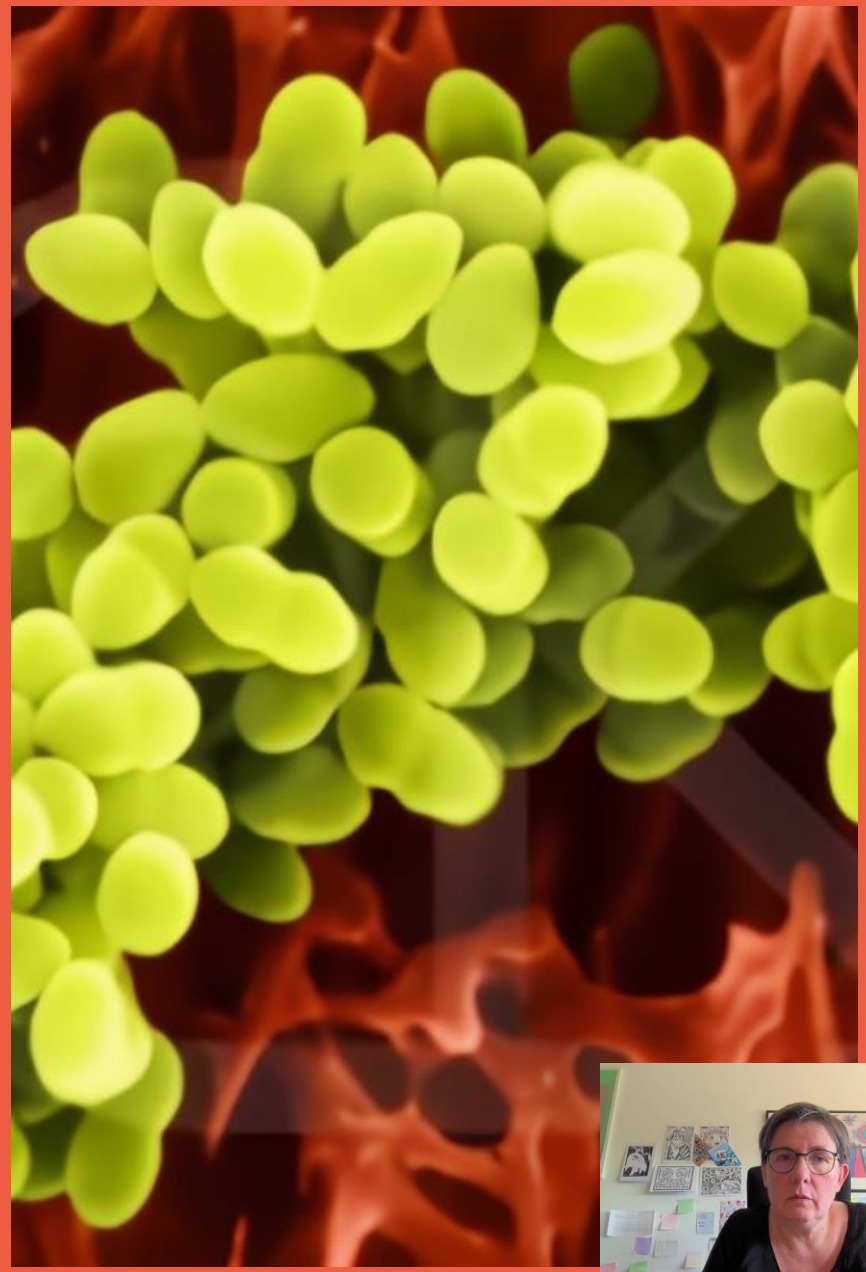
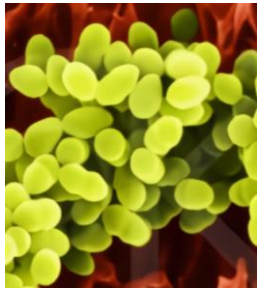
Apport de la microscopie électronique pour la caractérisation des nano-vésicules intracellulaires produites dans la bactérie *Lactococcus lactis*

