

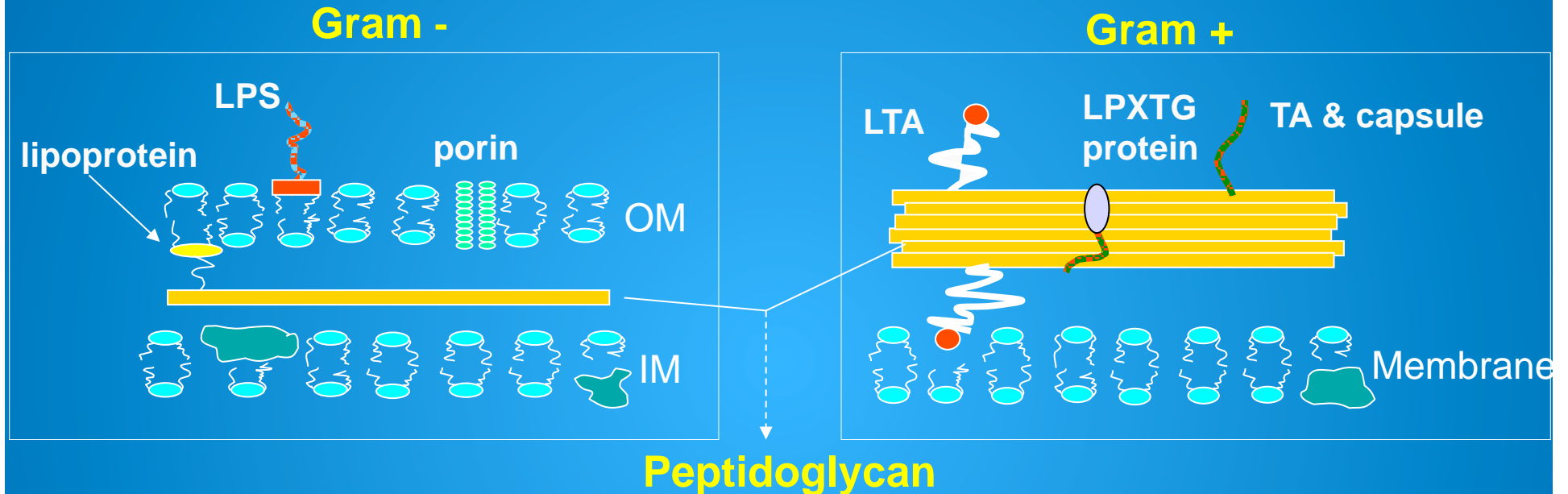
Pourquoi certaines bactéries ne deviennent pas résistantes aux antibiotiques ?

I. G. Boneca

Biology and Genetic of the Bacterial Cell Wall

Institut Pasteur

The peptidoglycan (PG): A major bacterial cell wall component



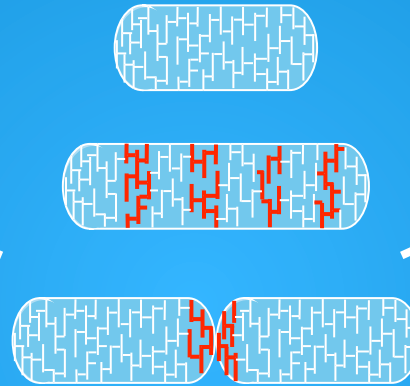
- Essential for cell integrity
- Major determinant of cell morphology
- Major target of different classes of antibiotics
- Important role in virulence

PG
a key component of bacterial physiology

Bacterial shape/
physiology



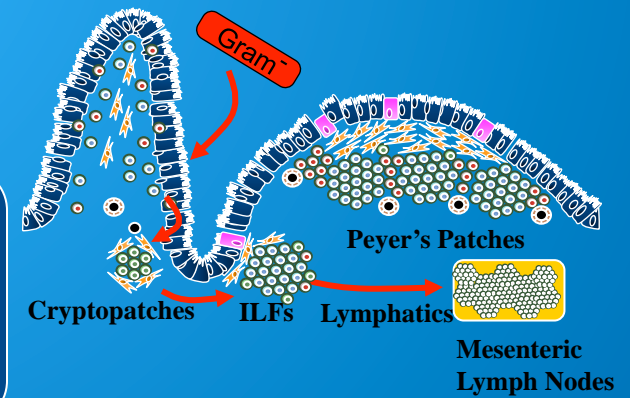
peptidoglycan



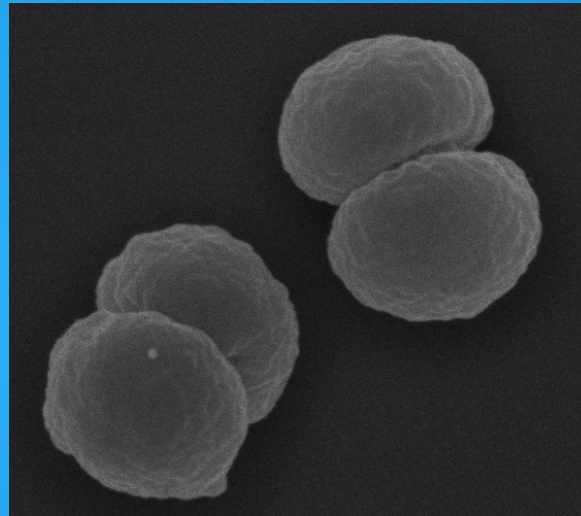
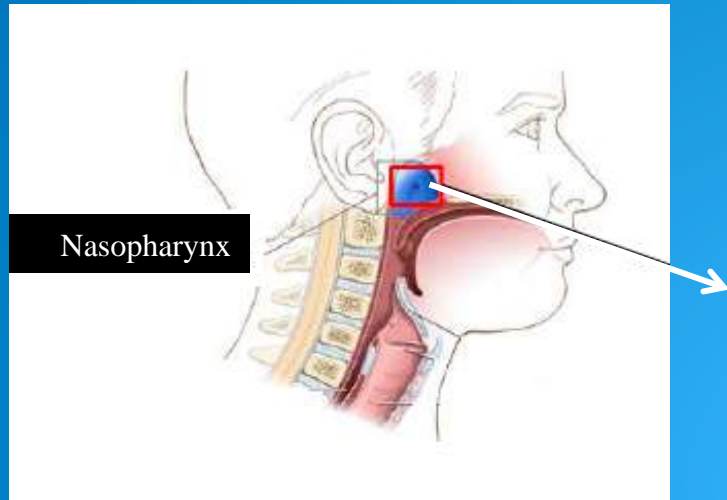
Antimicrobial
resistance



Host/Microbe
interactions

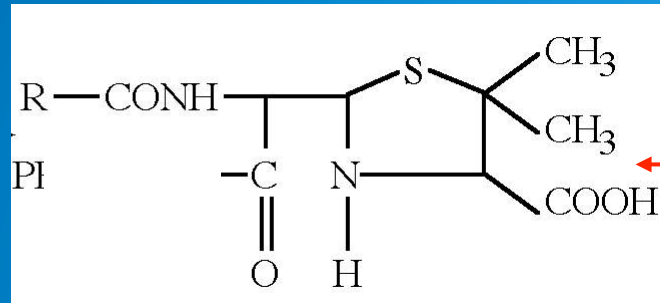


Introduction : *Neisseria meningitidis*

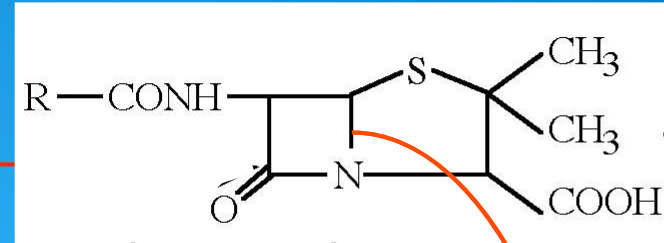


- Asymptomatic carriage : 5 to 20 % of human population
- Occasionnally invasive :
 - septicemia
 - meningitis

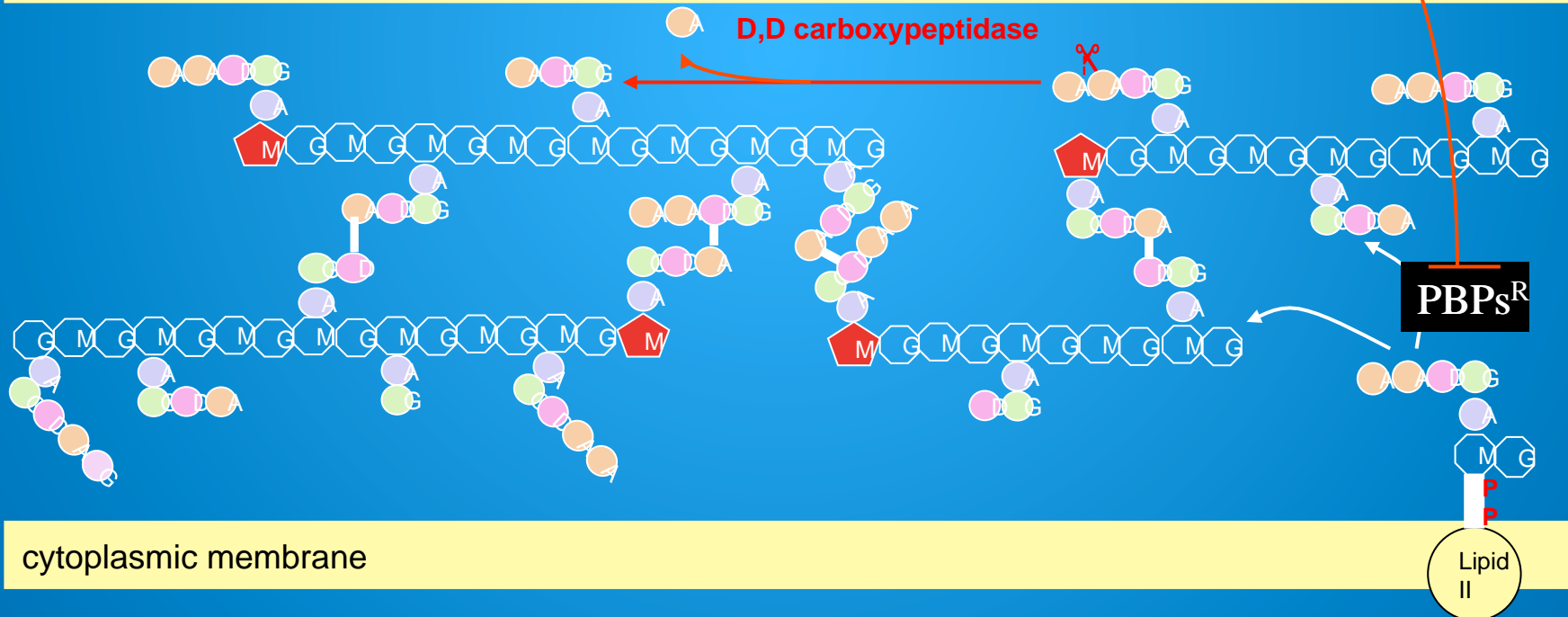
Mechanisms of resistance



β -lactamase



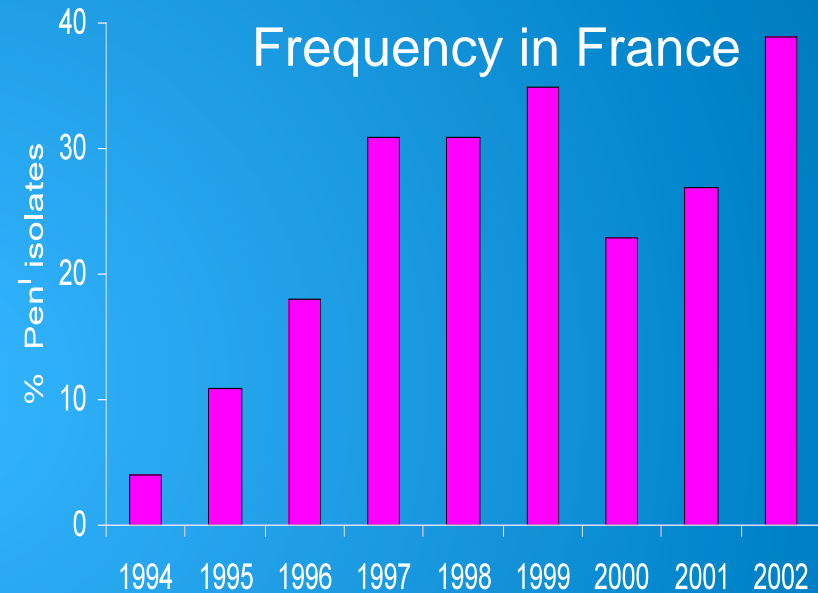
outer membrane



Penicillin G resistance in *N. meningitidis*

✓ *reduced susceptibility to penicillin G (Pen^I)* → $0.125 \leq MIC \leq 1 \mu\text{g/ml}$

- First isolation in 1985 in Spain
in 1994 in France
- Variable prevalence worldwide



✓ *resistant to penicillin G (Pen^R)* → $MIC > 1 \mu\text{g/ml}$

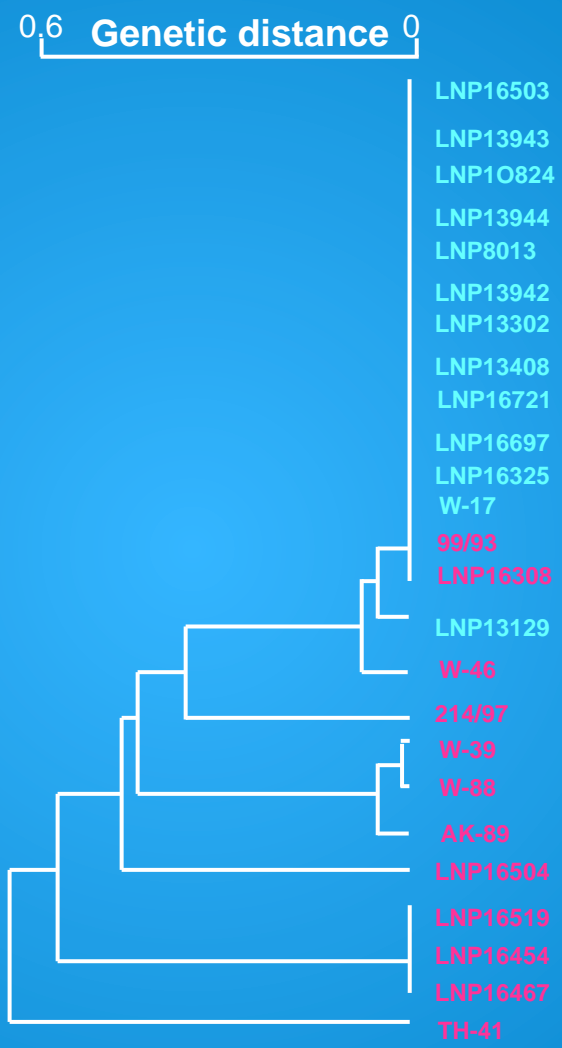
- So far no strains isolated

Why doesn't meningococci develop resistance to penicillin G?



