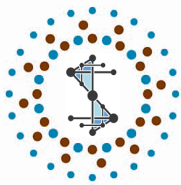


Isabelle BROUTIN

Laboratoire de Cristallographie et RMN biologique
CNRS UMR 8015 Université Paris Descartes, USPC
Faculté de pharmacie

Directeur : N. Leulliot (PR, UPD)
Directrice adjointe: C. Tisné (DR, CNRS)



Thèmes de Recherche et Techniques



Signalisation et
transport membranaire

I. Broutin

RNA interactions
RMN

C. Tisné

initiation
de la traduction
VIH

B. Sargueil

Cristallographie des
complexes moléculaires

Pr. N. Leulliot

Structure des Protéines
Virales par RMN

S. Bouaziz

Thématiques

Maladies infectieuses
VIH
Cancer
Ribosome
Signalisation

Objets

ARN
Protéines
Petites molécules
Complexes

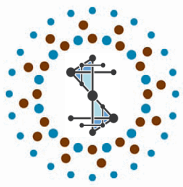
Techniques

Biologie moléculaire
Production /purification de protéines
(soluble et membranaire)

Biochimie
Biochimie de l'ARN
Synthèse de peptides

Biologie structurale
Cristallographie aux rayons X /
RMN / SAXS / Shape (2D)
Criblage de petites molécules (RMN)
Modélisation moléculaire

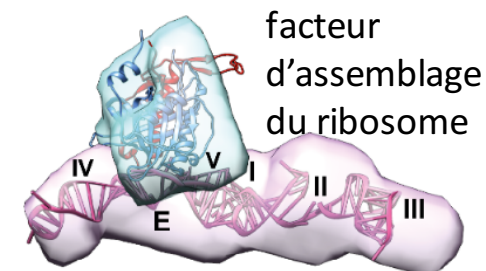
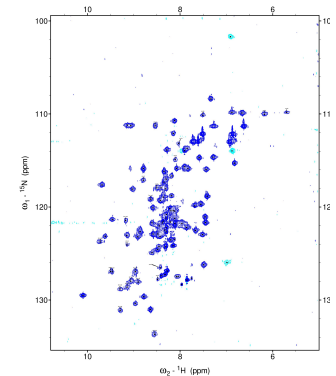
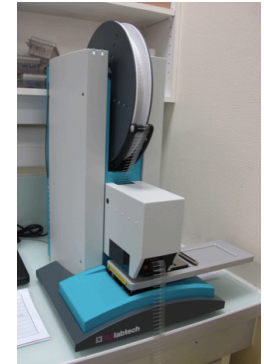
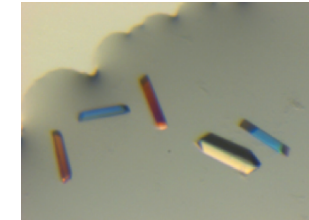
Biophysique
Fluorescence
Isothermal titration calorimetry (ITC)
Dichroïsme circulaire (CD)

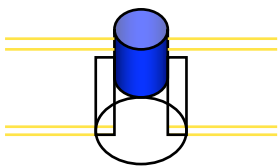


Infrastructure de Biologie Structurale



- **Plateforme robotique de cristallogénèse (3 robots)**
 - Criblage en plaques 96 puits, en nano-gouttes
 - Création de grilles d'optimisation
- **Porteur d'un BAG Synchrotron (Beamtime Allocation Groups) pour l'ESRF (Grenoble) et SOLEIL (Saclay)**
 - 7 laboratoires Parisiens
 - comprenant 5 équipes de l'USPC
- **RMN**
 - 2 Bruker 600MHz + 1 cryo-sonde
 - Cryofit insertion d'échantillons par injection
- **Biophysique** (CD, Fluo, Microcalorimétrie...)
- **2 Equipex** (SAXS et RMN)

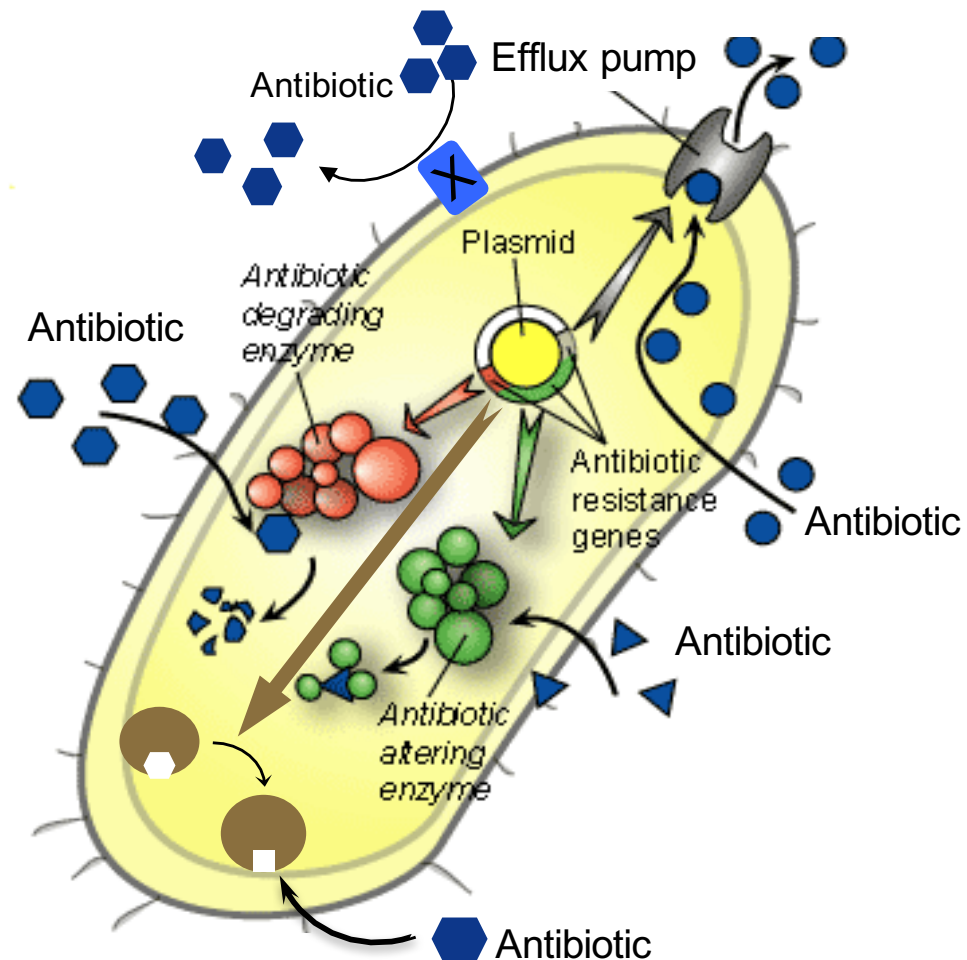


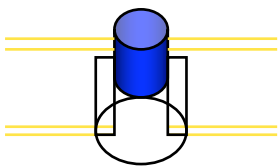


Four main mechanisms of resistance to antibiotics

- Antibiotic **modification** / **degradation** by altering enzymes
- **Modification** of the target
- **Permeability** of the cellular wall
- Active efflux

