



afmb
architecture et fonction
des macromolécules biologiques



New molecular tools for integrated structural biology.

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**Ecole Nationale de
Biologie Structurale
Intégrative**

**Juin 2019
Ile d'Oléron**



Système de Sécrétion de Type 9

Dernier système découvert

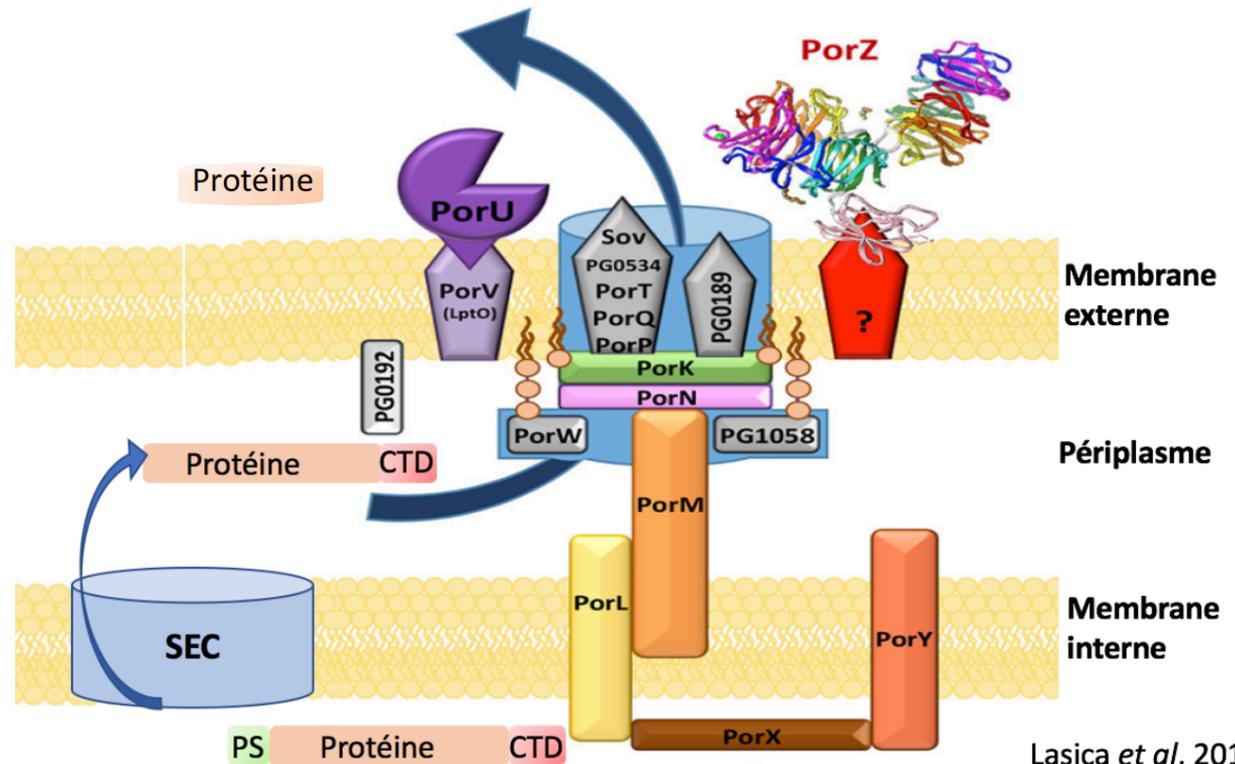
Mécanisme en 2 étapes:

PS : peptide signal

CTD: domaine C-ter conservé

~ 20 gènes identifiés

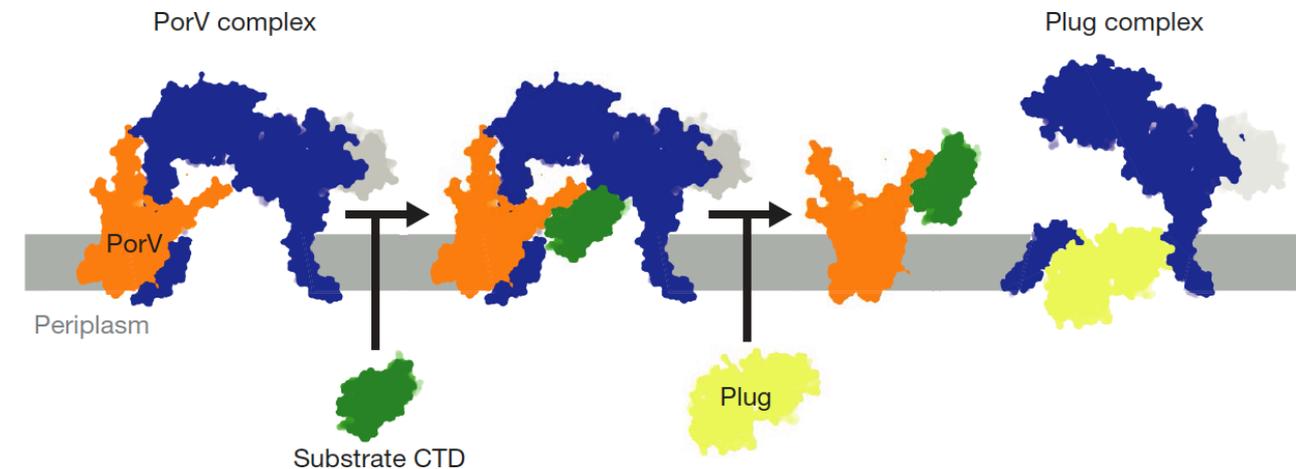
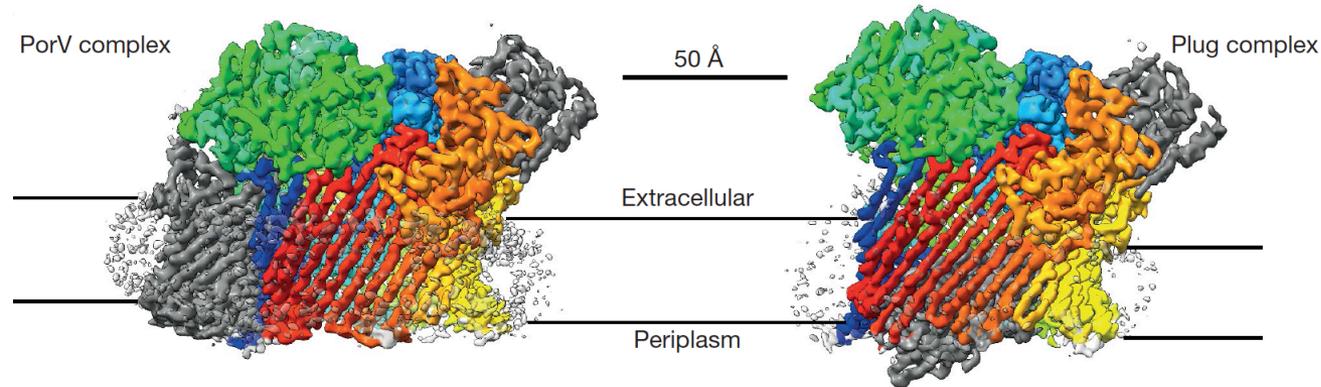
KLMN: Complexe cœur ?



Lasica et al, 2017

Type 9 secretion system structures reveal a new protein transport mechanism

Frédéric Lauber^{1,4}, Justin C. Deme^{2,3,4}, Susan M. Lea^{2,3*} & Ben C. Berks^{1*}



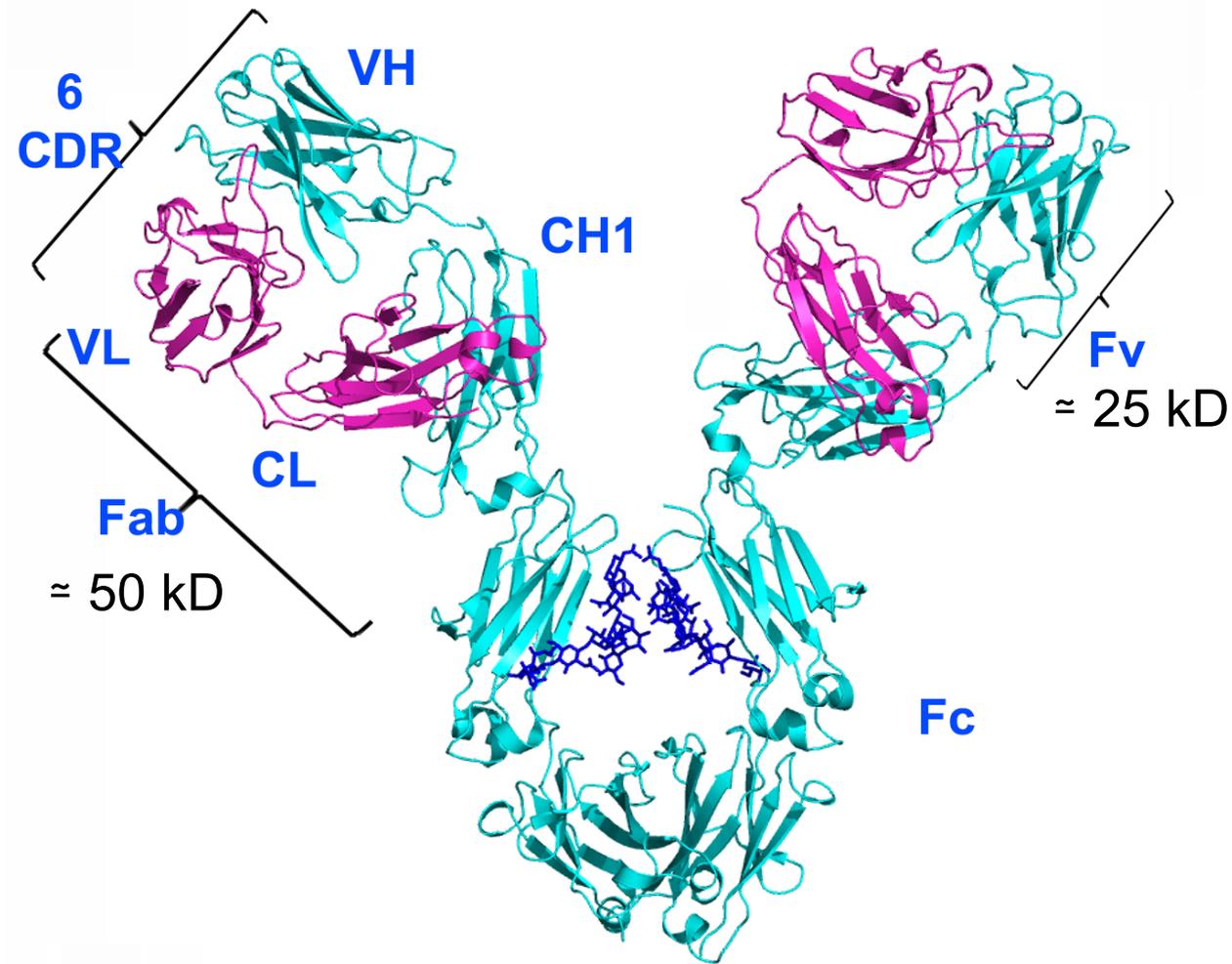
Methods:

We constructed a strain of the gliding bacterium *Flavobacterium johnsoniae* in which the SprA protein was fused to a Twin-Strep tag to permit affinity purification.