•Molecular typing:

•Combinaison of 3 restriction profiles
→ definition of *penA* alleles:



Nm Pen^S
↓
penA1 (1-1-1)
Highly related *penA* alleles

Nm Pen^I
↓
penA2 (1-6-1)
penA3 (1-2-1)
penA9 (6-8-7)
penA5 (2-3-3)
penA4 (2-3-2)
penA8 (5-7-6)
penA7 (4-5-5)
penA6 (3-4-4)
Various *penA* alleles

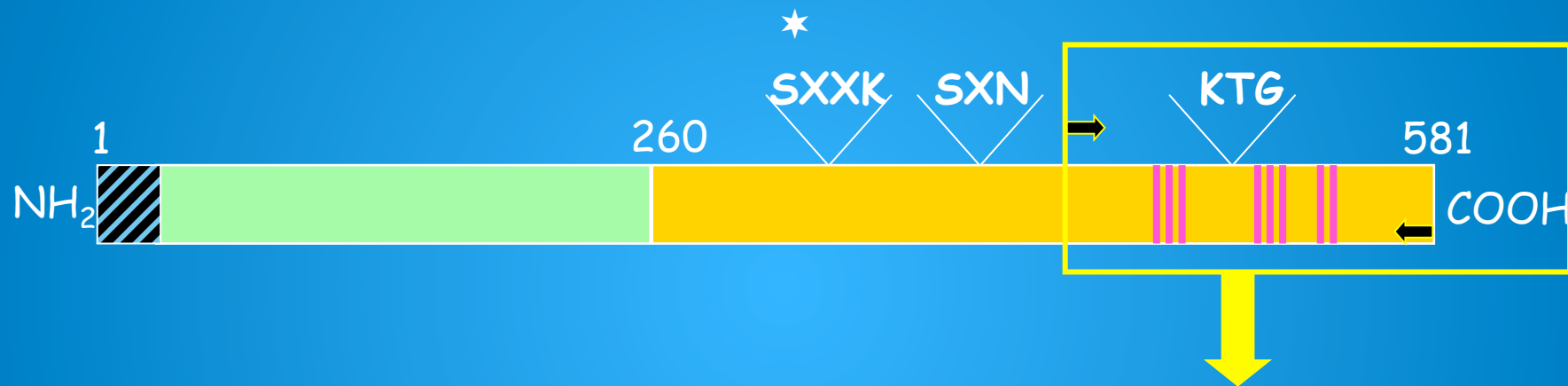


High degree of polymorphism in the *penA* gene correlated with reduced susceptibility to penicillin G



PBP2 modifications are required for the Pen^I phenotype in *N. meningitidis*

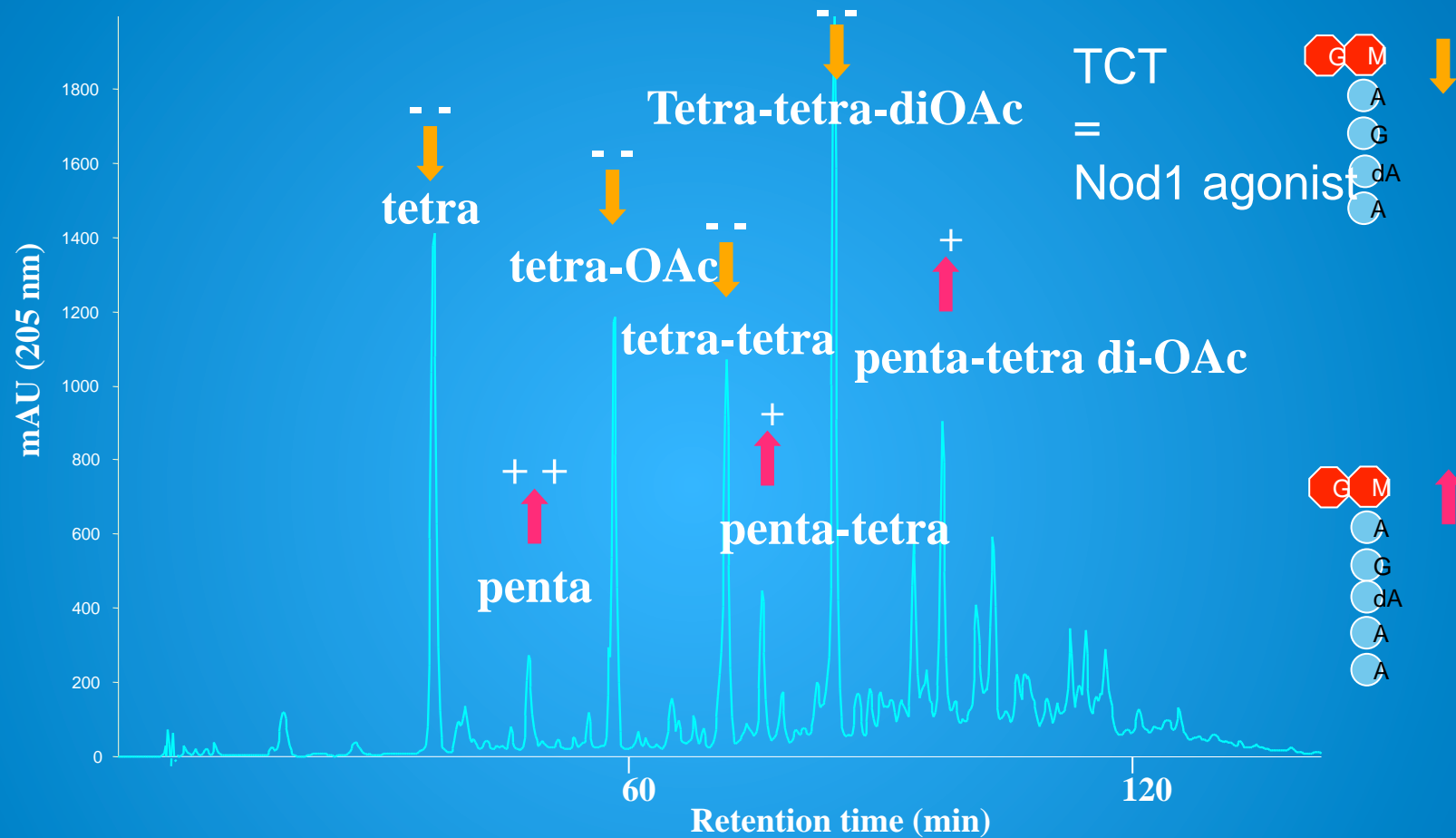
Aude Antignac
Muhamed Taha
Ivo Boneca



VLLPVSFEKQAVAPQGKRIFK**E**STAR**E**VR**N**LMVSVTEPGGTGTAGAVDGFVGA**K**T**G**TARK**F**VNGRY**A**DNK**H**I
↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓
A **Q/K** **E** **L** **V** **V**
ATFIGFAPAKNPRVIVAVTIDEPTA**H**GY**Y**GGV**V**AGPPFKKIMGGSLNILGISPTKPLTAAAVKTPS -COOH
↓ ↓
N **T**

Antignac *et al.* 2003. *J Biol Chem* 278. (34): 31521-31528
Antignac *et al.* 2003. *J Biol Chem* 278. (34): 31529-31535

PG modifications in Pen^I meningococcal strain



- Increase in mucopeptides carrying pentapeptide chains directly related to the eight aa substitutions



Distinct roles of the NOD proteins

Nod1



- expressed ubiquitously
- first line of defense in epithelial cells against infections
- senses mesoDAP-type peptidoglycan

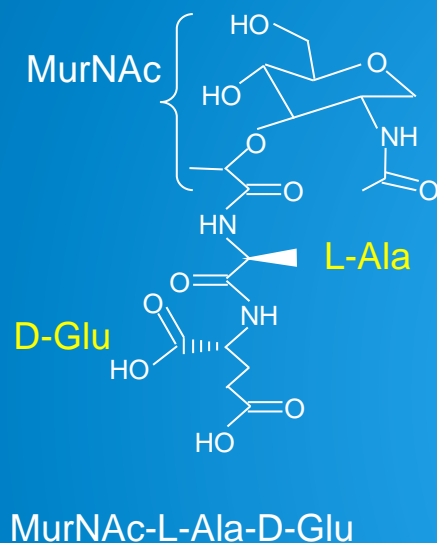
Nod2



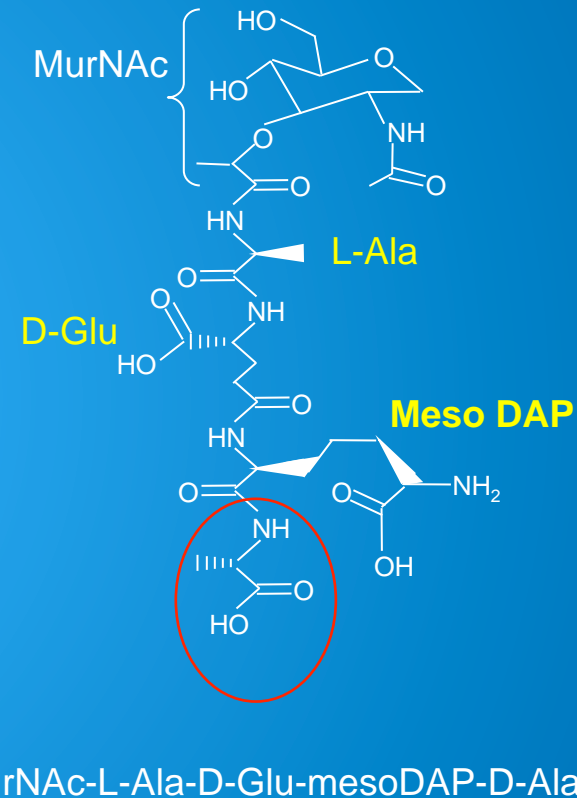
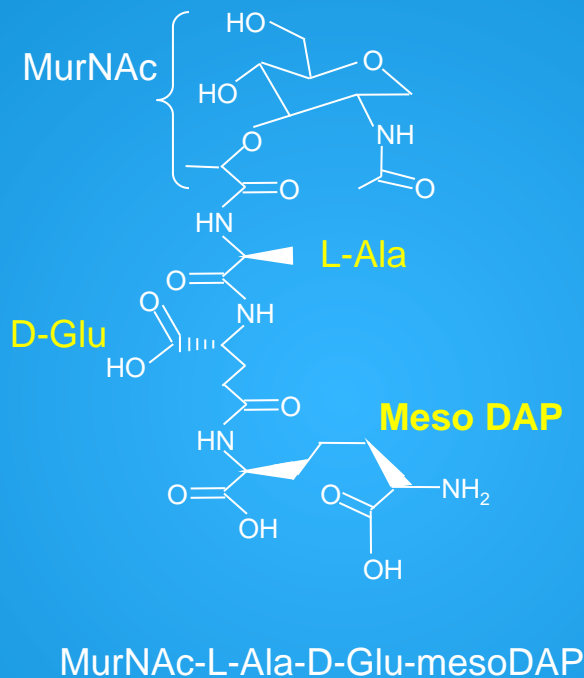
- mainly expressed in myeloid lineage
- can be upregulated in epithelial cells during inflammatory responses and infections
- first susceptibility gene in Crohn's disease and Blau syndrome
- large spectrum sensor of peptidoglycan



Nod2



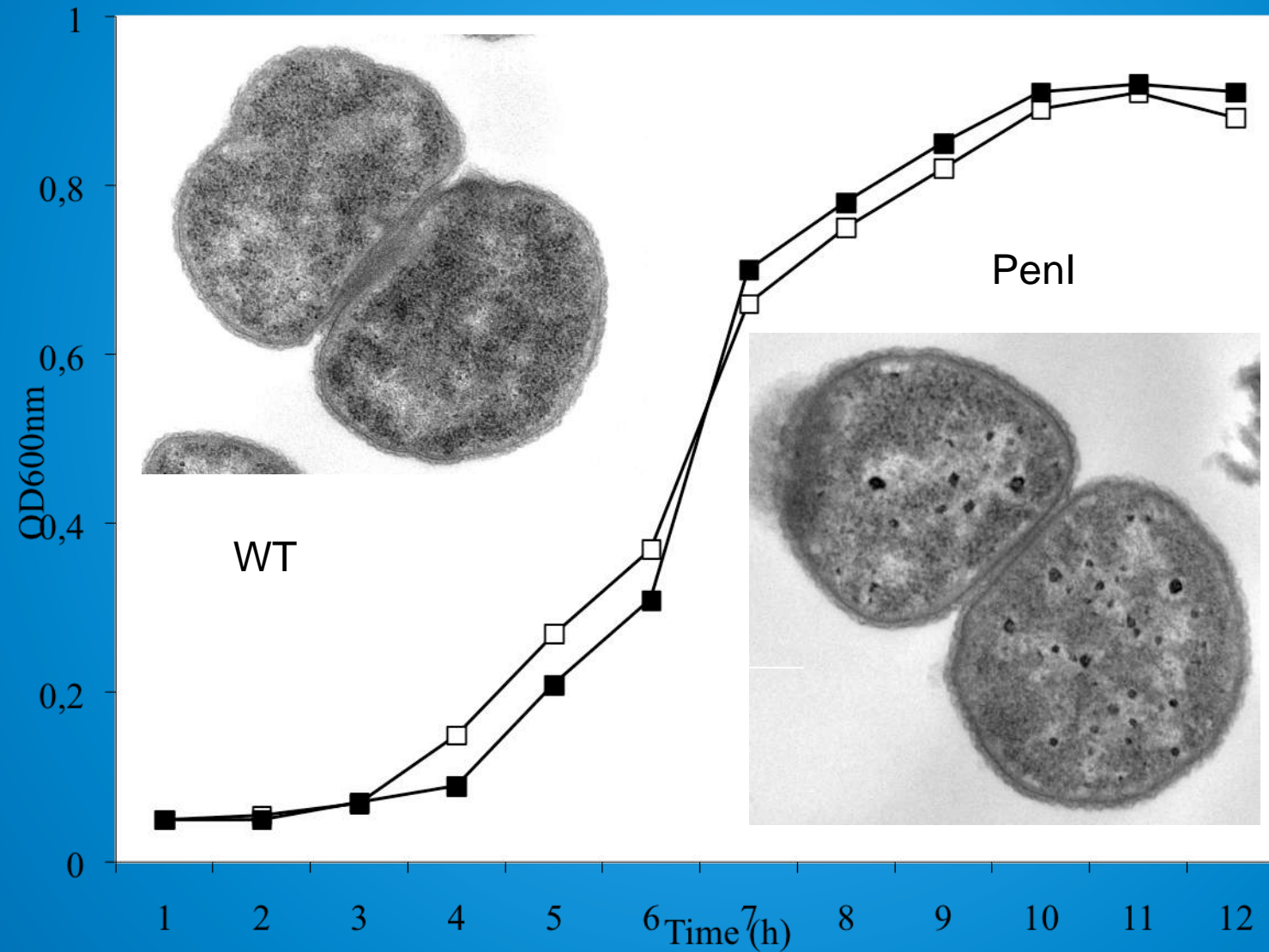
Nod1



Pen^I

- Girardin et al. 2003. *J. Biol. Chem.* 278(11):8869-72
- Girardin et al. 2003. *J. Biol. Chem.* 278(43):41702-8
- Girardin et al. 2003. *Science.* 300(5625):1584-7
- Magalhaes et al. 2005. *EMBO Rep.* 6(12):1201-7

Pen^l strains have normal growth and morphology



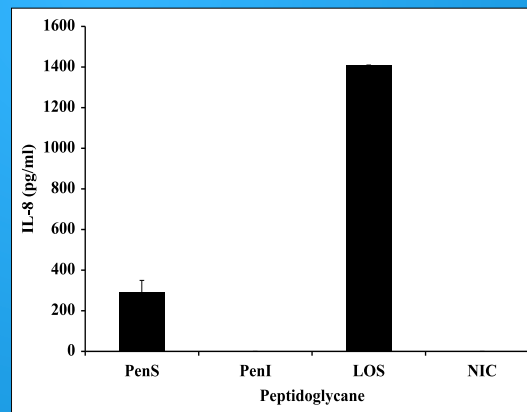
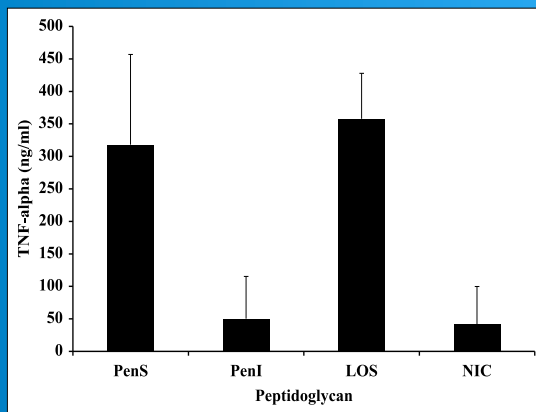
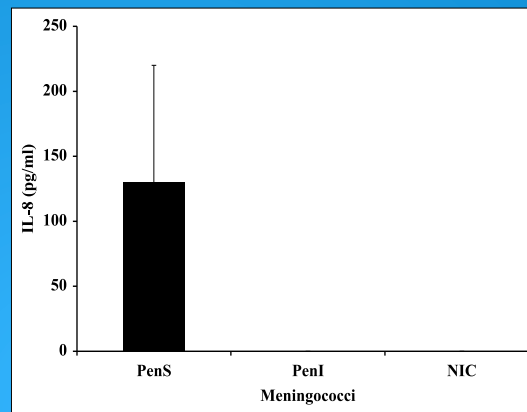
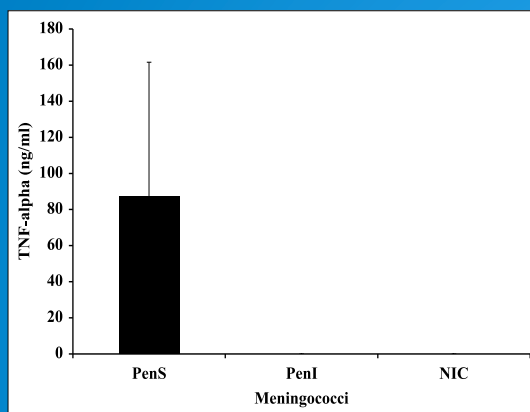


