New designed antimicrobial Polymyxin B analogs with a broader spectrum of activity and effective against resistant bacteria

Francesc Rabanal
Ariadna Grau-Campistany
Xavier Vila-Farrés
Javier Gonzalez-Linares
Montserrat Pujol
Miquel Borràs
Kirsty Hewitson
Jordi Vila
Mª Àngels Manresa
Yolanda Cajal

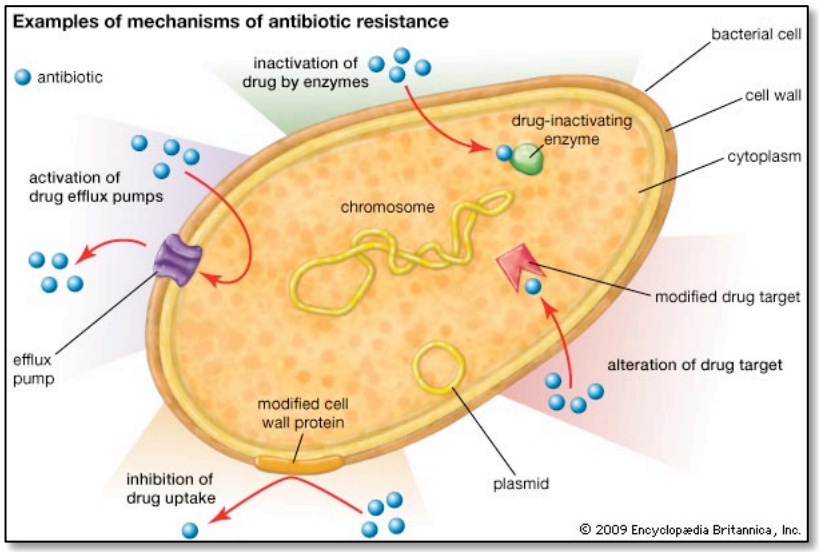
B:KC Barcelona Knowledge Campus

UNIVERSITAT DE BARCELONA

Fundació Bosch i Gimpera
Universitat de Barcelona

Introduction

- Need of new antibiotics
  - Infectious diseases → Second cause of death in the world (third in the developed world)
  - Inadequate use of antibiotics → Increase in resistant bacteria → Antibiotics loose effectiveness
  - Reduced pipeline of new antibiotics



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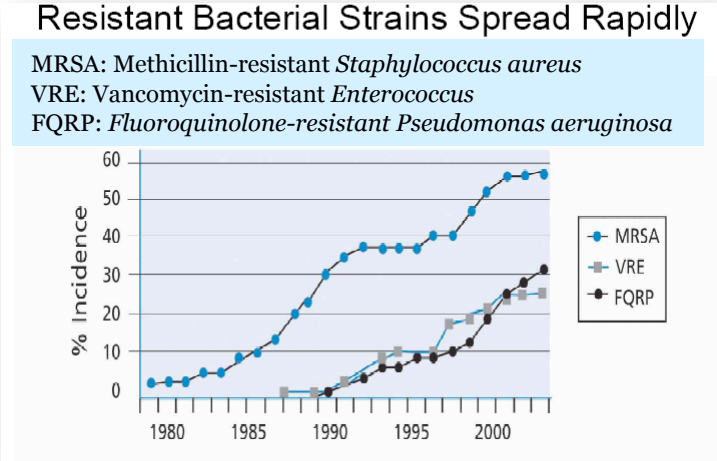
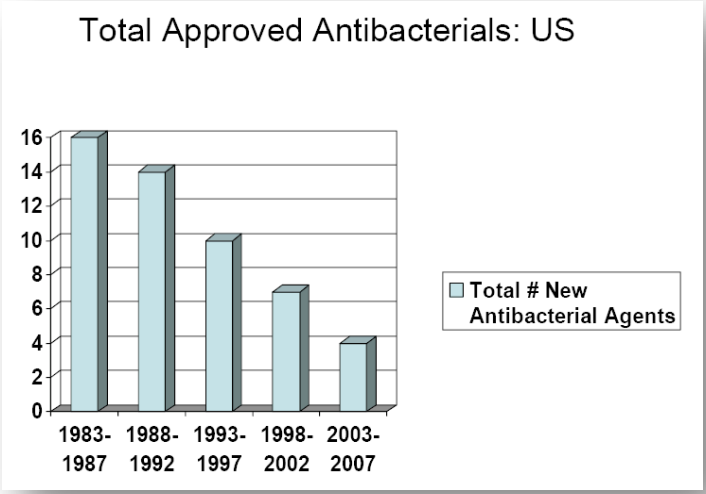
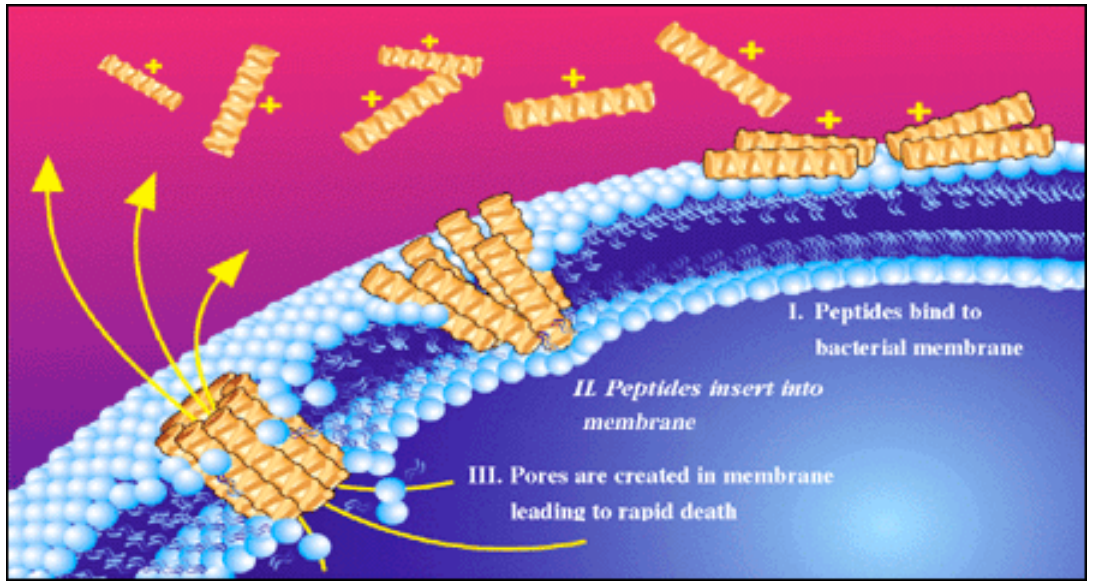