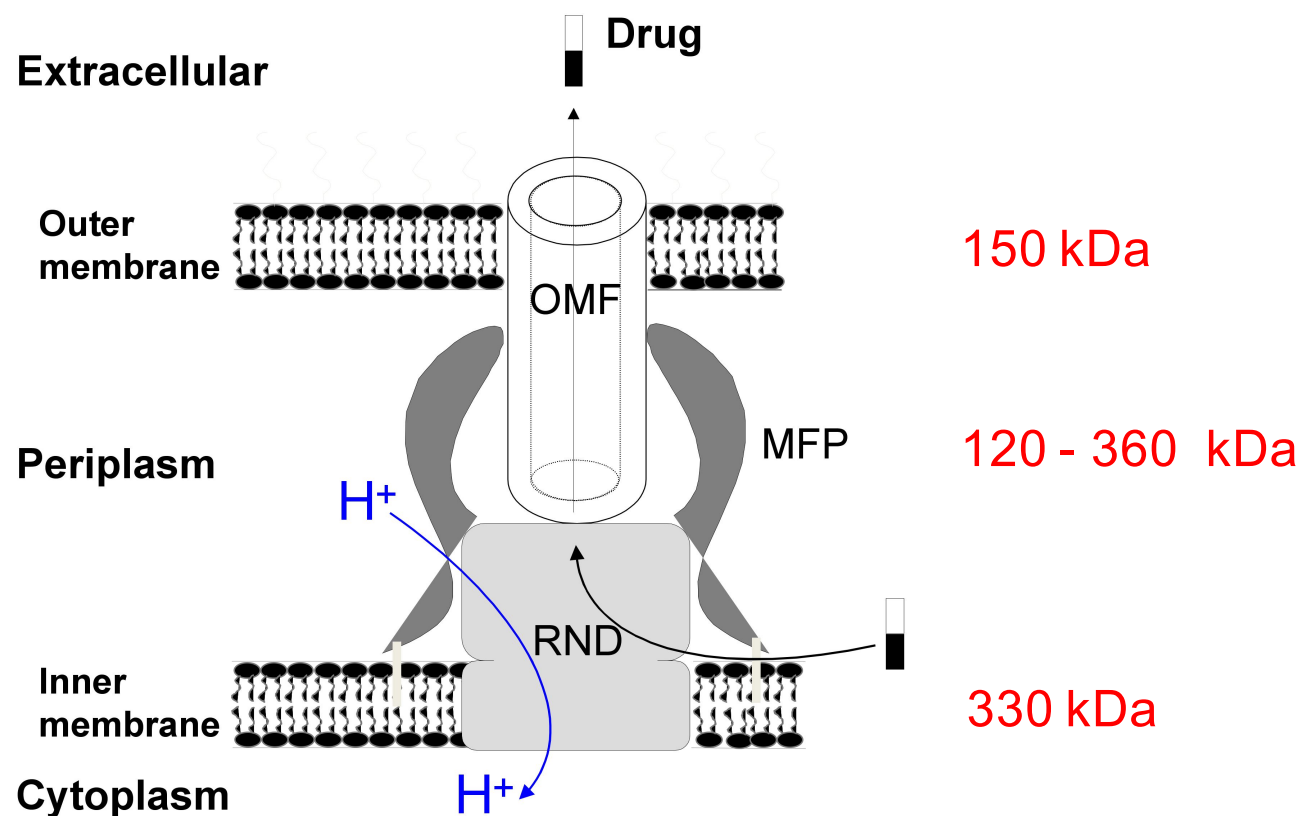
Easy spread of efflux genes between distant species du to transportable genetic elements as transposons or plasmids



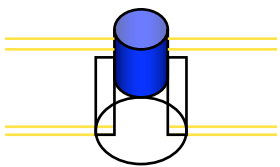


Efflux pumps from the RND family

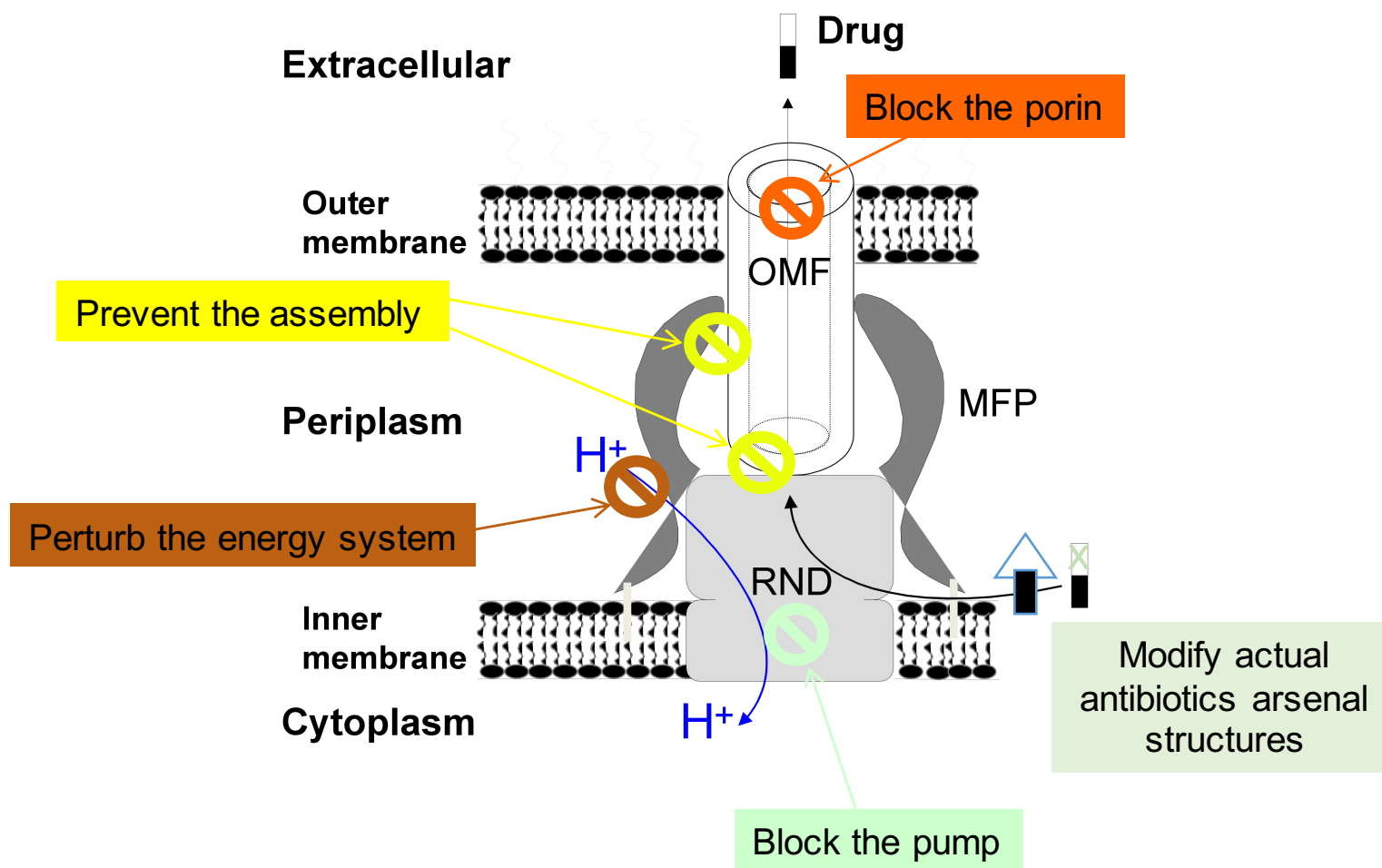
proteins complexes of 600 to 840 kDa

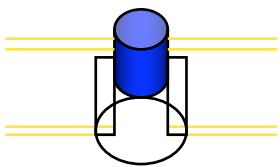


OMF = Outer Membrane Factor
MFP = Membrane Fusion Protein
RND = Resistance Nodulation Division

