Bacterial outer membrane vesicles (OMVs)

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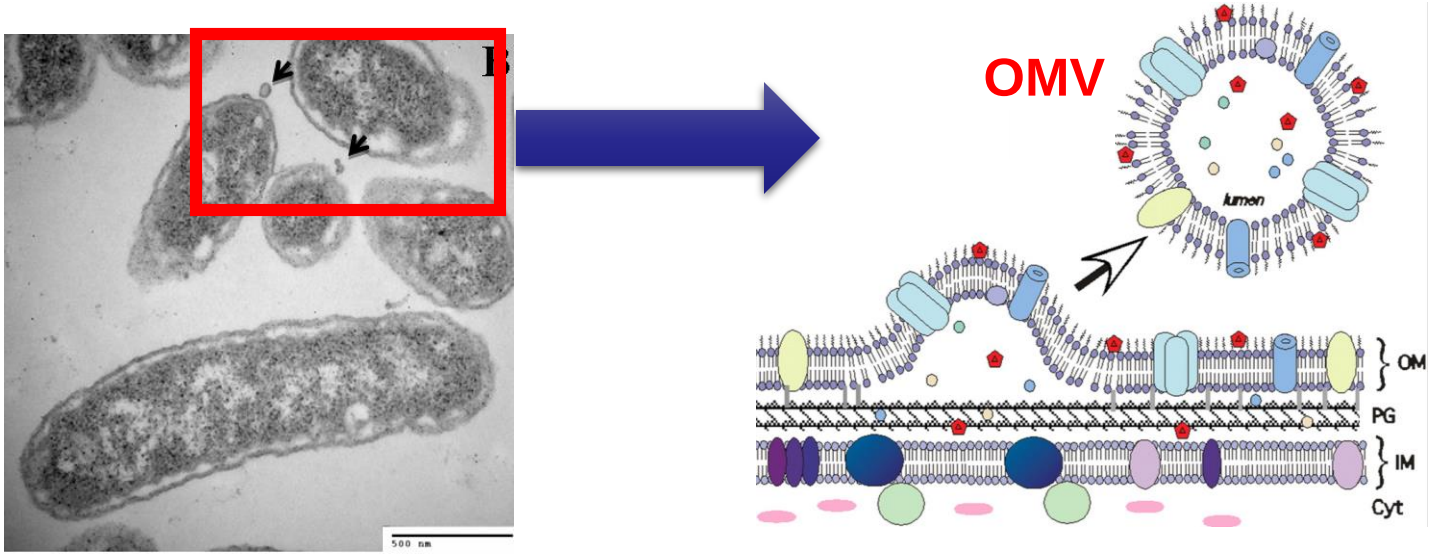
Outline

- Introduction of OMVs
- Formation mechanism and content of OMVs
- Entry mechanism into host cells of OMVs
- Functions of OMVs
- Application: OMV-based vaccines
- Conclusions and perspectives

Outer membrane vesicles (OMVs)

- **Bacterial OMVs**: nano-sized spherical structures 20-250 nm in diameter derived from the bacterial cell envelopes
- produced by of almost all Gram-negative bacteria during all growth phases and in all environmental conditions
  - ➢ Pathogenic and nonpathogenic bacteria



TEM image of OMVs in *Aeromonas hydrophila*

Meta J. Kuehn and Nicole C. Kesty. Genes Dev, 2005
Andrea Guerrero Mandujano, et al. EC Microbiology, 2015

How do bacteria produce OMVs?