Impaired inflammatory response of epithelial cells to Pen^I strains

Anna Skoczynska
Leticia Zarantonelli
Jean-Michel Alonso
Muhammed Taha
Ivo Boneca

TNF- α

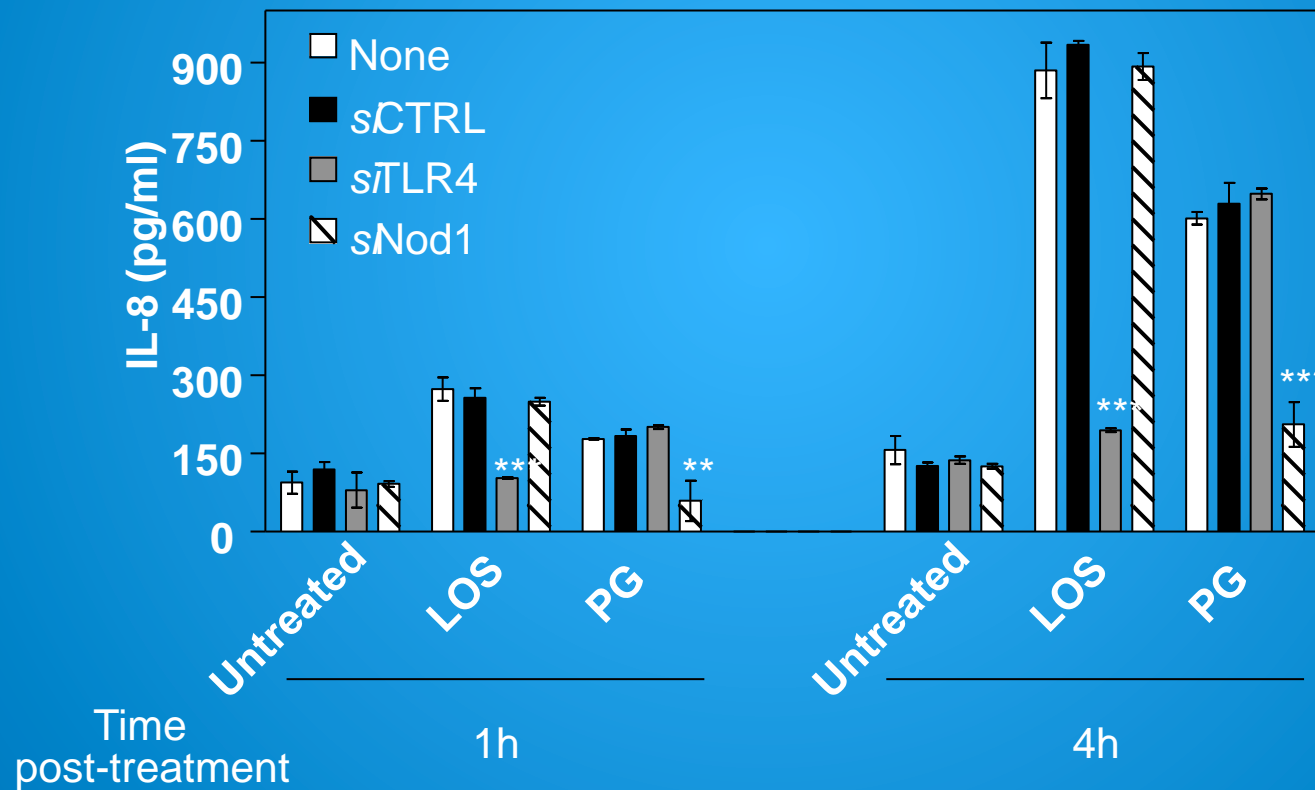
IL-8



Bacteria

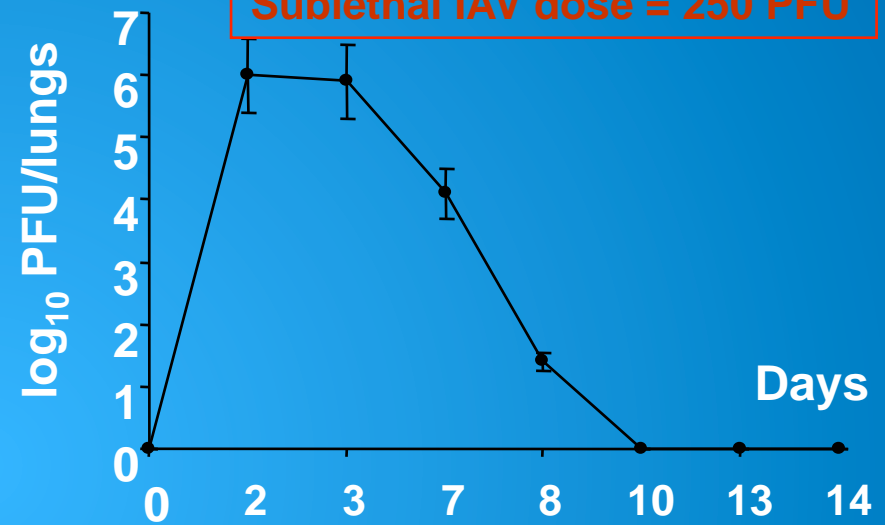
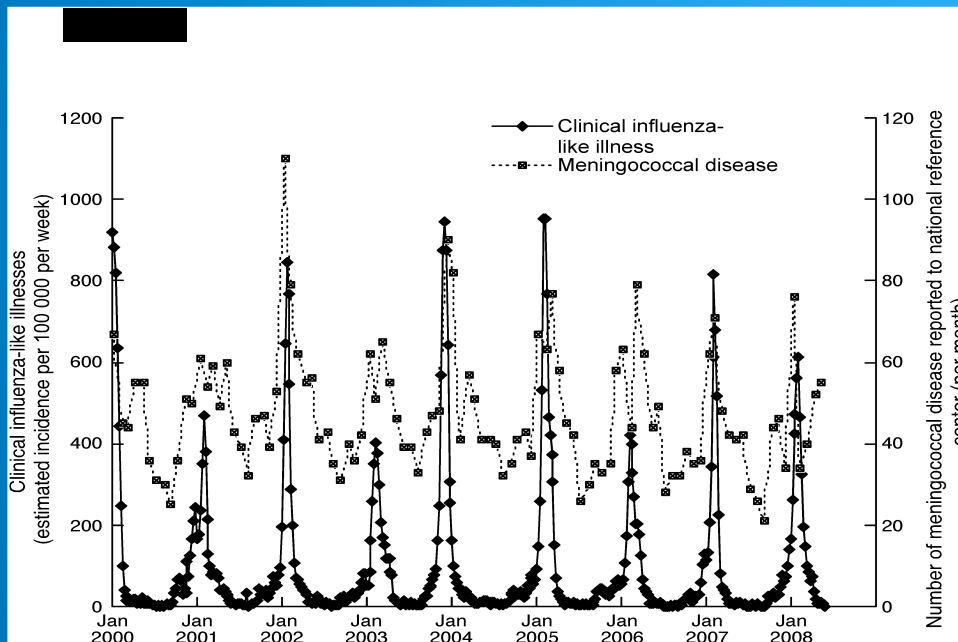
Pure ligands:
PG~ 10⁸ bact
LOS~8x10⁸ bact

Effects of TLR4 and Nod1 silencing in epithelial cells



Animal model for meningococcal virulence

Association between influenza and meningococcal disease



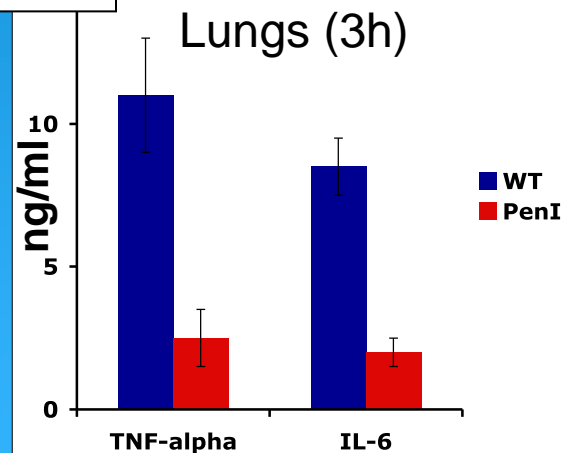
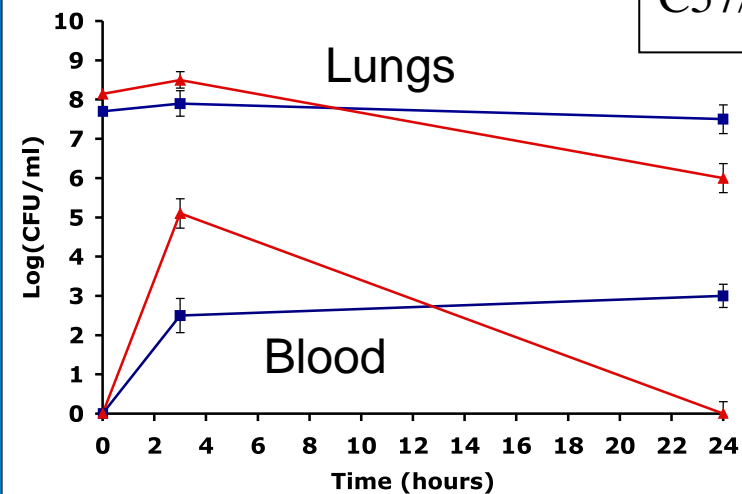
susceptibility to respiratory superinfection with *N. meningitidis*



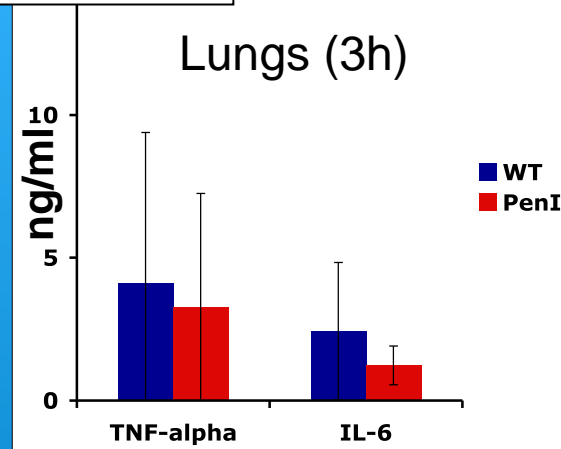
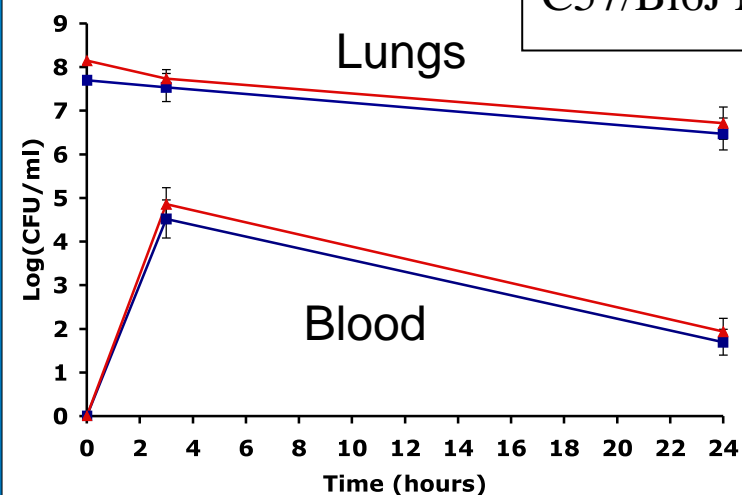
Impaired virulence of Pen^I strains