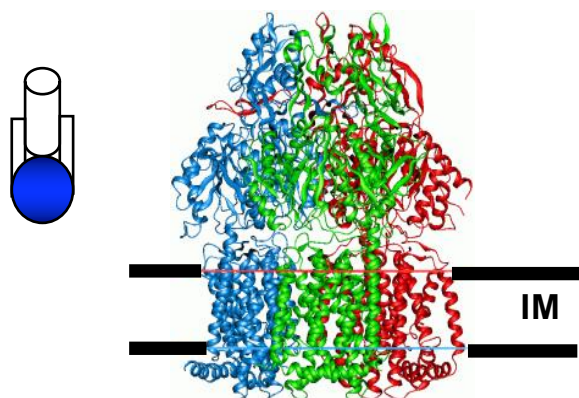


Strategies against bacterial antibiotic resistance

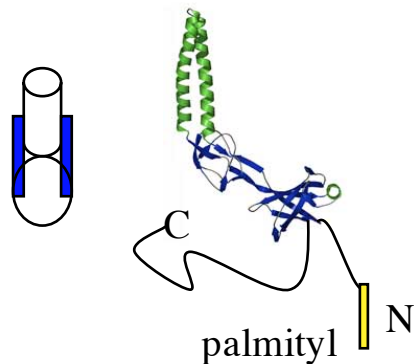




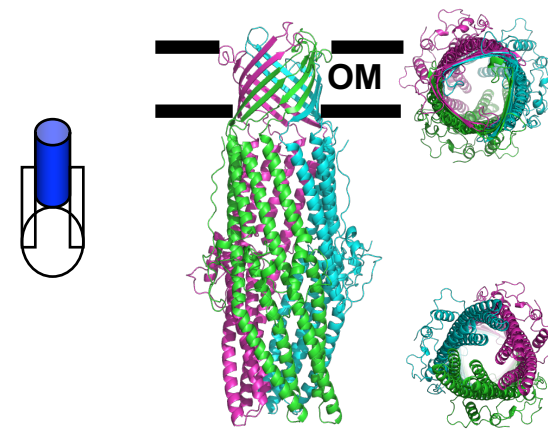
Structural analysis of the different protein partners



E. coli: AcrB
CusA



AcrA
CusB



TolC
CusC
CmeC

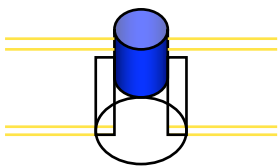
P. aeruginosa: MexB
MexD
MexF
MexY

MexA
MexC
MexE
MexX

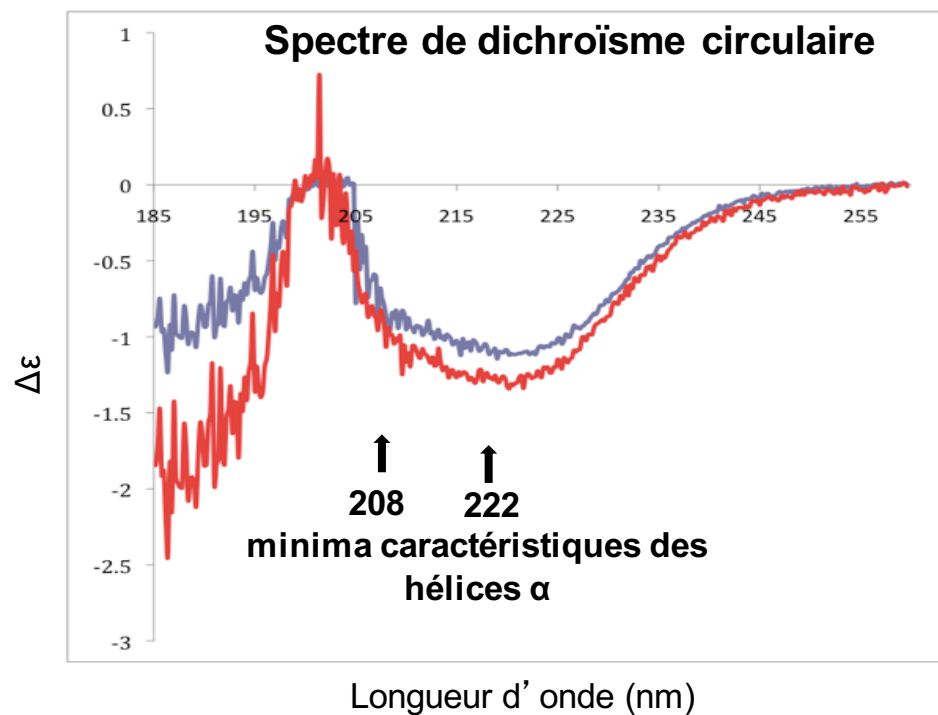
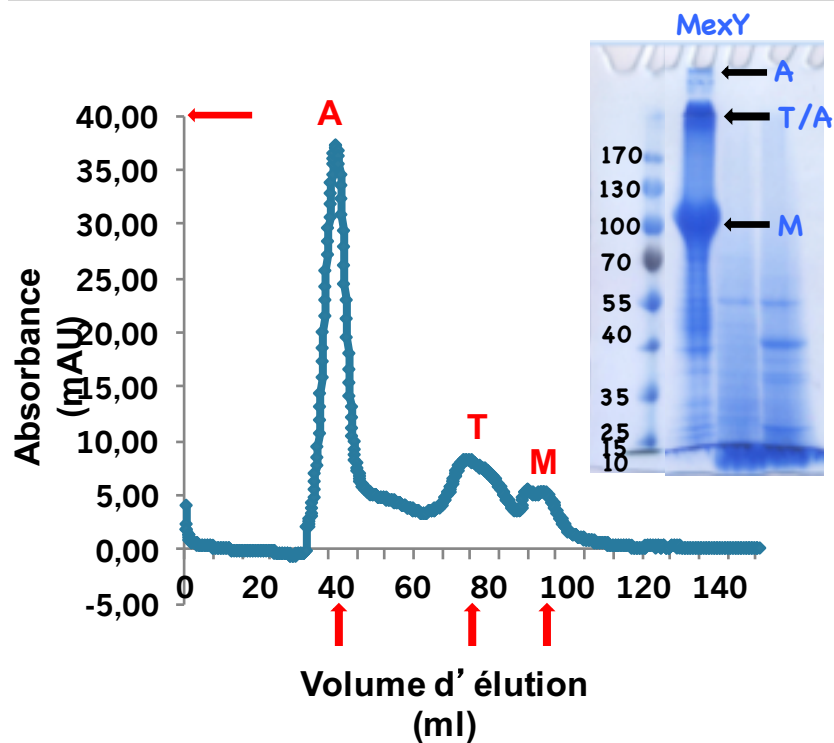
OprM
OprJ
OprN
OprM