----a general mechanism of OMVs formation

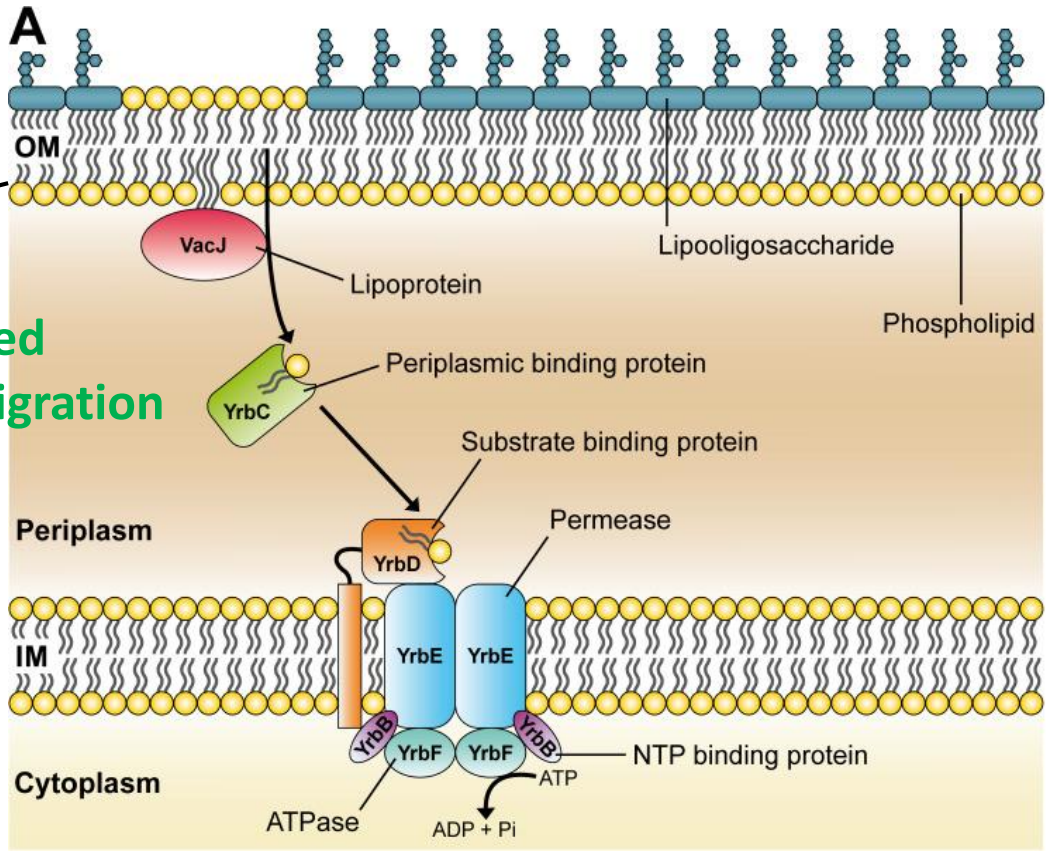
OMVs formation

VacJ/Yrb transport system:

- highly conserved in Gram-negative bacteria
- affects the migration of membrane phospholipids

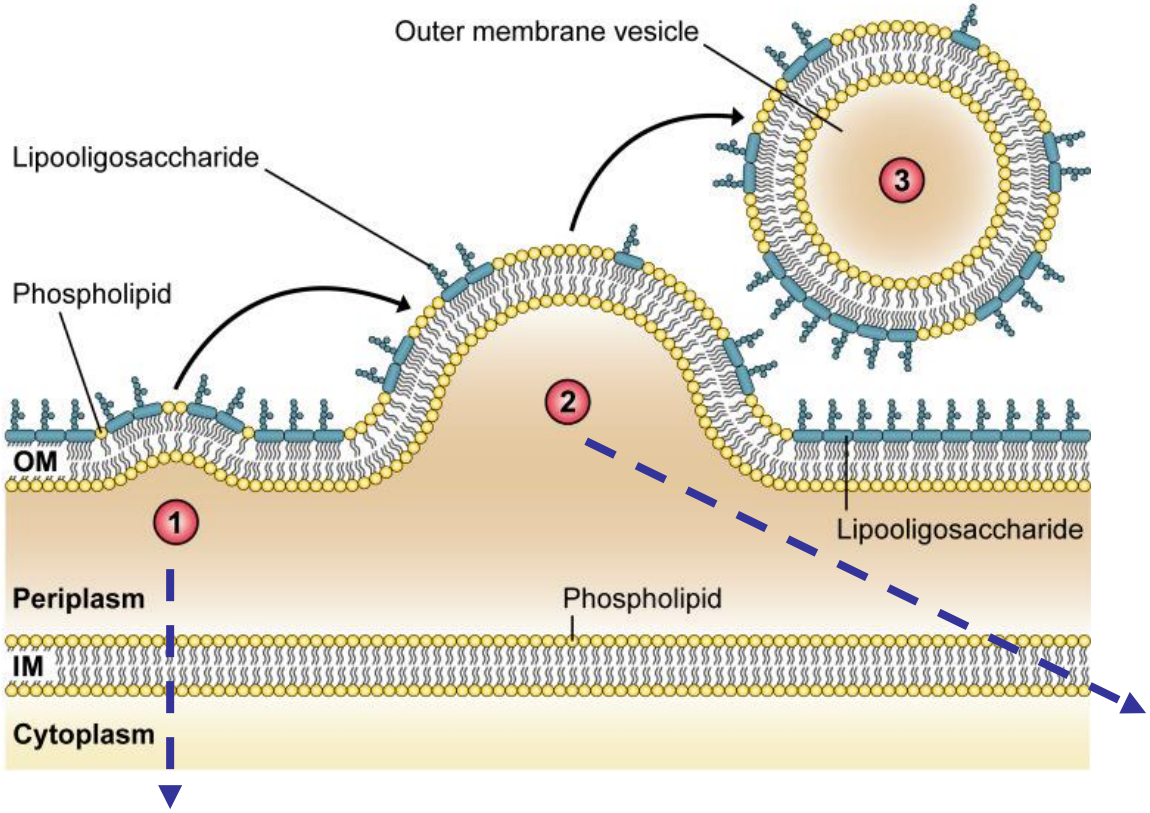
VacJ/Yrb-mediated phospholipids migration

Prevention of phospholipids accumulation in the outer leaflet of the outer membrane