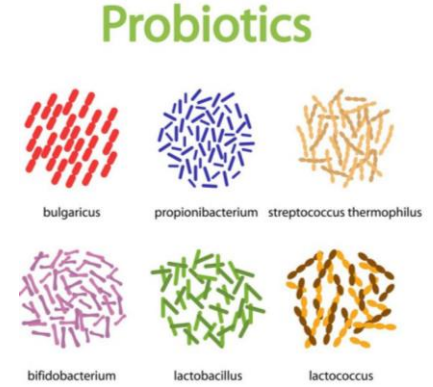
Annie FRELET-BARRAND

annie.frelet-barrand@femto-st.fr



The Gram⁺ bacteria *Lactococcus lactis*

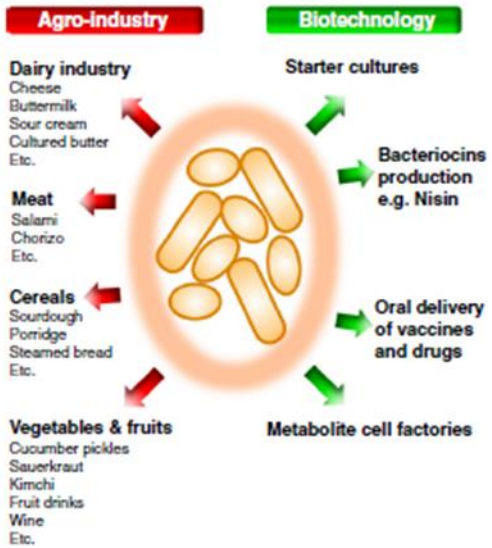




- Optimal growth at 30°C; size : 0,5-1,5 µm
- Naturally present in cheese and fermented food
- Growth under fermentation or respiration

- Recognized as « Generally regarded as safe » by FDA (QPS by EFSA)

→ agro industry + biotechnology (fermented food)



- Production of heterologous proteins

→ Antigen/therapeutic protein delivery - **MEDECINE**

→ <u>BIOTECHNOLOGY</u> = Soluble and membrane proteins (MPs)

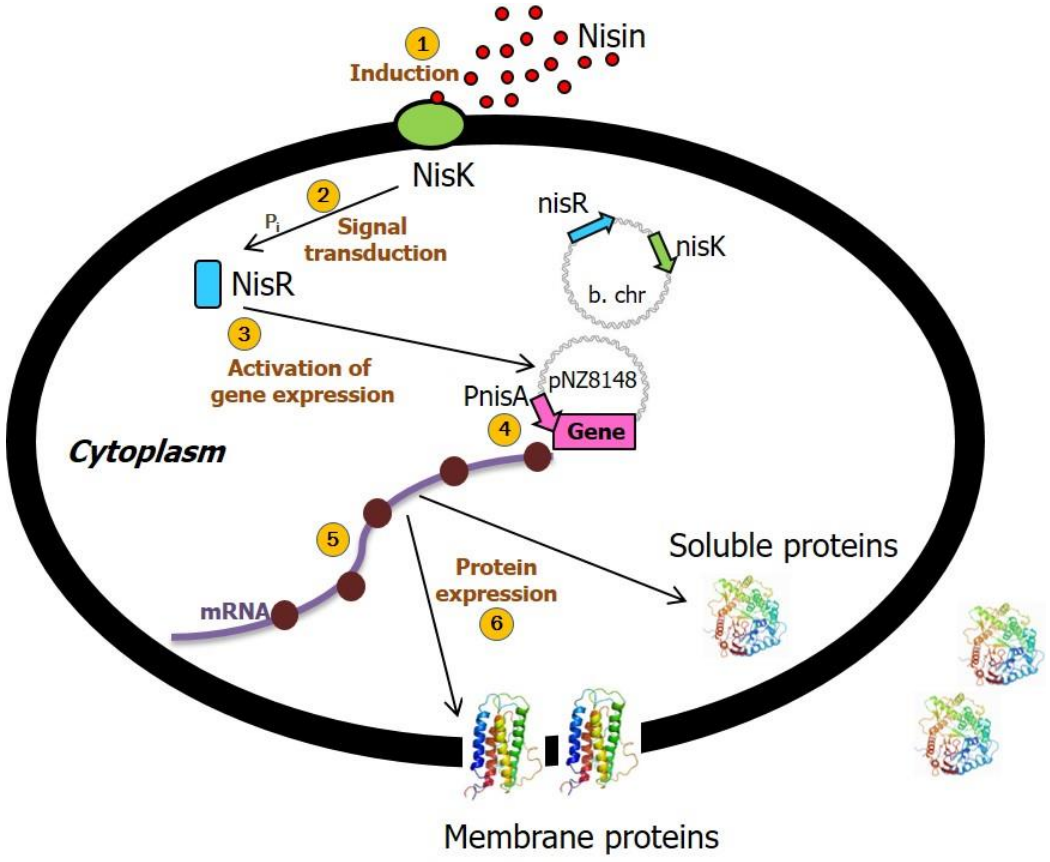
- Promoter-based expression (Pontes *et al.*, 2011)
- <u>No</u> inclusion bodies, <u>no</u> endotoxins, <u>no</u> proteases (advantages over *E. coli*)
- Expression of proteins under their native oligomeric state