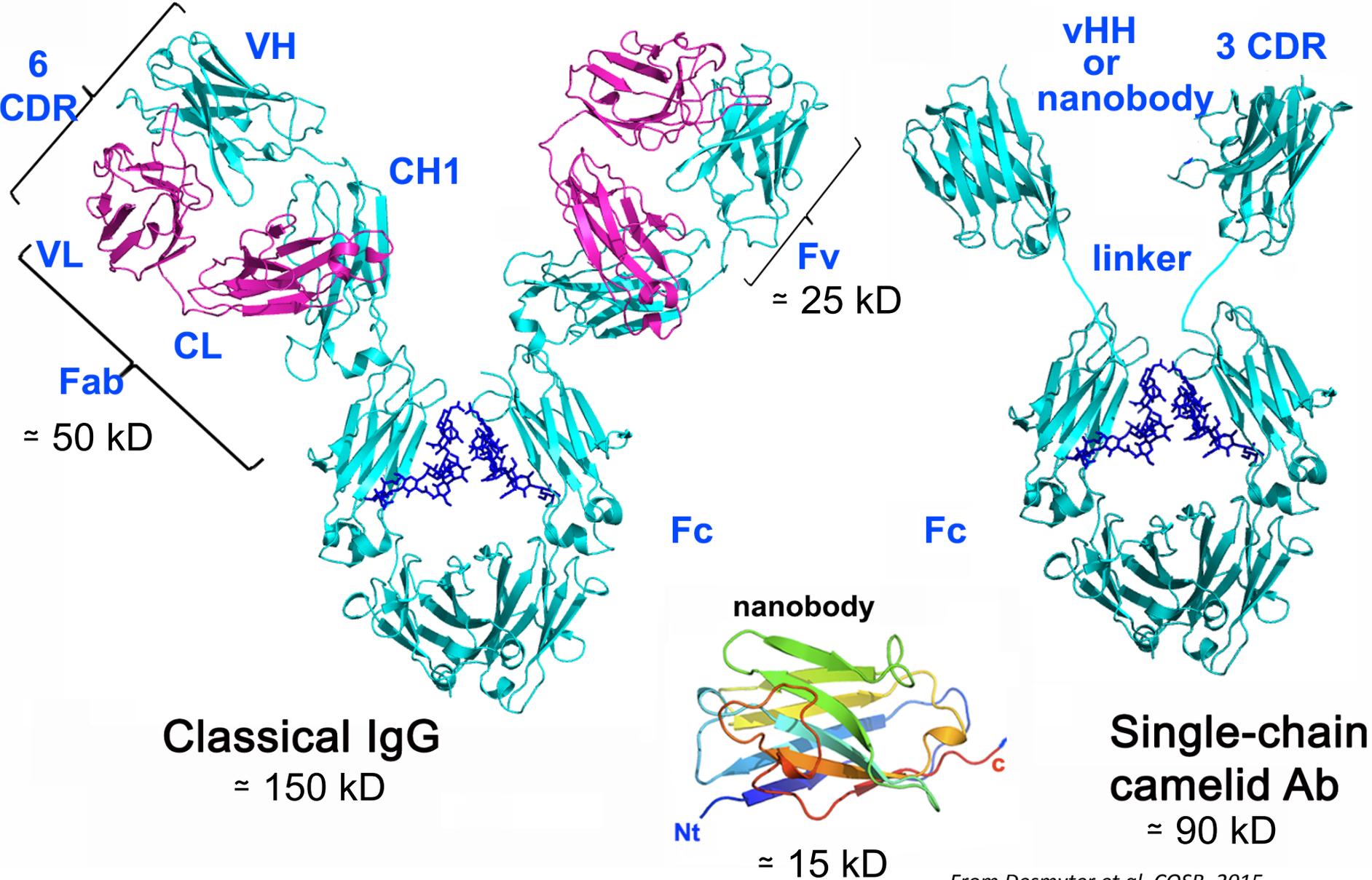
PorV and Plug was co-purified with SprA.

Heavy chain-only antibodies (HcAbs)



Classical IgG
≈ 150 kD

Heavy chain-only antibodies (HcAbs)



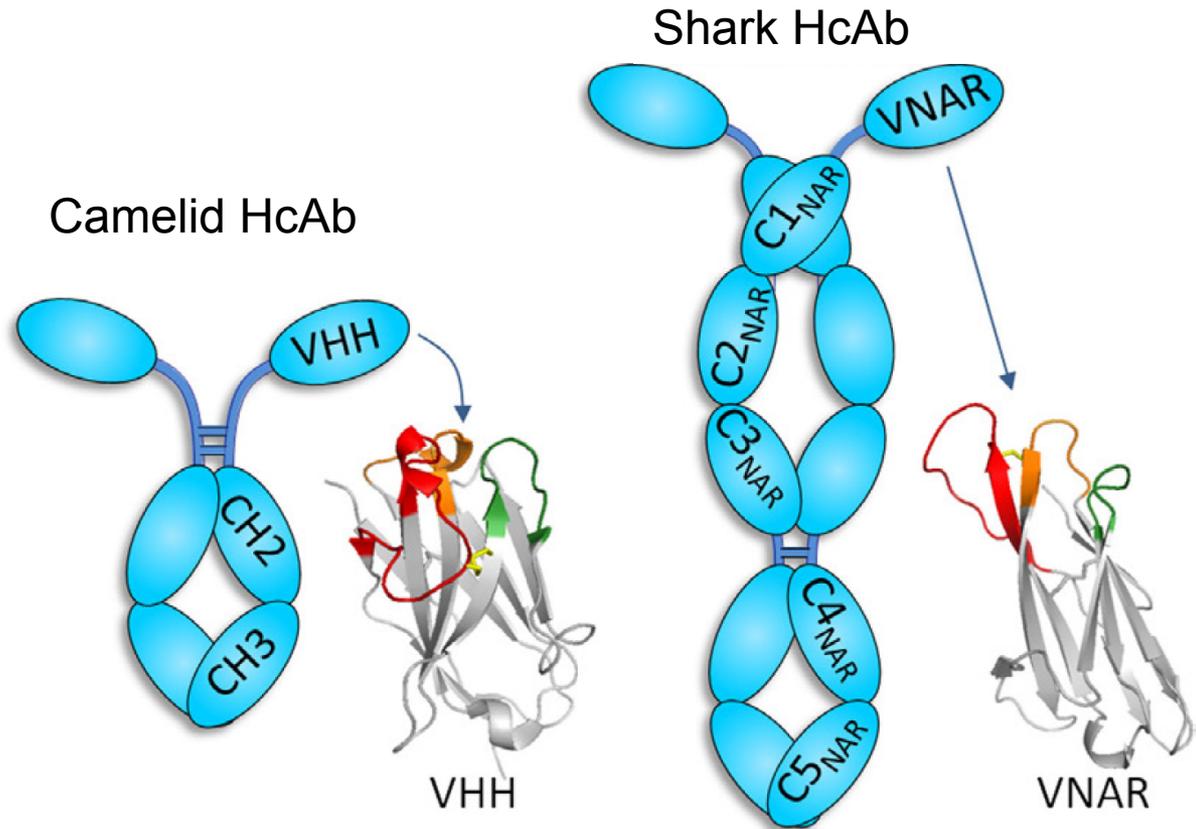
Classical IgG
≈ 150 kD

≈ 15 kD

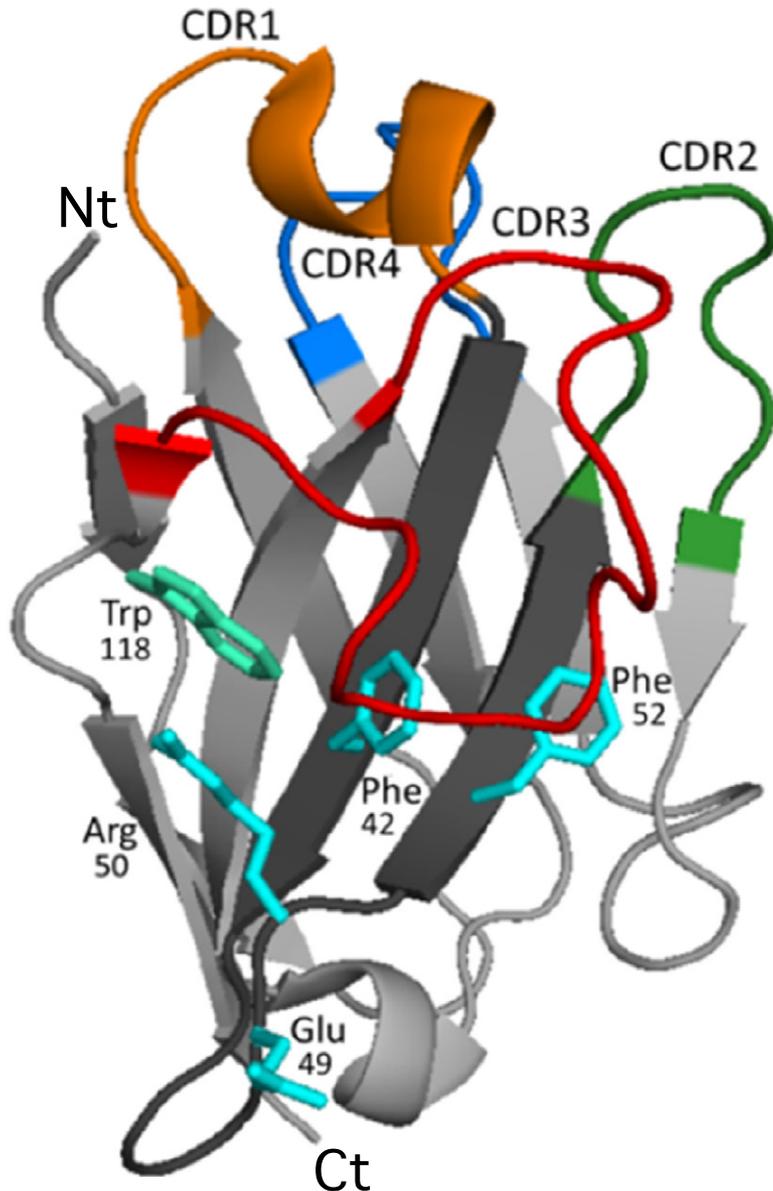
Single-chain camelid Ab
≈ 90 kD

From Desmyter et al, COSB, 2015

Heavy chain-only antibodies (HcAbs)



Structure and key characteristics of the nanobody domain

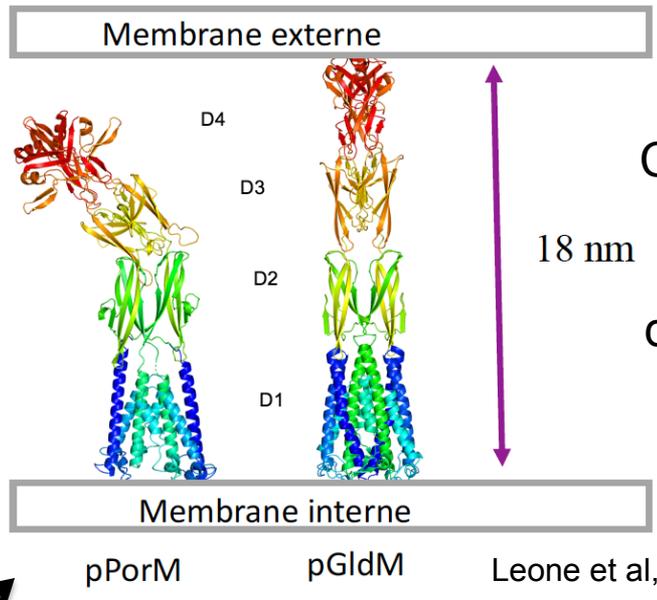
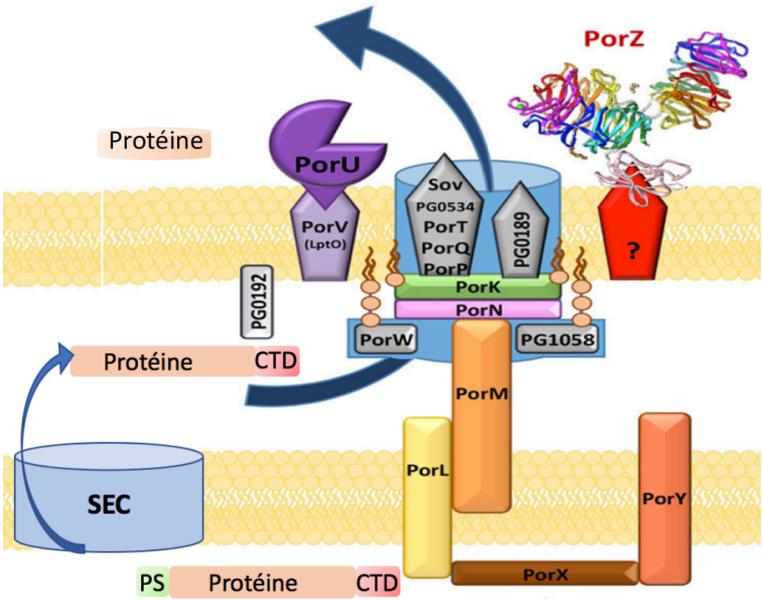