Leticia Zarantonelli
Jean-Michel Alonso
Muhammed Tahar
Ivo Boneca

C57/B16J

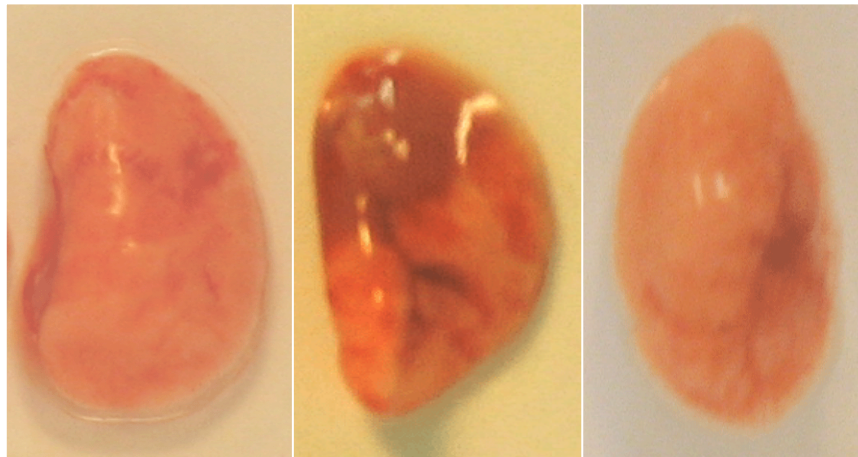


C57/B16J Nod1 -/-



Impaired virulence of Pen^I strains

C57B16/J Nod1^{+/+} mice

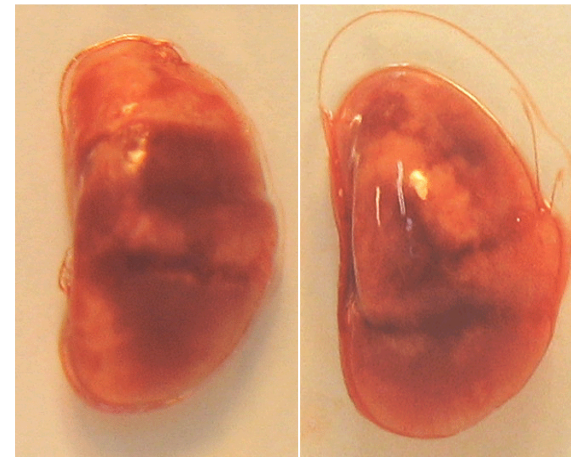


Non-infected

Pen^S

Pen^I

C57B16/J Nod1^{-/-} mice



Pen^S

Pen^I



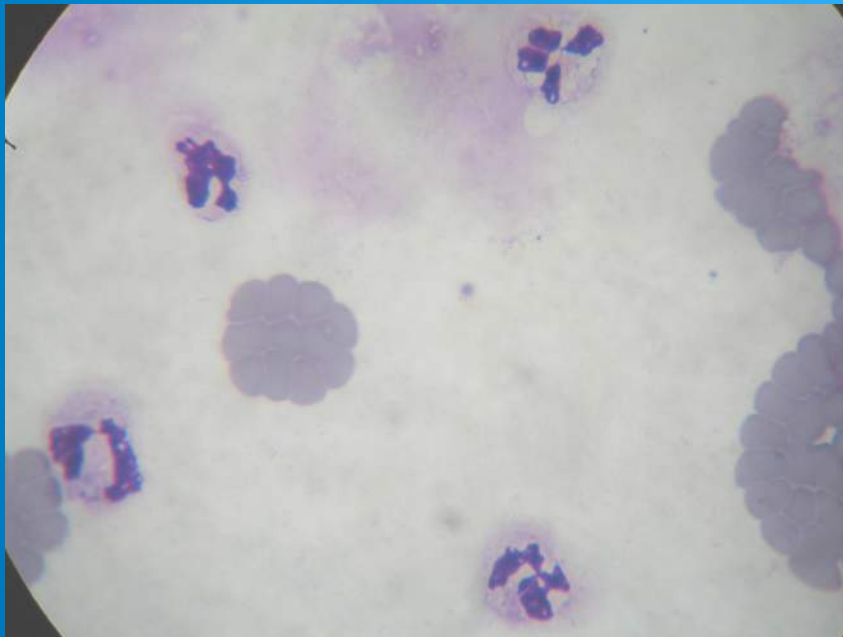


penI

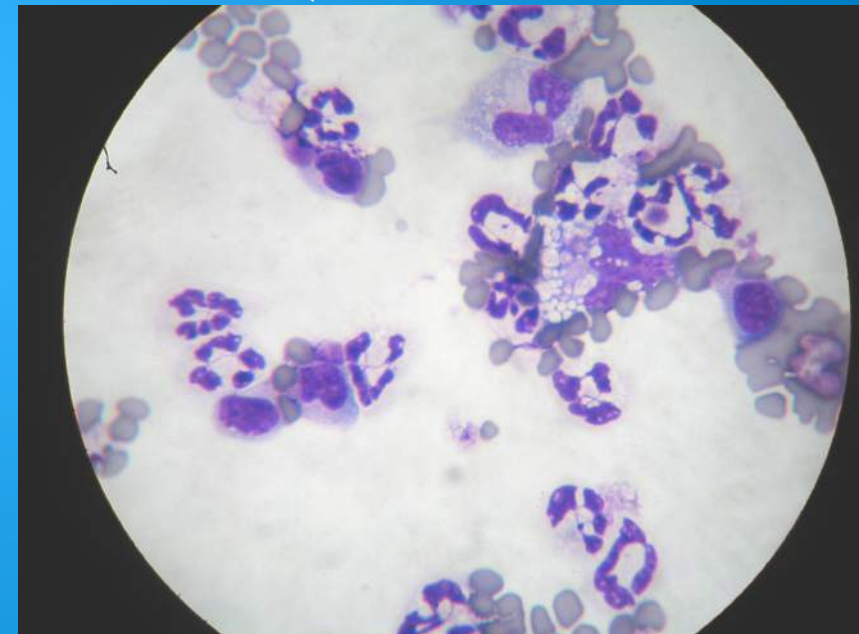
penS



LBA



3.7×10^5 cells/ml

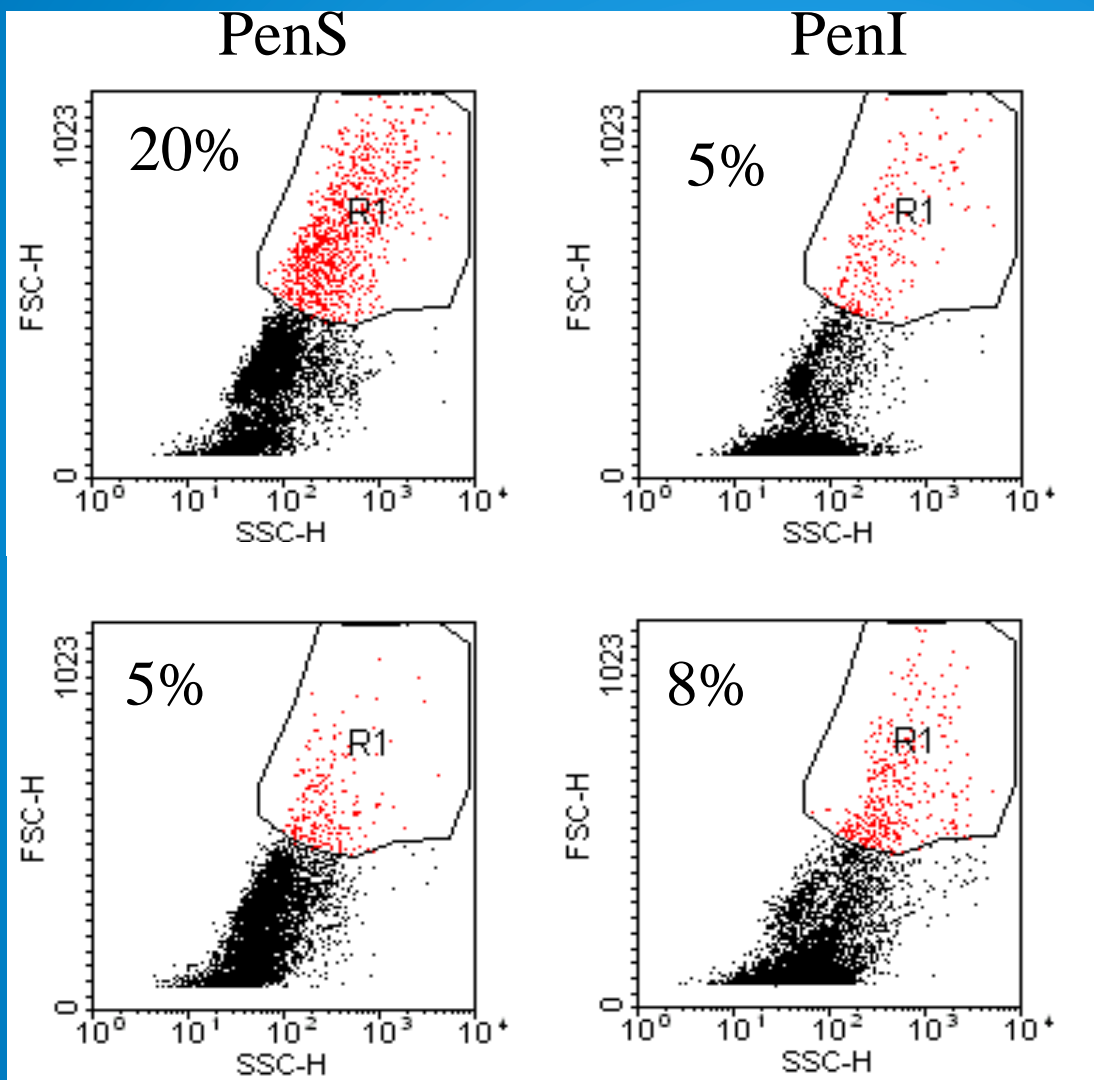


2.9×10^6 cells/ml

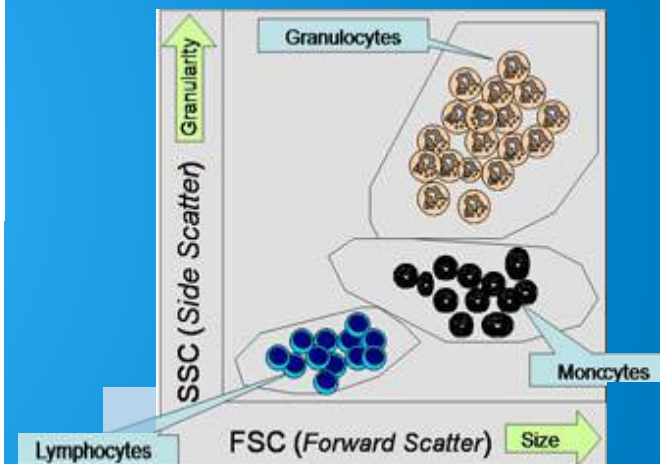


FACS analysis of bronchial lavage

WT mice

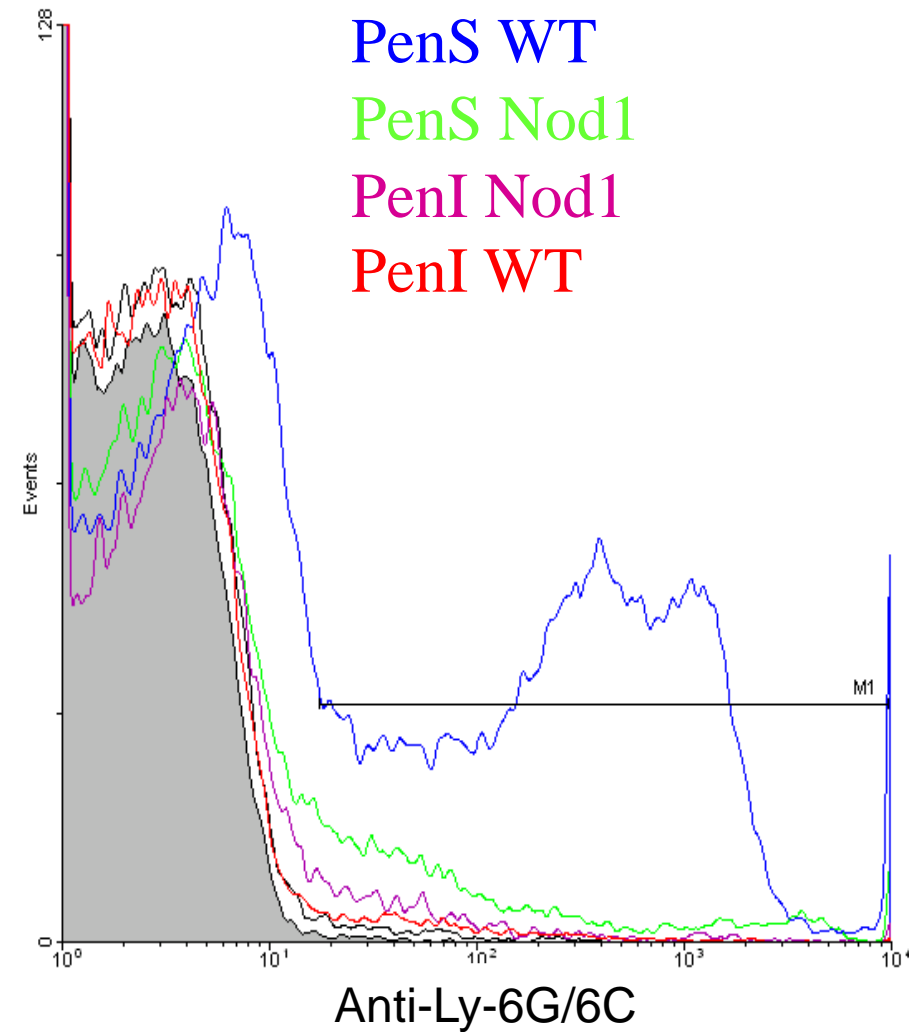
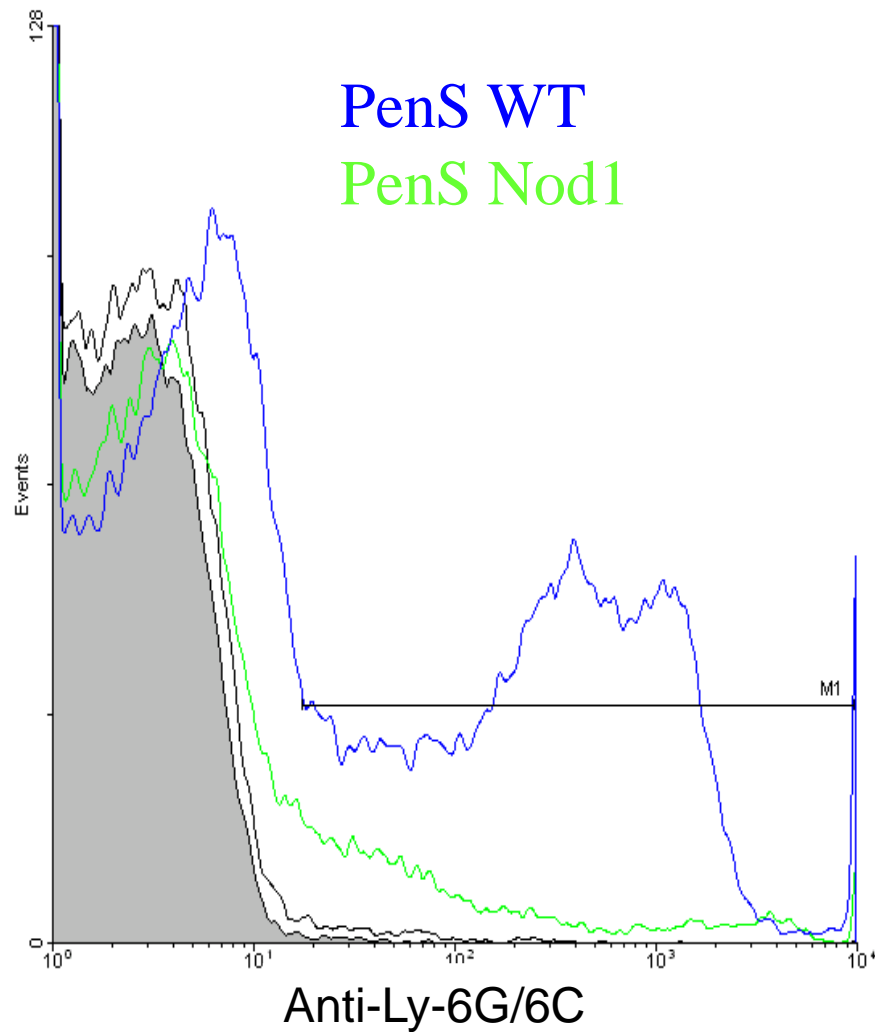


Nod1 KO



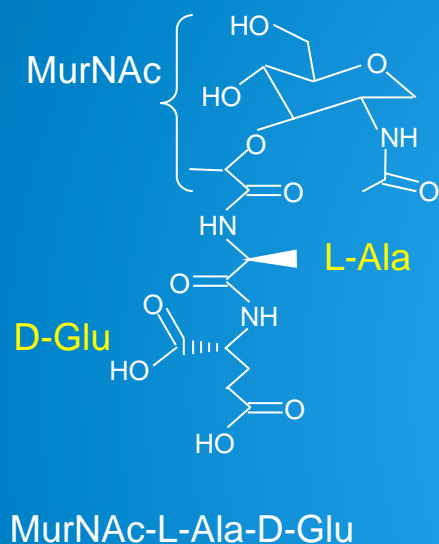


FACS analysis of bronchial lavage

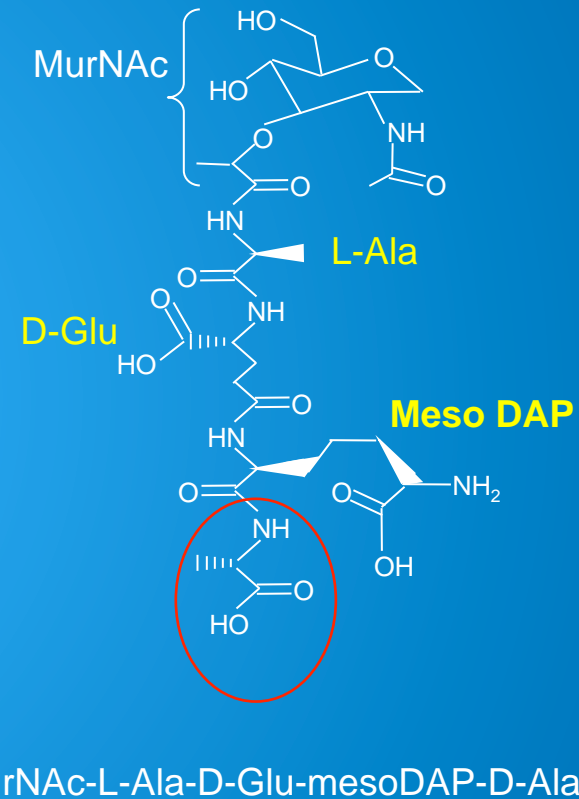
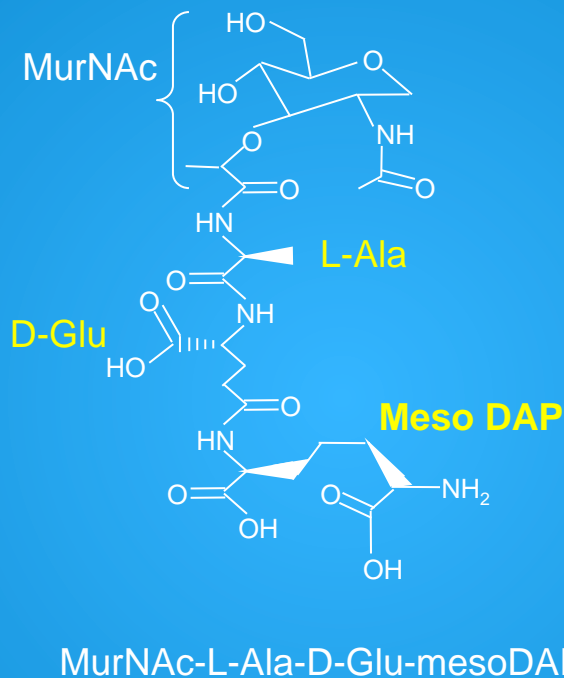