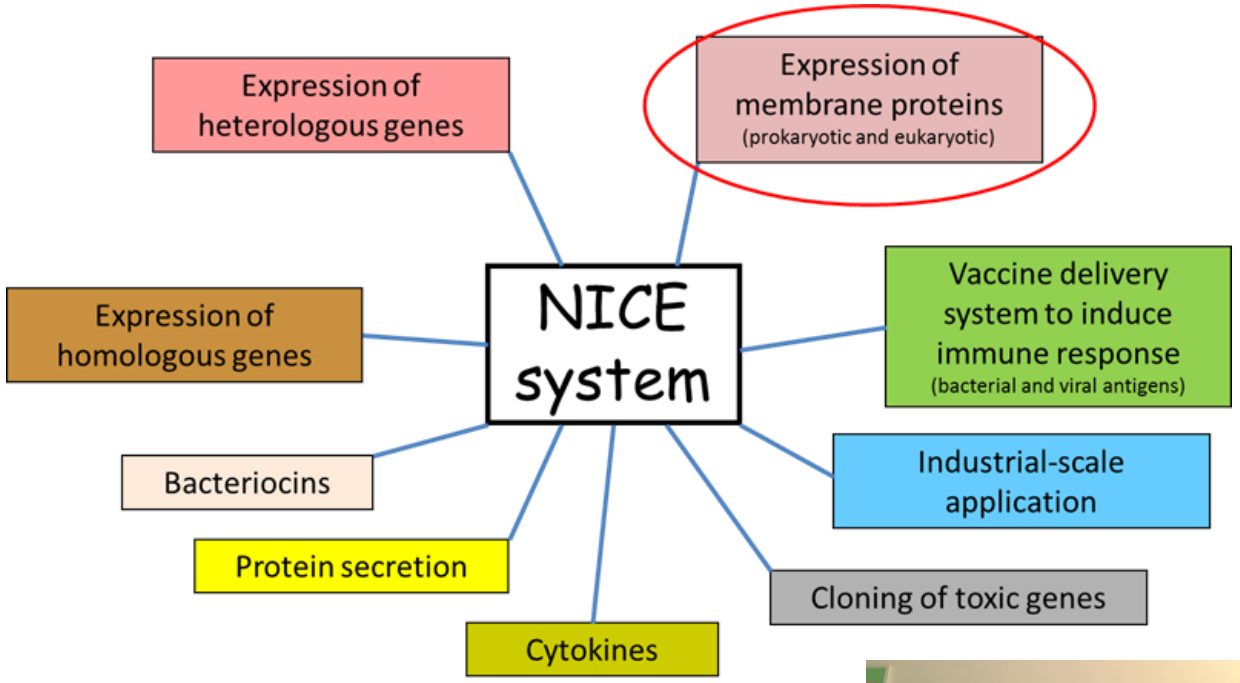


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Versatile and tightly controlled NICE system to express proteins

NICE : Nisin Induced Controlled gene Expression





Nisin : 34 AA polypeptide antibiotic // Induction with subinhibitory concentrations/ calibrated home-made

Frelet-Barrand, 2022, MDPI Biomolecules



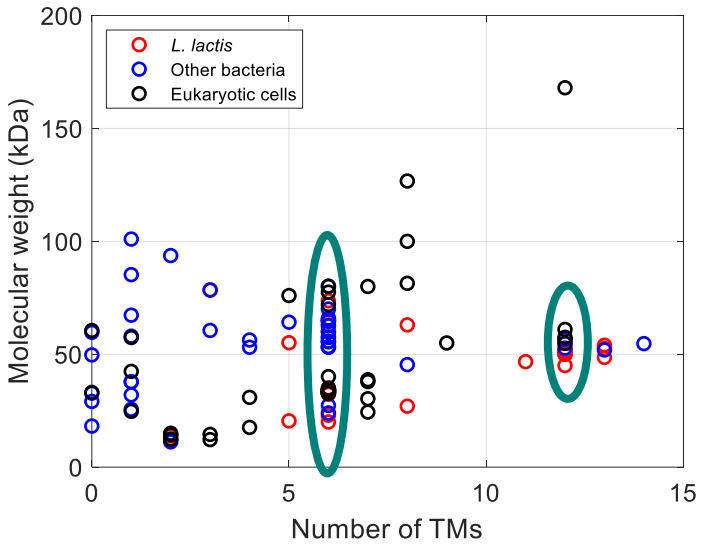
L. lactis has allowed to overexpress 115 MPs