Structure of nanobodies:

- ✓ monomeric Ig domain of ~120 residues
- ✓ one conserved disulphide bond
- ✓ framework mutations in residues involved in the VH-VL interaction (G49E, L50R)

Key characteristics of nanobodies:

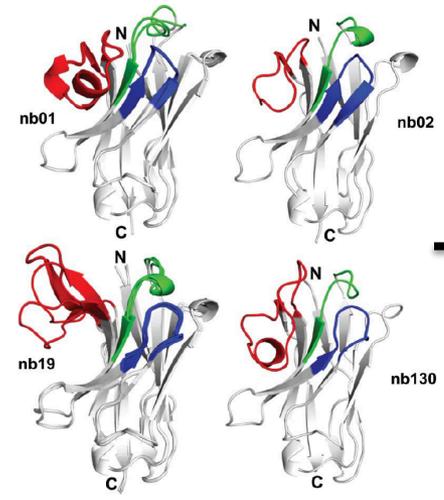
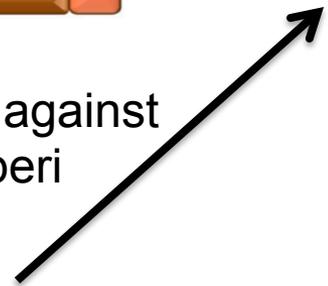
- ✓ high **affinity** and **specificity** (equivalent to conventional antibodies)
- ✓ high **thermostability**
- ✓ good **solubility** and strictly **monomeric** behavior
- ✓ **small size** (2.5 nm in diameter and about 4 nm in length; ~15 kDa)
- ✓ relatively low production cost
- ✓ ease of genetic engineering, format flexibility or **modularity**
- ✓ low immunogenicity, and a higher penetration rate into tissues



Crystal structure of the periplasmic domain of PorM and GldM

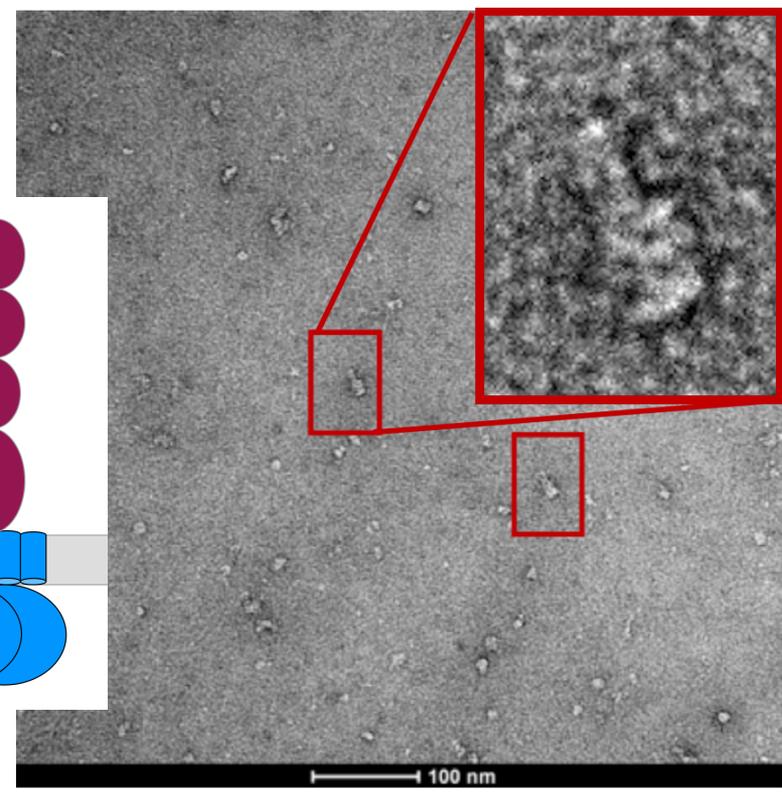
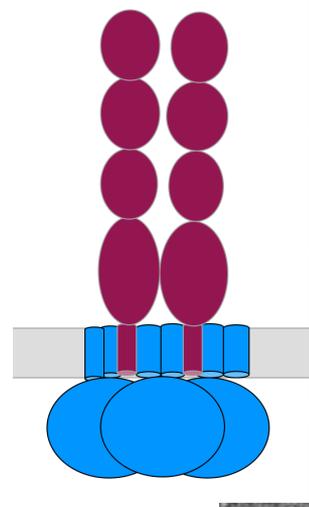
Leone et al, Nature Com., 2018

Generation of nanobodies against PorM-peri and GldM-peri



Duhoo et al, Acta Cryst F, 2017

Purification of the inner membrane complex GldM/GldL





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innate pharma

Extracellular vesicles (EVs) as a new platform to study cell surface protein structure and interaction with ligand

