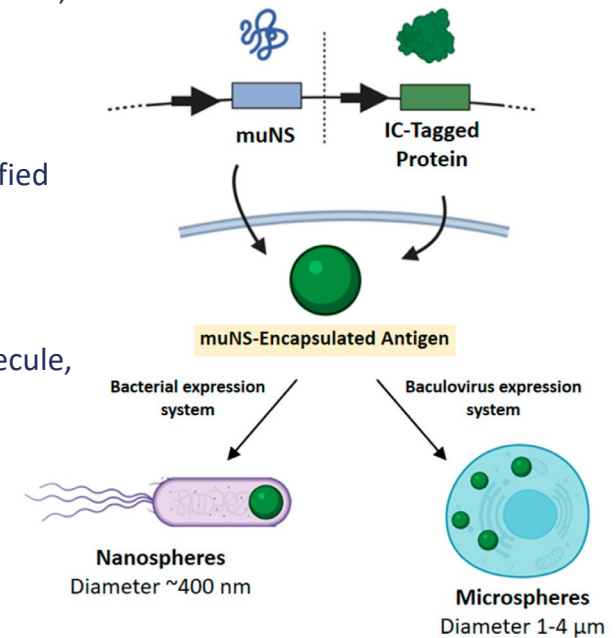


Intra-Cellular micro/nanospheres (MS/NS), loaded with your proteins, or glycoproteins, made in any expression system (baculovirus / insect cell expression system and/or bacteria)

- ✓ **Proteins are properly folded** inside the MS/NS (0,4 – 4 μm) and **completely functional**, admit quaternary interactions and complex enzymatic reactions.
 - Can also work inside ER: **glycoproteins**.
- ✓ **Simplicity cost effective & easy production**: big amounts of MS/NS are easily purified by simple mechanical methods, due to their size and stability.
 - Cells do the work: express & encapsulate.
- ✓ Allows highly specific covalent particle **surface modification** with any desired molecule, **unaffected the activity** and structure of the encapsulated protein.
 - Couple adjuvants, targeting, protect from degradation, etc.
- ✓ The MS/NS could be **relocated** to nucleus or cytoplasm.



Current areas of work:

- **Vaccines**. Already tested in animal models: AHSV, BTV. Ongoing: PRSV, CCHFV, RVFV, and one aquaculture-relevant virus.
- **Treatment of metabolic diseases**. Enzyme replacement therapy based on a stabilized version.
- **Cancer immunotherapy**. Melanoma mouse model.
- **Stabilization of industrial enzymes**. Laccase, Peroxygenase, MHTase, Cutinase...

1. New vaccine platform

[Vaccines 2022, 10\(7\), 1124](#)

[Vaccine, 2020, 38, 882-889](#)

[Antiviral Research, 2017, 142, 55](#)

- ✓ **Subunit vaccines with intrinsic adjuvancy**, overcoming drawbacks of inactivated or attenuated vaccines.
 - **Animal healthcare.** Concept demonstrated in recombinant vaccine incorporating Bluetongue (BTV) or African Horse Sickness viruses' epitopes has generated significant levels of neutralizing antibodies, protecting mice towards a lethal challenge.
 - **SARS-COV-2** vaccine project funded by European Vaccine Initiative (EVI), ISCIII Health Research Institute (Spain), Banco Santander.
- ✓ **Cancer Immunotherapy.** New vaccine approach based on protein microspheres
 - Proof of concept in collaboration with the UK National Institute for Biological Standards and Control (NIBSC)

2. Enzyme immobilization & stabilization

[Scientific Reports, 2021, 11, 2802](#)

- ✓ It works at **wider pH ranges** than the free enzyme.
- ✓ Covalent surface modification, with any desired molecule, **without affecting the activity** of the proteins.
- ✓ **Cascade reactions**, e.g., production of nanoparticles with unspecific peroxygenases & formate oxidase.
- ✓ Proof of concept: **CotA laccase.** Chemical & thermal stabilization, one-step expression and immobilization.
 - Activity almost unaffected after 30 min incubation at 90 °C, and 15 min at 95 °C.
 - The encapsulated laccase decolorates the recalcitrant dye RB19 at room temperature.

3. Toxic or difficult-to-express antigens/antibodies/proteins

[Scientific Reports, 2018, 16286](#)

- ✓ Completely **functional**, proteins are properly folded inside the MS.
- ✓ **Lyophilizable and easily purified** by simple mechanical methods due to their size and stability.
- ✓ Working inside the endoplasmic reticulum: properly **modified viral glycoproteins**.
 - Case 1: Membrane-bound IGRP, which expression remained elusive to date, it is an antigen of enormous interest for type 1 diabetes.
 - Case 2: Viral glycoprotein from Rift Valley Fever Virus. An unique case of expression of a Type II fusion protein on a prokaryotic system.