unknown

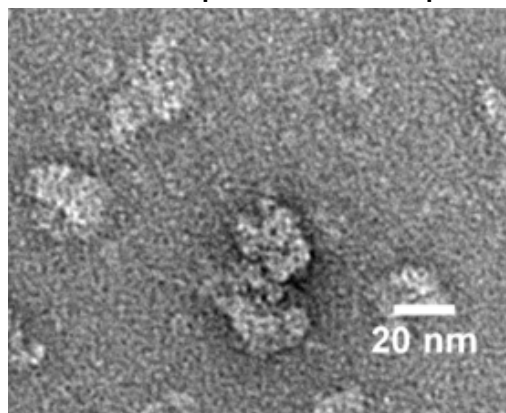
Solved by our team



Production du transporteur RND MexY



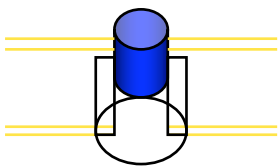
Microscopie électronique



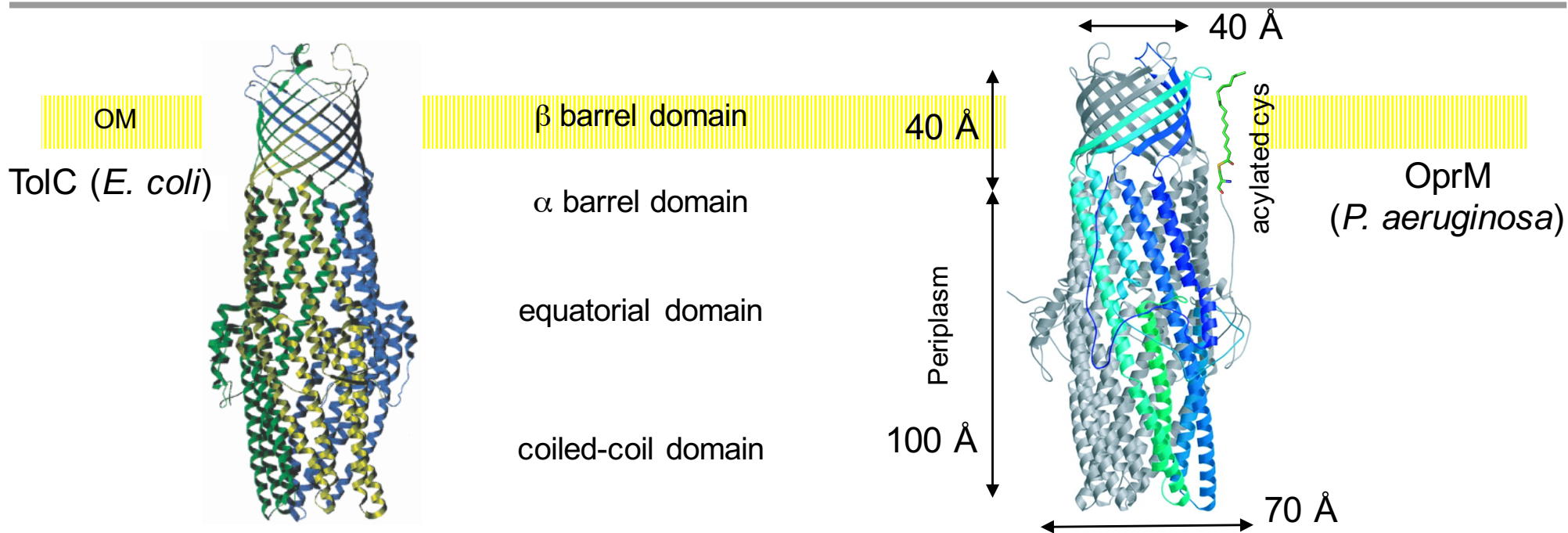
Collaboration
 O. Lambert
 Bordeaux

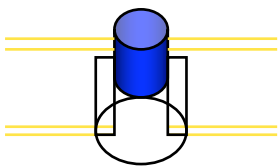
Machine CD6 Jobin Yvon



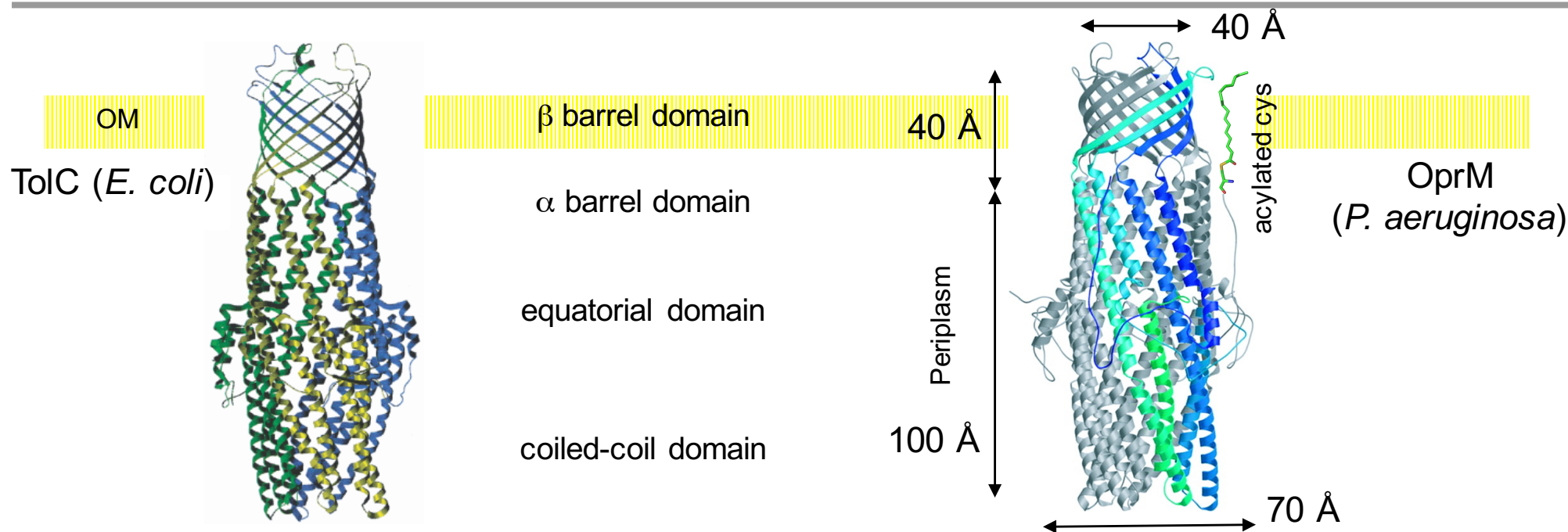


The OMF protein structure





The OMF protein structure

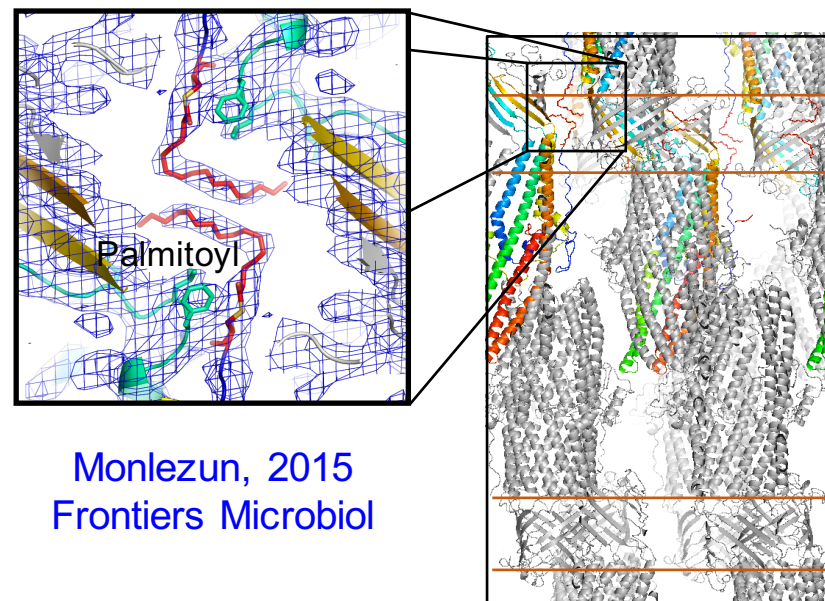


Use of fluorophores to identify which site is occupied on the N-terminal cysteine (revelation on our UV transilluminator)

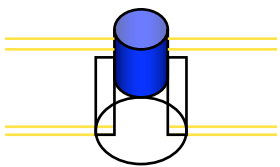
4-chloro-7-nitrobenzofurazan (NBD-Cl) that reacts with N-terminal amines ($\lambda = 475$ nm)

N-(2-Methanethiosulfonyl)-7-methoxycoumarin-4-acetamide (MTS-EMCA) that covalently attaches to a reduced cysteine via a disulfide bond ($\lambda = 312$ nm)

Mass analysis by 3P5 platform (Cochin)



Monlezun, 2015
Frontiers Microbiol



Opening mechanism: normal modes analysis combination of 2 modes

Phan (2010) structure

Initial state: closed

final state: open

2 Å

Twist Mode

8 Å

Extension Mode

weighted sum of vectors

β-barrel domain

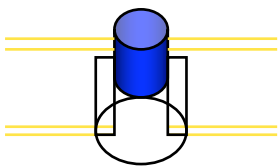
α-barrel domain

Equatorial domain

Coiled-coil domain

Formation of a cleft
Could it be
MexA binding
site ?