Introduction

- Need of new antibiotics
- AMPs



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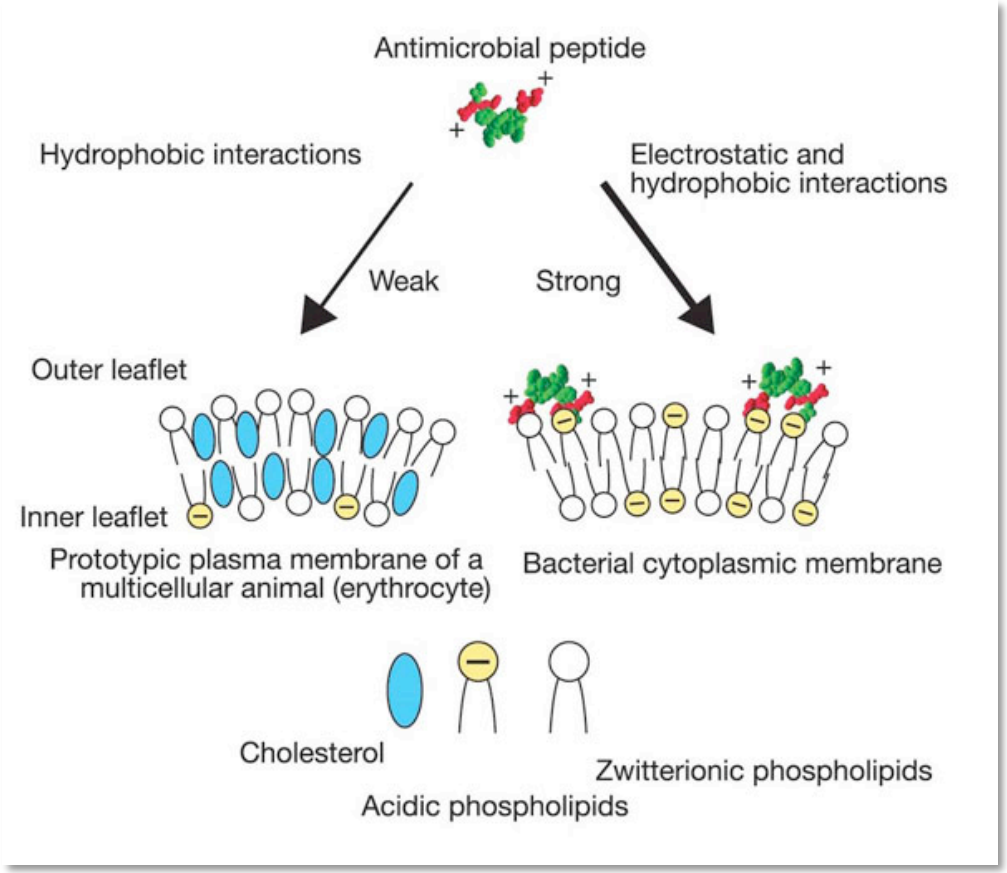
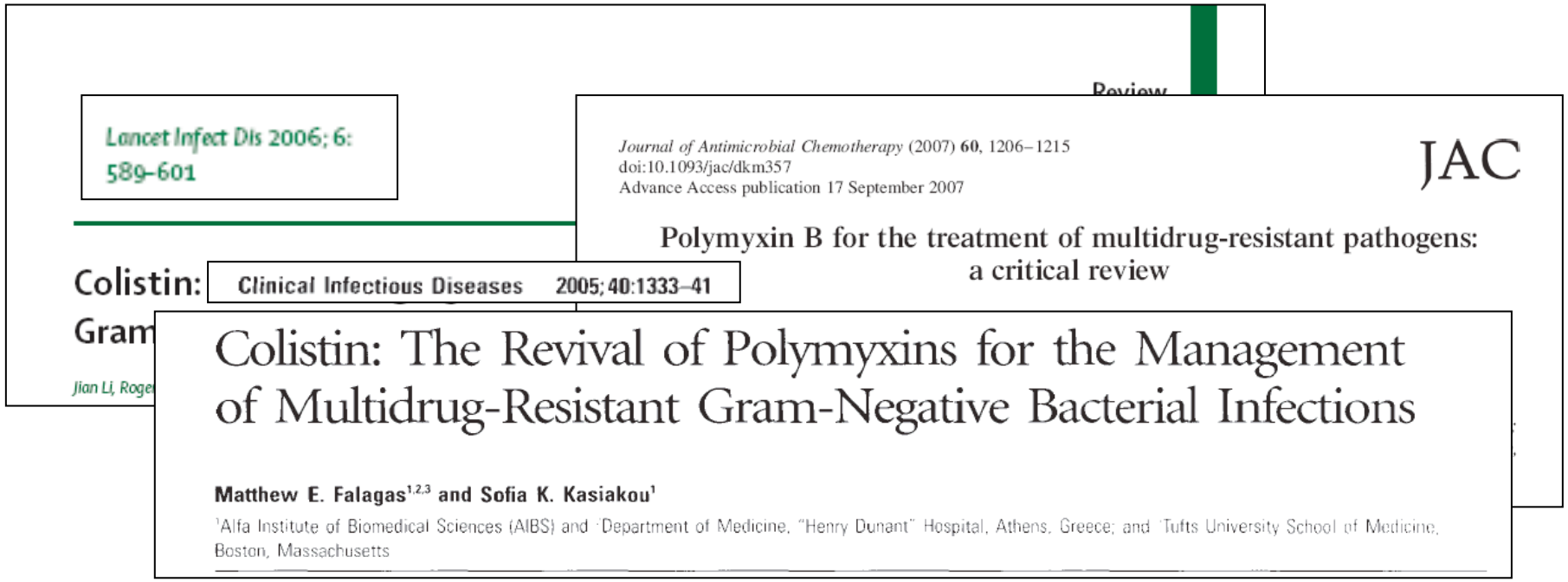
- AMPs