Sandro Roier, et al. Nature Communications, 2016

OMVs formation mechanism



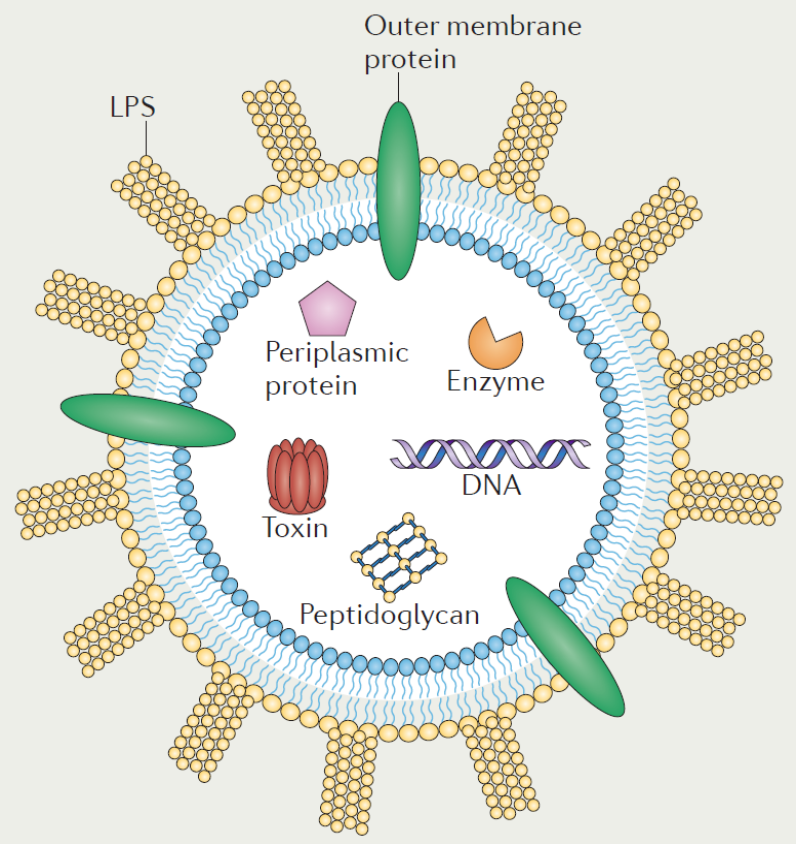
Step 3:
The released OMV is enriched in phospholipids incorporated into the outer leaflet of the vesicle membrane.

Step 2:
Further enrichment of positive and negative curvature-inducing phospholipids in both leaflets supports the budding of the outer membrane, which finally pinches off to form an OMV.

Step 1:
Inhibition of VacJ/Yrb system-related genes expression results in phospholipids accumulation in the outer leaflet of the outer membrane, thus initiates an outward bulging of the outer membrane through the asymmetric expansion of the outer leaflet.

Sandro Roier, et al. Nature Communications, 2016

Structure and content of OMVs



Detailed proteomic and biochemical analyses have shown that OMVs contain diverse components derived from the parent bacteria:

lipopolysaccharide (LPS), periplasmic and membrane-bound proteins, enzymes, toxins, DNA, RNA

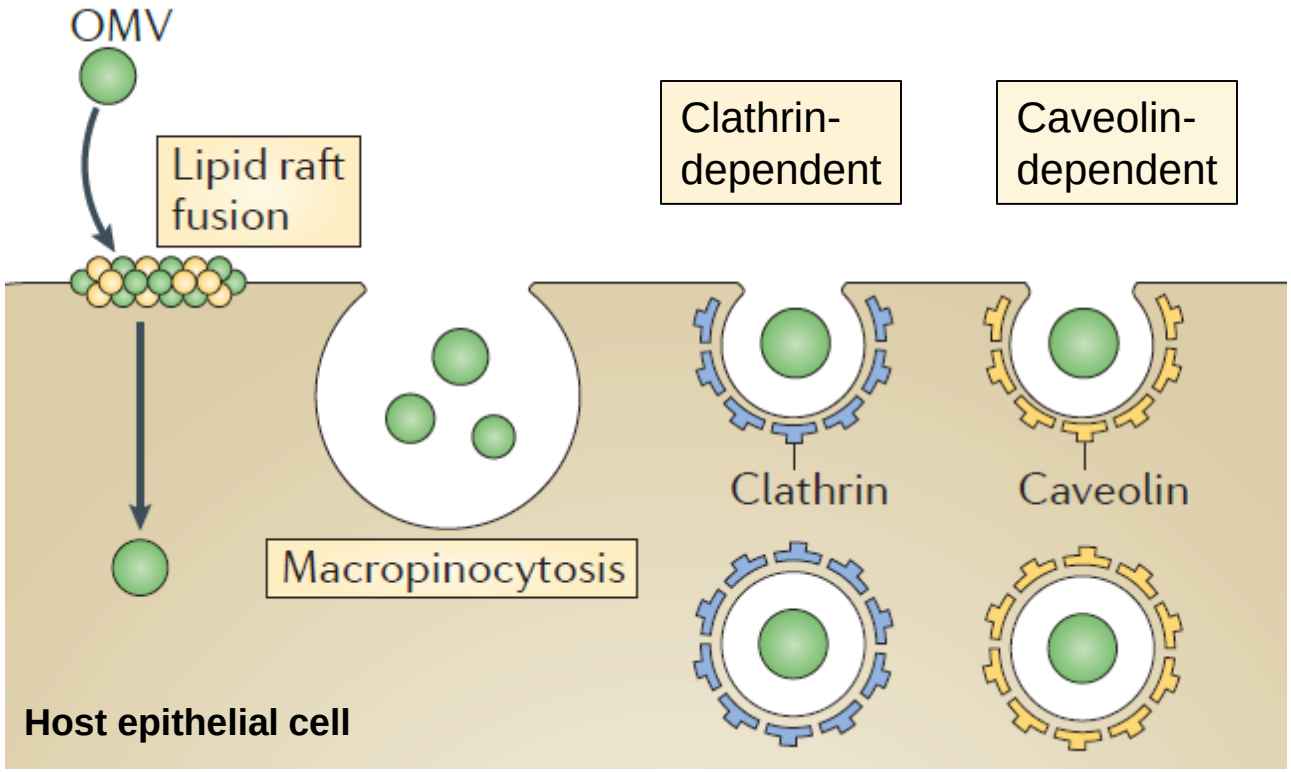
A secretion and delivery system

Maria Kaparakis-Liaskos and Richard L. Ferrero.
Nature Reviews Immunology, 2015

How do OMVs enter host cells?