Vincent Delauzun

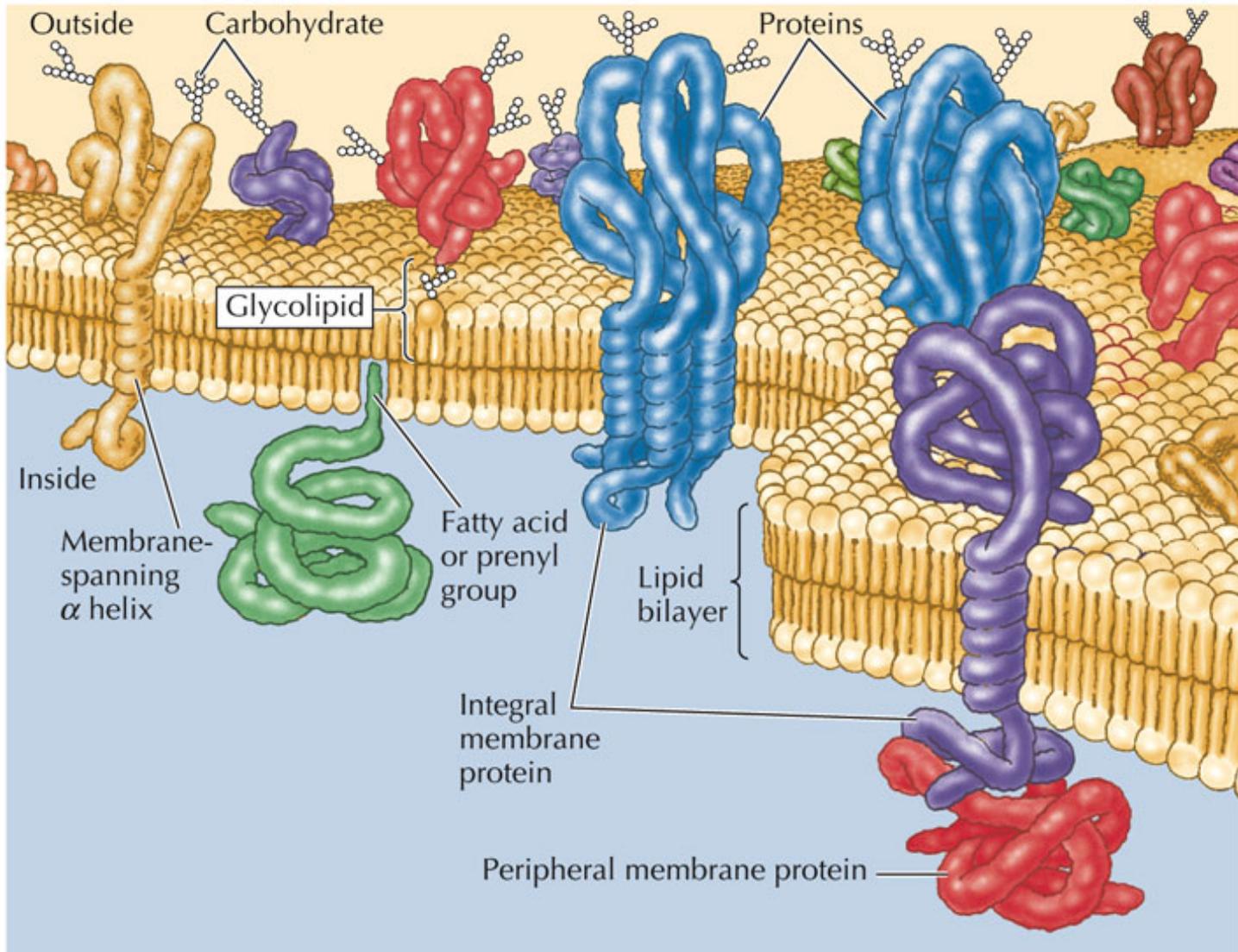
PhD student

2019 / 05 / 22

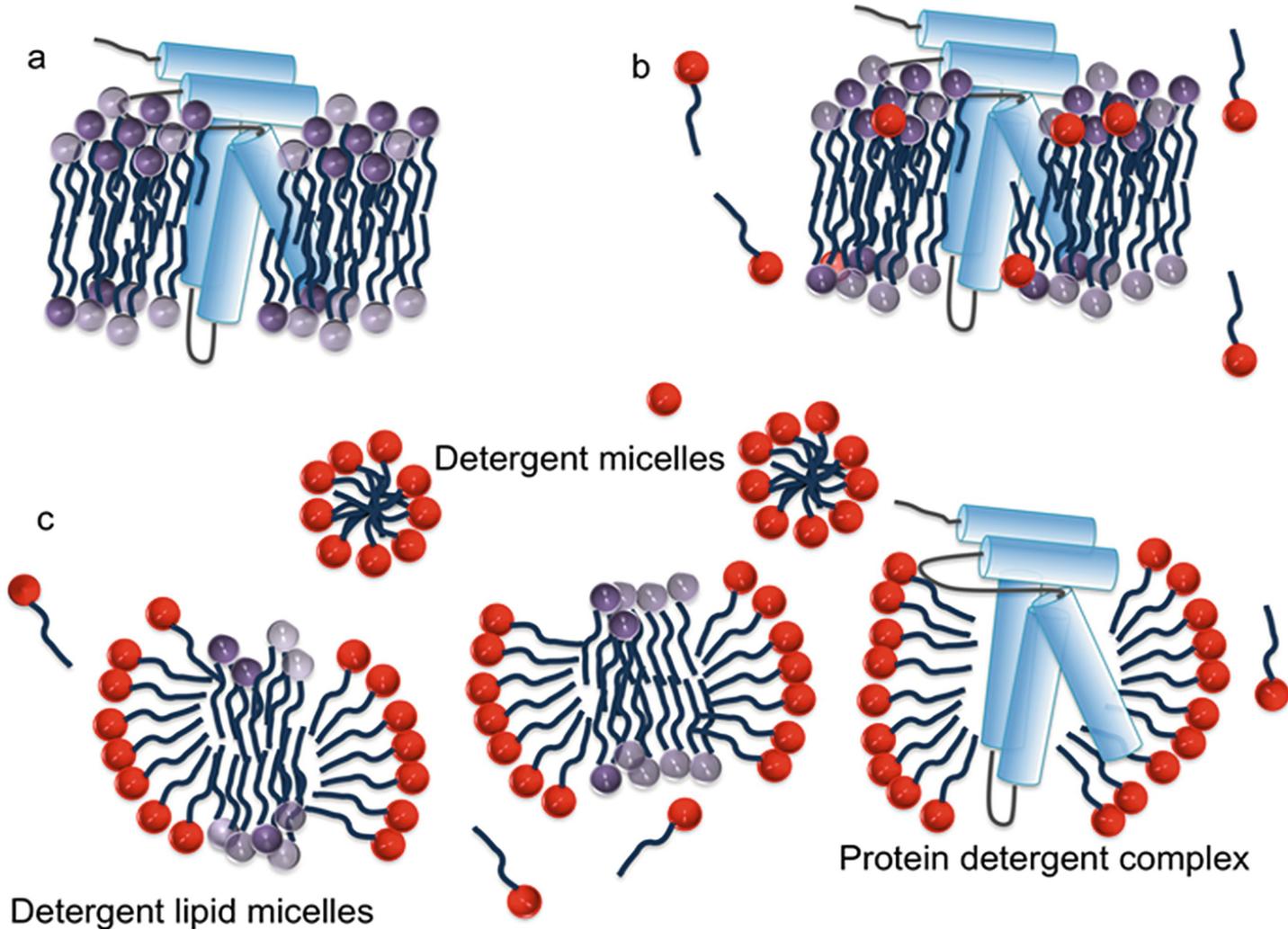
Director : Dr A. Roussel



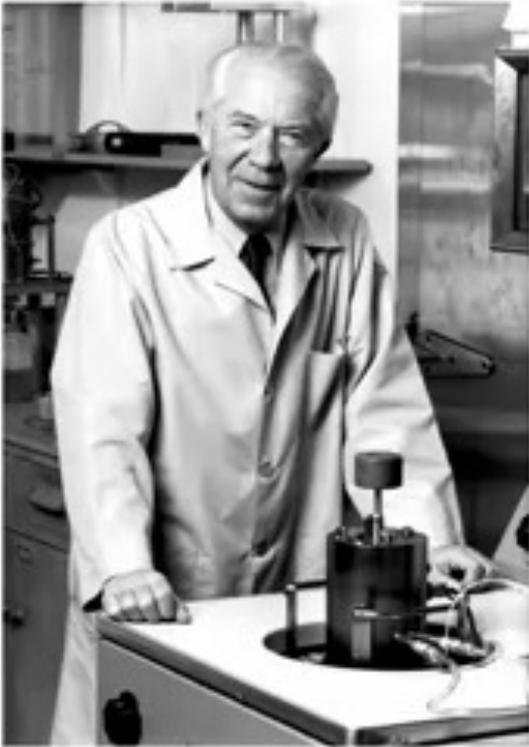
Membrane proteins



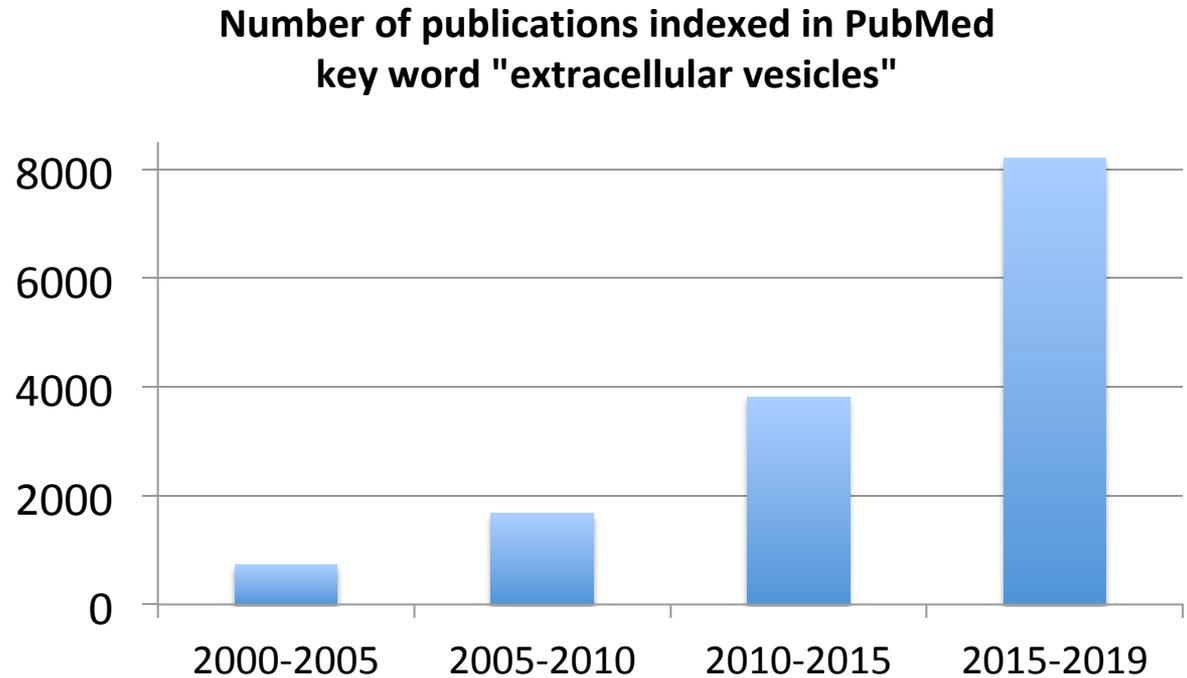
The challenging task of membrane protein purification



History of EVs



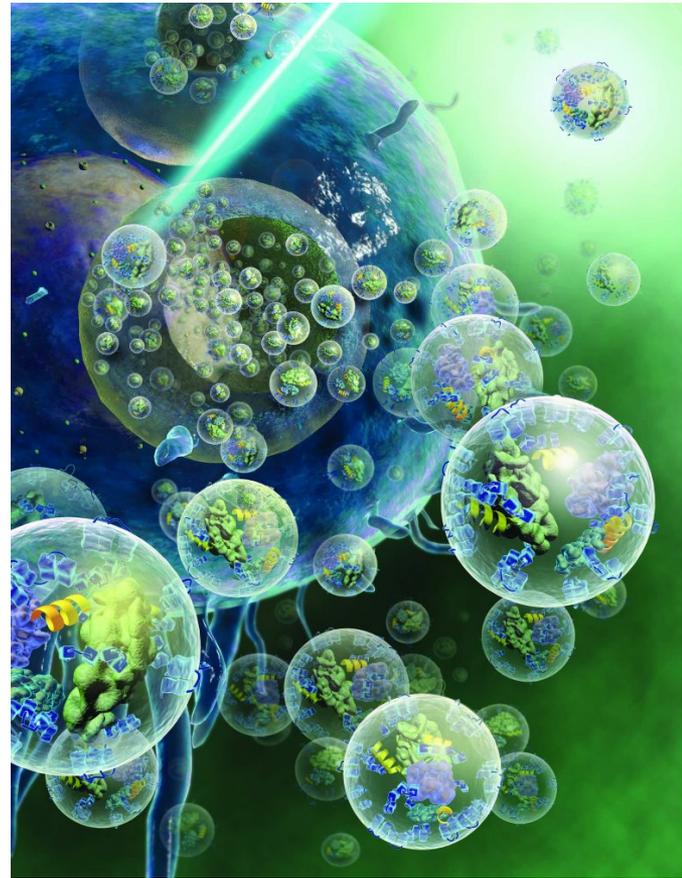
Christian de Duve
1917-2013
Nobel Prize 1974
Discovery of Lysosomes



EVs are everywhere

Every cells release vesicles in their environment

- Plasma
- Urine
- Milk
- Sweat
- Saliva
- Tears

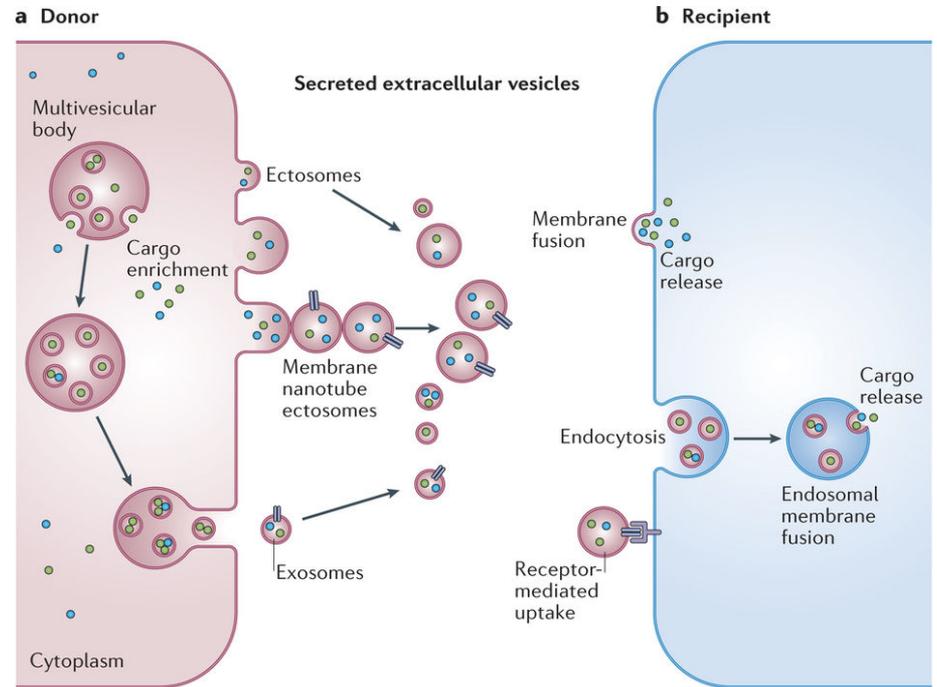


From UKC Washington DC

What are EVs for ?

- Cell-to-cell communication

- immune system
- angiogenesis
- inflammation
- gene transfer
- tumor growth
- metastasis