Introduction

- Need of new antibiotics
- AMPs
- Resurgence of Polymyxin

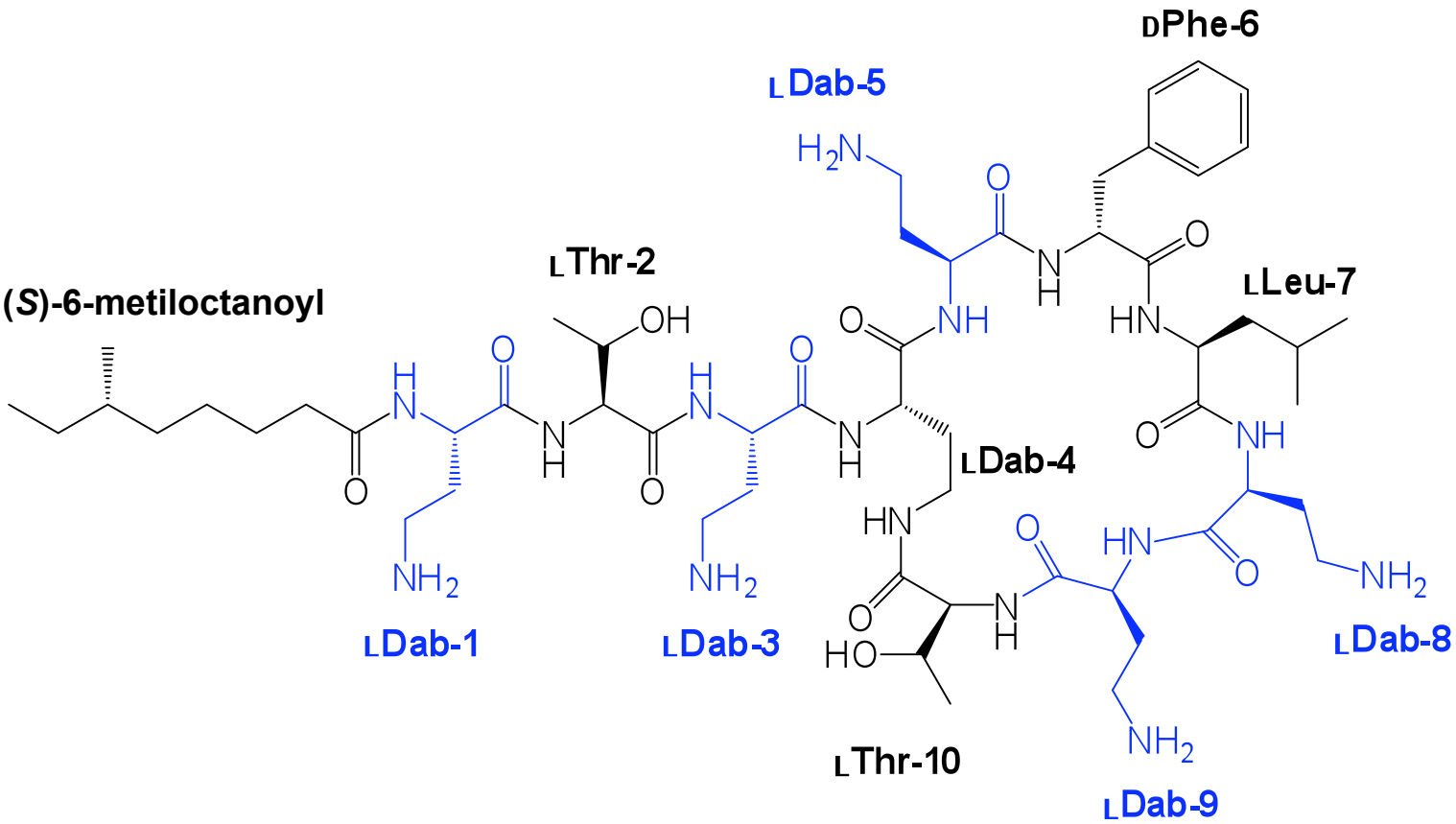


Objectives

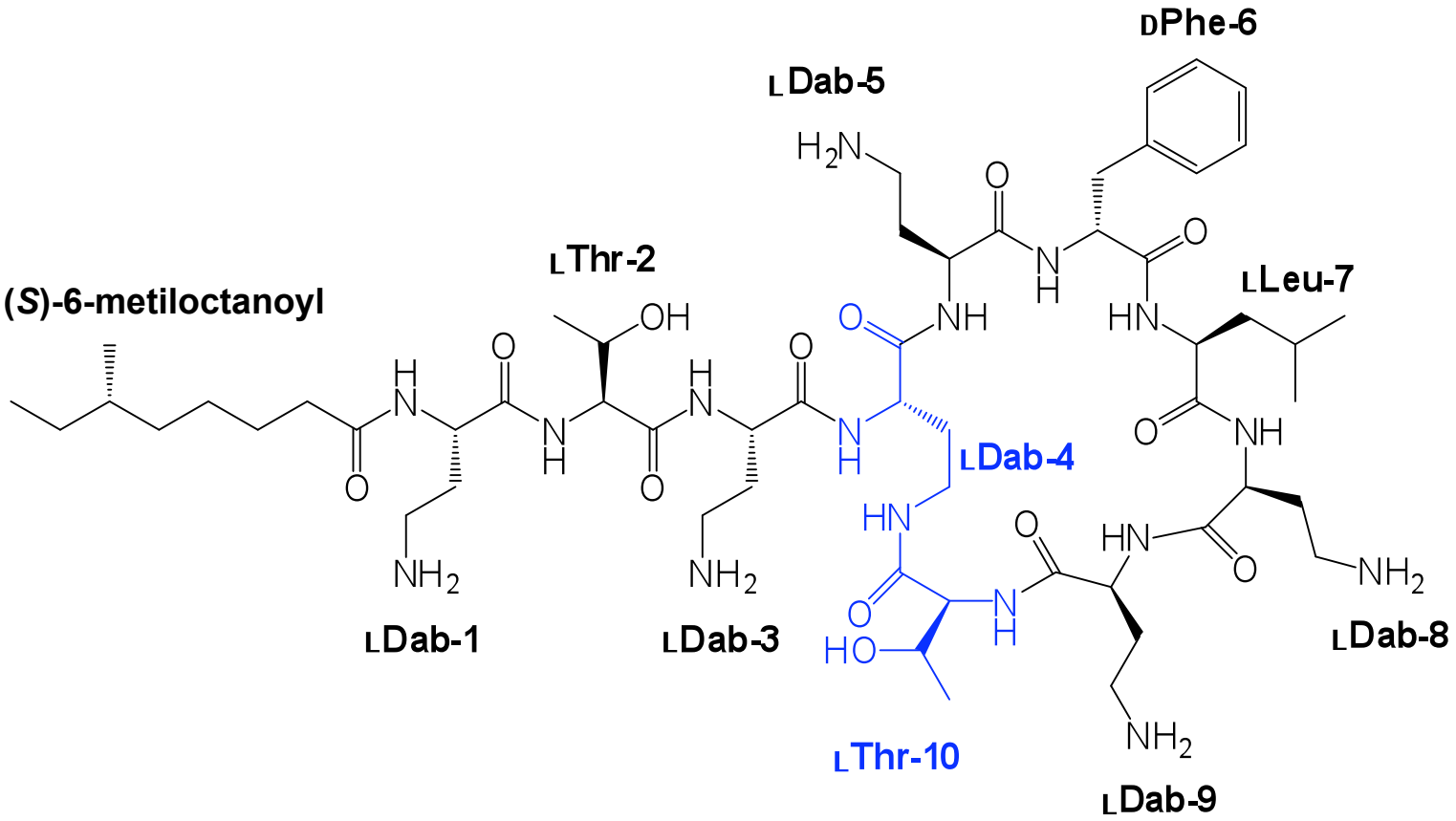
- Design and synthesize new lipopeptides based in the structure of Polymyxin

  - Lower toxicity
  - Activity in resistant and multi-resistant bacteria
  - Wider spectrum of activity
  - Easy synthesis

Polymyxin B


(S)-6-metiloctanoyl
LDab-1
LThr-2
LDab-3
LDab-4
LDab-5
DPhe-6
LLeu-7
LDab-8
LDab-9
LThr-10

Polymyxin B


(S)-6-metiloctanoyl
LThr-2
LDab-5
DPhe-6
LLeu-7
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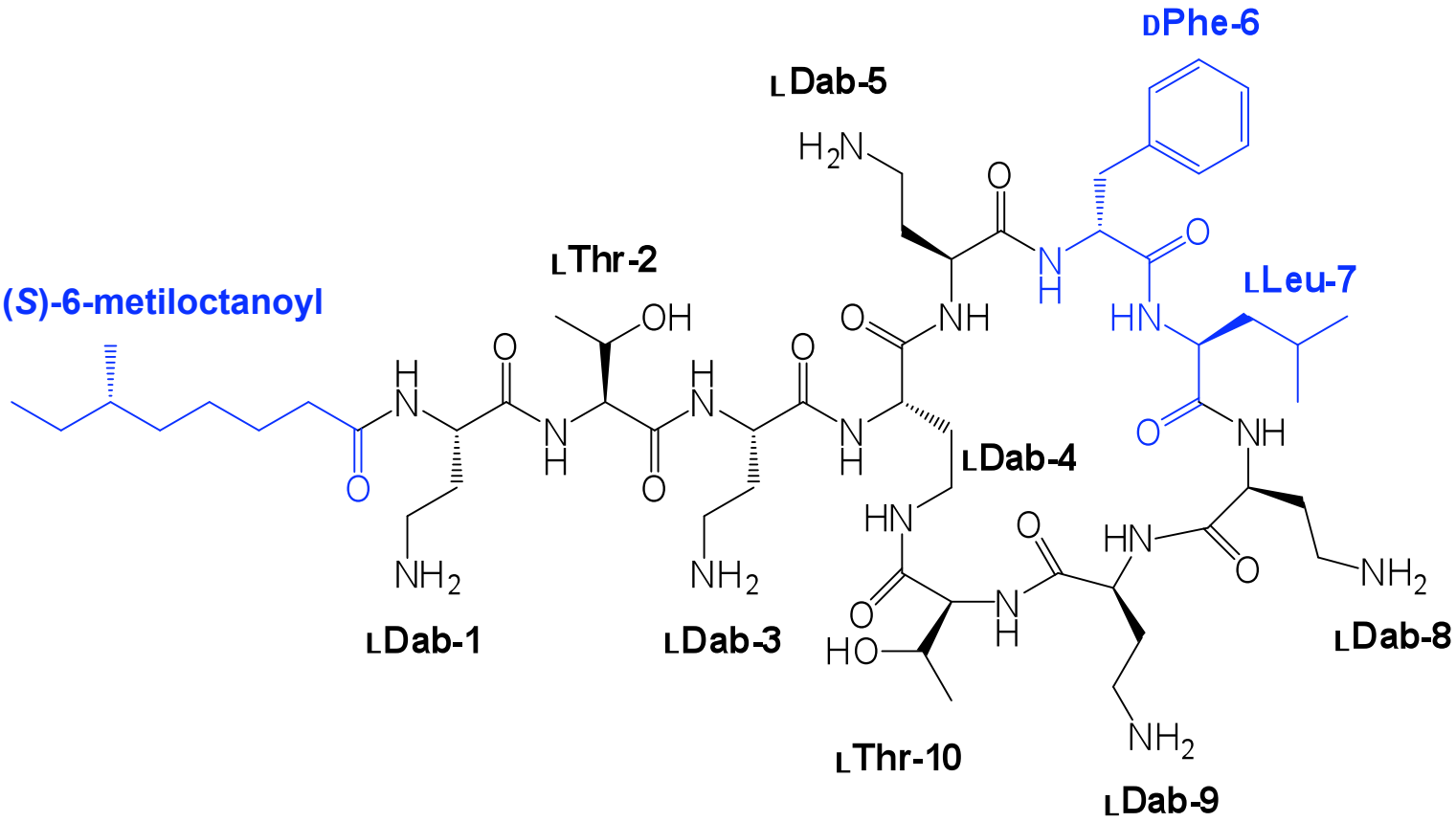
LDab-3
LThr-10
LDab-9
LDab-8