4 Å



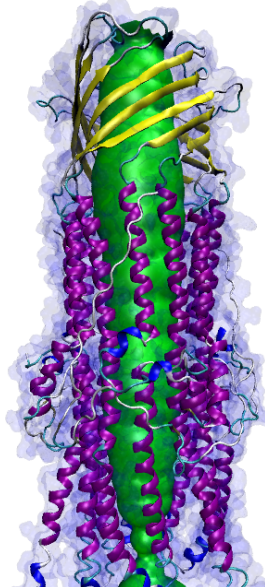
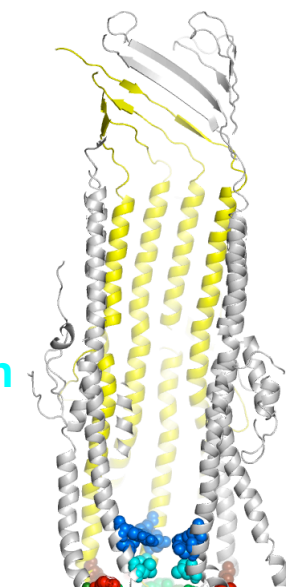
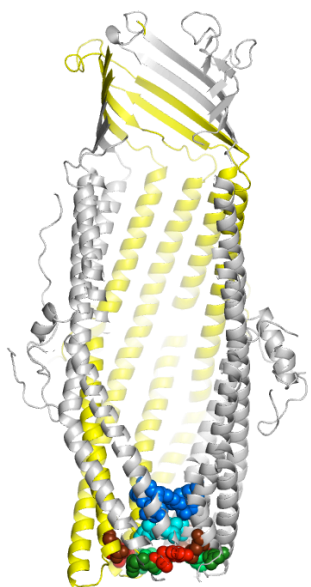
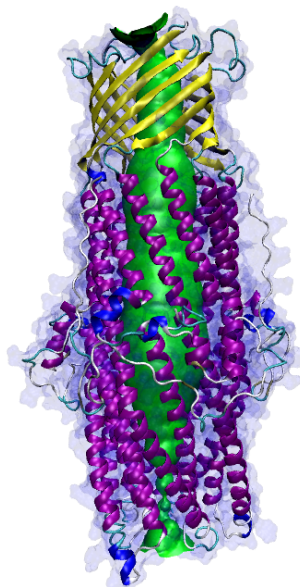
Opening of the lock by mutagenesis

closed

open

OprM

TolC



D416*- R419*
 salt bridges inter-
 monomers

L412

smallest constriction
 (Van der Waals)

D205-Y404*

H-bond $\alpha 4 - \alpha 7$
 intra-monomer

D409-R403*

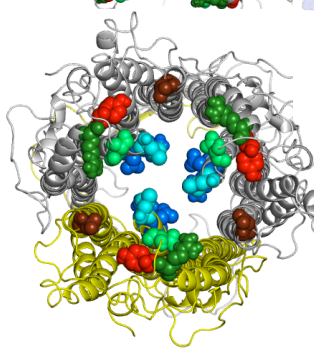
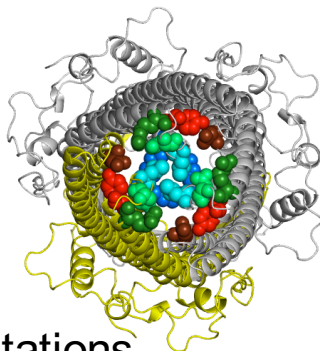
salt bridge $\alpha 8 - \alpha 7$
 intra-monomers

D371-D374
 Cross link D374
 → export
 abolishment

G365

D153-Y362
 Break of H bonds
 → increased
 sensibility

R367-T152



Choice of mutations

D416A

R403L

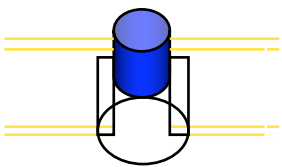
R419A

Y404F

D416A-R419A

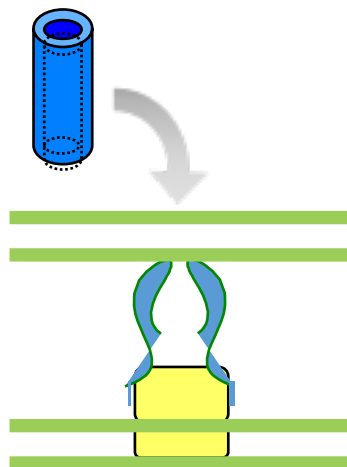
R403L-Y404F

R403L-Y404F-D416A-R419A (Quad)



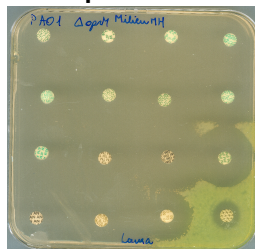
Structural and functional study of the OprM mutants

Complementation *in vivo*
In *P. aeruginosa* delta-OprM

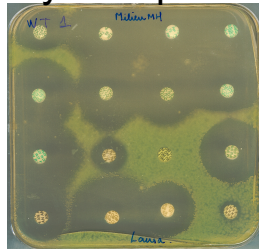


antibiogrammes

Δ OprM-PAO1



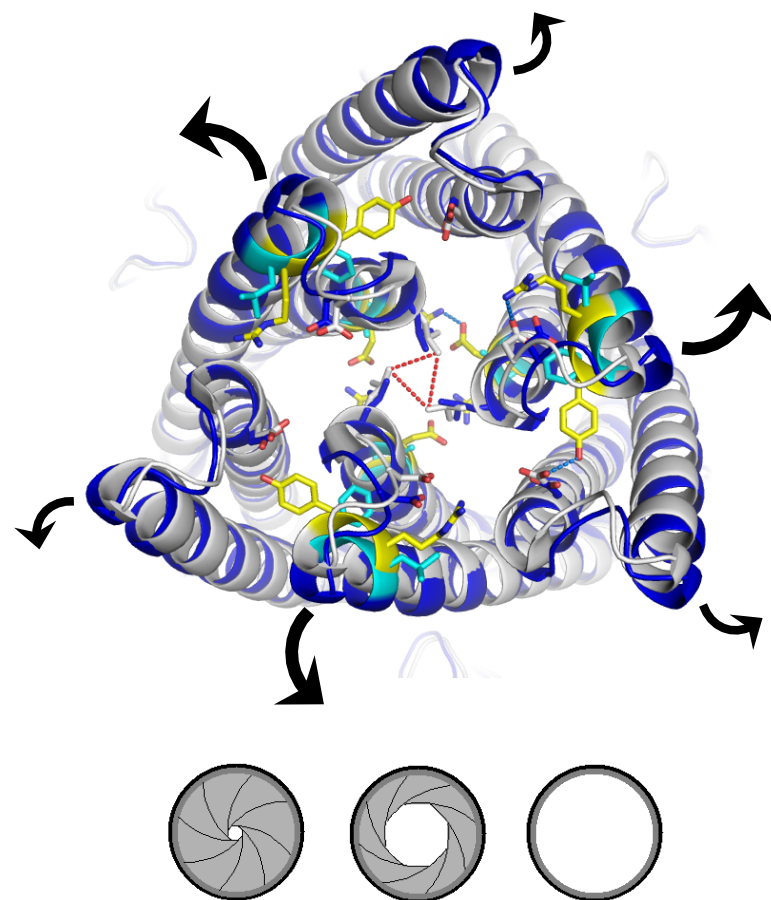
complemented
by wt OprM

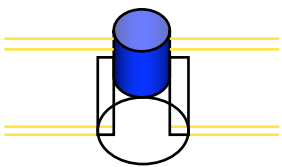


Welcomed in the laboratory of
Pr Marie-José Butel, Faculté de
Pharmacie, Paris Descartes