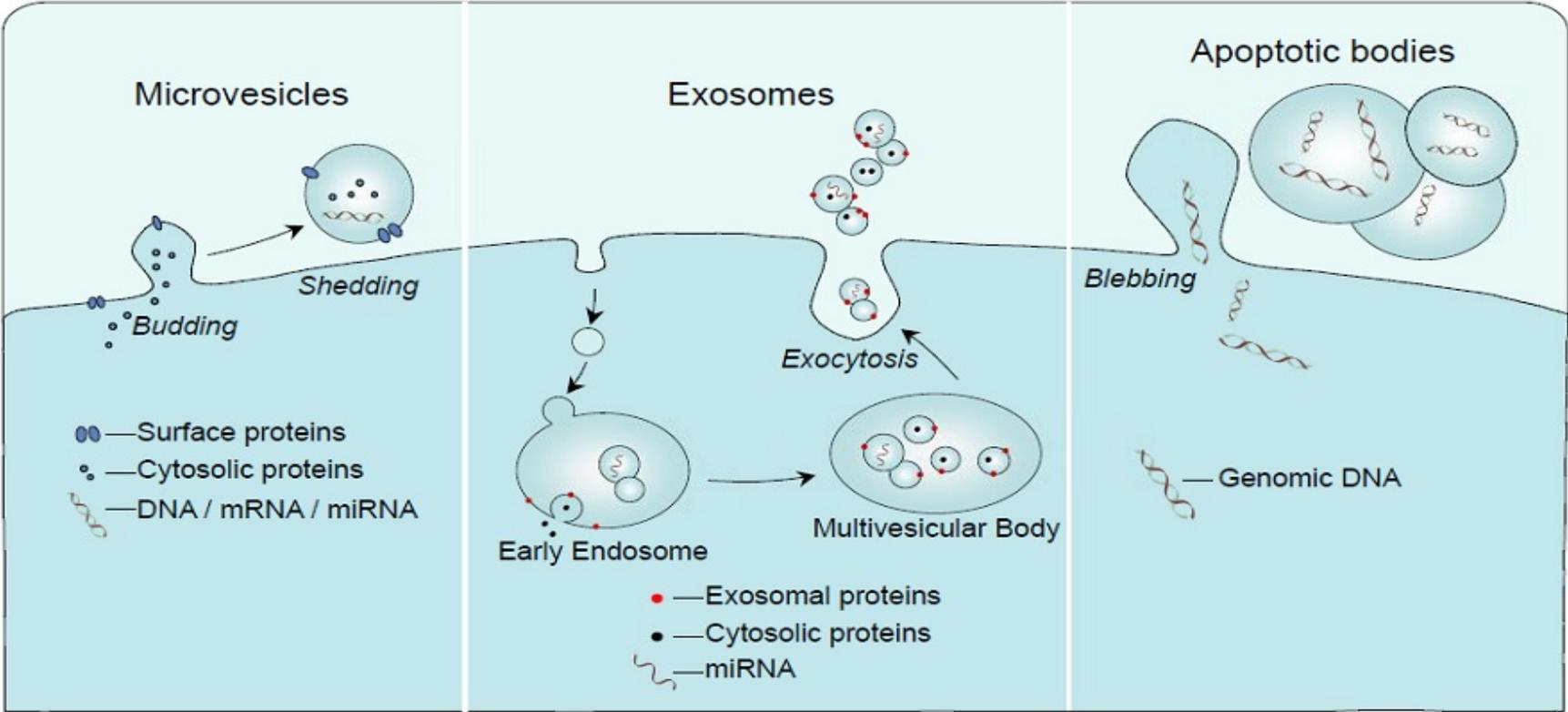


Nature Reviews | Microbiology

- Cell adhesion

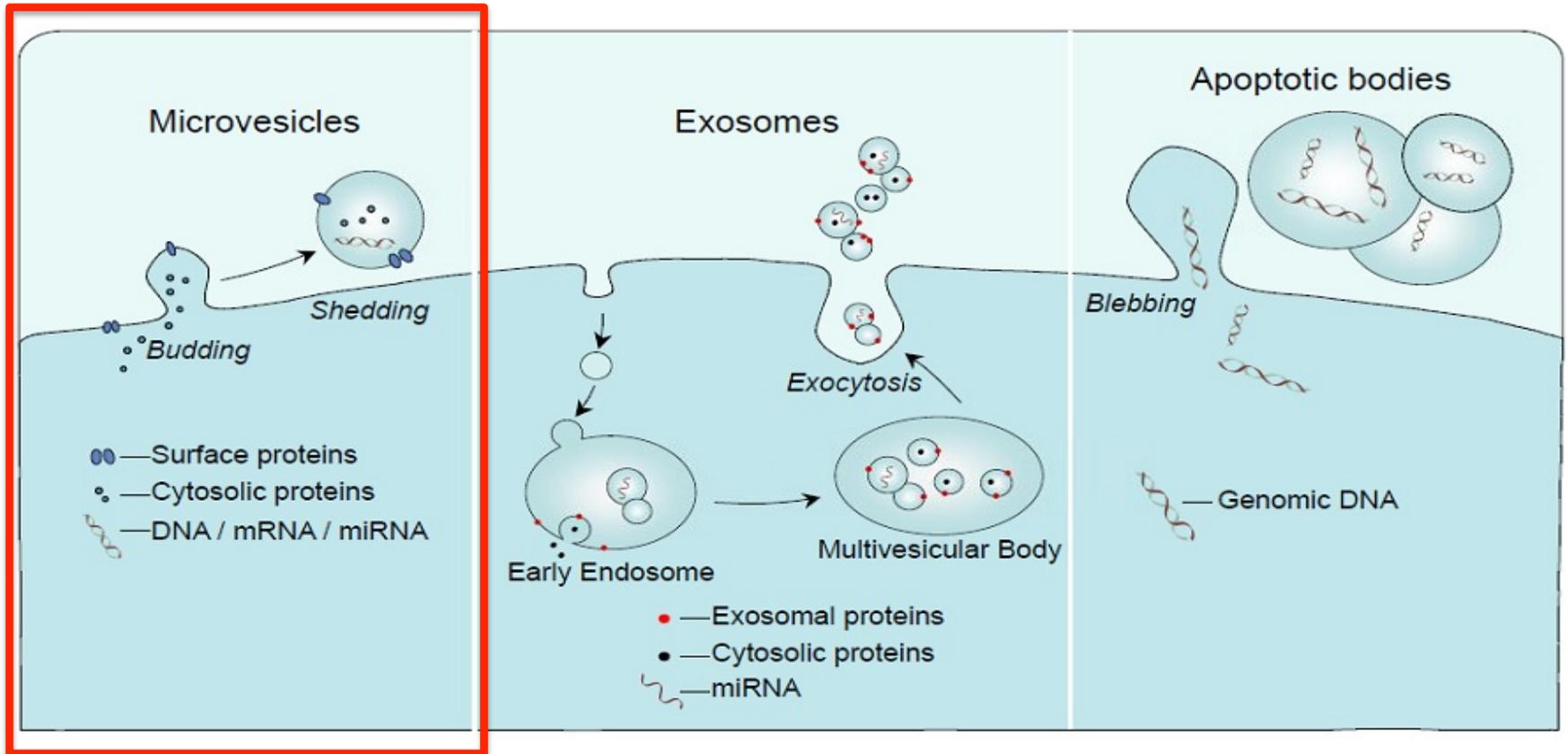
- Waste management

Classification of EVs



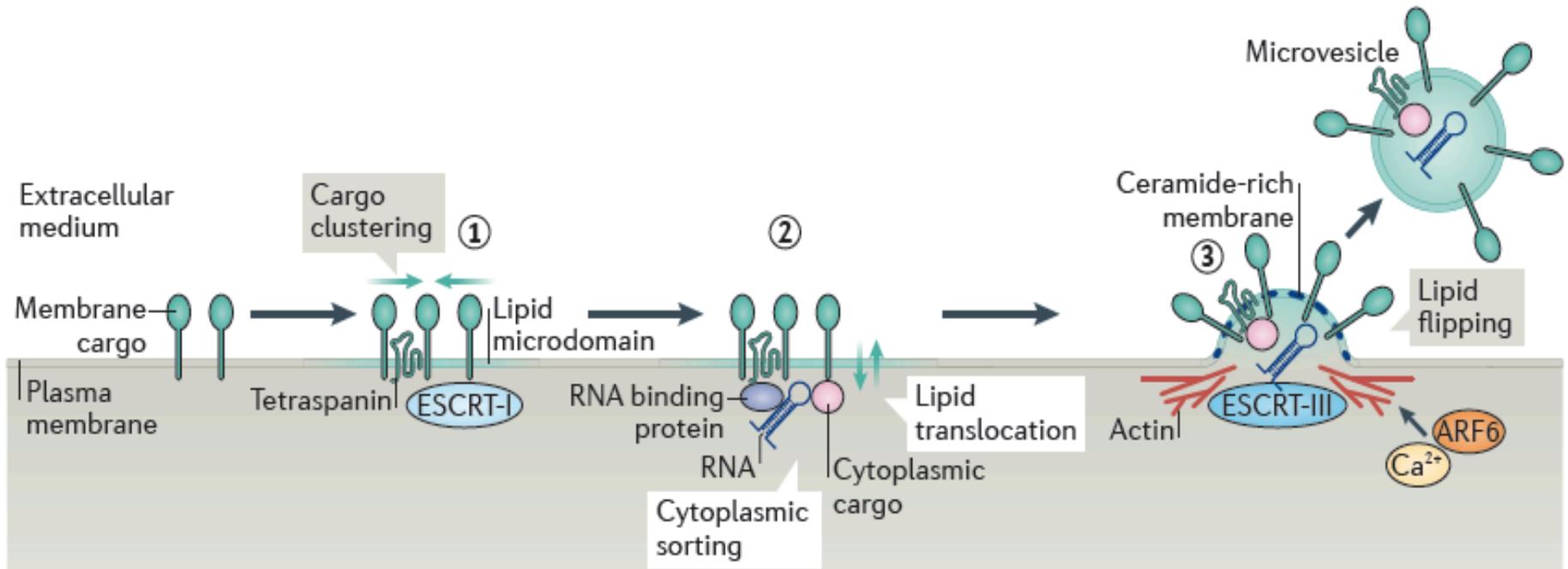
Charlotte Lawson et al, *Journal of Endocrinology*, 2016

Classification of EVs



Charlotte Lawson et al, *Journal of Endocrinology*, 2016

Biogenesis of EVs (microvesicles)



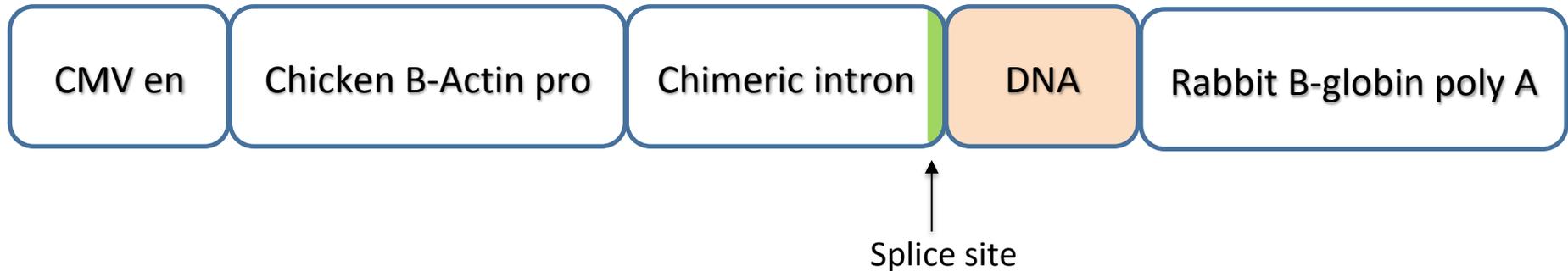
Guillaume Van Niel et al, Molecular Cell Biology, 2018

Workflow process

DNA Cloning



pCAGGS vector



Pre-mRNA must be spliced to produce recombinant EVs

Workflow process

DNA Cloning

**EVs
Production**



Cell Line (HEK293)

Transfection (Lipofectamine 2000)

Serum-free medium (Freestyle 293)

Expression time course (96 h)

Workflow process

DNA Cloning

**EVs
Production**

**EVs
Purification**



Differential centrifugations (500 g / 3000 g / 10 000 g)

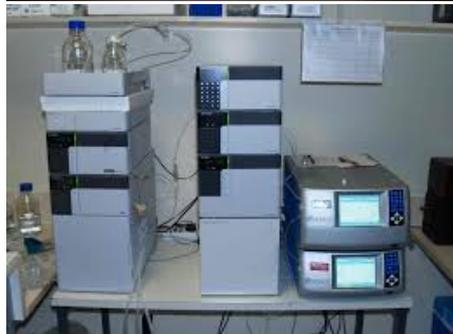
Ultracentrifugation 1 (110 000 g)

Ultracentrifugation 2 (washing step 110 000 g)

Size Exclusion Chromatography

S200 10/300, 0.3 mL/min

Dynamic Light Scattering



Workflow process

DNA Cloning

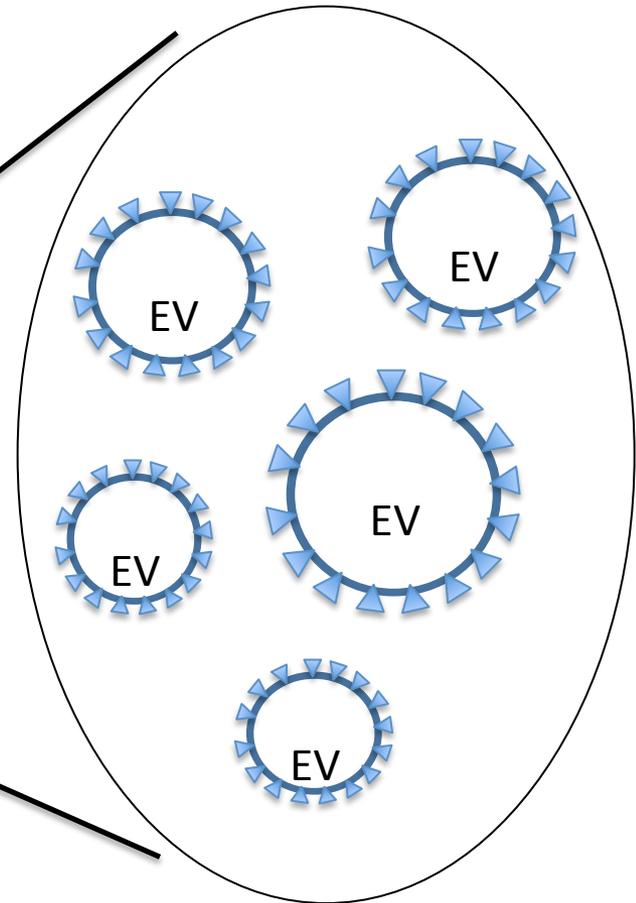
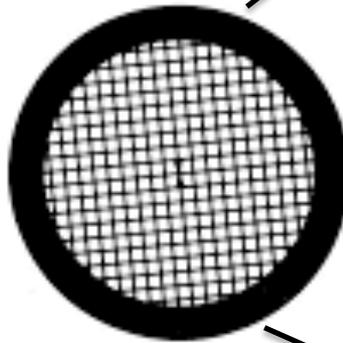
EVs
Production