Polymyxin B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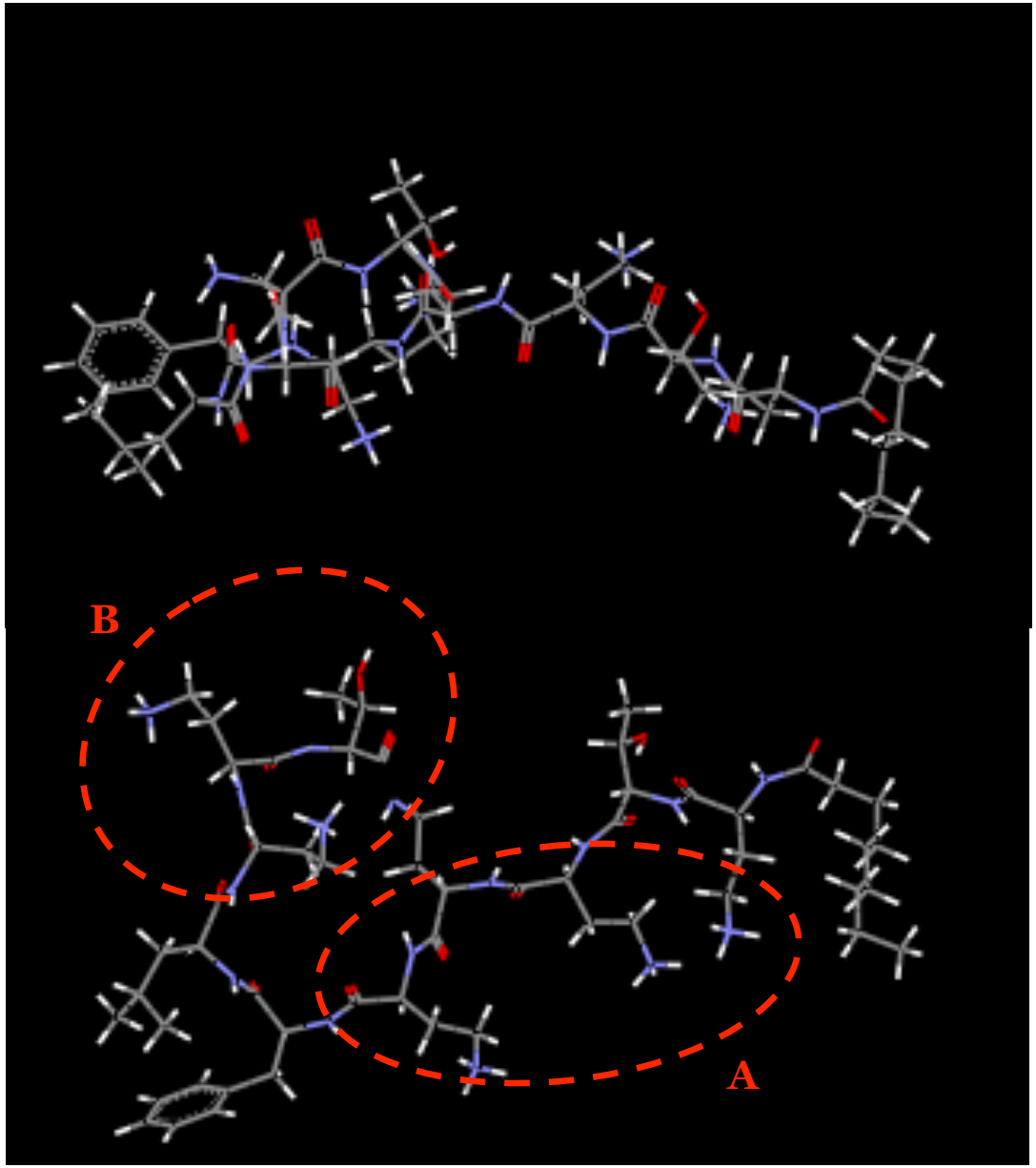
LThr-2
LDab-5
DPhe-6
LLeu-7
LDab-4
LDab-1
LDab-3
LThr-10
LDab-9
LDab-8

Polymyxin B

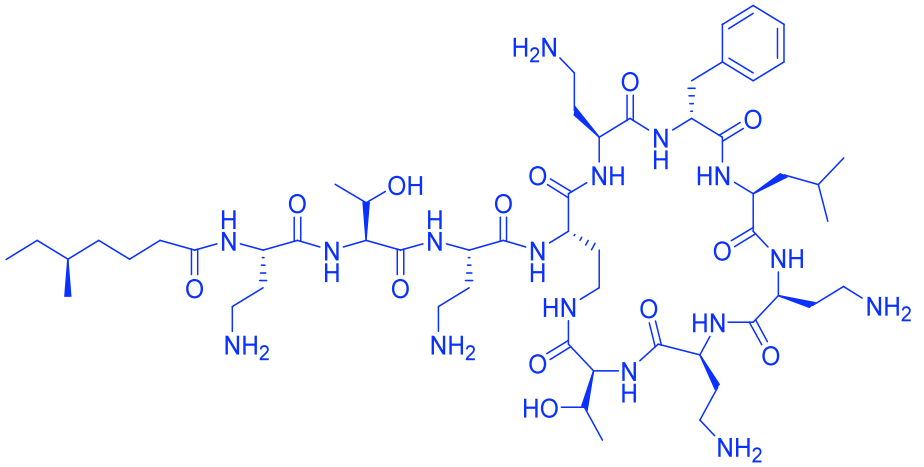
- Fixed: Phe-6, Leu-7, Thr-10
- Flexible: Dab-4, Dab-8, Dab-9
- Binding domain to anionic groups:
  - Site A: Dab-1, Dab-3, Dab-5
  - Site B: Dab-8, Dab-9, Thr-10
- Hydrophobic binding site
  - Acyl chain, Phe-6, Leu-7