Mechanisms of OMVs entry

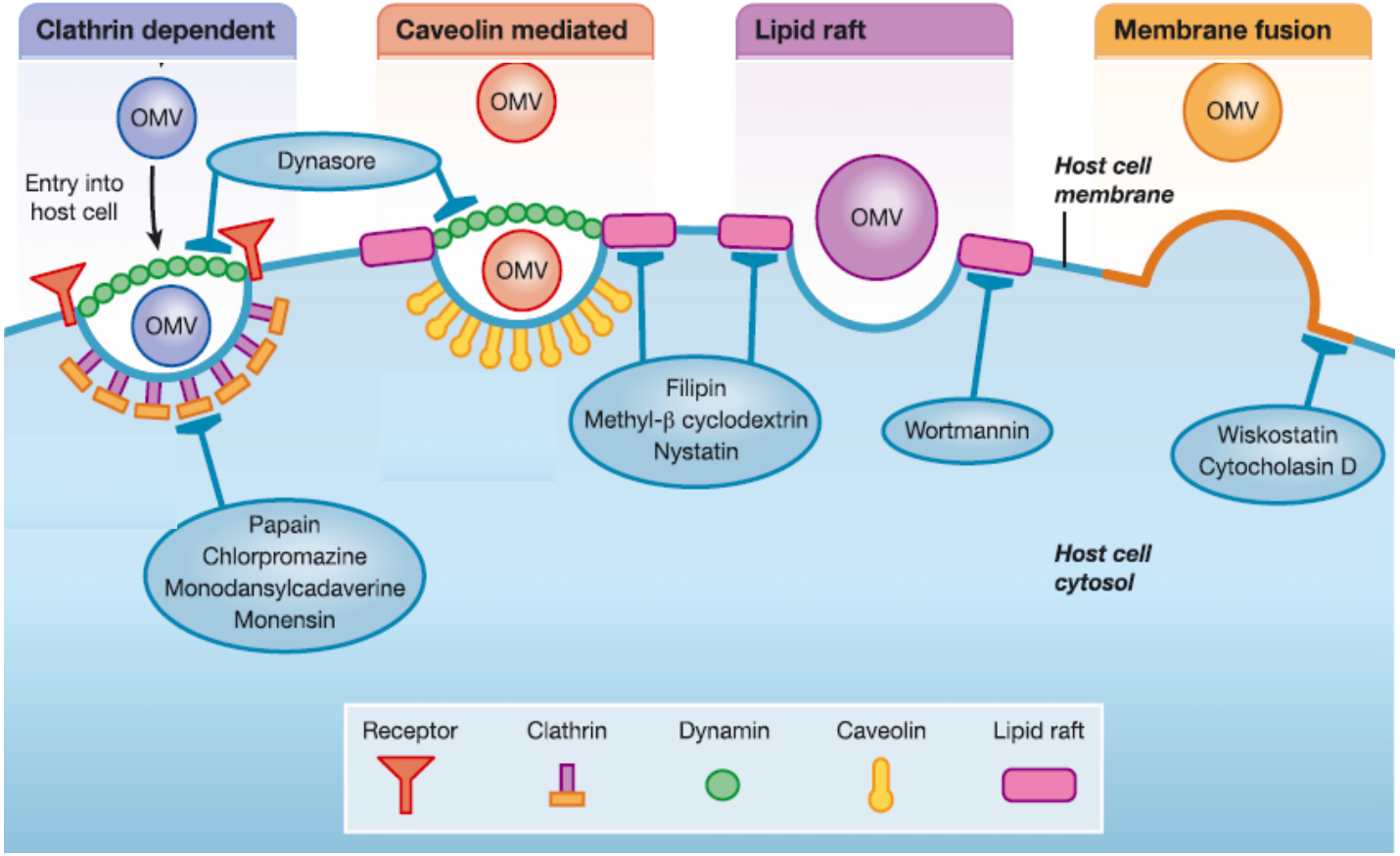
Four main pathways of **endocytosis** have all been implicated in mediating OMVs entry into host cells.