EVs
Purification

Quality
Control

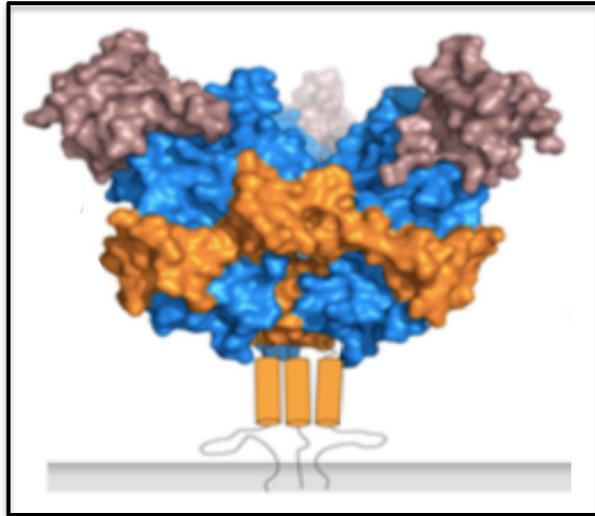


300 mesh, Cu
Negative stain
1 % Uranyl acetate

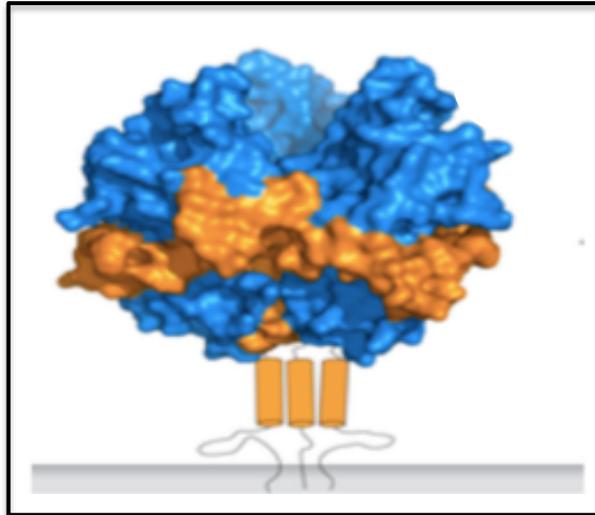




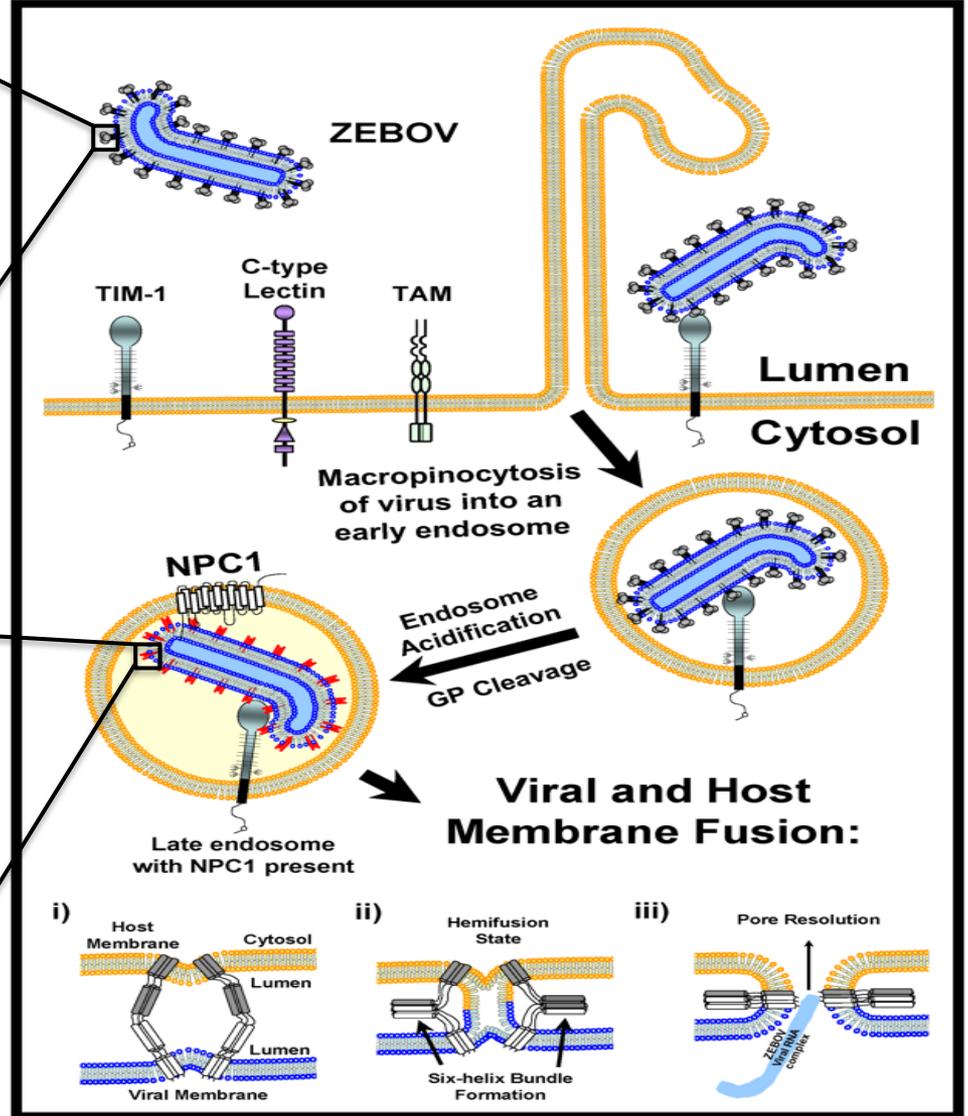
gpEbola



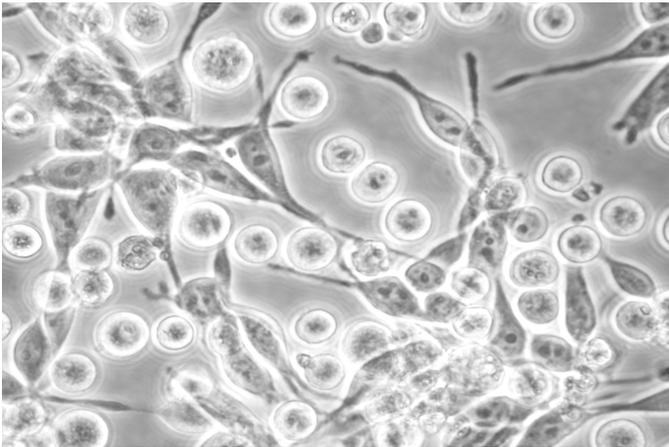
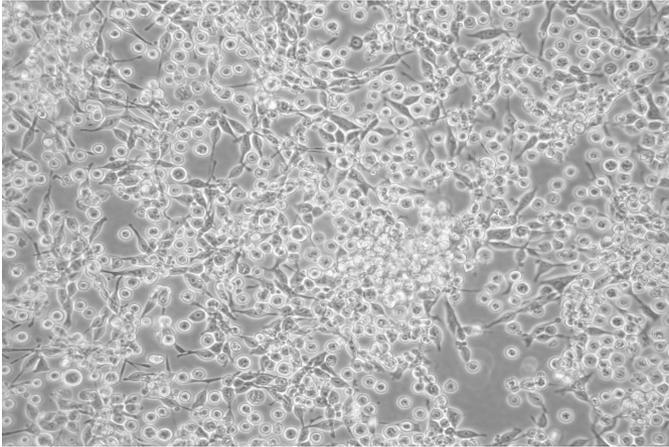
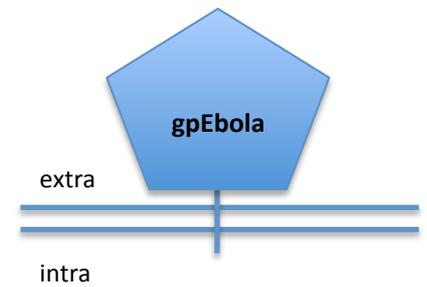
gpEbola full length