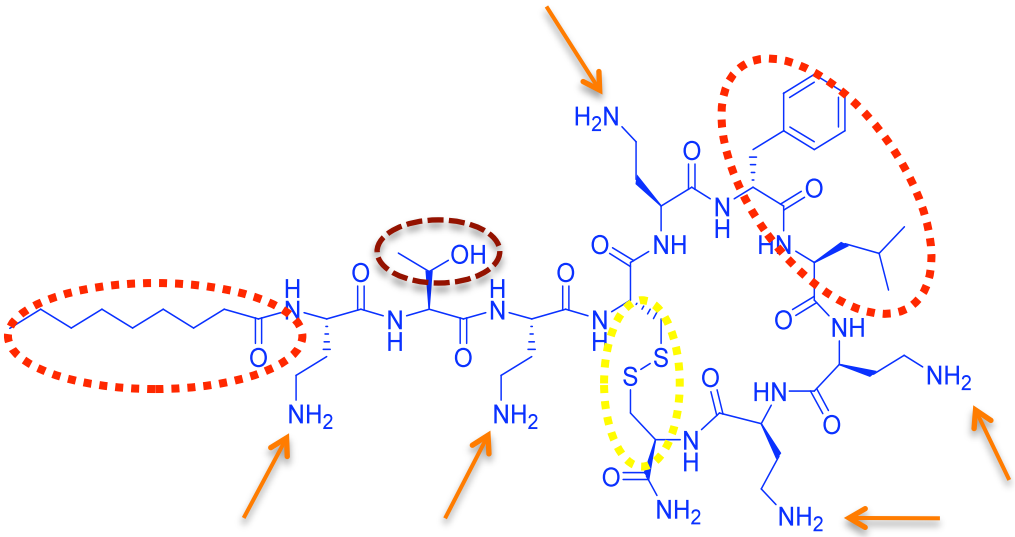
Tony Velkov, Philip E. Thompson, Roger L. Nation, and Jian Li, *J. Med. Chem.* **2010**, 53, 1898-1916

Analogs Design

- Polymyxin

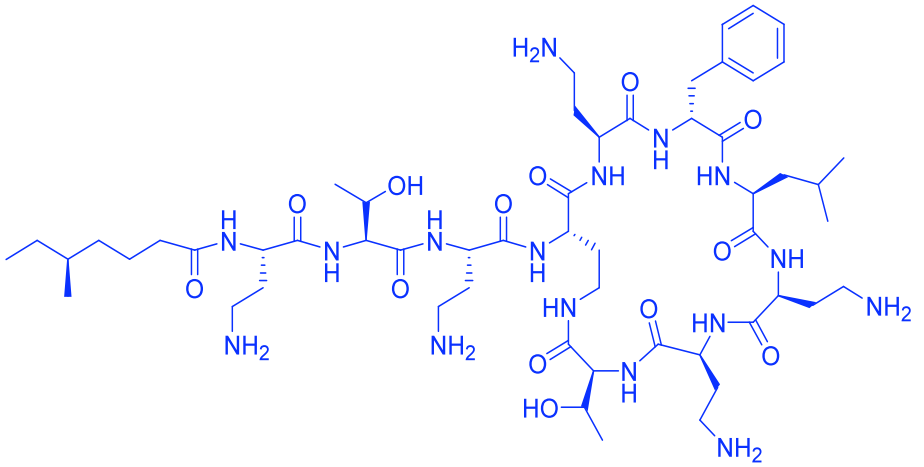


- Designed peptide



Analogs Design

- Polymyxin



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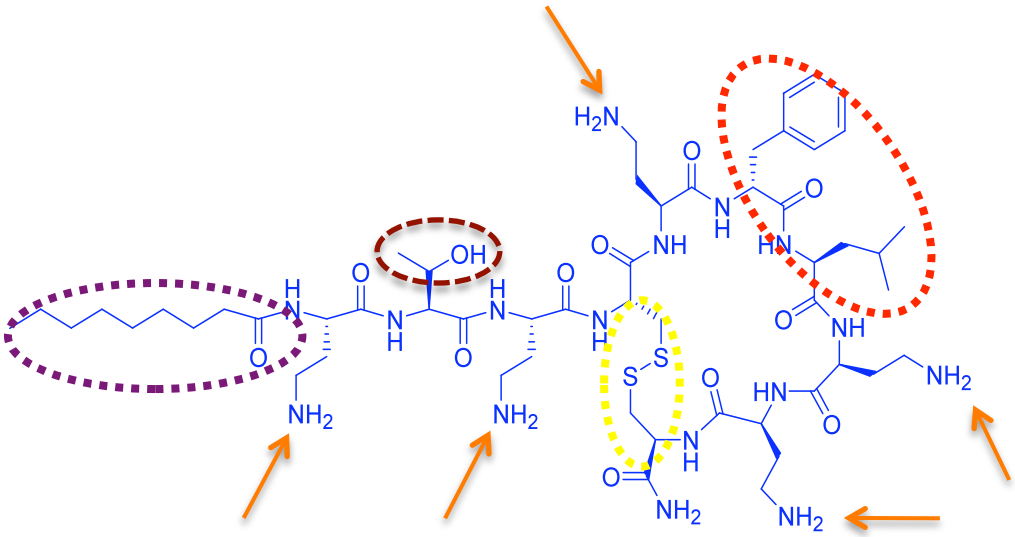
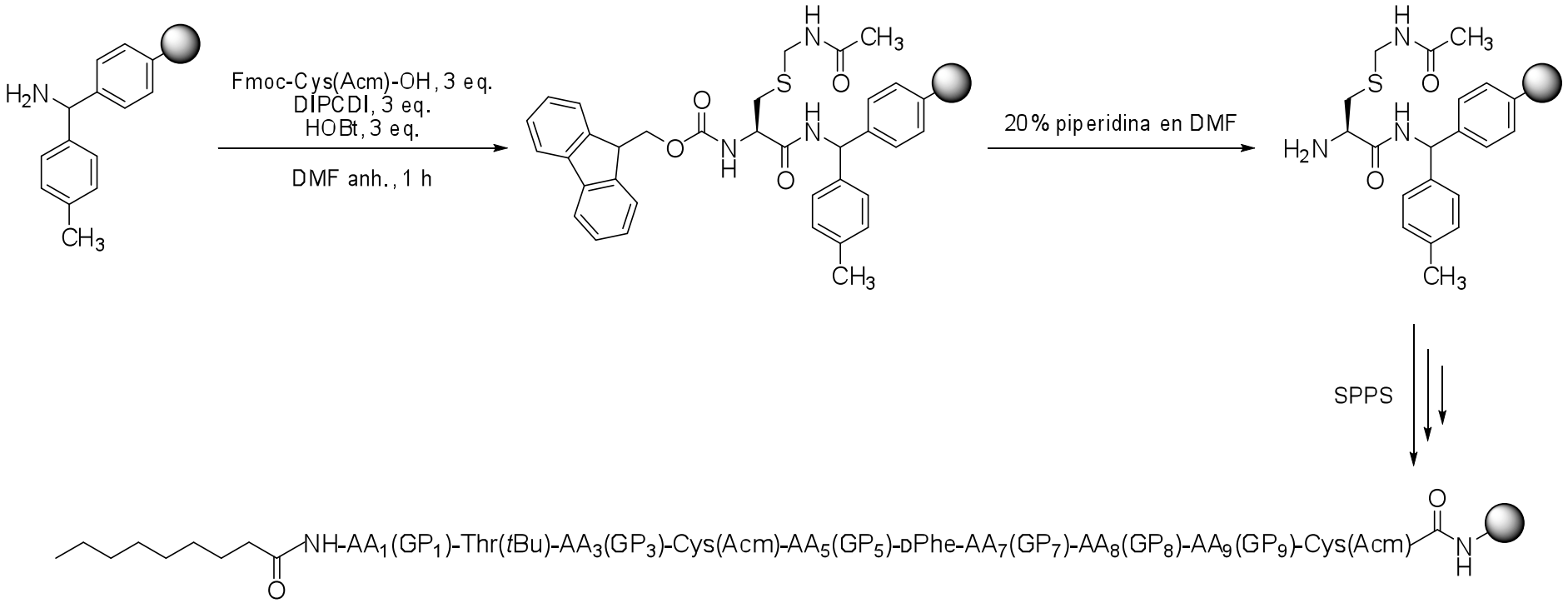