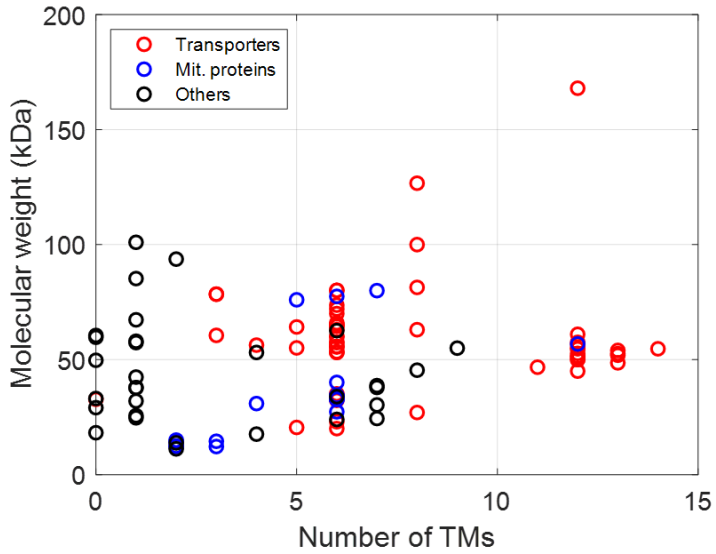
MPs expressed in *L. lactis* (2003-2022)		
23	prokaryotic	*L. lactis*
45	prokaryotic	Other bacteria
47	eukaryotic	Yeast/plant/ human

From 0 to 14 TM helices
From 12 to 170 kDa

2 main clusters

In their native oligomeric state





Mainly transporters including the large family of known ABc transporters + some mitochondrial proteins, mainly from *S. cerevisiae*

- ✓ Yields : 0.1 to 30% of Total Membrane Proteins // 5-10 times less than *E. coli*
- ✓ Already 3D structures of MP obtained using L. lactis as expression host

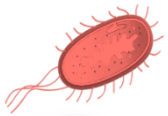


Frelet-Barrand, 2022, MDPI Biomolecules

Expression of an eukaryotic MP= caveolin

ANR
CAVEOTANK
(2017-2021)