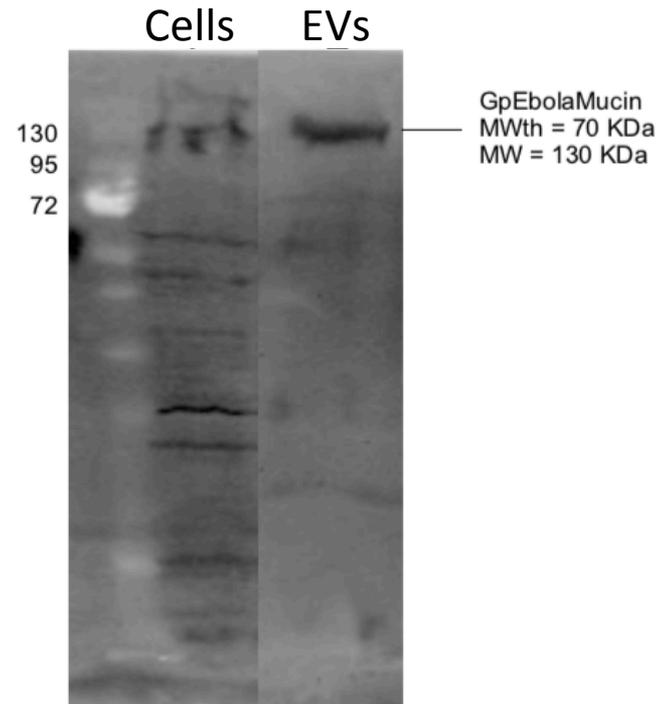
gpEbola Δ Mucin



gpEbola

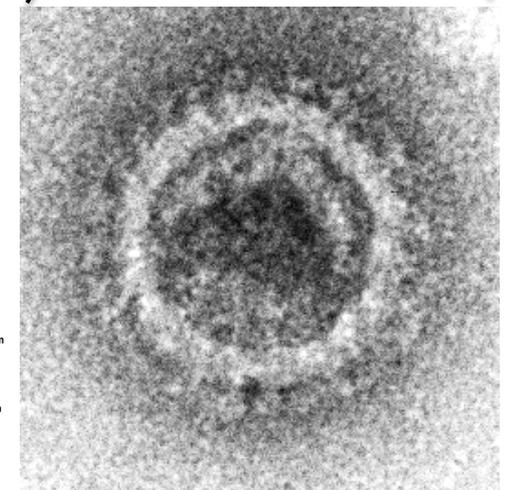
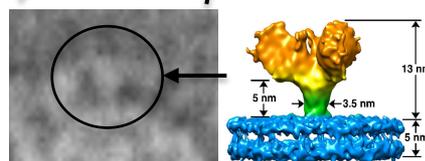
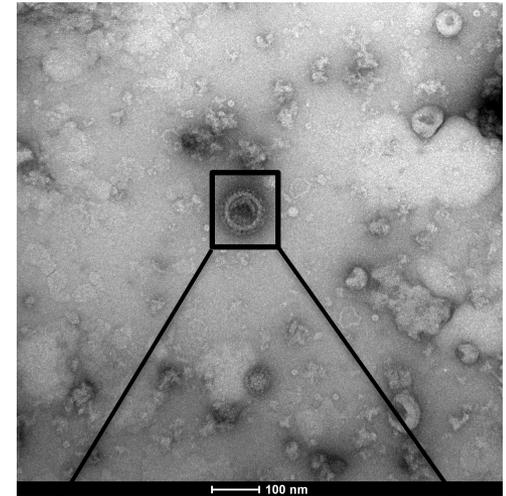
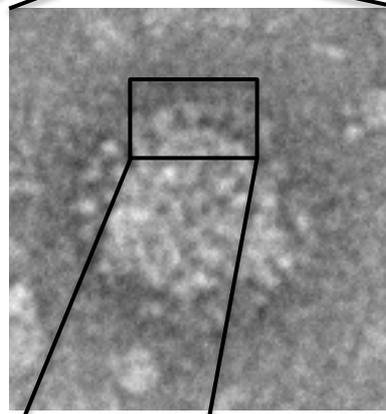
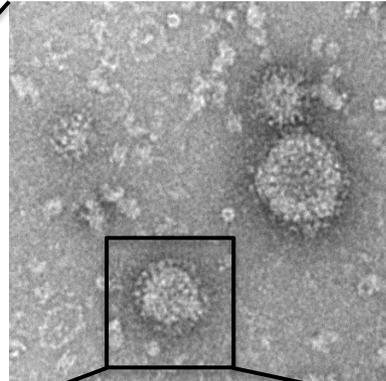
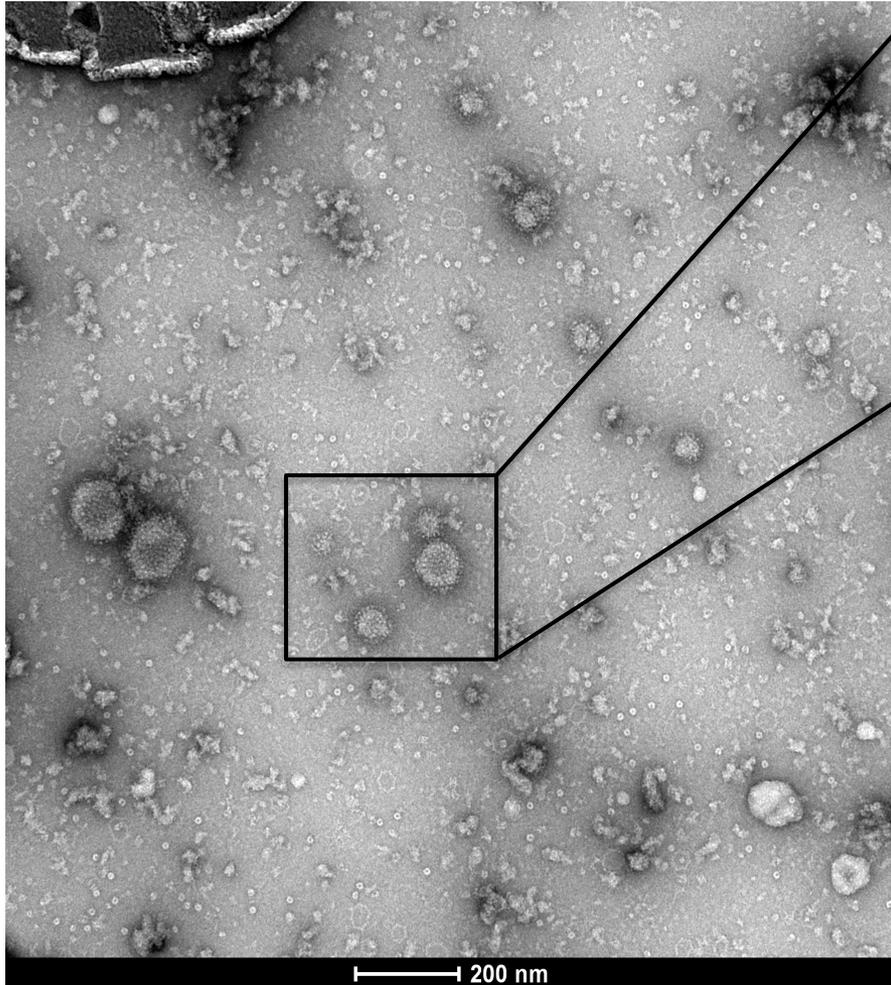
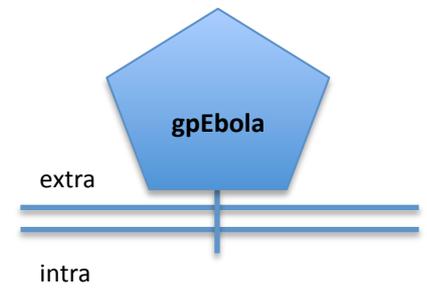


HEK293

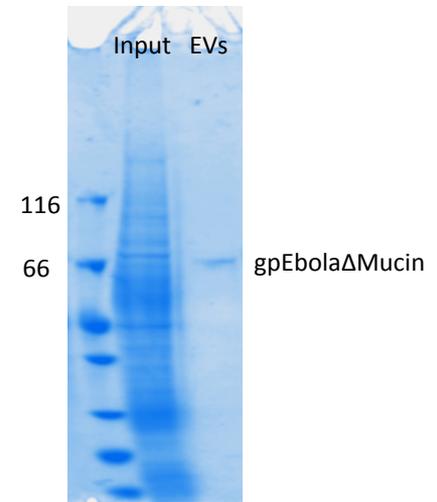
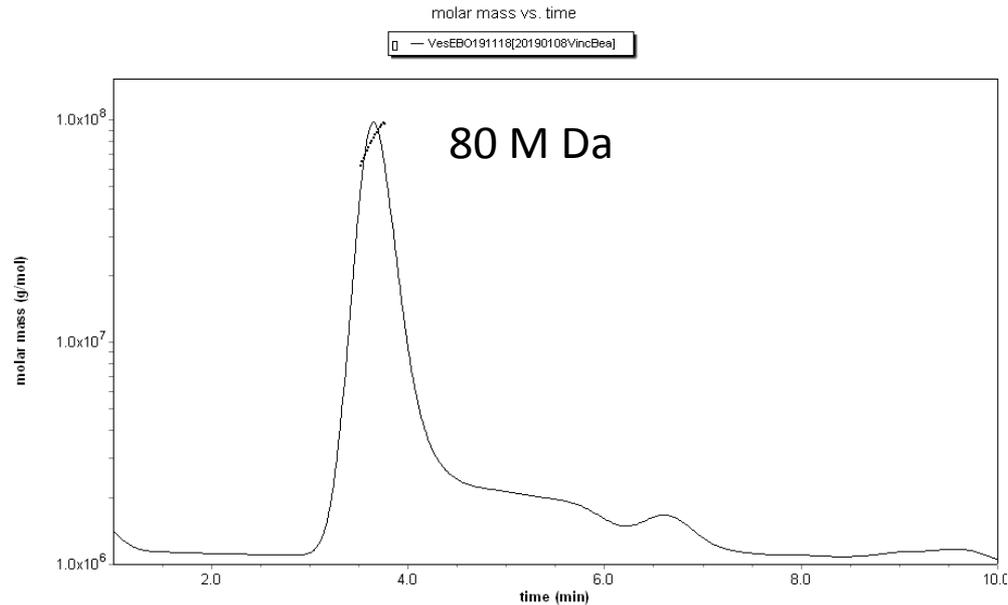
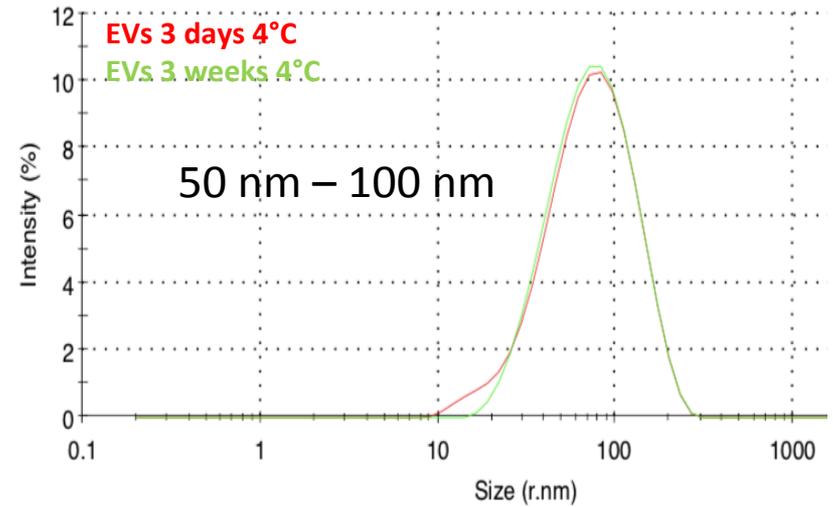
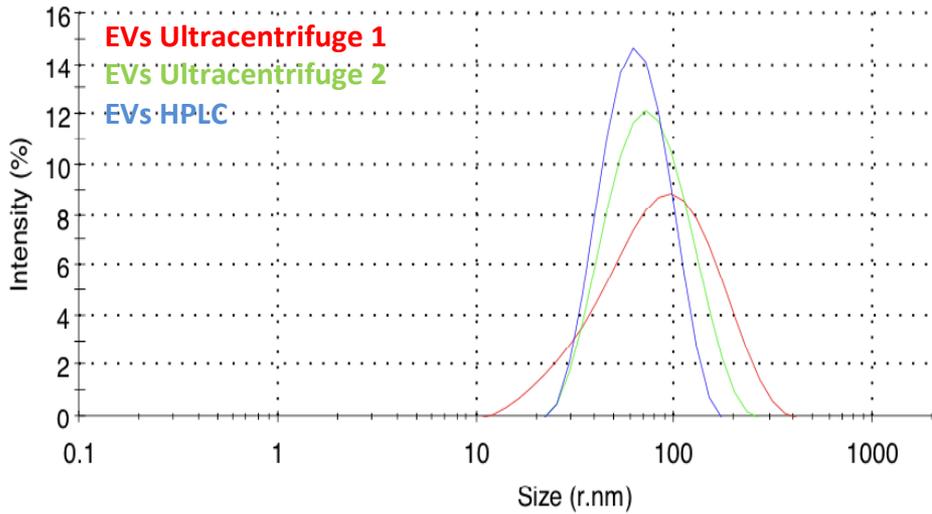