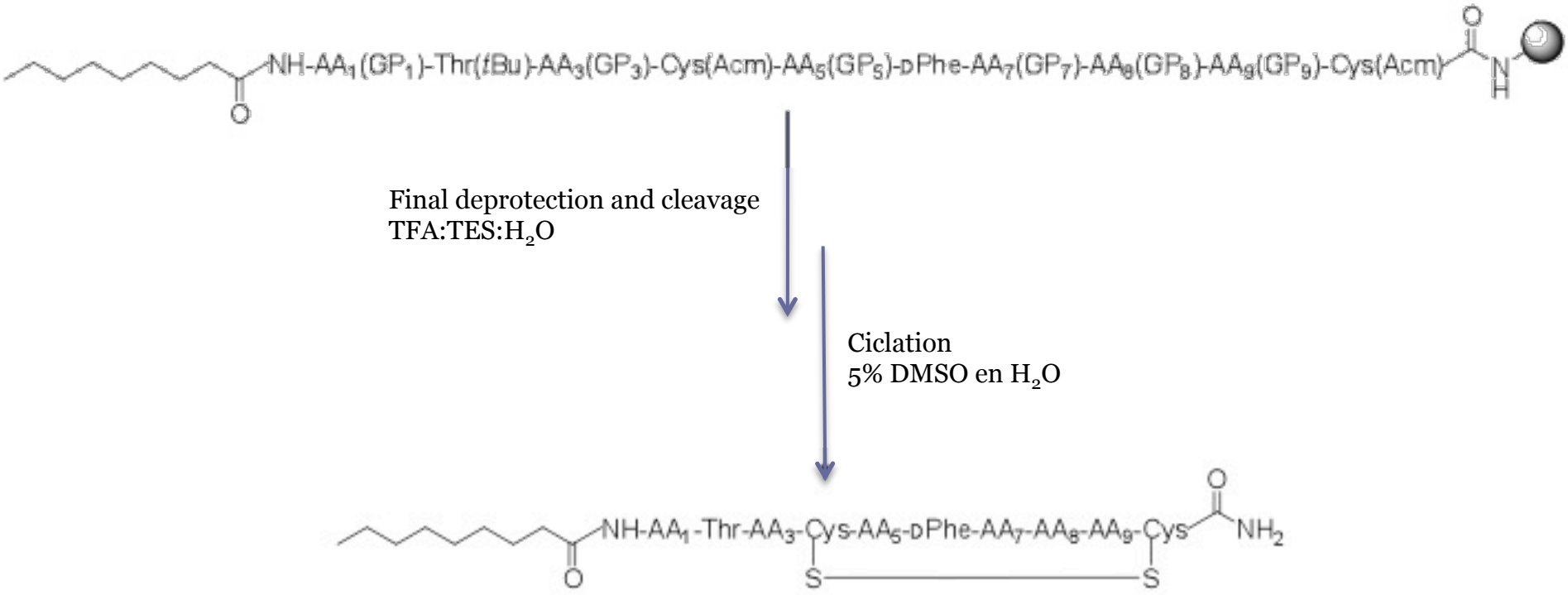
Synthesis

- Synthetic strategy: Fmoc/*t*Bu
- Resin: MBHA, BHA
- Protective groups: Acm (Cys), Boc (Dab), O (Met), Pbf (Arg), *t*Bu (Thr)

Synthesis

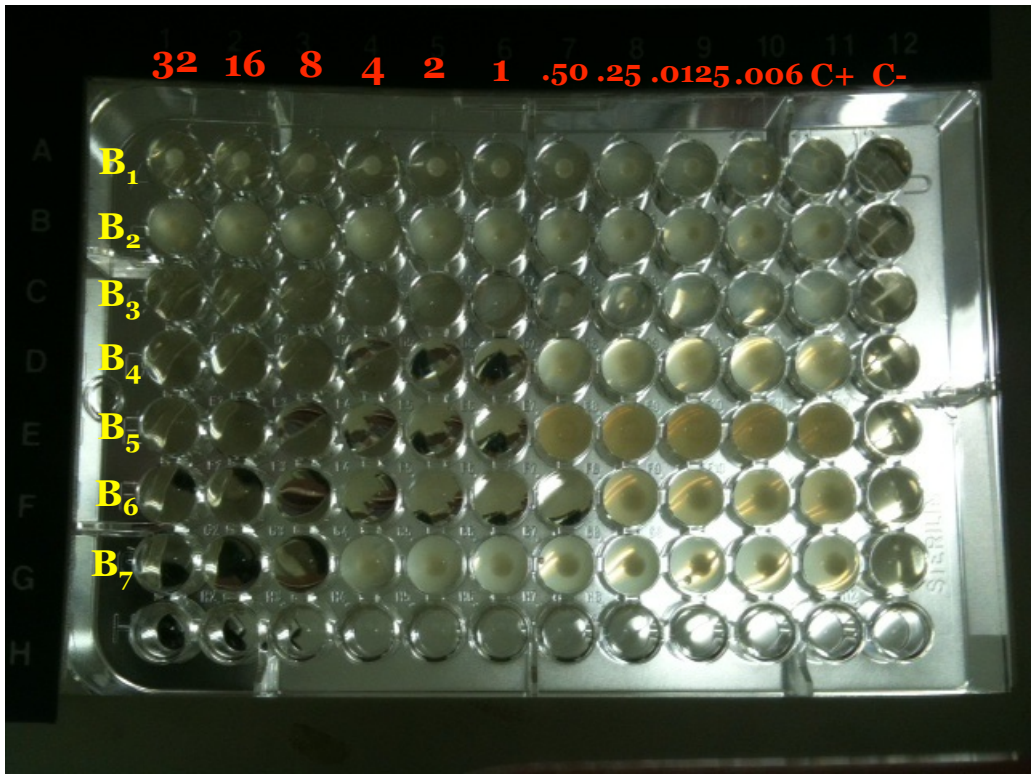

Fmoc-Cys(Acm)-OH, 3 eq.
DIPCDI, 3 eq.
HOBt, 3 eq.
DMF anh., 1 h
20% piperidina en DMF
SPPS
NH-AA1(GP1)-Thr(tBu)-AA3(GP3)-Cys(Acm)-AA5(GP5)-DPhe-AA7(GP7)-AA8(GP8)-AA9(GP9)-Cys(Acm)

Synthesis


NH-AA1(GP1)-Thr(tBu)-AA3(GP3)-Cys(Acm)-AA5(GP5)-DPhe-AA7(GP7)-AA8(GP8)-AA9(GP9)-Cys(Acm)
Final deprotection and cleavage
TFA:TES:H2O
Ciclation
5% DMSO en H2O
NH-AA1-Thr-AA3-Cys-AA5-DPhe-AA7-AA8-AA9-Cys
NH2

Antibacterial Activity

- Minimum Inhibitory Concentration (µg/mL)