Nod2



Nod1



Pen^I

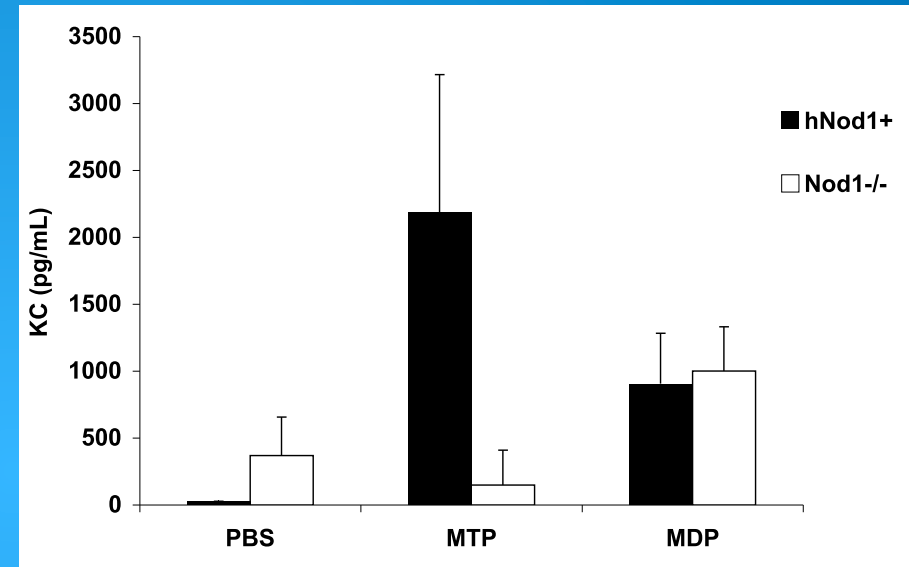
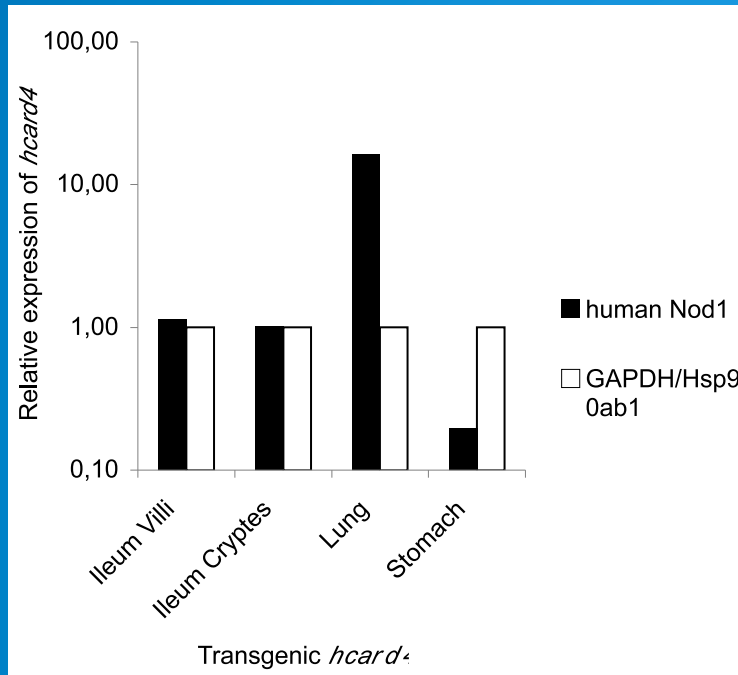


human

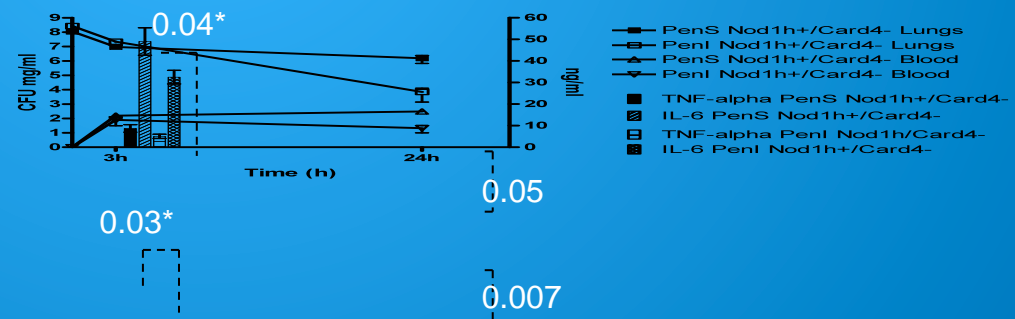
mouse



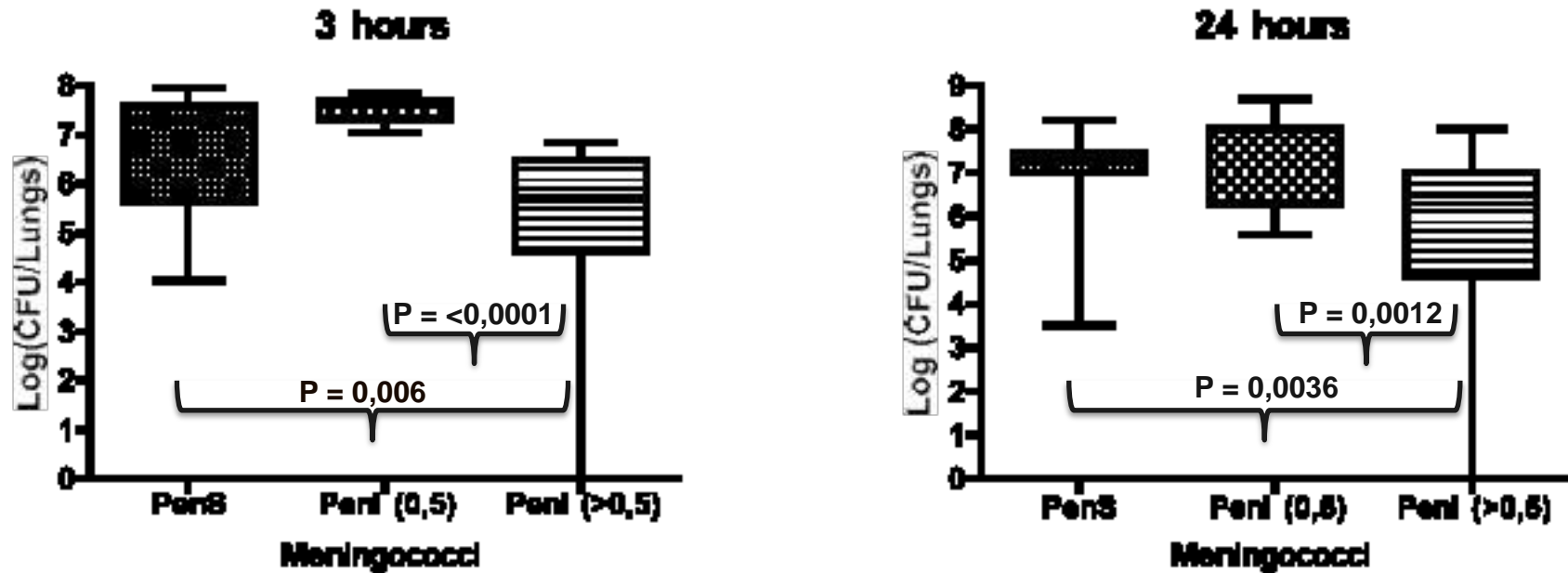
Impaired virulence of Pen^I strains in humanized hNod1 mice



C57BL/6J hNod1⁺ mice



Comparative virulence of Pen^S and Pen^I meningococcal isolates belonging to the ST11 clonal complex



Bacterial shape/
physiology

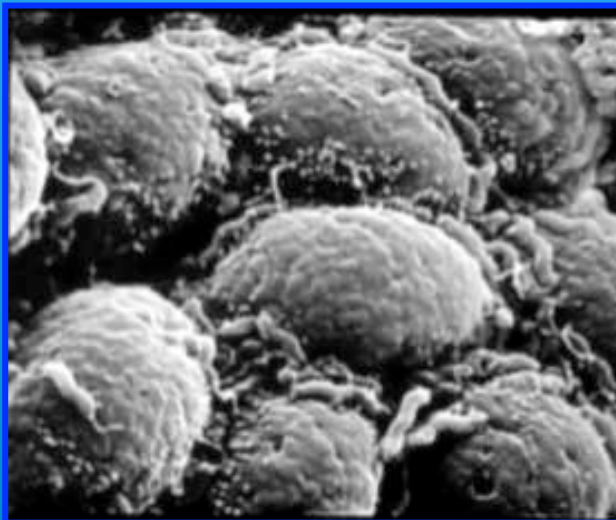
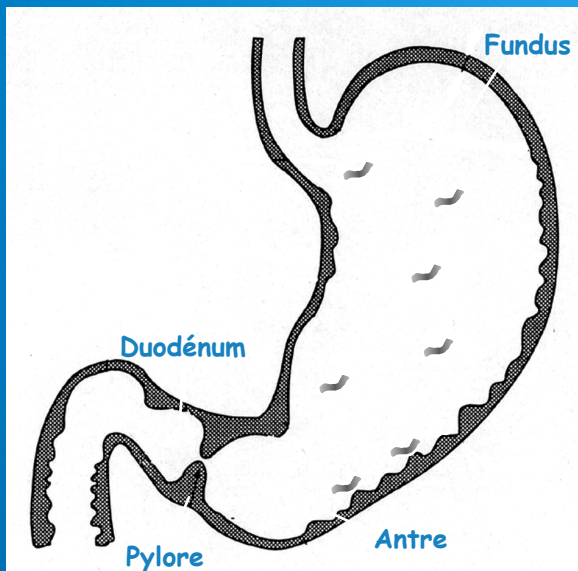
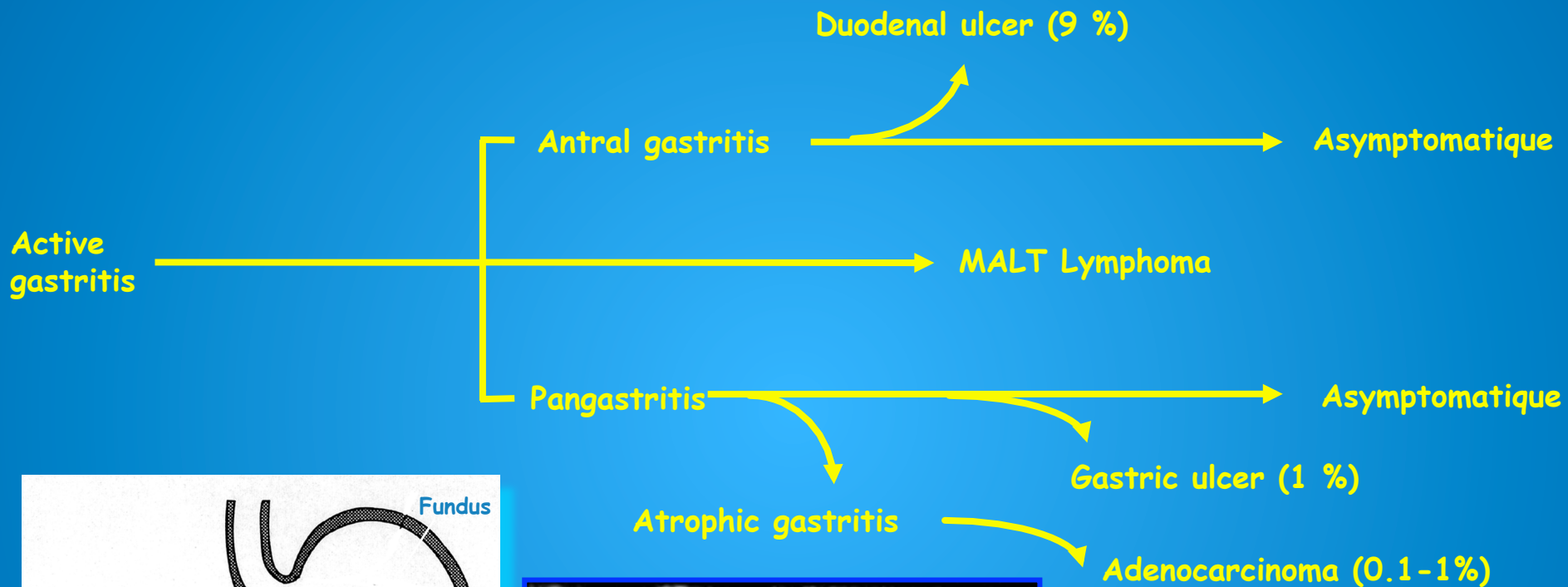
Antibiotics
resistance

PG metabolism

Inflammation
Virulence



Pathologies associated with *H. pylori* infection



Therapy used against *Helicobacter pylori*

Tritherapy:

- a proton pump inhibitor
- plus a combination of two antibiotics out of clarithromycin, metronidazole and amoxicillin

Emergence of resistance:

Resistance to clarithromycin (~20%) and metronidazole (~40%)



Resistance to amoxicillin is still sporadic (<1%)



Aims of the study

- Evaluate the degree of natural variations of amoxicillin targets
- Anticipate the emergence of amoxicillin resistance

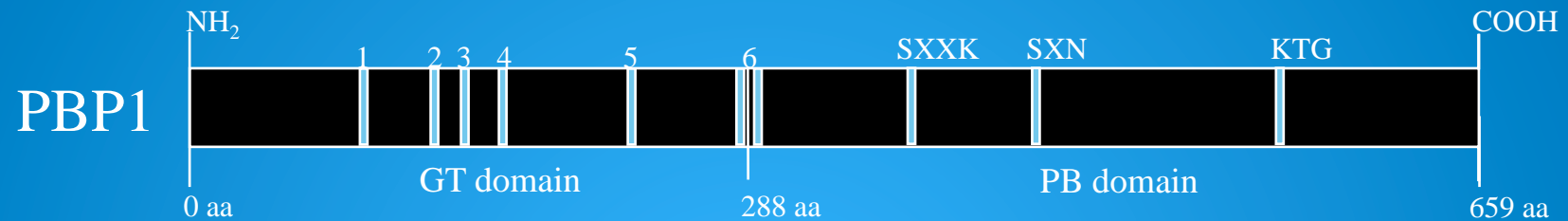
Rational for the strain selection

- 70% of the Portuguese population carries *H. pylori*
- High rate of self-medication with antibiotics
- A highly homogeneous human population