Maria Kaparakis-Liaskos and Richard L. Ferrero.
Nature Reviews Immunology, 2015

Inhibition of OMVs entry

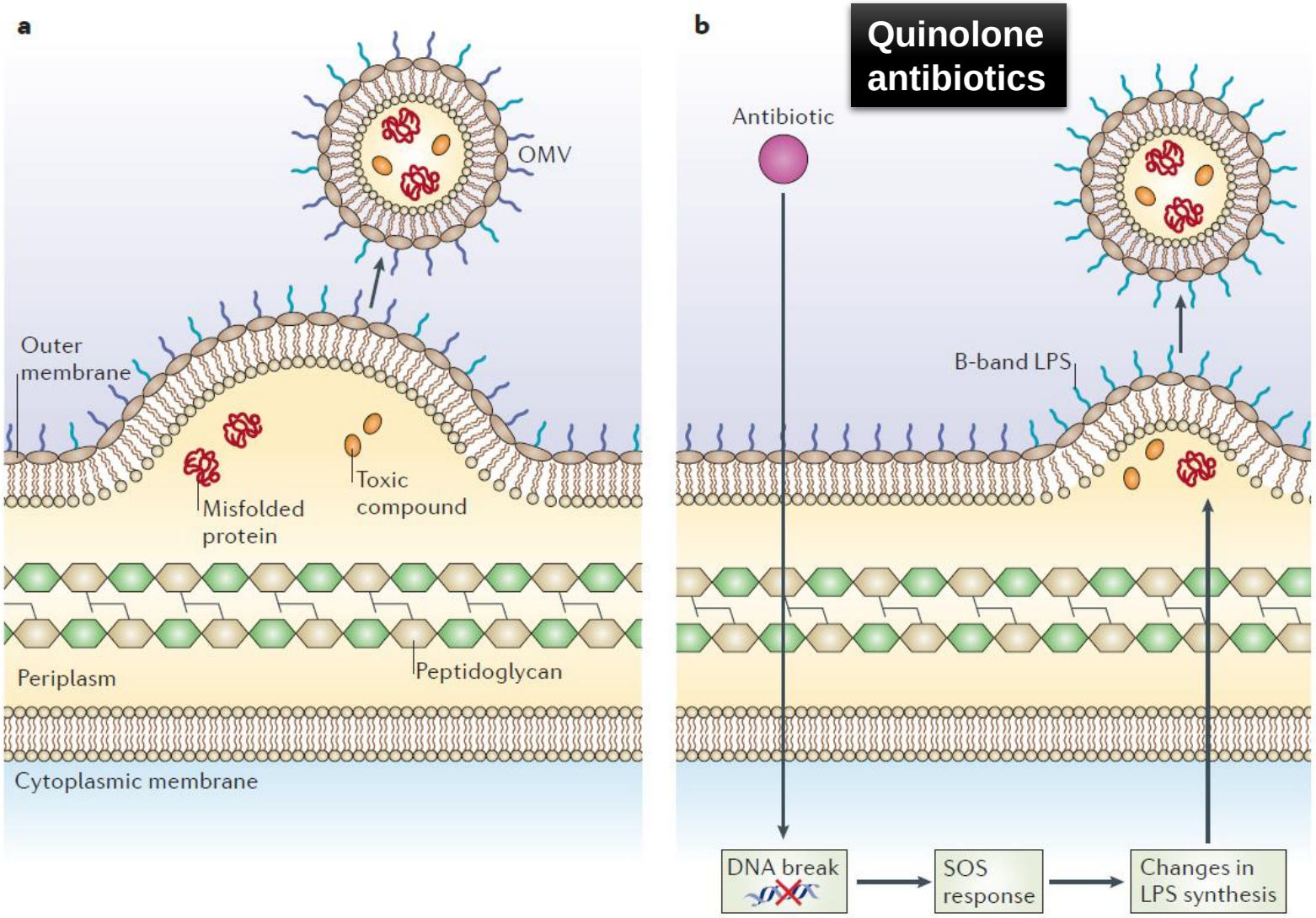
Pharmacological inhibition of key components of the endocytic pathways prevents OMVs entry:



Eloise J. O'Donoghue and Anne Marie Krachler.
Cellular Microbiology, 2016

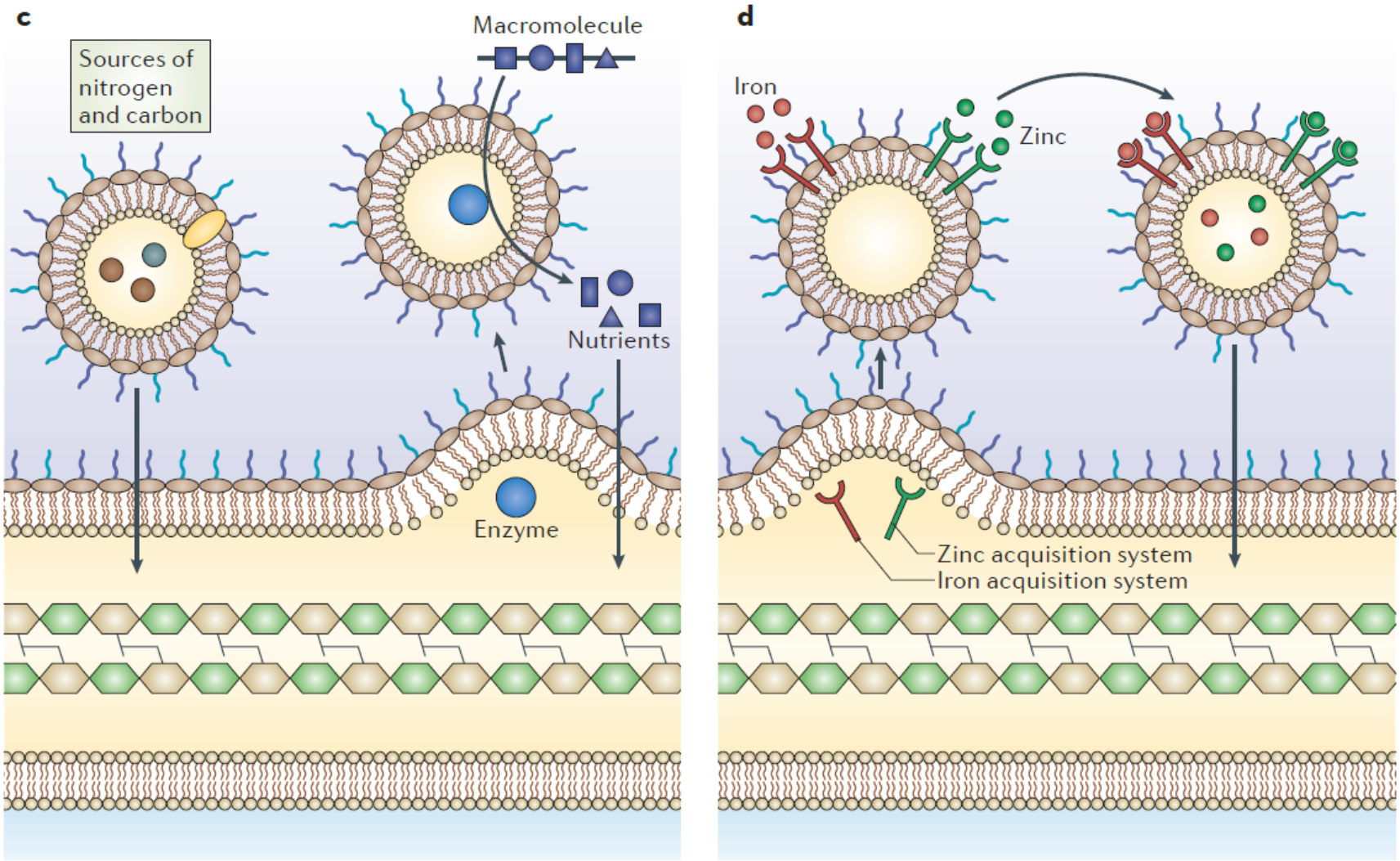
Functions of OMVs in bacterial physiology and pathogenesis

● Stress response >>>> bacterial survival



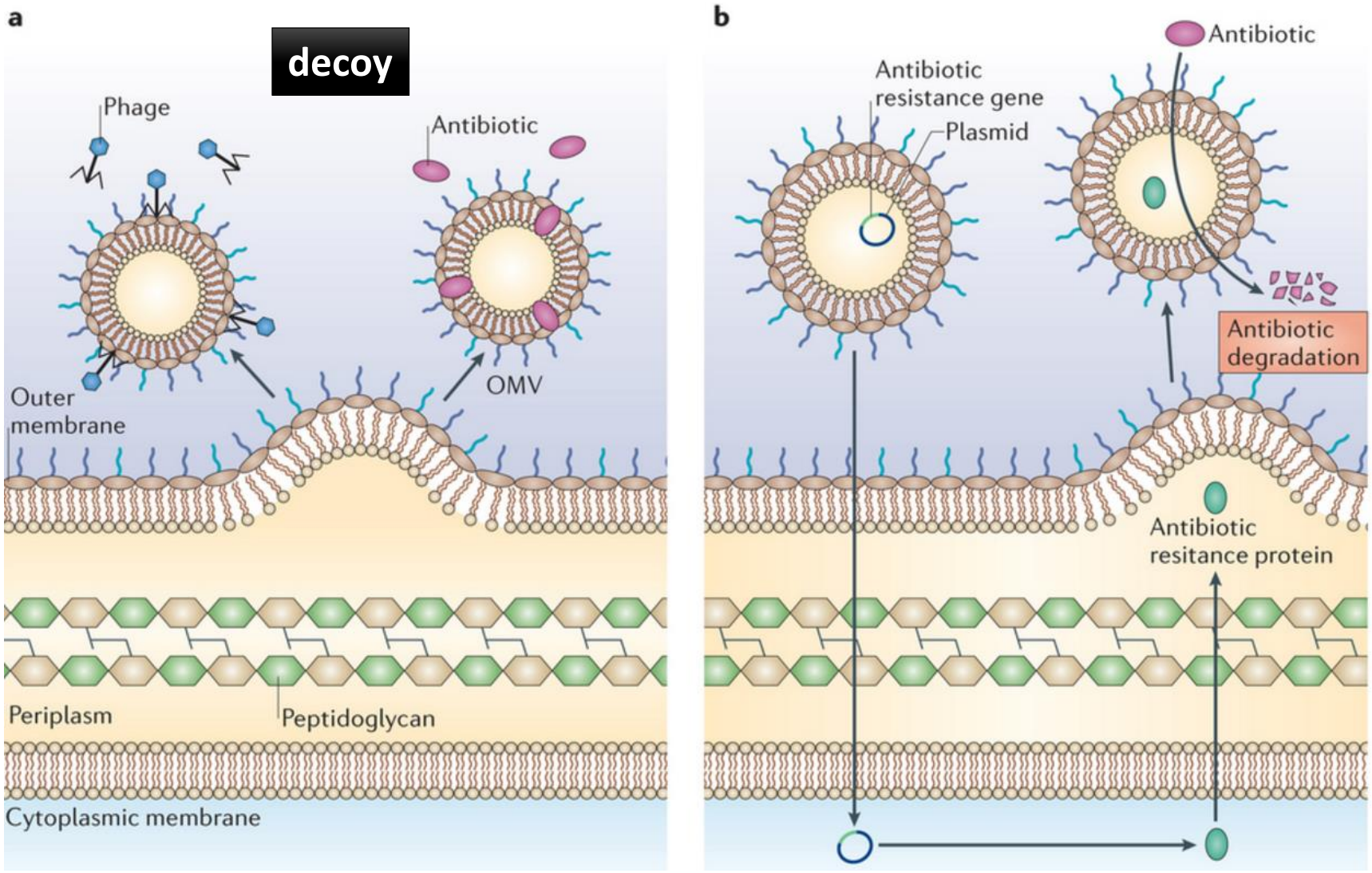
Carmen Schwechheimer and Meta J. Kuehn.
Nature Reviews Immunology, 2015