Escherichia coli

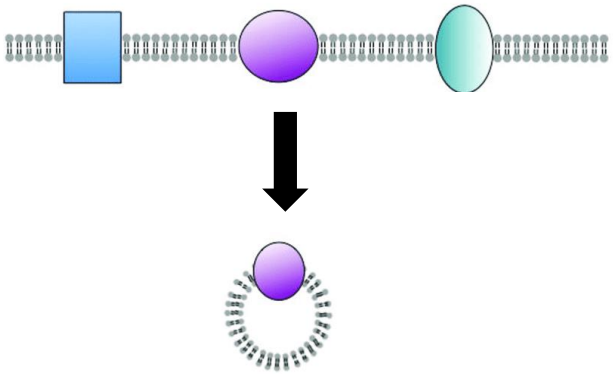


Lactococcus lactis



Insect cells *Sf*21

MP within a more
favorable native-like

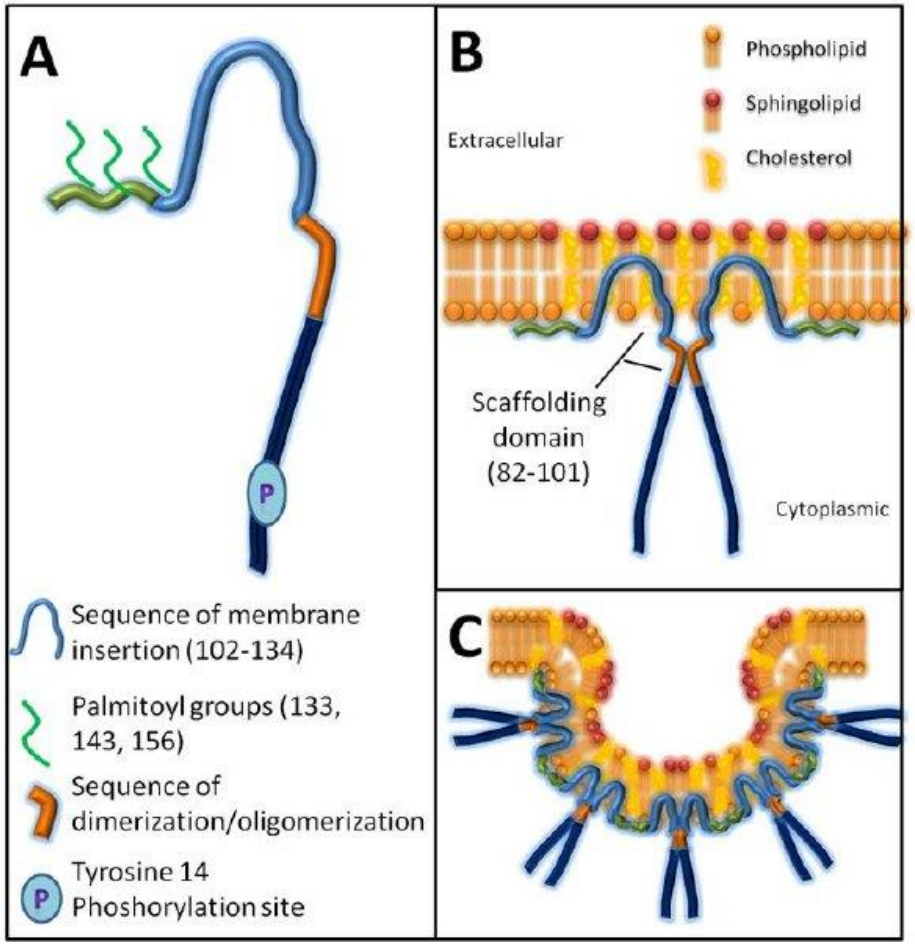


⚠ Steps ⚠
perturbing/denaturing protein
= solubilisation/purification &
proteoliposome reconstitution

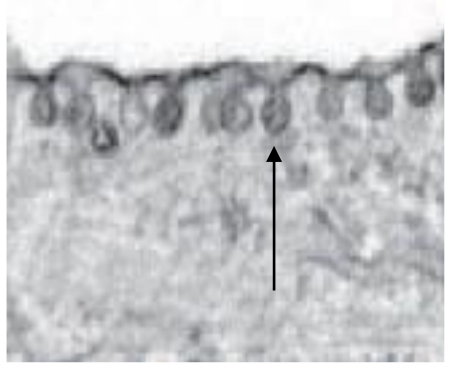


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Context of the study – Expression of caveolin



- Caveolin (23 kDa)
- Functional oligomeric state
- Essential for caveolae formation

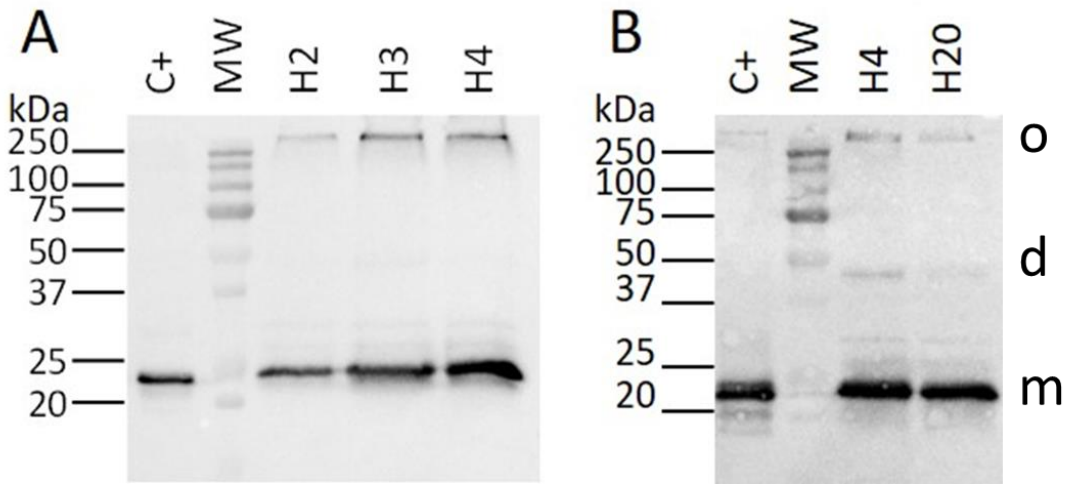


Caveolae in adipocytes

Lobos et al., 2011, DOI: 10.5772/21829/InTech



Caveolin is expressed in *L. lactis* under its native form



- Caveolin (23 kDa) expressed with maximum at 4h post induction
- High yield = 25% of total membrane proteins PMT
- Under its native oligomeric state
- Still present at relatively high yield after 20h