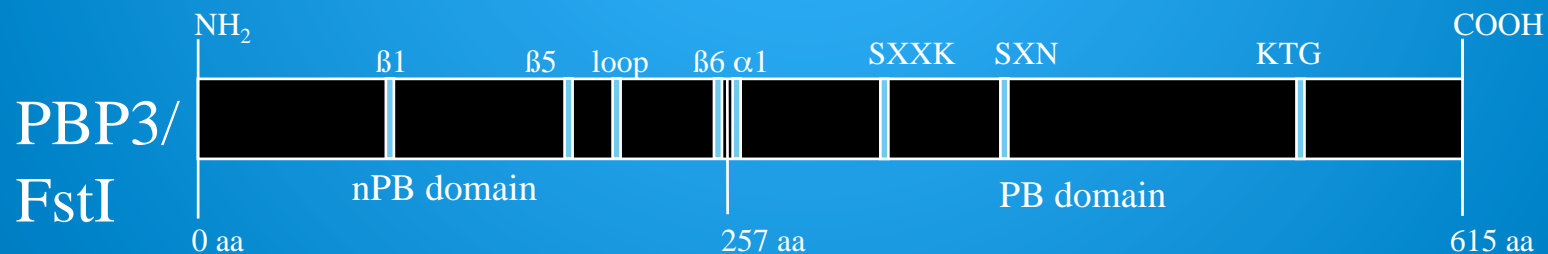
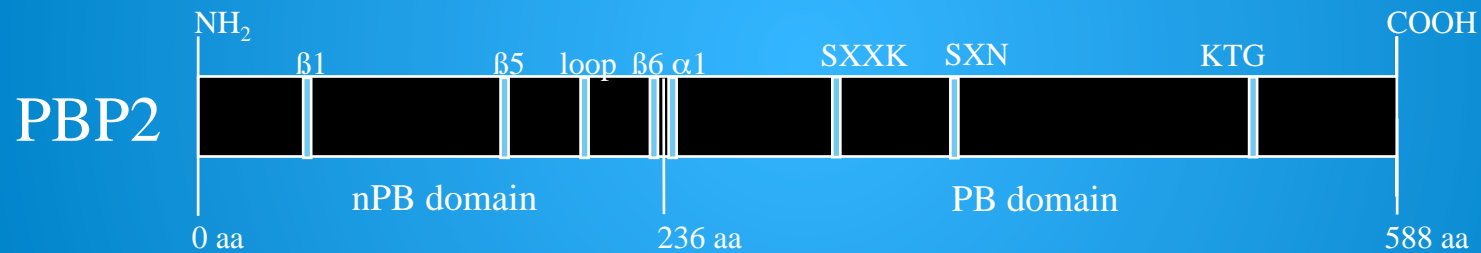


Three penicillin-binding proteins in *H. pylori*

High Molecular Weight (HMW) class A:



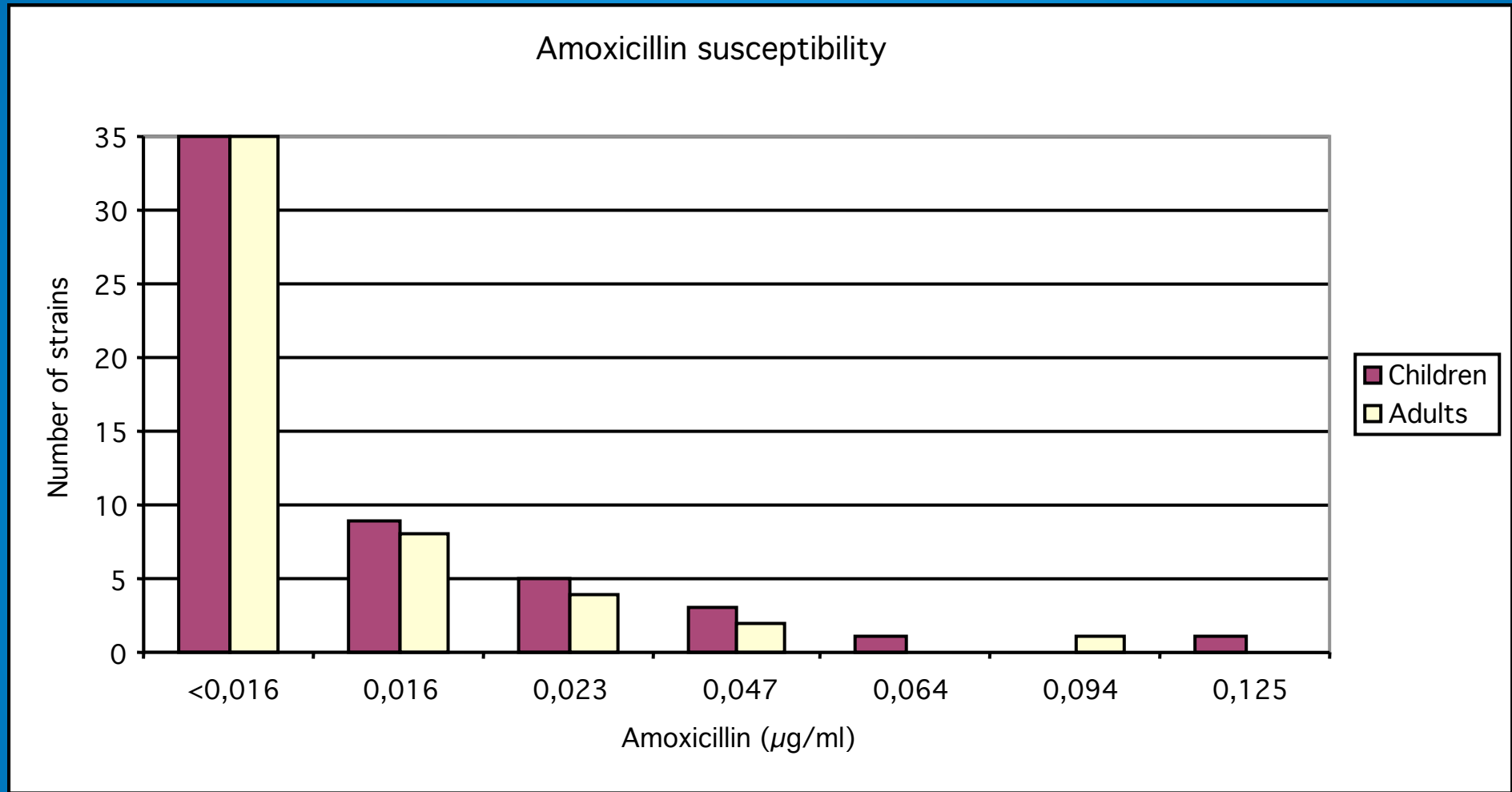
HMW class B:



➔ No low molecular weight PBP



Results



Amoxicillin resistance (MIC \geq 0,5 µg/ml)



No resistant strains

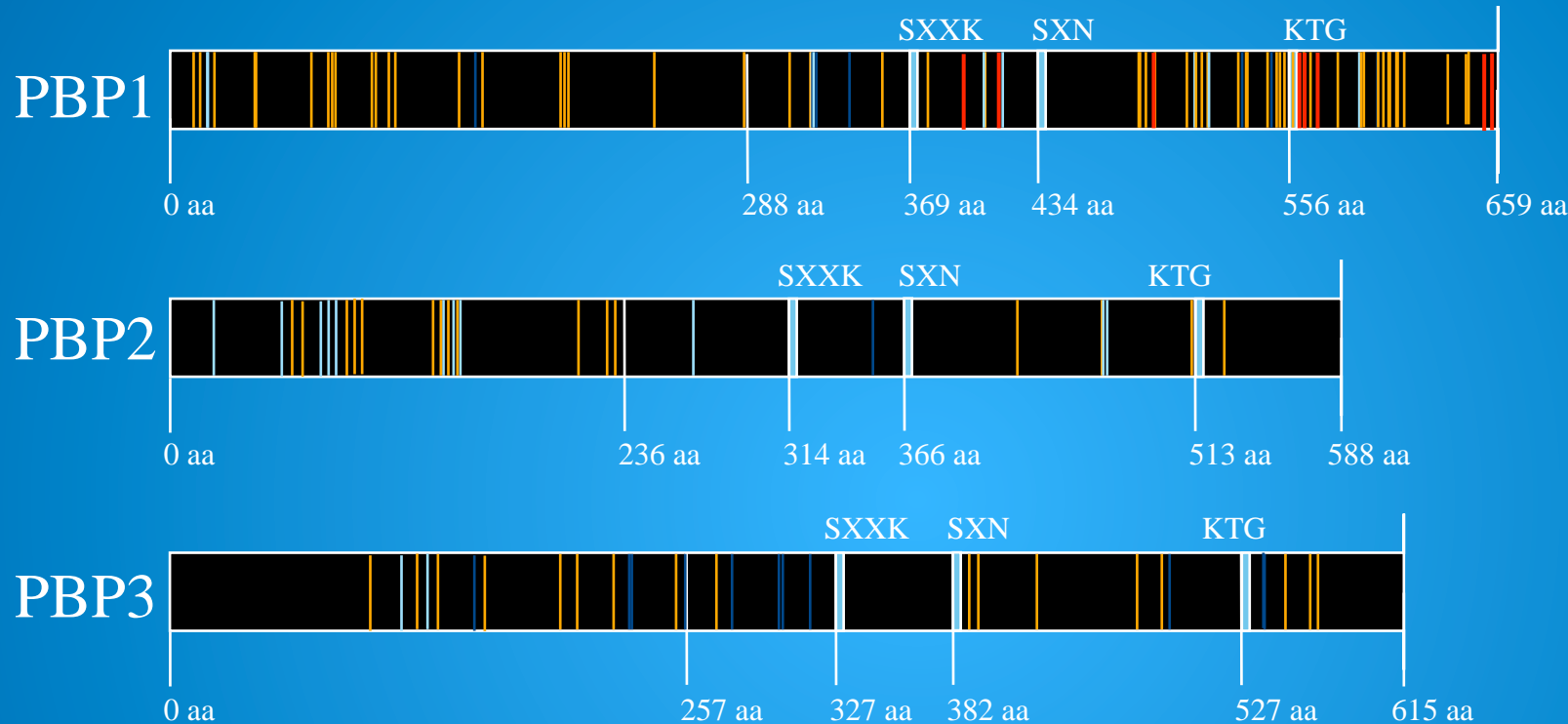
Results

- *pbp1* gene: 5 distinct RFLP profiles for *Hind*III
4 for *Hae*III
- *pbp2* gene: 4 distinct RFLP profiles for *Hind*III
2 for *Hae*III
- *pbp3* gene: 2 distinct RFLP profiles for *Hind*III
4 for *Hae*III



Sequencing of the *pbp1*, *pbp2* and *pbp3* genes of representatives of each RFLP profile among the highly susceptible strains (MIC ≤ 0,016 µg/ml)

Natural polymorphism analysis



- | amino acid position substituted at least in one strain
- | amino acid position substituted in ~50% of the strains
- | amino acid position substituted in <50% of the strains
- | **substitutions unique to Am^I or Am^R strains^{a,b}**

S403G, K483E & I564V reduced susceptibility
(0,125-0,5 µg/ml)

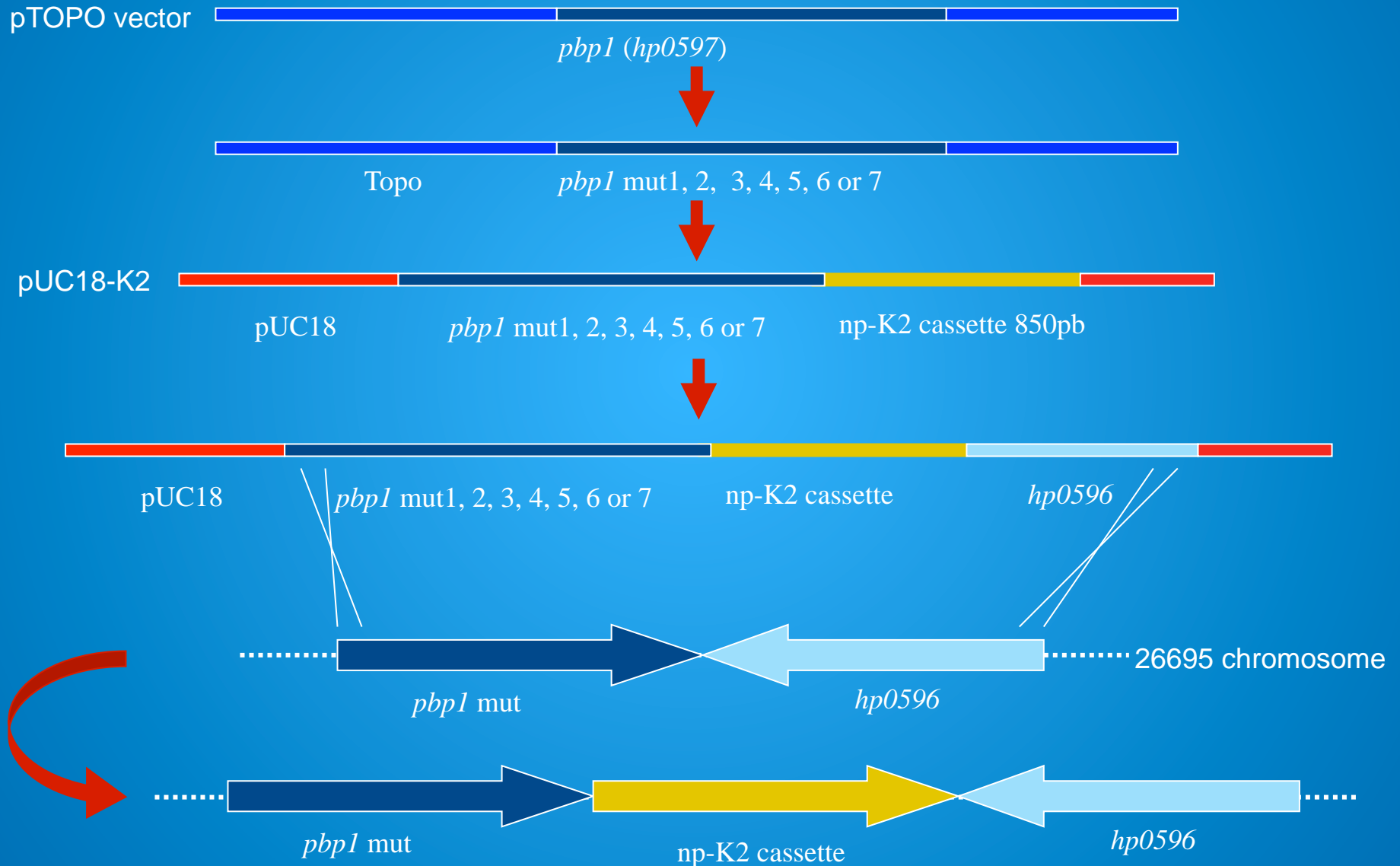
S414R^a &/or T558S^a for low resistance
(0.5-1 µg/ml)

N562Y^b, R597P^b &/or R650K for high
resistance (8 µg/ml)



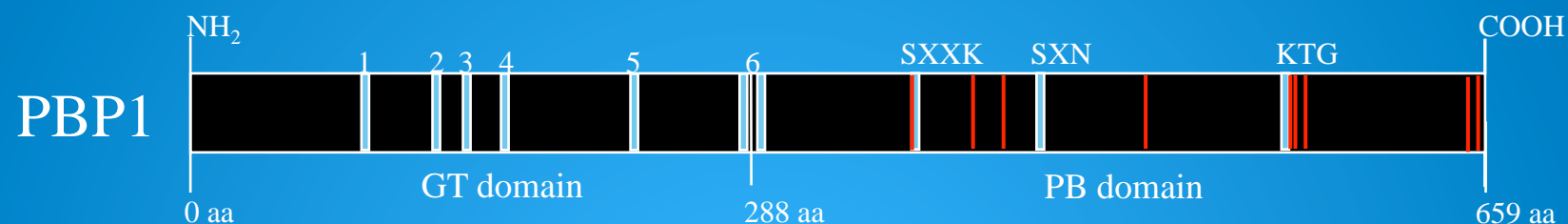
^a Paul et al. *Antimicrobial Agents Chemotherp.* 2001. ; Gerrits et al. *Antimicrobial Agents Chemotherp.* 2002.
^b Kwon et al. *Antimicrobial Agents Chemotherp.* 2003.