Antibacterial Activity

- 100 analogs synthesized and tested

Antibacterial Activity

Nº	Peptide sequence	*Gram+*	*Gram-*	
		S. aureus	*P. aeruginosa*	*E. coli*
pxb	CH3-octanoyl-Dab-Thr-Dab-Dab-Dab-*Phe*-Leu-Dab-Dab-Thr	**>32**	**2**	**1**
0	nonanoyl-Dab-Thr-Dab-Cys-Dab-*Phe*-Leu-Dab-Dab-Cys	**>32**	**8**	**4**
1	nonanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	**32**	**16**	**8**
2	nonanoyl-Arg-Thr-Arg-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	**8**	**8**	**4**
3	nonanoyl-Dab-Thr-Dab-Cys-Dab-*Phe*-Met-Dab-Dab-Cys	**32**	**16**	**4**
34	nonanoyl-Arg-Thr-Dab-Cys-Dab-*Trp*-Leu-Arg-Dab-Cys	**8**	**8**	**4**
79	decanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	**16**	**8**	**8**
85	dodecanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	**32**	**8**	**8**
16	nonanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	**16**	**8**	**4**
93	decanoyl-Lys-Thr-Arg-Cys-Lys-*Trp*-Leu-Arg-Lys-*Cys*	**16**	**>64**	**64**
100	decanoyl-Arg-Thr-Arg-Cys-Dab-*Trp*-Nle-Arg-Dab-*Cys*	**4**	**64**	**8**
101	nonanoyl-Dab-Thr-Arg-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	**8**	**16**	**2**
103	----------	**4**	**2**	**1**
104	----------	**4**	**1**	**4**
105	----------	**4**	**1**	**2**

Antibacterial Activity

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		S. aureus	*P. aeruginosa*	*E. coli*
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0	nonanoyl-Dab-Thr-Dab-Cys-Dab-*Phe*-Leu-Dab-Dab-Cys	>32	8	4
1	nonanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	32	16	8
2	nonanoyl-Arg-Thr-Arg-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	8	8	4
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85	dodecanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	32	8	8
16	nonanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	16	8	4
93	decanoyl-Lys-Thr-Arg-Cys-Lys-*Trp*-Leu-Arg-Lys-*Cys*	16	>64	64
100	decanoyl-Arg-Thr-Arg-Cys-Dab-*Trp*-Nle-Arg-Dab-*Cys*	4	64	8
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103	----------	4	2	1
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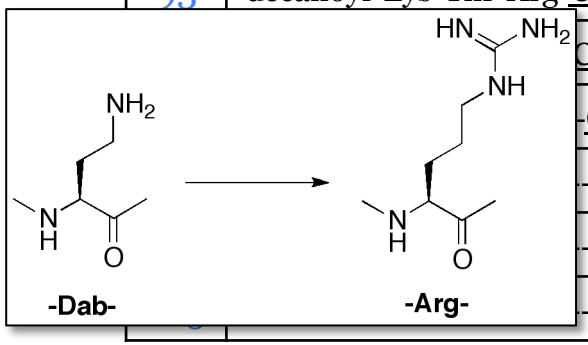
93	decanoyl-Lys-Thr-Arg-Cys-Lys-*Trp*-Leu-Arg-Lys-*Cys*	16	>64	64
	Cys-Dab-*Trp*-Nle-Arg-Dab-*Cys*	4	64	8
	-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	8	16	2
	----------	4	2	1
	----------	4	1	4
	----------	4	1	2



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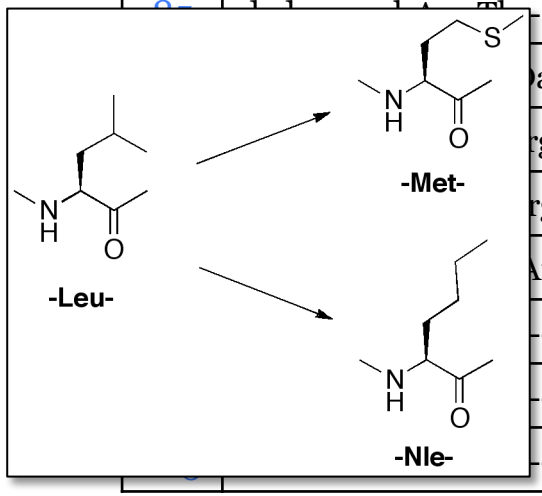
	-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	32	8	8
	ab-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	16	8	4
	g-Cys-Lys-*Trp*-Leu-Arg-Lys-*Cys*	16	>64	64
	g-Cys-Dab-*Trp*-Nle-Arg-Dab-*Cys*	4	64	8
	rg-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	8	16	2
	----------	4	2	1
	----------	4	1	4
	----------	4	1	2



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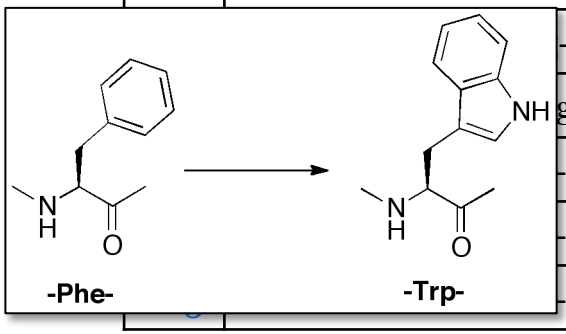
	-Cys-Dab-*Trp*-Nle-Arg-Dab-*Cys*	4	64	8
	g-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	8	16	2
	----------	4	2	1
	----------	4	1	4
	----------	4	1	2



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3	nonanoyl-Dab-Thr-Dab-Cys-Dab-*Phe*-Met-Dab-Dab-Cys	**32**	**16**	**4**
34	nonanoyl-Arg-Thr-Dab-Cys-Dab-*Trp*-Leu-Arg-Dab-Cys	**8**	**8**	**4**
79	decanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	**16**	**8**	**8**
85	dodecanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-Cys	**32**	**8**	**8**
16	nonanoyl-Arg-Thr-Dab-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	**16**	**8**	**4**
93	decanoyl-Lys-Thr-Arg-Cys-Lys-*Trp*-Leu-Arg-Lys-*Cys*	**16**	**>64**	**64**
100	decanoyl-Arg-Thr-Arg-Cys-Dab-*Trp*-Nle-Arg-Dab-*Cys*	**4**	**64**	**8**
101	nonanoyl-Dab-Thr-Arg-Cys-Dab-*Phe*-Leu-Arg-Dab-*Cys*	**8**	**16**	**2**
103	----------	**4**	**2**	**1**
104	----------	**4**	**1**	**4**
105	----------	**4**	**1**	**2**

Antibacterial Activity

Species, MIC (µg/mL)		Analog 103	Analog 104	Analog 105	PxB G(-) control	Vancomycin G(+) control	Daptomycin G(+) control
GRAM -	*Pseudomonas aeruginosa*	**2**	**1**	**1**	**2**	**-**	**>32**
	Escherichia coli	**1**	**4**	**4**	**1**	**-**	**>32**
GRAM +	*Mycobacterium phlei*	**4**	**4**	**2**	**16-32**	**-**	**>32**
	Staphylococcus aureus	**4**	**4**	**4**	**>32**	**1**	**2**

Antibacterial Activity

Species, MIC (µg/mL)		Analog 103	Analog 104	Analog 105	PxB G(-) control	Vancomycin G(+) control	Daptomycin G(+) control
GRAM -	*Pseudomonas aeruginosa*	**2**	**1**	**1**	**2**	**-**	**>32**
	Escherichia coli	**1**	**4**	**4**	**1**	**-**	**>32**
GRAM +	*Mycobacterium phlei*	**4**	**4**	**2**	**16-32**	**-**	**>32**
	Staphylococcus aureus	**4**	**4**	**4**	**>32**	**1**	**2**

Antibacterial Activity

Resistant and multi-resistant Gram negative bacteria			Analog 103	Analog 104	Analog 105
P. aeruginosa	**40b**	Carbapenem-resistant strain	**4**	**2**	**4**
	38a	Highly-resistant strain	**0,5**	**1**	**16**
E. coli	**MAC 21a**	Intermediate resistance to quinolones	**0,5**	**1**	**2**
	VAL 10	Intermediate resistance to quinolones	**0,5**	**0,5**	**1**
	VAL 5	Intermediate resistance to quinolones	**0,5**	**0,5**	**1**
	NDM	Highly-resistant strain	**0,5**	**0,5**	**1**