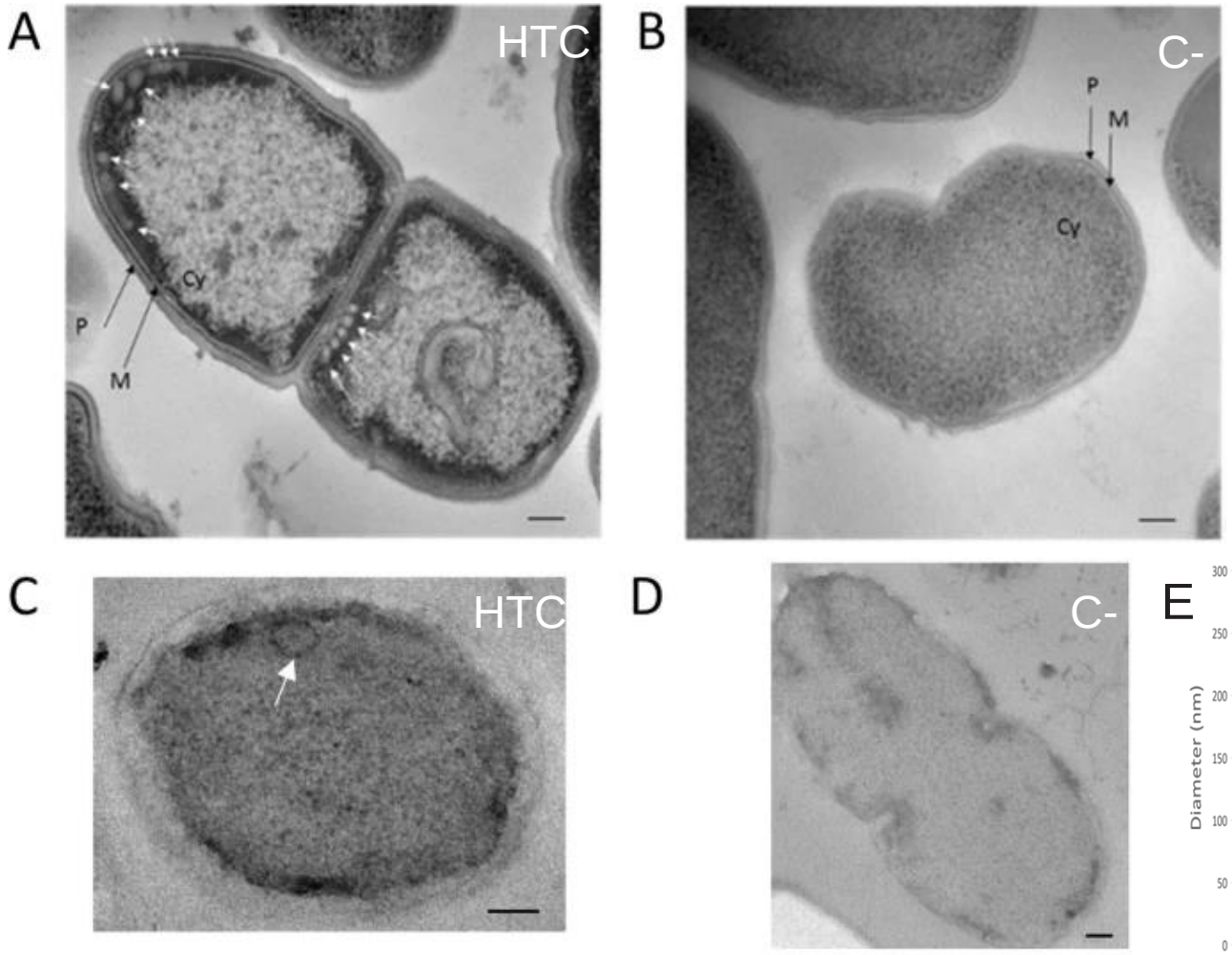
✓ *Lactococcus lactis* is able to express caveolin under its functional oligomeric state



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Flourieusse et al., 2022, MCF

TEM (Transmission Electron Microscopy) revealed caveolae within *L. lactis* bacteria



Immunolabelling (C/D)

Scale = 100 nm.

Transfer of recombinant bacteria after 20h of induction maintained at 30°C directly to aluminium sample holders then immediate cryoimmobilization using a Leica High-Pressure Machine (HPM 100, Leica Microsystems, Vienna, Austria) and transferred to liquid nitrogen.

A-B TEM on bacteria and C-D Immuno-electron microscopy (IEM) with antibody specific to caveolin (1/75e) and a secondary antibody goat anti-mouse conjugated with 5-nm colloidal gold (1/25)