● Nutrient acquisition >>>> bacterial survival



Carmen Schwechheimer and Meta J. Kuehn.
Nature Reviews Immunology, 2015

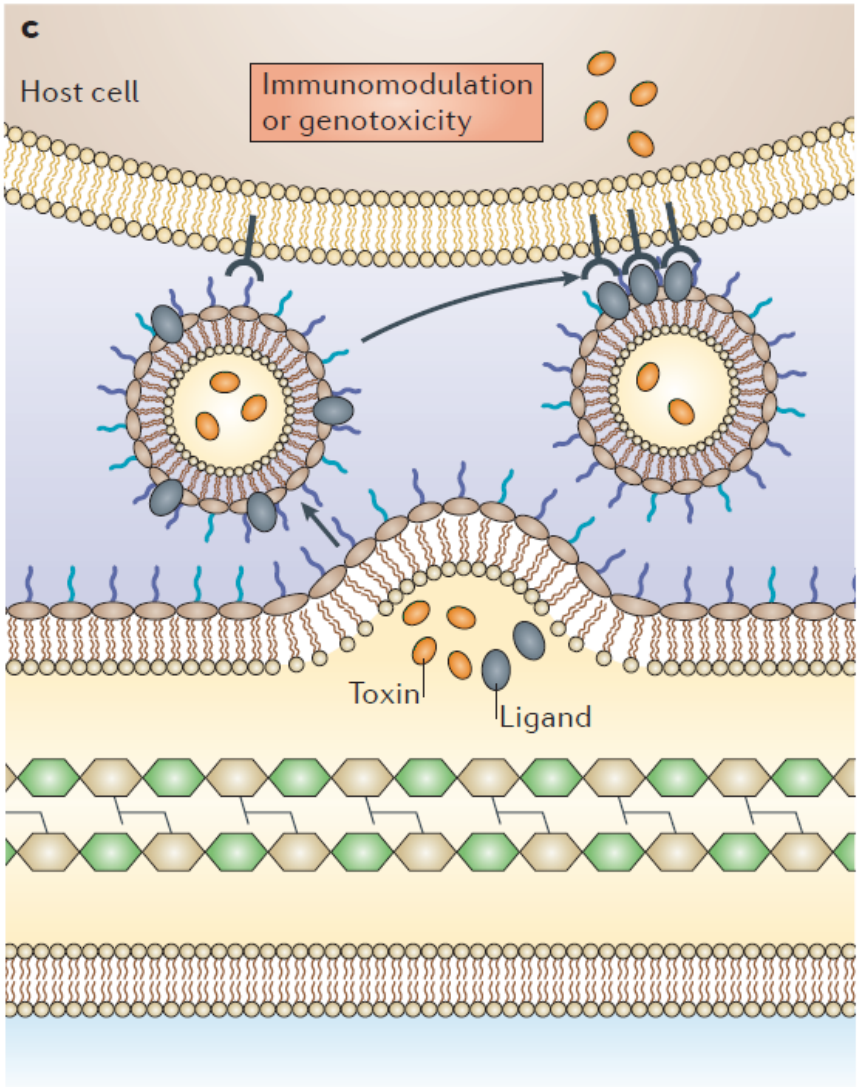
Defense and resistance >>>> bacterial pathogenicity



Carmen Schwechheimer and Meta J. Kuehn.
Nature Reviews Immunology, 2015

- **Delivery of virulence factors >>>> bacterial pathogenicity**

Pathogenic bacteria can use OMVs to mediate the delivery of virulence factors, such as toxins, into host cells, including immune cells.