OprM WT : grey/**yellow**

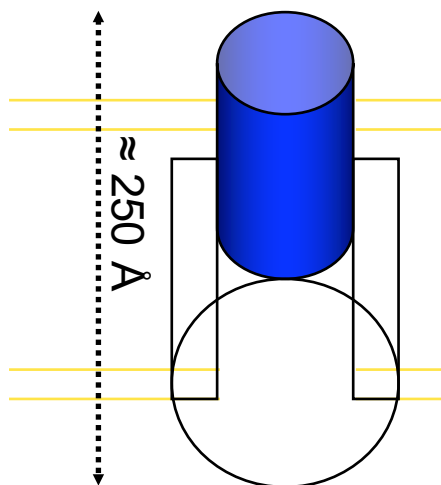
OprM Quad: **blue**/**cyan**



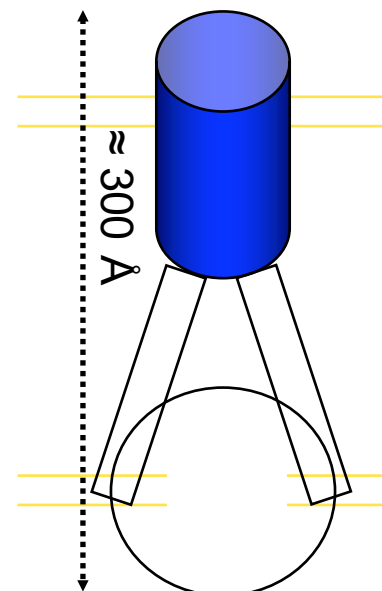
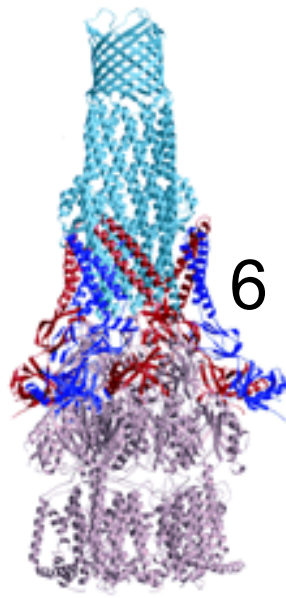
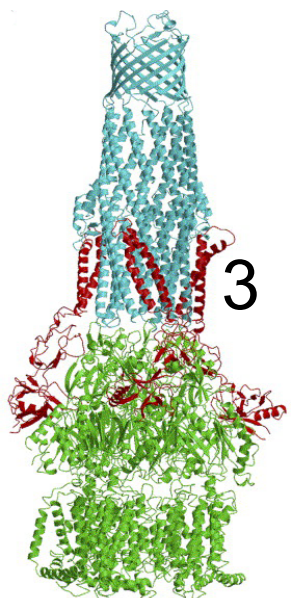


Assembly which model? Which Stoichiometry for the MFP?

Collaboration: Cathy Etchebest, INTS, Université Paris-Diderot



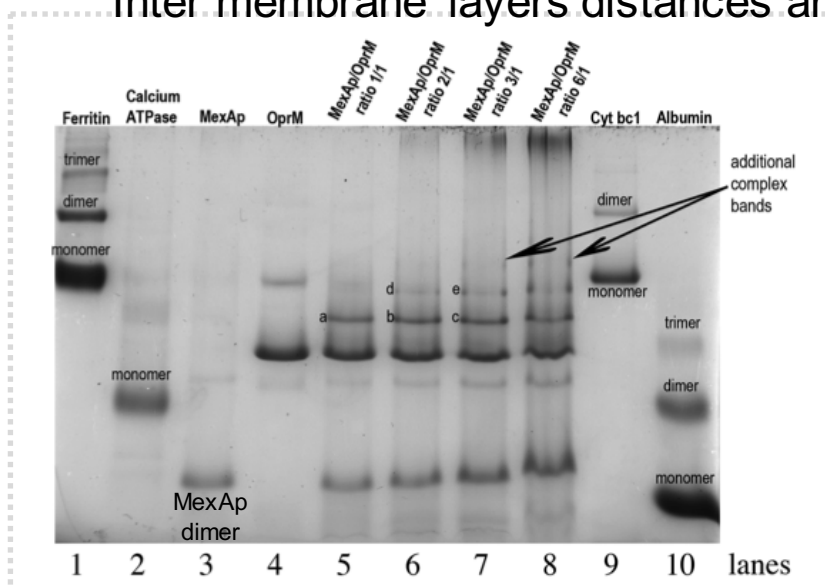
Model « short »



Model « long »



Inter membrane layers distances are compatible with observed cell wall fluctuations

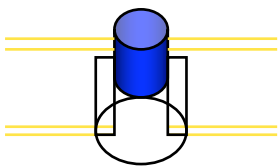


BN-PAGE

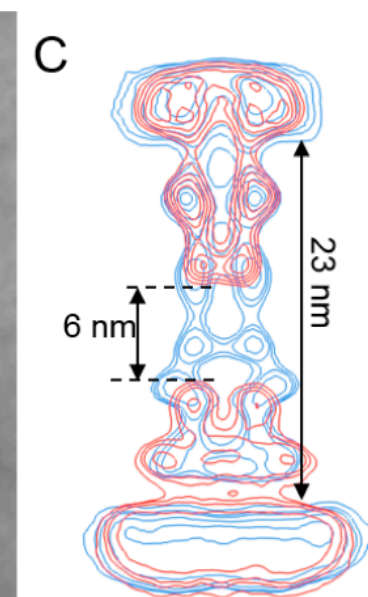
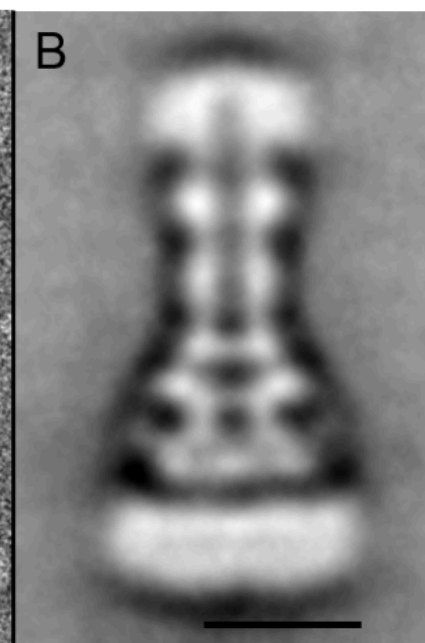
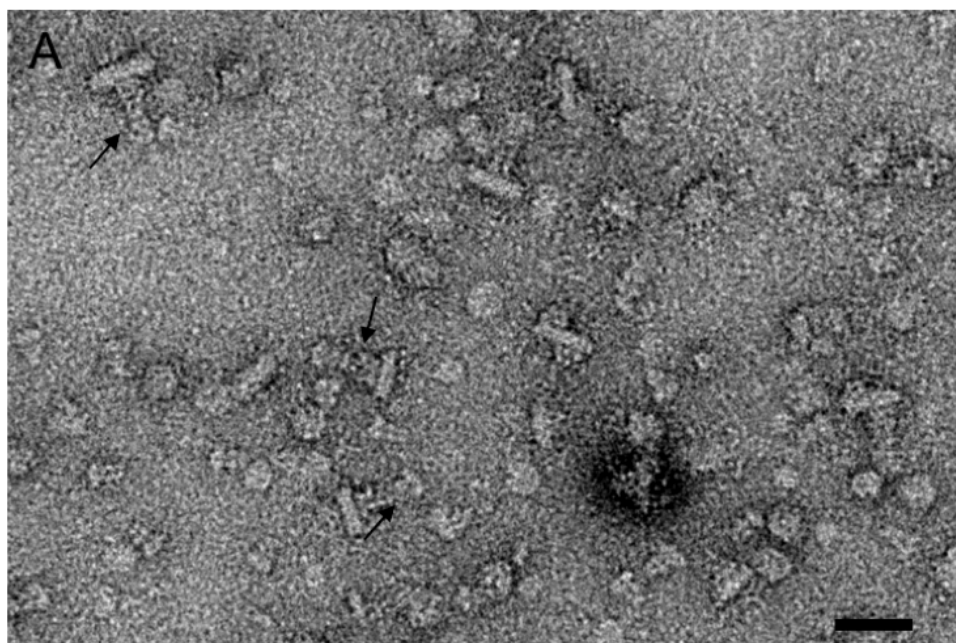
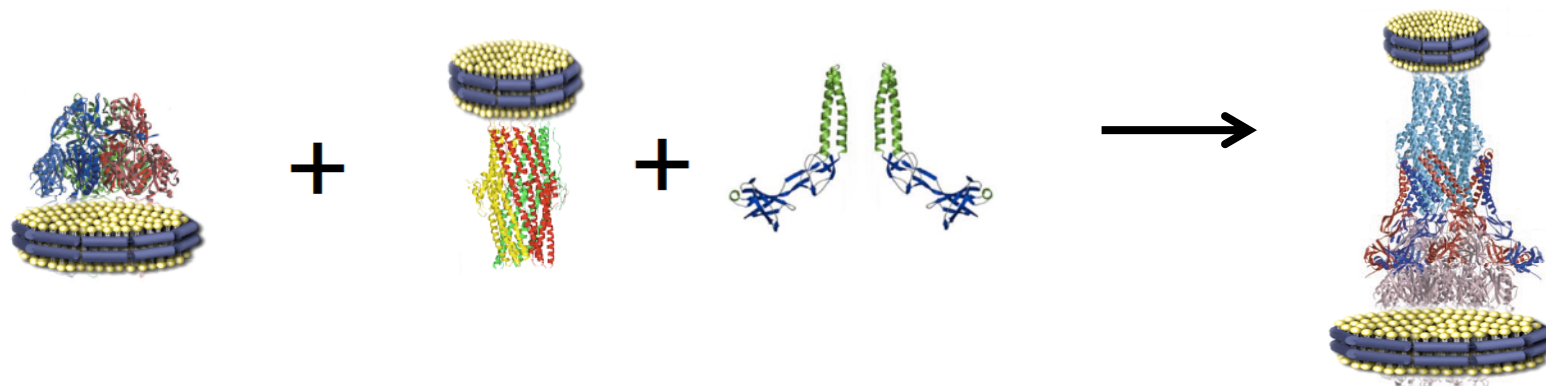
MexAp-OprM => 2, 4, 6 MexA per trimer of OprM
non-palmitylated MexA does not form complex with OprM