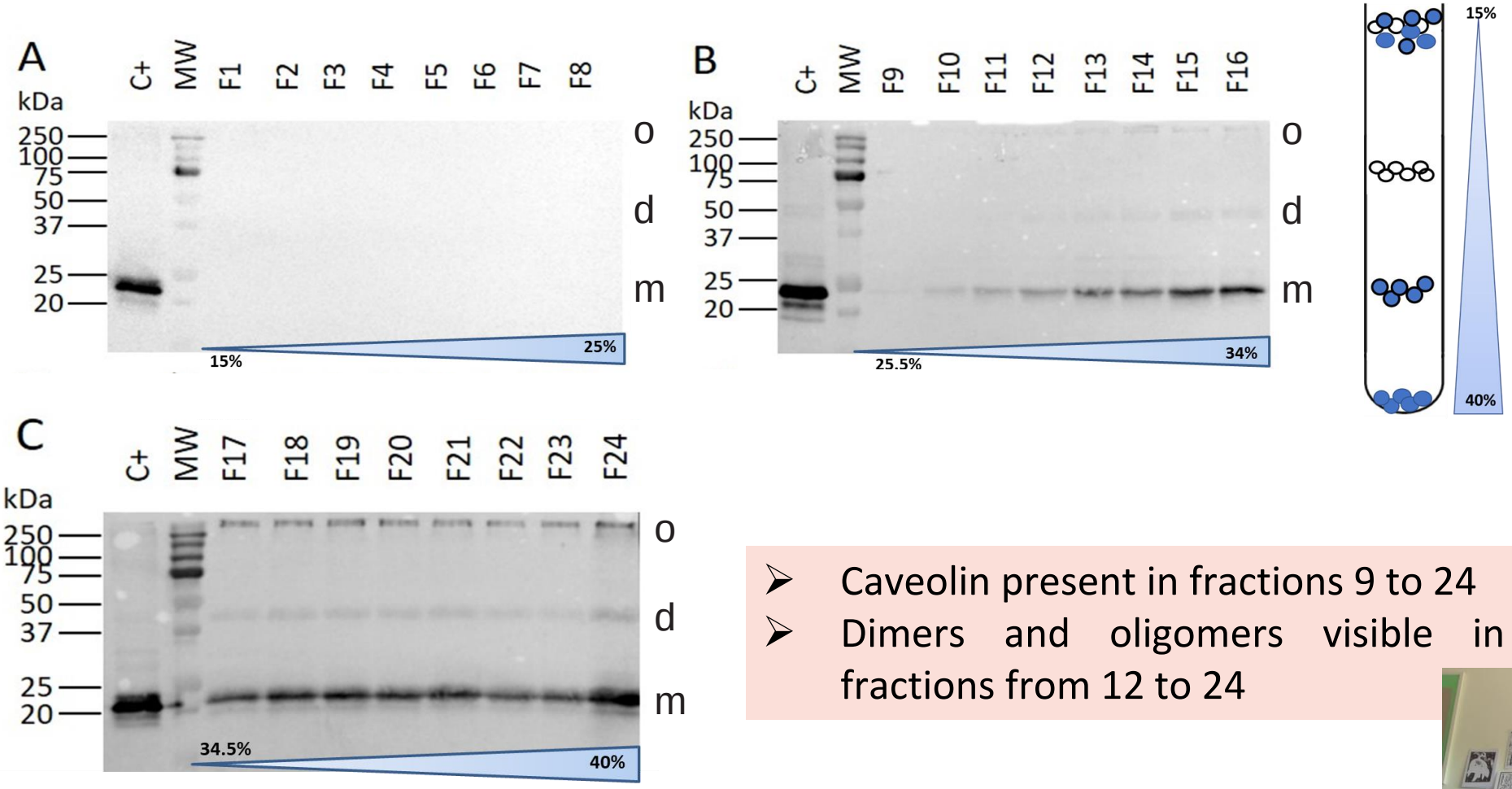
- Caveolae at the apex in HTC recombinant *L. lactis,* absents in negative control
- Mean diameter measured of 40,2 nm (n=390/E)



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Flourieusse et al., 2022, MCF

Sucrose density gradient allowed caveolae isolation (1)

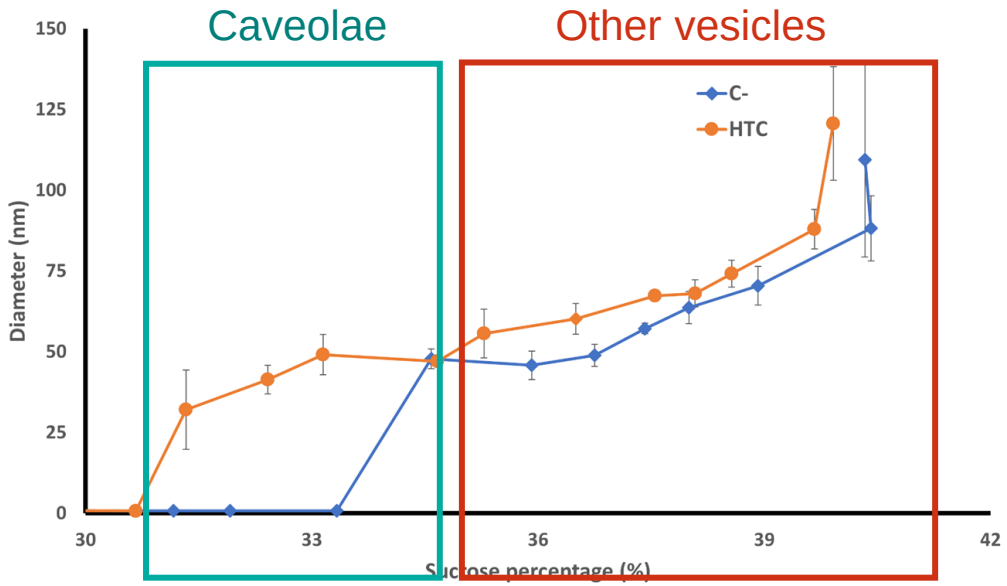


- Caveolin present in fractions 9 to 24
- Dimers and oligomers visible in fractions from 12 to 24