Carmen Schwechheimer and Meta J. Kuehn.
Nature Reviews Immunology, 2015

OMVs-related virulence factors

OMV-associated proteins	Species	Activity	Reference
Apx toxin	*Actinobacillus pleuropneumoniae*	Hemolysis, Cytolysis	(59)
BabA, SabA	*Helicobacter pylori*	Adhesin	(60)
CagA	*Helicobacter pylori*	Cytotoxicity-associated immunodominant antigen	(61)
Cholera toxin (CTX)	*Vibrio cholera*	Adenylate cyclase activation	(62)
Cif	*Pseudomonas aeruginosa*	Cystic fibrosis transmembrane conductance regulator (CFTR) inhibition	(63)
Cytolethal distending toxin (CDT)	*Aggregatibacter actinomycetemcomitans*, *Campylobacter jejuni*, *Escherichia coli*	DNA damage, Cell death	(64, 65)
Cytotoxic necrotizing factor type 1 (CNF1)	Uropathogenic Escherichia coli	Cytotoxic	(66)
Cytotoxin ClyA	*Escherichia coli* O111:H-	Pore-forming	(48)
Gingipains (RgpA, RgpB, Kgp)	*Campylobacter jejuni*, *Porphyromonas gingivalis*	Trypsin-like cysteine proteinases	(22, 67)
Heat-labile enterotoxin (LT)	Enterotoxigenic *Escherichia coli*	Enterotoxic and vacuolating activities	(68)
HmuY	*Porphyromonas gingivalis*	Sequestering heme from host carriers	(19)
HtrAb	*Borrelia burgdorferi*	Proteolytic activity	(69)
IpaB, IpaC, IpaD	*Shigella flexneri*	Invasins	(70)
Leukotoxin (Ltx)	*Aggregatibacter actinomycetemcomitans*, *Campylobacter jejuni*	Pore-forming	(71, 72)
NarE	*Neisseria meningitidis*	Iron-containing ADP-ribosyltransferase	(73)
OmpA	*Aggregatibacter actinomycetemcomitans*	Adhesin/invasion, immune evasin, biofilm formation	(71)
OspA, OspB	*Borrelia burgdorferi*	Outer membrane surface antigens	(74)
PaAP	*Pseudomonas aeruginosa*	Aminopeptidase	(36)
PagC	*Salmonella enterica* serovar Choleraesuis	Required for survival	(75)
PagJ, PagK1, PagK2	*Salmonella enterica* serovar Typhimurium	Required for survival	(45)
PorA	*Neisseria meningitidis*	Outer membrane protein (OMP) antigens	(73)
RTX (repeat-in-toxin) toxin	*Vibrio cholera*	Cross-linking of actin cytoskeleton	(76)
Serralysin	*Pseudomonas aeruginosa*	Extracellular protease	(60)
Shiga toxins (Stx, Stx1, Stx2)	*Pseudomonas aeruginisa*, *E. coli*	Protein synthesis inhibition	(13, 77)
UspA1, UspA2	*Moraxella catarrhalis*	Surface adhesion protein	(39)
VacA	*Helicobacter pylori*	Vacuolating cytotoxin	(78)
α-Hemolysin (HlyC)	Enterohemorrhagic *E. coli*	Acyltransferase inducing hemolysin	(68)
β-Lactamase	*Pseudomonas aeruginosa*	Antibiotics resistance	(79)

Over 30 virulence factors……

Sangyong Lim and Hyunjin Yoon. J Bacteriol Virol, 2015

- **association with biofilm formation >>>> bacterial pathogenicity**

Species	Factor	Effect
Helicobacter pylori	22-kDa protein	Plays an important role in biofilm formation.
Francisella	OMV	Involved in biofilm formation and forming part of biofilm matrix.
Pseudomonas aeruginosa	CPA	Its absence causes structural defects which limit the development of mature biofilms.
Vibrio cholerae	OMV-associated protein DegP	Required for the secretion of biofilm matrix components and the activity strongly influences biofilm formation.
Pseudomonas putida	OMV	Lead to an increased hydrophobicity of cells surface which enhanced their ability to form biofilms

OMVs have been shown to participate in biofilm formation.

Wendong Wang, et al. FEMS Microbiology Letters, 2015
Carmen Schwechheimer and Meta J. Kuehn. Nature Reviews Immunology, 2015

Application: OMV-based vaccines

OMVs are attractive vaccine candidates:

1. high immunogenicity
   closely reflect the native conformation of their parent bacteria that induce both innate and adaptive immunity after entering eukaryotic cells
2. non-replicative and hence safe

✓ **A success**: OMV-based vaccine against serogroup B *Neisseria meningitides* (approved by the European Commission)

- The OMV-based 4CMenB vaccine contains three highly immunogenic proteins that induce protective antibody responses.

Maria Kaparakis-Liaskos and Richard L. Ferrero. Nature Reviews Immunology, 2015
Sophie M Andrews and Andrew J Pollard. Lancet Infect. Dis, 2014

Conclusions and perspectives

1. The production of OMVs in Gram-negative bacteria allows bacteria to interact with a wide area of their environment.

2. OMVs play important roles in bacterial physiology and pathogenesis, ranging from secretion and delivery of biomolecules over stress response and biofilm formation to immunomodulation and adherence to host cells.

3. Due to the multifunctional activities, OMVs have been an attractive platform for bioengineering applications.
   (OMV-based vaccines, drug delivery vehicles)

Conclusions and perspectives

Some aspects of OMVs need to be illustrated

- What is the energy source for the vesiculation process in Gram-negative bacteria?
- How do bacteria regulate OMVs formation?
- How is protein cargo selected?

➢ Gram-positive bacteria also release membrane vesicles: *Staphylococcus aureus* and *Mycobacterium tuberculosis*…….

  ◆ biogenesis mechanisms?

  ◆ functions in bacterial physiology and pathogenesis??

Thank you