PhD Open Days

Purification of small heat shock protein nanocages through a chromatographic approach

PhD Programme in Biotechnology and Biosciences (BIOTECnico)

Jorge João[1,2] (jorgejoao@tecnico.ulisboa.pt)

[1]iBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal

[2]Associate Laboratory i4HB – Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal

Motivation and Background

- Protein nanocages are versatile vehicles for drug delivery.
- These nanometer scale architectures feature an inner cavity that can be loaded with different therapeutic molecules.
- The clinical development of protein nanocages requires large amounts of pure, well-folded assemblies.

- **The currently used purification 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 clinical applications.**

Objective of this work:

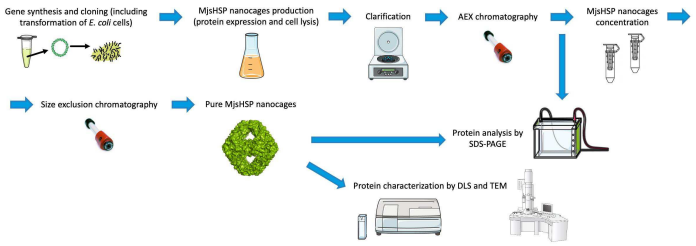
Development of scalable and cost-effective processes for the biomanufacturing of protein nanocages with particular emphasis in the downstream steps.

- Nonviral naturally-occurring protein nanocages that have been used to deliver therapeutic drugs include the 16.5 kDa **small heat shock protein from *Methanococcus jannaschii* (MjsHSP)**, which was used as model in this work.
- The *in vivo* assembly of 24 units of MjsHSP originates 12-15 nm nanocages with octahedral symmetry, demarcated exterior and interior surfaces and 3 nm pores that facilitate the traffic of molecules in and out.

Here, we propose a purification strategy that combines an intermediate purification using an anion exchange (AEX) chromatography and a polishing step using a size exclusion chromatography (SEC), to achieve a highly purified product.

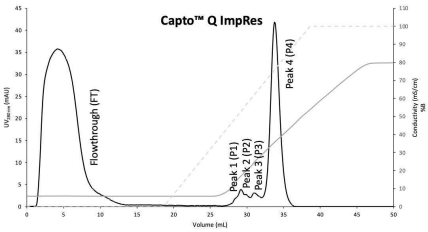
Quaternary amine AEX resins and a monolith with different properties were evaluated and compared with traditional agarose chromatographic supports.

Materials and Methods



Results

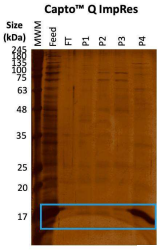
1. Anion Exchange Chromatography



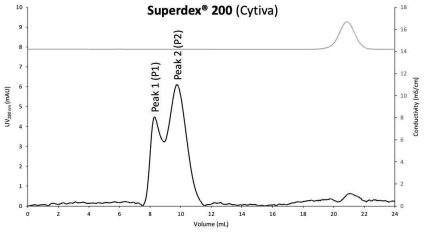
Capto™ Q ImpRes (Cytiva) is a strong anion exchange resin that has a quaternary amine as functional group.

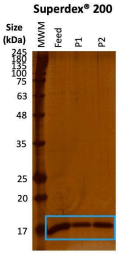
The other strong AEX chromatographic supports tested were:

- HiTrap® Q FF (Cytiva);
- POROS™ HQ (Thermo Scientific);
- CIMmultus™ QA (BIA Separations).



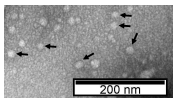
2. Size Exclusion Chromatography





Dynamic light scattering (DLS) allowed to estimate an average value in terms of hydrodynamic diameter for the obtained MjsHSP nanocages of approximately 13.34 nm (SEC-P1) and 9.62 nm (SEC-P2).

Negative Staining TEM



Conclusions and Future Work

- ✓ **Initial results showed that a downstream processing strategy based on two chromatography steps could be an efficient platform to obtain pure protein nanocages for eventual pre-clinical/clinical applications.**
- ➢ An analytical strategy needs to be developed in order to assess and quantify the purity of the MjsHSP nanocages as well as to determine the respective yields of the purification process.
- ➢ Additional analytical methodologies can be used to subsequently characterize and confirm the final structure of the purified protein nanocages. Examples in consideration are atomic force microscopy, circular dichroism spectroscopy and fluorescence correlation spectroscopy.

Acknowledgments:

The author acknowledges the funding received for the iBB-Institute for Bioengineering and Biosciences from FCT-Fundação para a Ciência e a Tecnologia (Project UIDB/04565/2020 and UIDP/04565/2020) and from Programa Operacional Regional de Lisboa 2020 (Project N. 007317) and for the i4HB-Associate Laboratory Institute for Health and Bioeconomy (Project LA/P/0140/2020). Funding from FCT through the PhD fellowship to Jorge João (PD/BD/150335/2019, BIOTECnico PhD Programme) is also acknowledged.

Supervisor: Professor Duarte Miguel F. Prazeres

PhD Programme in Biotechnology and Biosciences

phdopendays.tecnico.ulisboa.pt