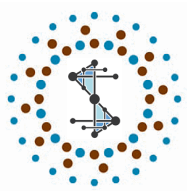
[Ferrandez et al. \(2012\) Electrophoresis](#)

extraction of each band, migration on SDS gel,
quantitative densitometry of each band relative
to a reference quantity lanes



OprM-MexA-MexB reconstitution

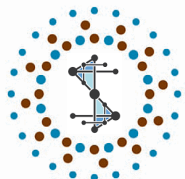




Compétences recherchées:



- Microscopie électronique
- Production de protéines dans des systèmes non bactérien
- Criblage *in silico*
- Synthèse d'inhibiteurs
- Spectroscopie de masse sur les ARN
- Spectroscopie de masse en condition non dénaturante
- Spectroscopie de masse de protéines membranaires
- Imagerie cellulaire localisation, interaction
- collaborations

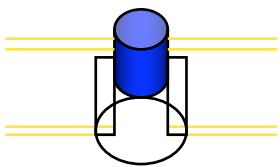


Equipes du Laboratoire LCRB



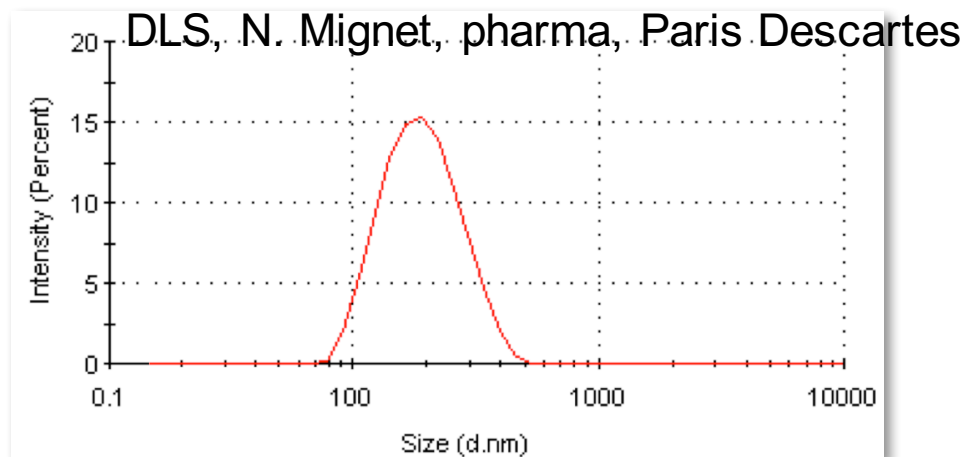
<http://lcrbw.pharmacie.univ-paris5.fr/>

Biogenèse des ribosomes	VIH, interaction	Résistance aux	Réplication du VIH	Synthèse peptidique
Biomimétiques	ARN/protéine	antibiotiques	Structure ARN	Peptides viraux
Petites molécules	Criblage RMN	Protéines membranaires		Perforateurs de membrane
« Cristallographie des complexes moléculaires »	« Structure des ARN, interactions et anti-infectieux »	« Signalisation et transport membranaire »	« Mécanismes moléculaires de l'initiation de la traduction »	« Structure et mécanisme d'action des protéines virales »
Chefs d'équipe :	Chef d'équipe :	Chef d'équipe :	Chef d'équipe :	Chef d'équipe :
LEULLIOT Nicolas	TISNE Carine	BROUTIN Isabelle	SARGUEIL Bruno	BOUAZIZ Serge
BLAUD Magali	BARRAUD Pierre	BENAS Philippe	CHAMOND Nathalie	CORIC Pascale
CHIADMI Mohamed	BRACHET Franck	DUCRUIX Arnaud	ULRYCK Nathalie	GINCEL Edith
DELBOS Lila	CATALA Marjorie	GARNIER Cyril		LAUGAA Philippe
DESCHAMPS Patrick	DARDEL Frédéric	GAUCHER Jean-François	AMEUR Melissa	
LEBARON Simon	DEGUT Clément	LASCOMBE Marie-Bernard		KARA Hesna
LEMOINE Pascale	LARUE Valéry	PHAN Gilles		
PRANGE Thierry	NONIN-LECOMTE Sylvie	SALEM Michèle		
SEIJO Bili	PONCHON Luc			
SELKTI Mohamed		ADRIEN Vladimir		
TOMAS Alain	MOUHAND Assia	HOUSSEINI-B-ISSA Karim		
		NTOGO Yvette		
MADRU Clément				

