Simulation



Negative Staining TEM

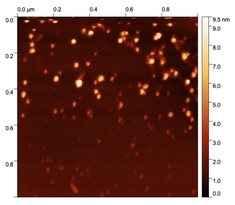
→ Estimation for the **hydrodynamic diameter** of the MjsHSP nanocages: **10.2 ± 0.9 nm**



Atomic Force Microscopy (AFM)

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

→ **MjsHSP nanocages**



Dynamic Light Scattering (DLS)

Hydrodynamic diameter* (nm)	
MjsHSP nanocages	
P1-SEC	**29.0**
P2-SEC	**12.2**

*Intensity Distribution

Fluorescence Correlation Spectroscopy (FCS)

→ **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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