Flourieusse et al., 2022, MCF

Dynamic Light Scattering analysis of caveolae



Measurements on 3 independent experiments
3 triplicates of each

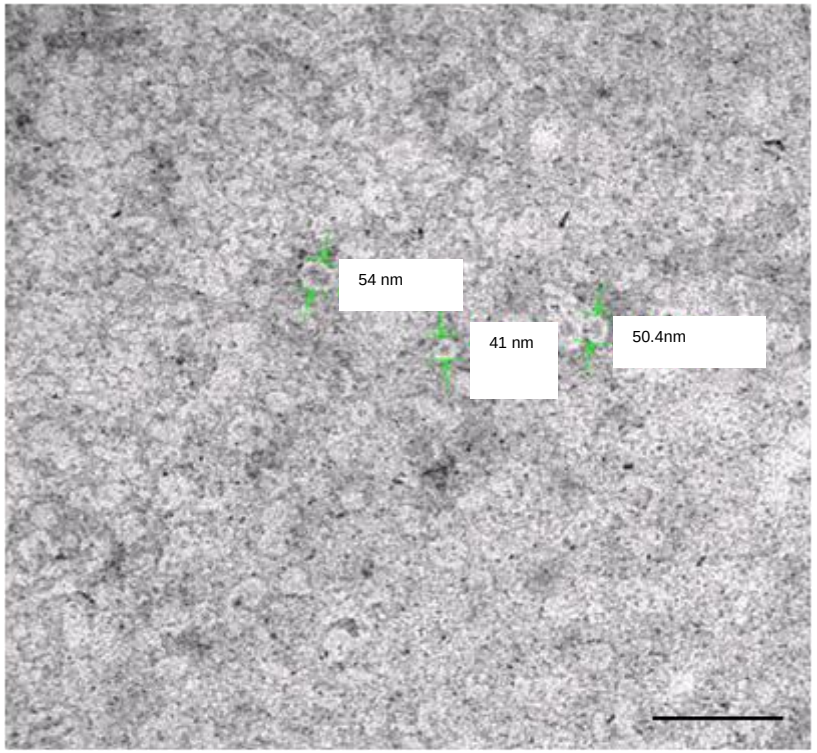
- Light fractions (31 to 34.5%) : size comparable to caveolae, coïncidence of vesicles with appearance of caveolin oligomers on WB analysis
- Heavy fractions (> 35%): vesicles with higher diameter (> 50 nm)

- *Lactococcus lactis* is able to produce « heterologous caveolae » of 30-50 nm diamet



Flourieusse et al., 2022, MCF

TEM (Transmission Electron Microscopy) of isolated caveolae



Negative staining electron microscopy of caveolae after isolation on sucrose gradient. Samples placed on an effluved carbon formvar grid and allowed to rest for 20 min before blotting with filter paper. Then negatively stained with commercial solution (Uranyless EMS, USA) during 3 min before blotting and air drying. Transmission electron microscopy (TEM) images were taken with a Hitachi H7800 at an acceleration voltage of 100 kV and an AMT camera.

✓ *L. lactis* produce heteologous caveolae of 30 to 50 nm diameter



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Flourieusse et al., 2022, MCF

Take home message and outlook

- ✓ *Lactococcus lactis* = alternative and promising host for expression of proteins including membrane proteins (of pharmaceutical interest) and caveolin
- ✓ NICE system for tightly regulated expression system of proteins
- ✓ Functional and structural characterizations of proteins

Other skills / projects at FEMTO-ST institute:
Bioengineering, bioproduction and biochemical and biophysical (of which MADLS) qualifications of elements :

- soluble & membrane proteins
- prokaryotic and eukaryotic native and synthetic vesicles (including extracellular vesicles),
- $Gram^+$ and $Gram^-$ bacteria and mammalian cells

in connection with developments of detection and monitoring devices



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Acknowledgements

CEA Grenoble

2006-2009

iRTSV/LPCV

iBS/LPM et LIM





CEA Saclay

2009-2015

I2BC/B3S/LSOD

Institute FEMTO-ST (since 2015)

Dep. MN2S / Team BIND

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