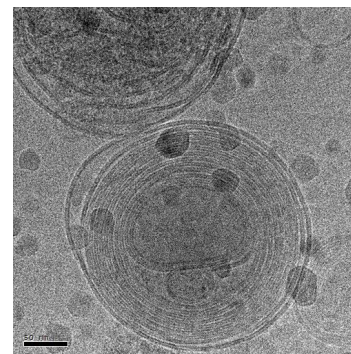


In vitro reconstitution and functional test

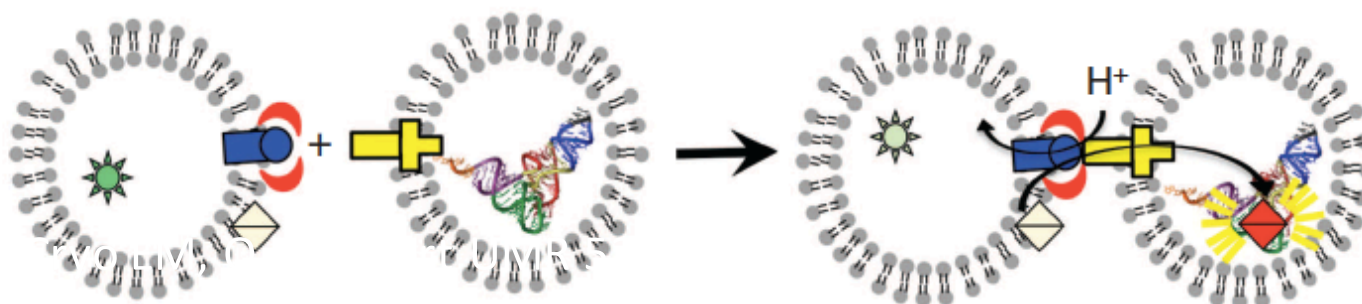
Reconstitution in liposomes



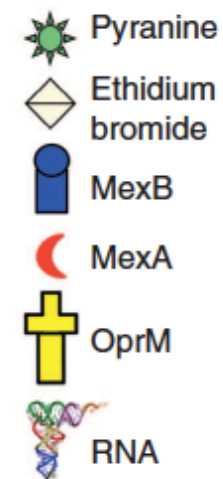
Microscopy, O. Lambert, Bordeaux



Transport =



Acidification, followed by the fluorescence variation of pyranine
Increase of substrate fluorescence



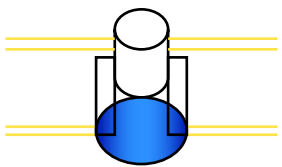
Fluorimeter Safas



Verchère (2015) Nat Comm

Picard (2012) & Verchère (2013) Anal Biochem

Verchère (2012) Scientific Reports



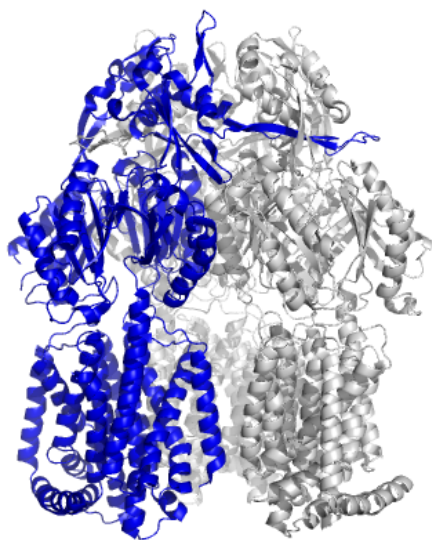
The RND protein structure

First AcrB structure 3.5Å

space group **R32**

1 molecule / u.a.

Murakami, Nature 2002



LLL

Space group **C2** 2.8 Å

Murakami, Nature 2006

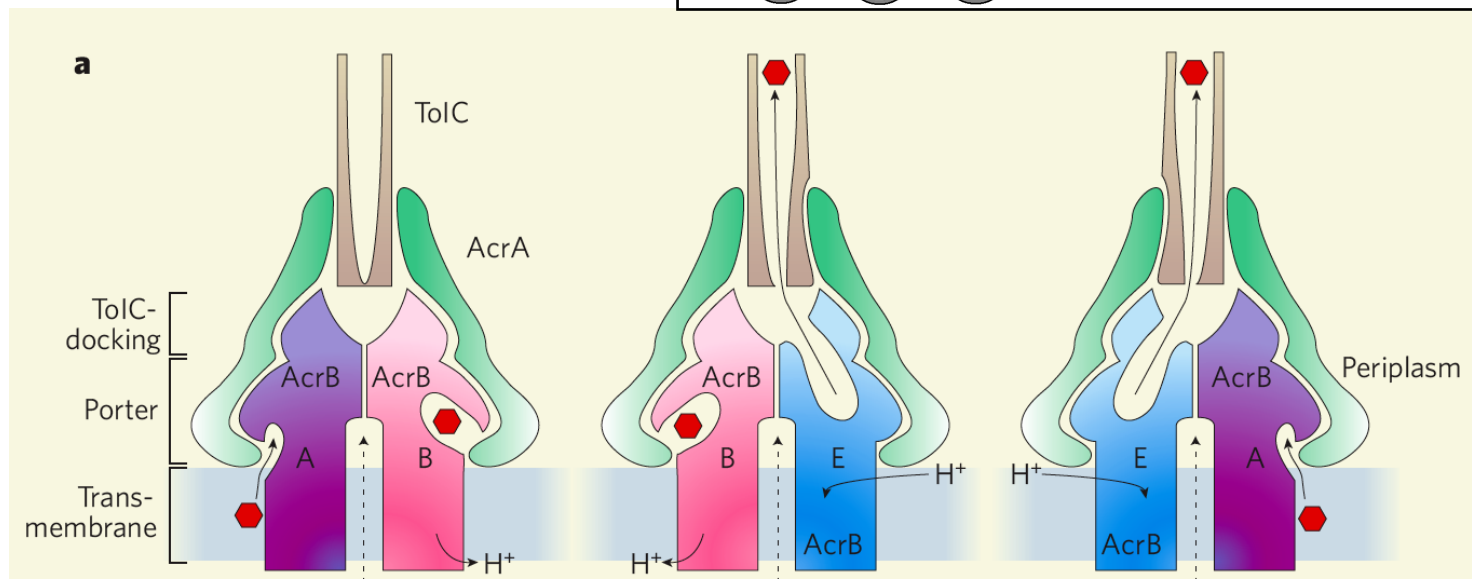
Seeger, Science 2006

LTO

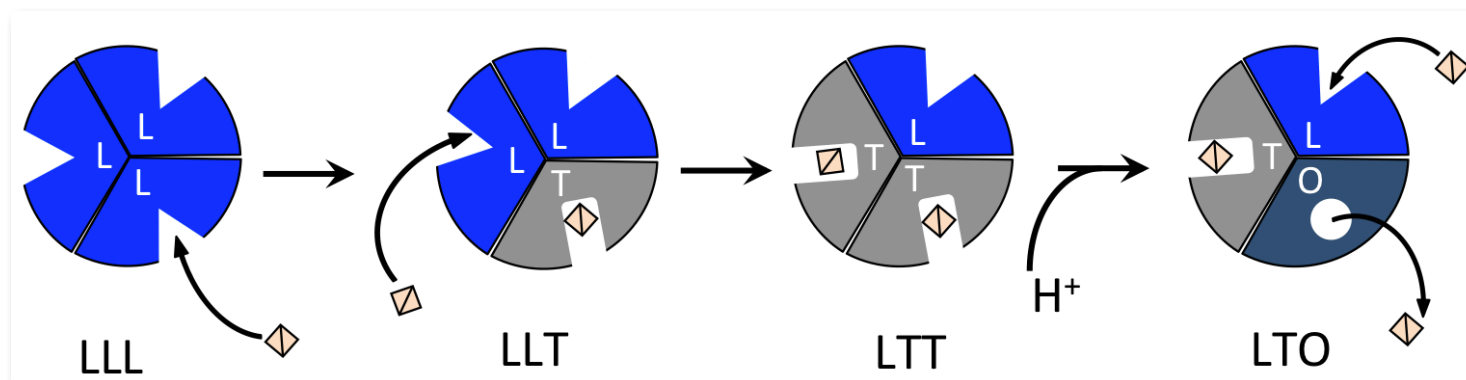
Peristaltic mechanism of transport



Schuldiner, Nature 2006



«Access(A)/Loose (L)» «Binding(B)/Tight(T)» «Extrusion(E)/Open(O)»



Cross-linking studies: LLT, LTT (Seeger 2008 Nat. Struc. Mol. Biol.)