Westernblot anti-gpEbola (Lama serum)
anti-Lama HRP

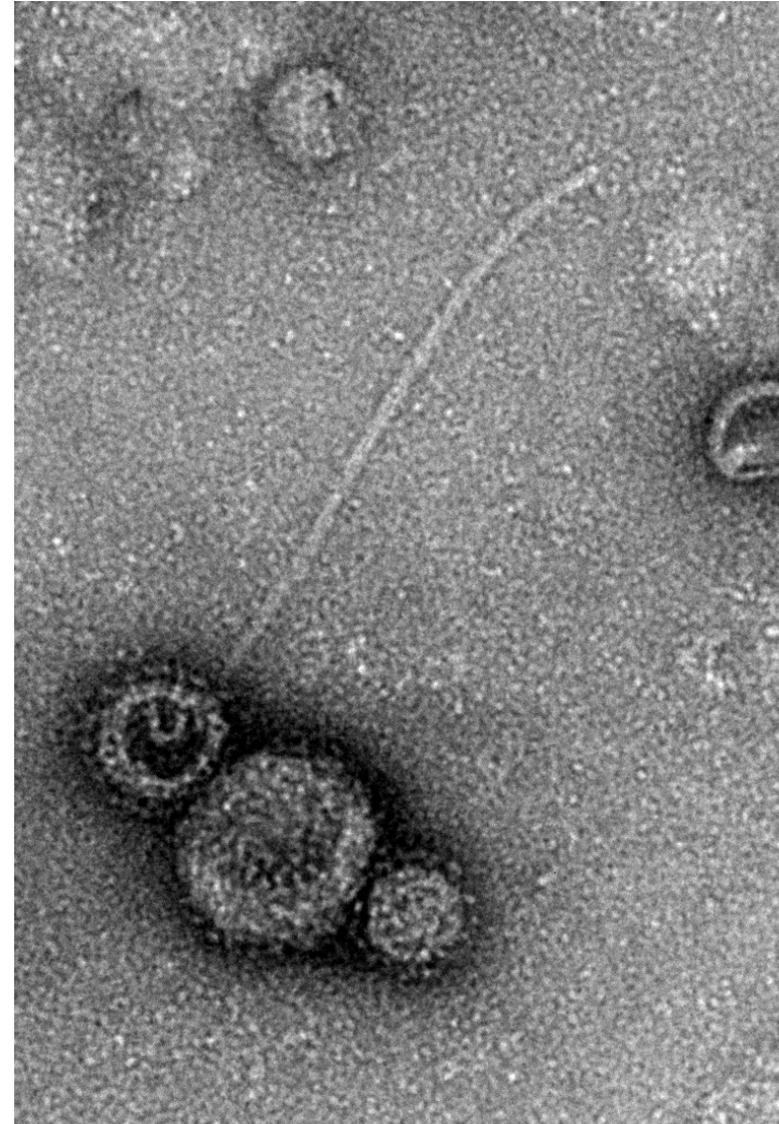
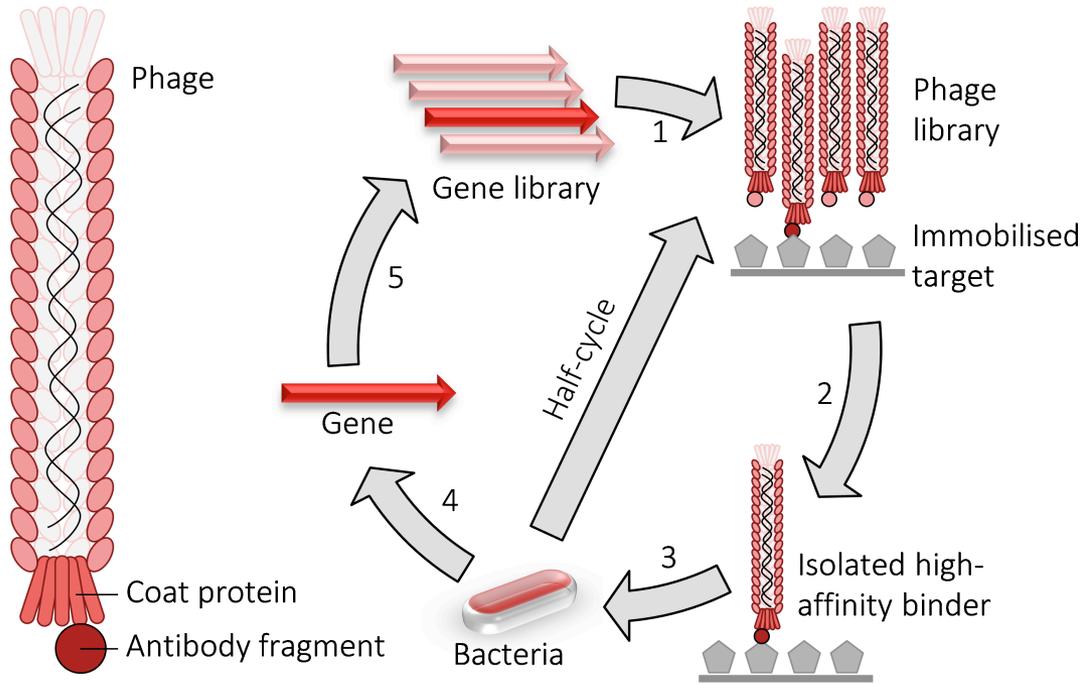
gpEbola



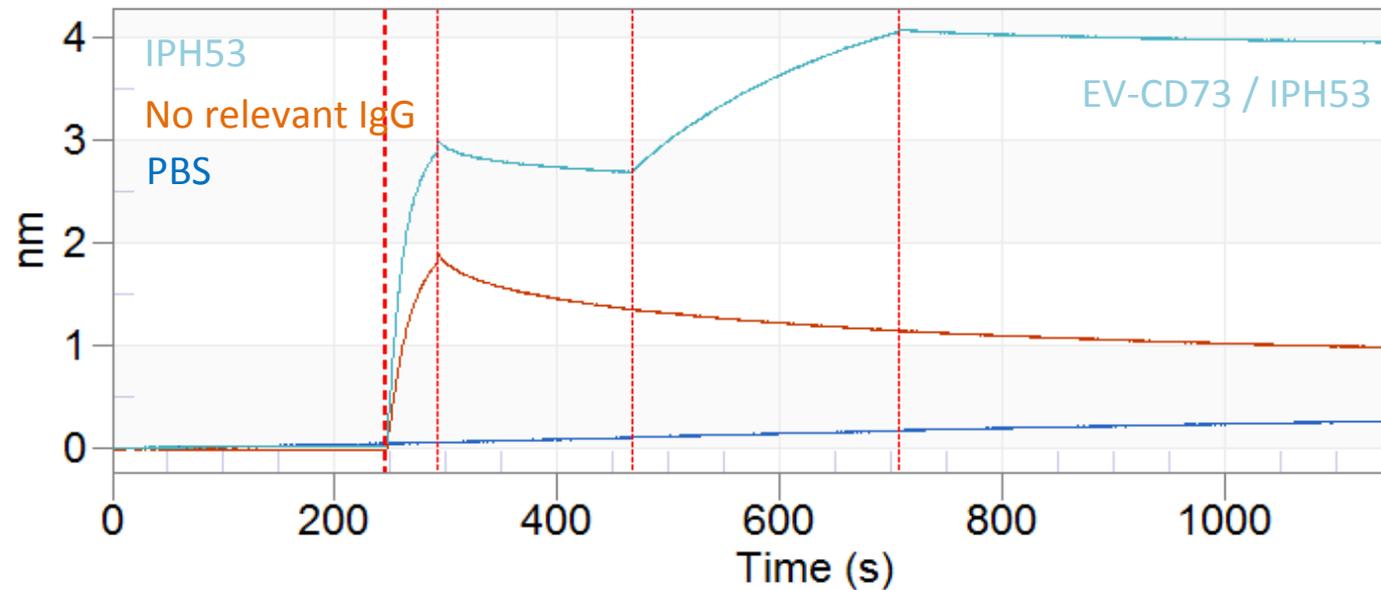
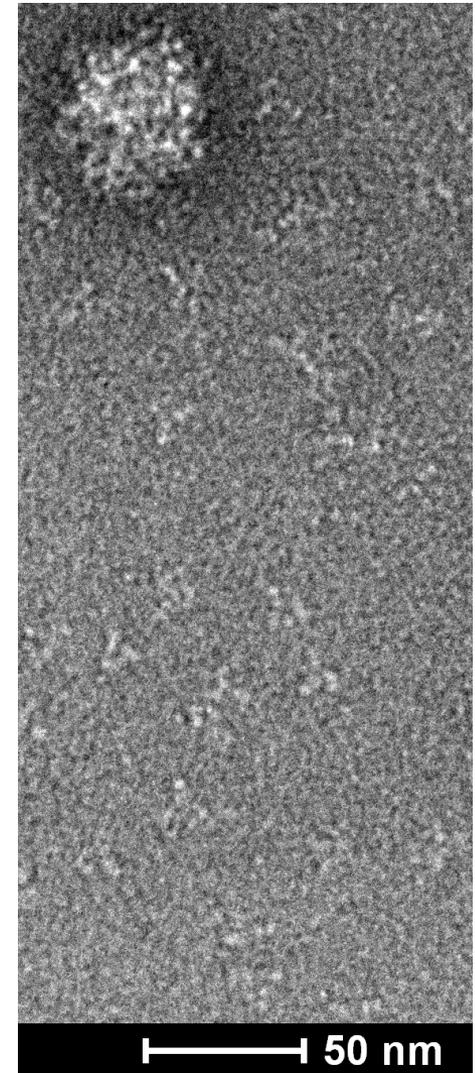
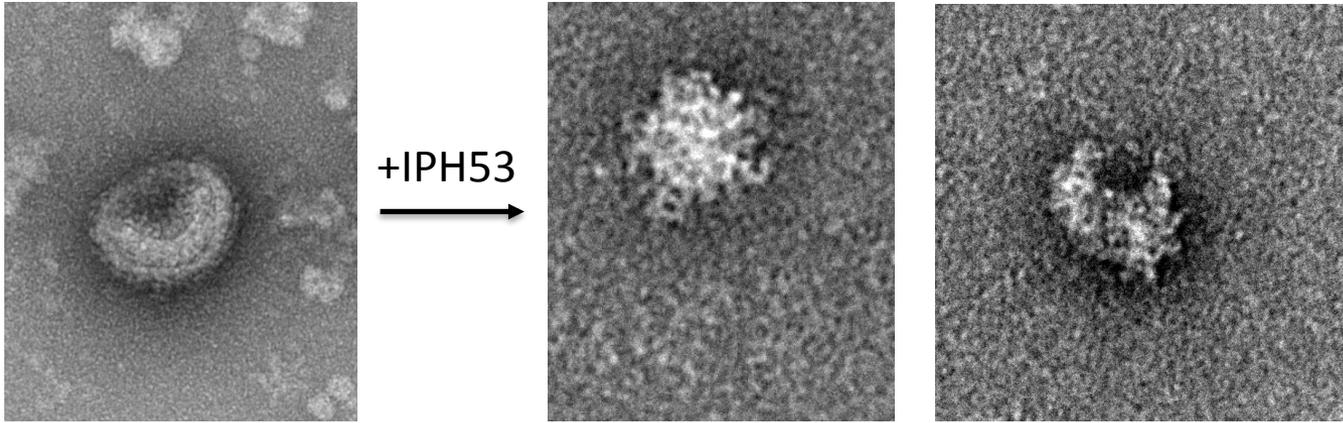
DLS & SEC-MALS analysis



Evs and phage display



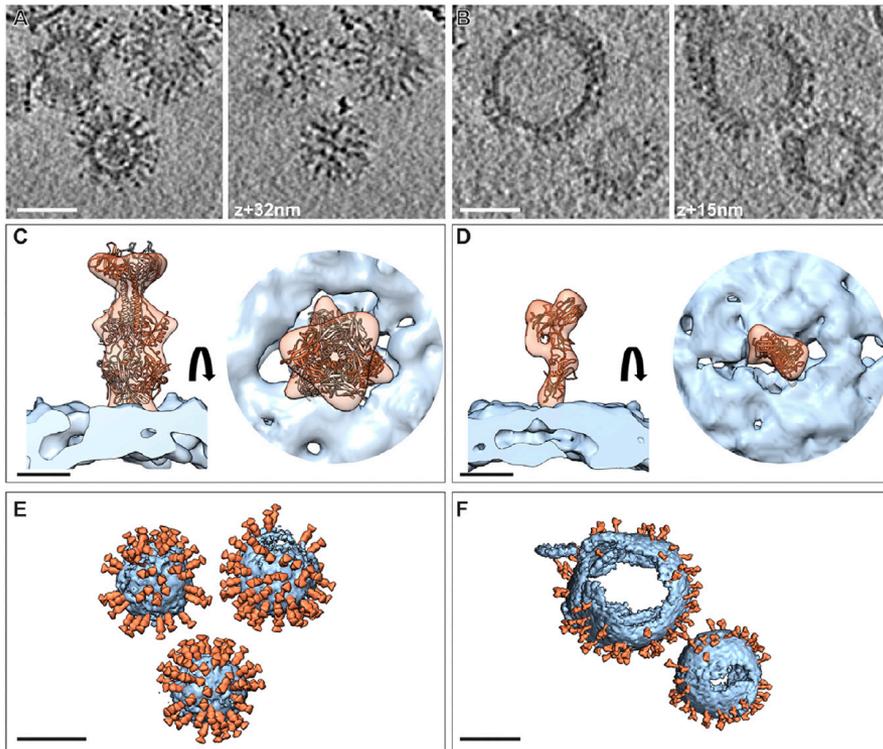
CD73 + IPH53 (IgG)



Extracellular Vesicles: A Platform for the Structure Determination of Membrane Proteins by Cryo-EM

Tzviya Zeev-Ben-Mordehai,¹ Daven Vasishtan,¹ C. Alistair Siebert,¹ Cathy Whittle,¹ and Kay Grünewald^{1,*}
¹Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK

Structure 22, 1687–1692, November 4, 2014



3D EM Reconstruction of the Proteins on the Membrane

(A and B) Central and tangential slices through a tomogram of MPEEVs displaying gB (A) and EFF-1 (B). Scale bar represents 50 nm.
 (C and D) Isosurface representation of the subvolume reconstruction of gB with the trimer crystal structure (PDB: 2GUM) fitted (C) and EFF-1 (PDB: 4OJC) (D). Scale bar represents 5 nm.
 (E and F) Isosurface representations of the tomograms shown in (A and B). Scale bar represents 50 nm.

Transfer from EVs to nanodisc