pbp1 site directed mutagenesis



Site directed mutagenesis of *pbp1*

High Molecular Weight (HMW) class A:



substitutions unique to Am^I or Am^R resistant strains^{a,b}

S403G, K483E & R597P^b	MIC 0,016 µg/ml
I564V	MIC 0,032 µg/ml
S414R^a	MIC 0,047 µg/ml
N562Y^b	MIC 0,094 µg/ml
S414R^a/ N562Y^b	MIC 0,094 µg/ml

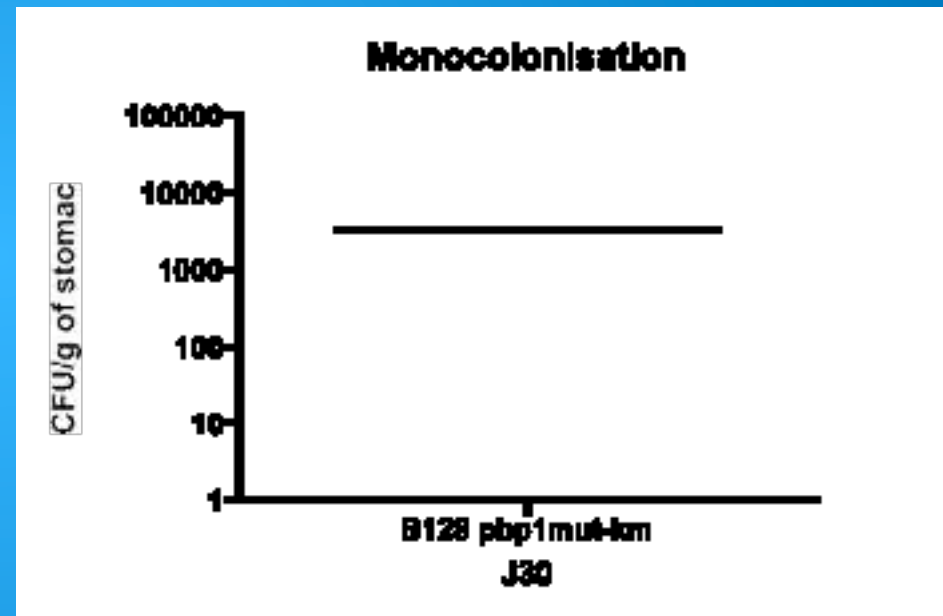
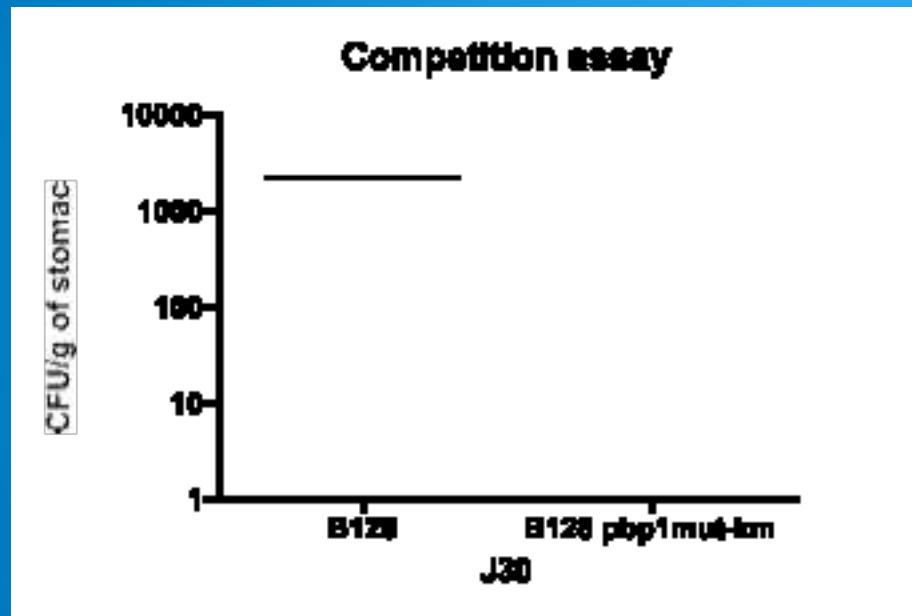
^a Paul et al. *Antimicrobial Agents Chemotherp.* 2001. ; Gerrits et al. *Antimicrobial Agents Chemotherp.* 2002.
^b Kwon et al. *Antimicrobial Agents Chemotherp.* 2003.

T558S^a &/or R650K^b are lethal in 26695

S369T is essential for PBP1 activity



Impact of S414R/ N562Y mutations *in vivo*



Conclusions

1 - *pbp1* mutates more frequently because it is subjected to a higher degree of external selective pressure (amoxicillin monotherapy?)

2 - *pbp1* mutations unique to Am^I or Am^R strains do not confer reduced susceptibility or resistance to amoxicillin

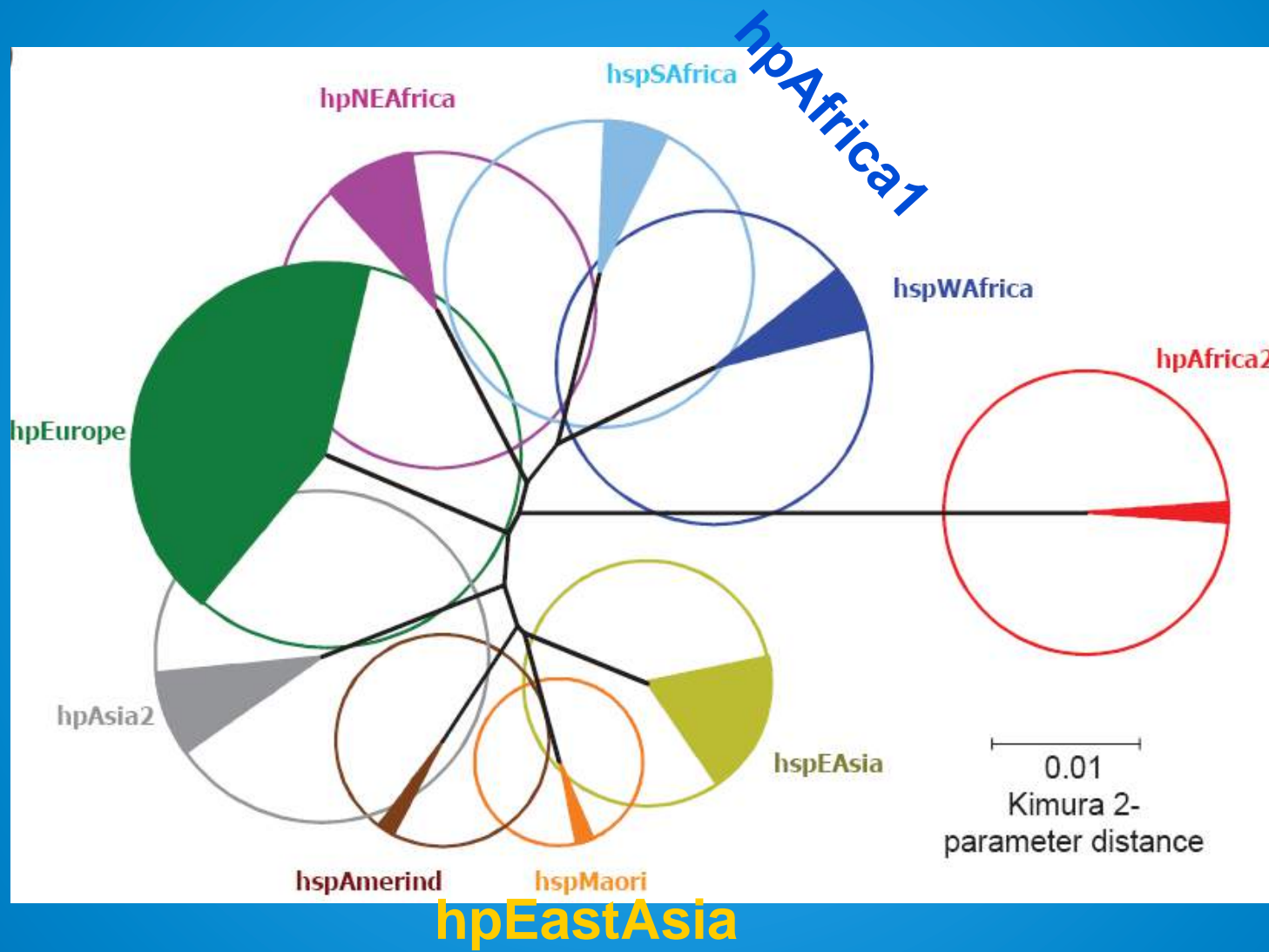
3 - some *pbp1* mutations unique to Am^R strains are essential for amoxicillin susceptible *H. pylori* strains either in vitro or in vivo

Reprothesies

PBP mediated resistance to amoxicillin carries a highly regionally conserved *pbp1* in ancient HpAfrica2 strains

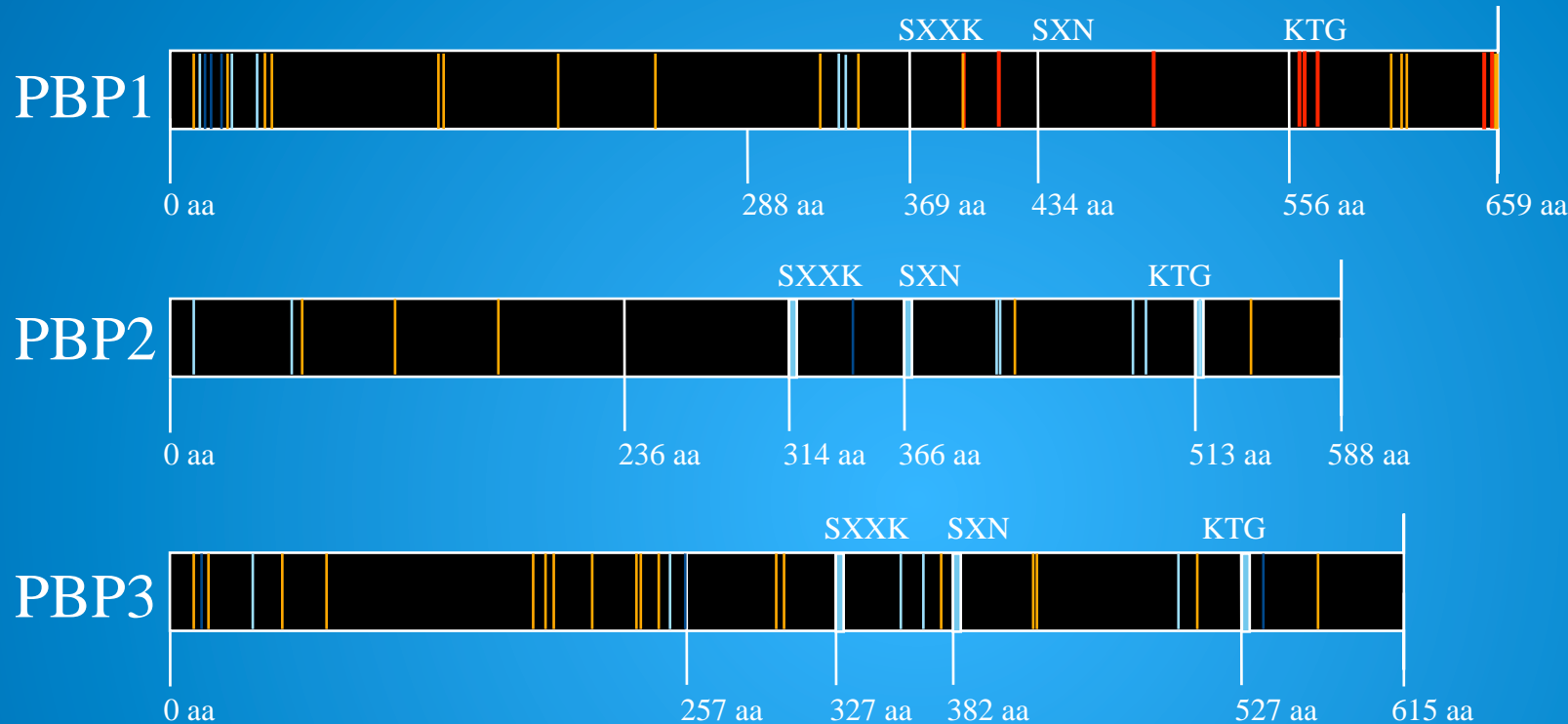


Global population structure of *H. pylori* within a collection of 769 strains from 51 sources



Linz et al., Nature 2007

Natural polymorphism analysis in HpAfrica2



- | amino acid position substituted at least in one strain
- | amino acid position substituted in ~50% of the strains
- | amino acid position substituted in <50% of the strains
- | **substitutions unique to Am^I or Am^R strains^{a,b}**

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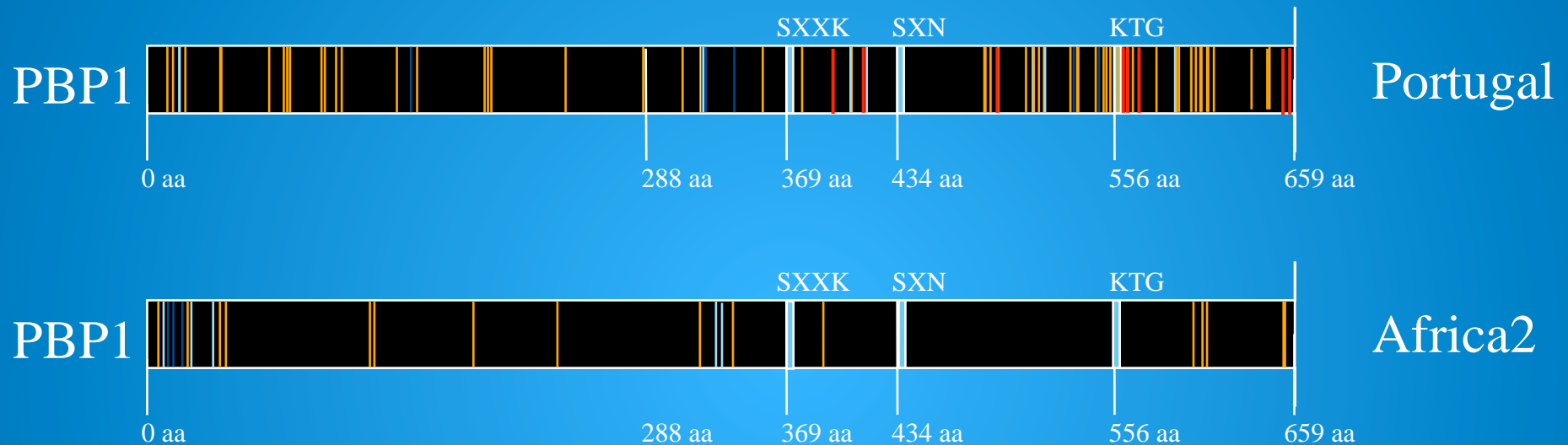
S414R^a &/or T558S^a for low resistance
(0.5-1 µg/ml)

N562Y^b, R597P^b &/or R650K for high
resistance (8 µg/ml)



^a Paul et al. *Antimicrobial Agents Chemotherp.* 2001. ; Gerrits et al. *Antimicrobial Agents Chemotherp.* 2002.
^b Kwon et al. *Antimicrobial Agents Chemotherp.* 2003.

Comparison of natural polymorphism analysis



- | amino acid position substituted at least in one strain
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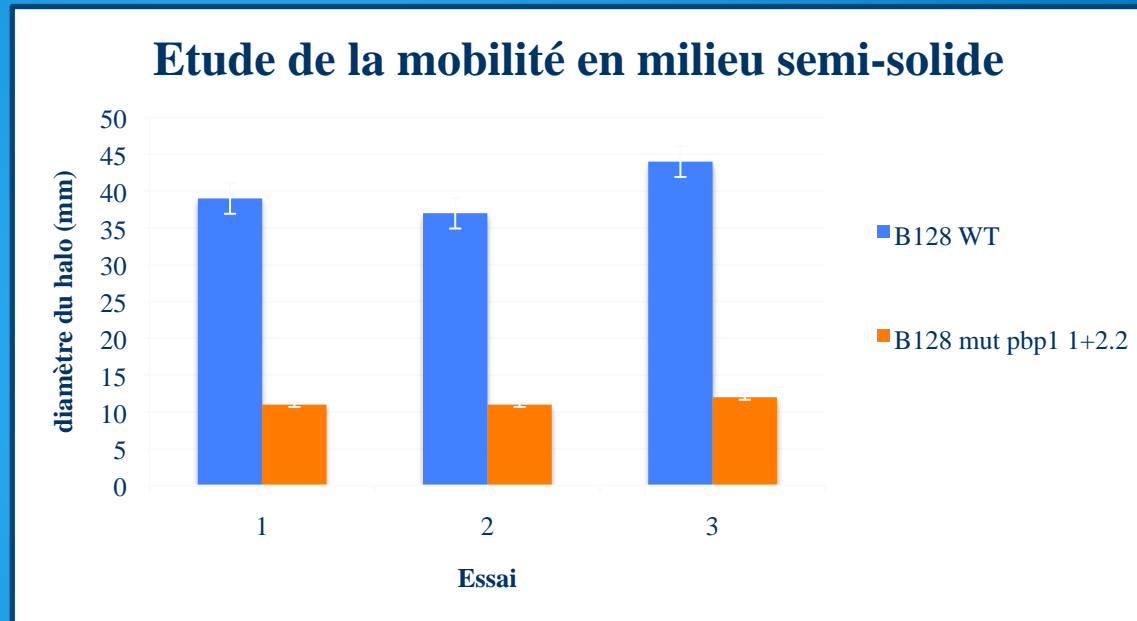
Mechanism of the biological cost

Motility



B128 mutant pbp1
1+ 2.2

B128 WT



Important impact on the motility of the double point mutation



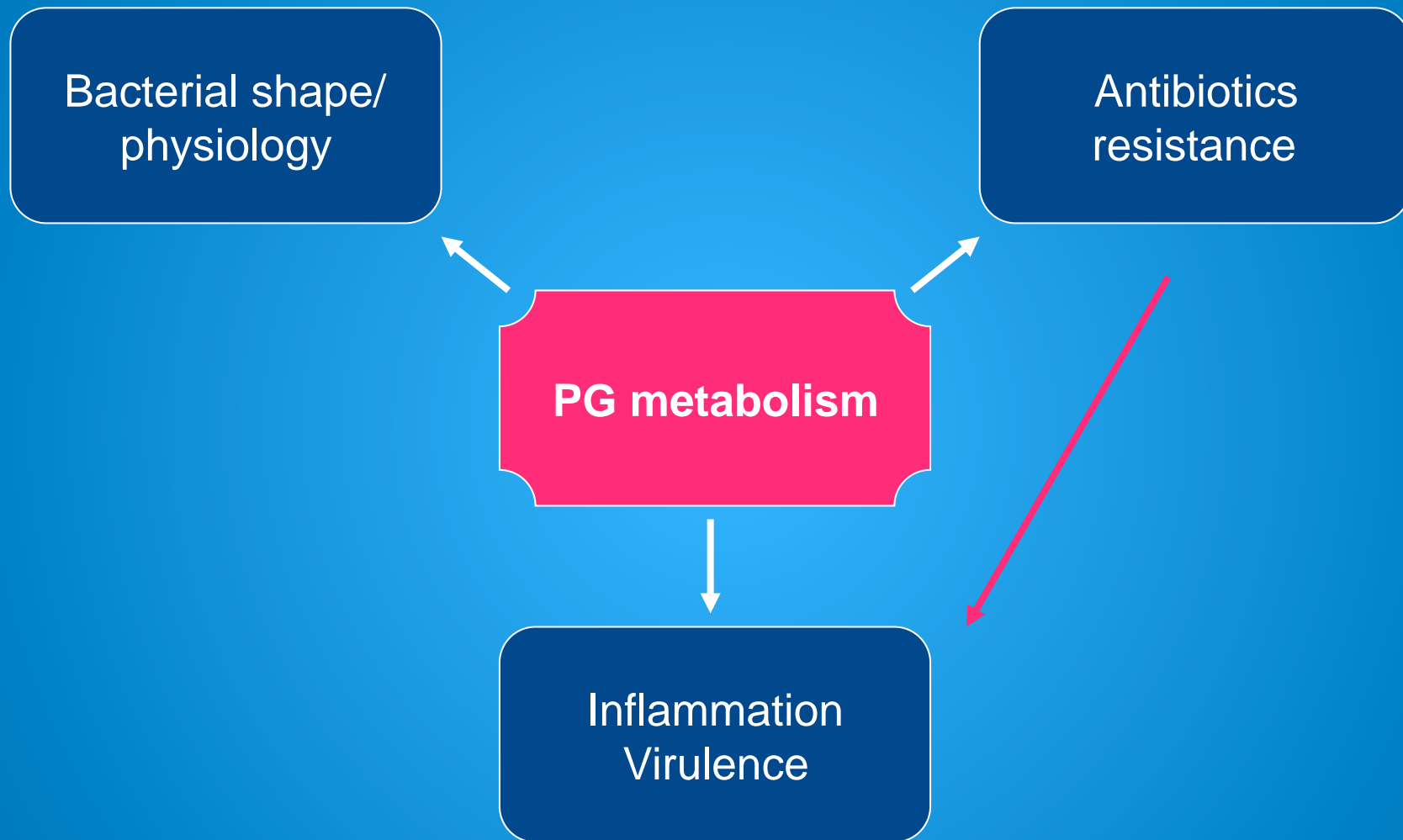
Probable cause of the fitness disadvantage of the mutant

Bacterial shape/
physiology

Antibiotics
resistance

PG metabolism

Inflammation
Virulence



Biology and genetics of the bacterial cell wall



Joel BERRY
Christiane BRENNER
Nienke BUDELMEIJER
Olivier DANOT
Chantal ECOBICHON
Martine FANTON D' ANDON
Elise GASIOROWSKI
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Hannover Medical School

Sebastian SUERBAUM

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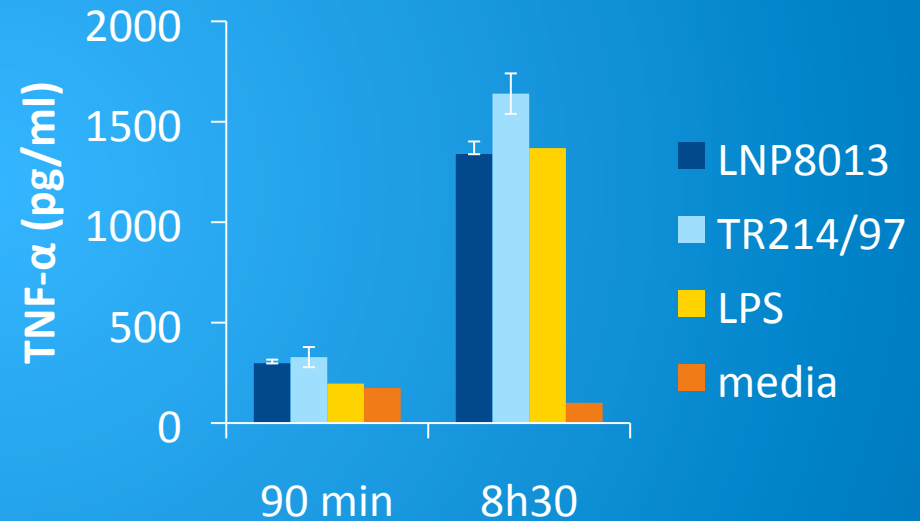
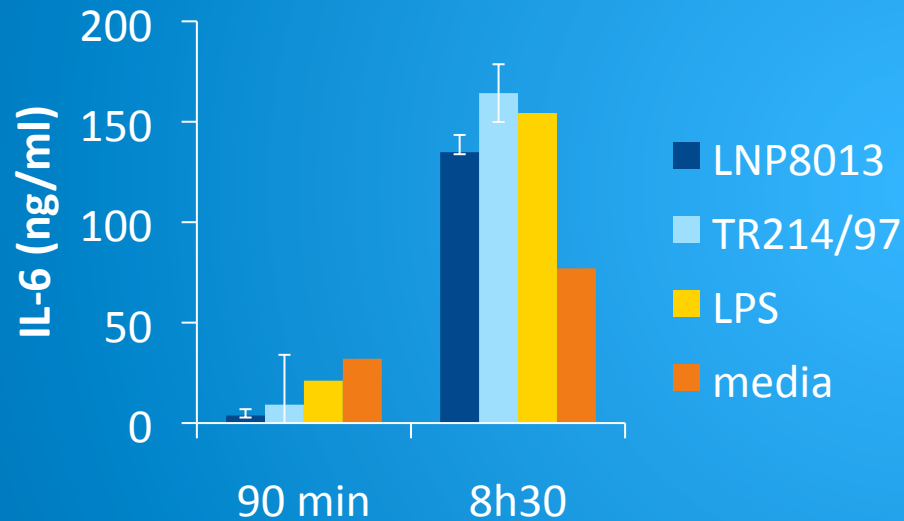
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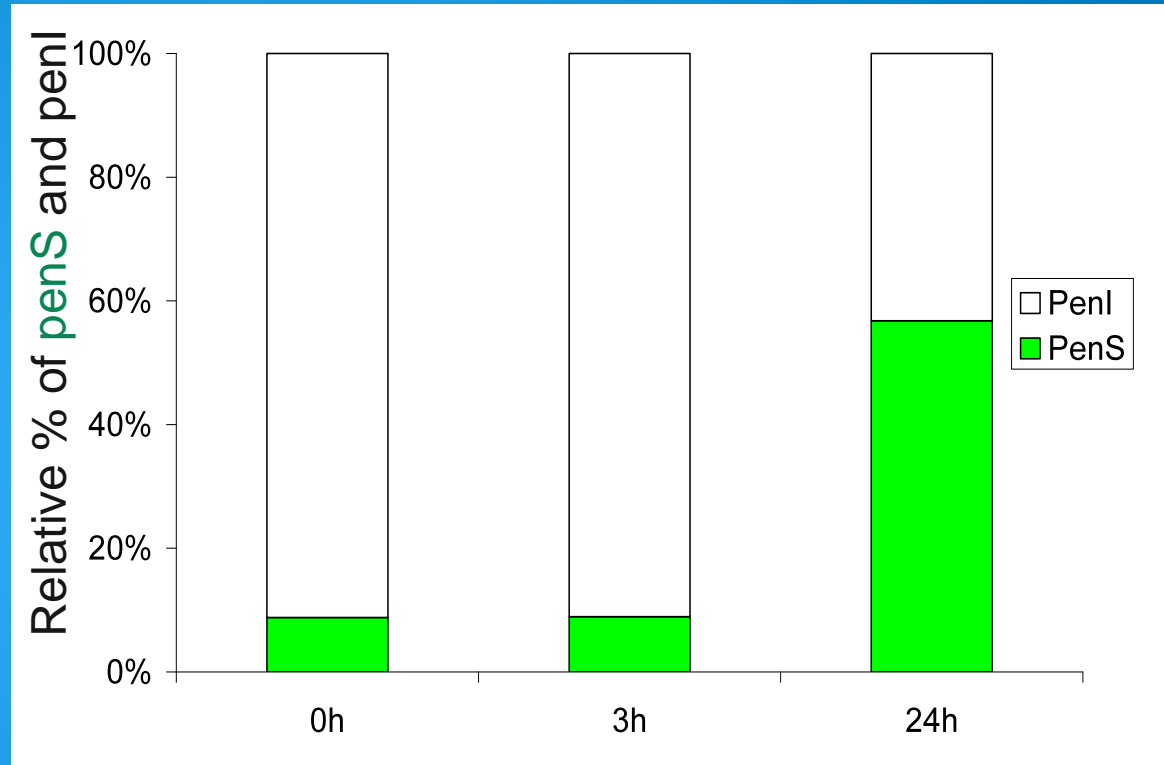
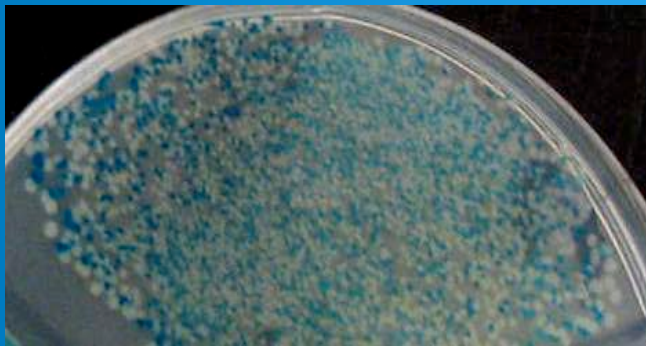
Macrophages respond normally to Pen^I strains



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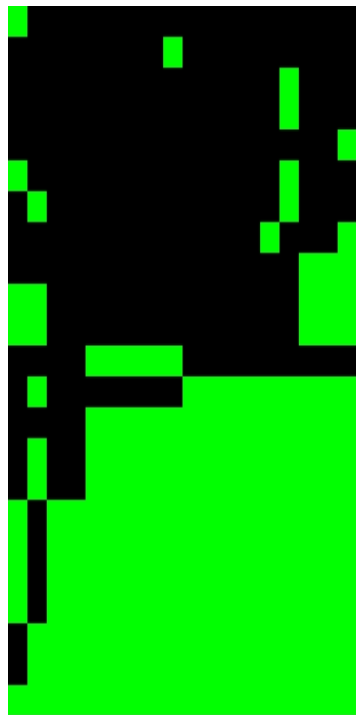
In vivo fitness of Pen^S vs Pen^I



Mice were infected IN by both Pen^I and Pen^S strains



Viral-mediated down-regulation of TLR4 and immune suppression



PYD and CARD domain containing
 indoleamine 2,3-dioxygenase 1
 CD180 antigen
 kiningogen 1
 tumor necrosis factor receptor superfamily, member 4
 Mediterranean fever
 toll-like receptor 4 ← Zscore -2.3
 chemokine (C-C motif) receptor 5
 tumor necrosis factor (ligand) superfamily, member 4
 interleukin 6
 tumor necrosis factor
 platelet-activating factor receptor
 chemokine (C-X-C motif) ligand 9
 chemokine (C-X-C motif) ligand 13
 similar to Small inducible cytokine B11 precursor (CXCL11) (Interferon-inducible T-cell alpha chemoattractant) (I-TAC); chemokine (C-X-C motif) ligand 11
 chemokine (C-X-C motif) ligand 10; similar to Small inducible cytokine B10 precursor (CXCL10) (Interferon-gamma-induced protein CRG-2) (Gamma-IP10) (IP-10) (C7)
 chemokine (C-C motif) ligand 12; similar to monocyte chemoattractant protein-5
 chemokine (C-C motif) ligand 2
 chemokine (C-C motif) ligand 7
 chemokine (C-C motif) ligand 8
 chemokine (C-C motif) ligand 4
 chemokine (C-C motif) ligand 3
 chemokine (C-C motif) ligand 5

mm0040660: Cytokine-cytokine receptor interaction
 GO:0005125: cytokine activity
 GO:0006952: defense response
 GO:0006954: inflammatory response
 GO:0006955: cytokine receptor binding
 GO:0006956: chemokine receptor binding
 GO:0006957: chemokine activity
 GO:0006958: interleukin-8-like chemokine activity
 GO:0006959: interleukin-8-like chemokine activity
 GO:0006960: interleukin-8-like chemokine activity
 GO:0006961: interleukin-8-like chemokine activity
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 GO:0007000: interleukin-8-like chemokine activity

Category	Term	Count	%	PValue
GOTERM_B P_FAT	GO: 0006952~defen se response	61	10.15	3.57E-22
GOTERM_B P_FAT	GO: 0006954~infla mmatory response	40	6.7	1.29E-18