- Resistance panel:
  - 40b (clinical isolate): resistance to IMP
  - 38a (clinical isolate): resistance to CAZ, CIP , IMP, P/T
  - NMD, New Delhi metallo-beta-lactamase: Amoxicillin 256 mg/L; Amoxicillin clavulanate 32 mg/L ; Piperacillin/tazobactam 256 mg/L; Cefoxitin 256 mg/L; Cefotaxime 256 mg/L; Ceftazidime 256 mg/L; Cefepime 256 mg/L; Imipenem 8 mg/L; Meropenem 16 mg/L; Doripenem 6 mg/L; Ertapenem 24 mg/L; Aztreonam 256 mg/L; Gentamicin 8 mg/L; Amikacin 32 mg/L, Tobramycin 8 mg/L; Ciprofloxacin 32 mg/L
  - AMI, amikacin; CAZ, Ceftazidime; CIP, Ciprofloxacin; IMP, Imipenem ; P/T, Piperacillin-tazobactam

Antibacterial Activity

Resistant and multi-resistant Gram negative bacteria			Analog 103	Analog 104	Analog 105
P. aeruginosa	**40b**	Carbapenem-resistant strain	**4**	**2**	**4**
	38a	Highly-resistant strain	**0,5**	**1**	**16**
E. coli	**MAC 21a**	Intermediate resistance to quinolones	**0,5**	**1**	**2**
	VAL 10	Intermediate resistance to quinolones	**0,5**	**0,5**	**1**
	VAL 5	Intermediate resistance to quinolones	**0,5**	**0,5**	**1**
	NDM	Highly-resistant strain	**0,5**	**0,5**	**1**

- Resistance panel:
  - 40b (clinical isolate): resistance to IMP
  - 38a (clinical isolate): resistance to CAZ, CIP , IMP, P/T
  - NMD, New Delhi metallo-beta-lactamase: Amoxicillin 256 mg/L; Amoxicillin clavulanate 32 mg/L ; Piperacillin/tazobactam 256 mg/L; Cefoxitin 256 mg/L; Cefotaxime 256 mg/L; Ceftazidime 256 mg/L; Cefepime 256 mg/L; Imipenem 8 mg/L; Meropenem 16 mg/L; Doripenem 6 mg/L; Ertapenem 24 mg/L; Aztreonam 256 mg/L; Gentamicin 8 mg/L; Amikacin 32 mg/L, Tobramycin 8 mg/L; Ciprofloxacin 32 mg/L
  - AMI, amikacin; CAZ, Ceftazidime; CIP, Ciprofloxacin; IMP, Imipenem ; P/T, Piperacillin-tazobactam

Antibacterial Activity

Colistin-Resistant Acinetobacter baumannii			Colistin	Analog 103	Analog 104	Analog 105
A. baumannii	**ATCC-wt**	Low resistance strain	**1**	**8**	**2**	**4**
	ATCC	In vitro mutant of ATCC with resistance to colistin	**256**	**64**	**16**	**32**
	77778	In vitro mutant of a clinical strain with resistance to colistin	**256**	**256**	**128**	**128**
	Ab 10	Clinical isolate resistant to colistin	**512**	**32**	**16**	**8**
	Ab 19	Clinical isolate resistant to colistin	**512**	**32**	**16**	**16**

Antibacterial activity in colistin-resistant Acinetobacter baumannii suggests an alternative mechanism of action

Mechanism of action

- Biophysics

Mechanism of action

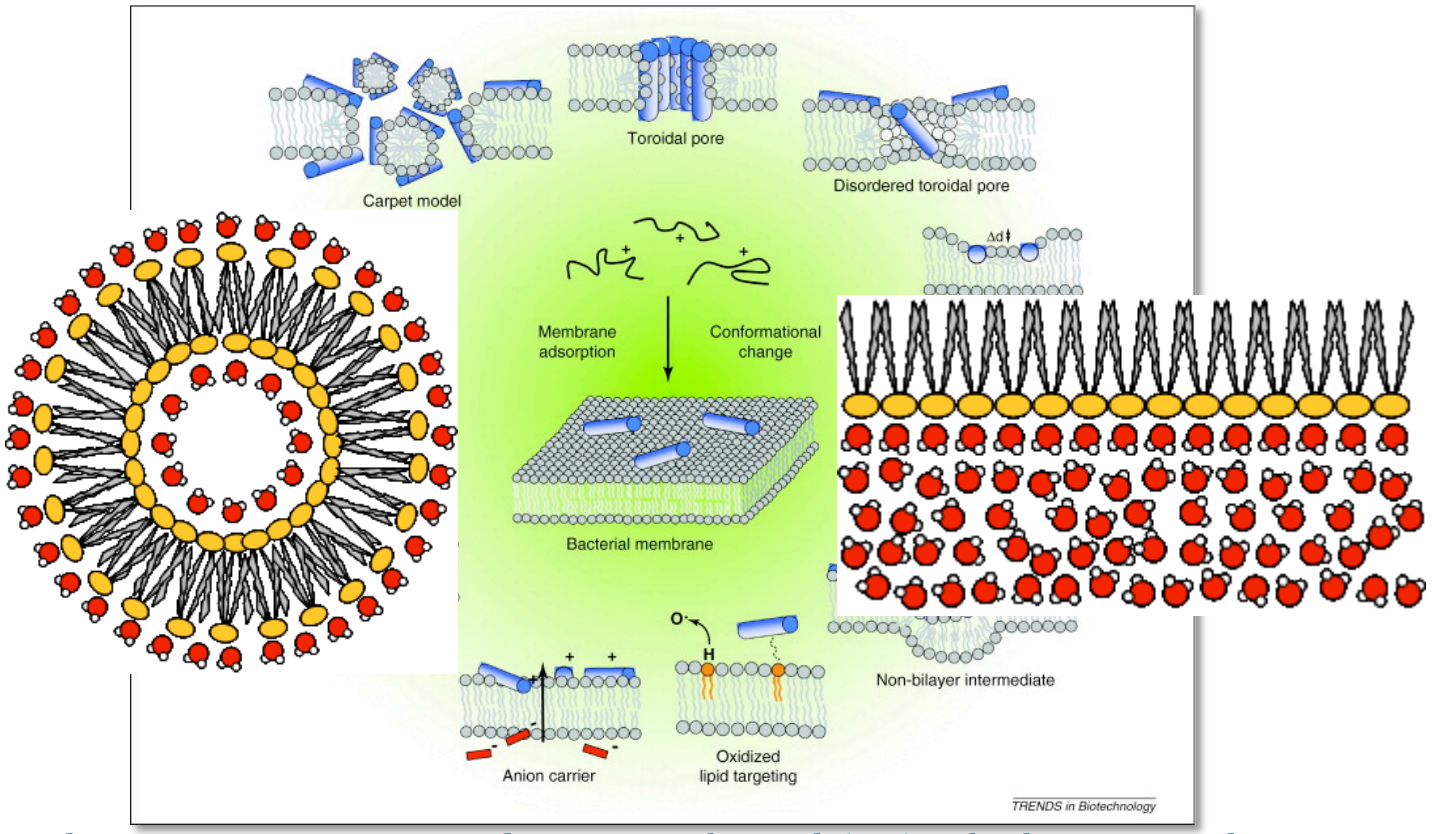
- Biophysics
- Flow cytometry

Mechanism of action

- Biophysics
- Flow cytometry
- Transmission Electron Microscopy (TEM)

Mechanism of action

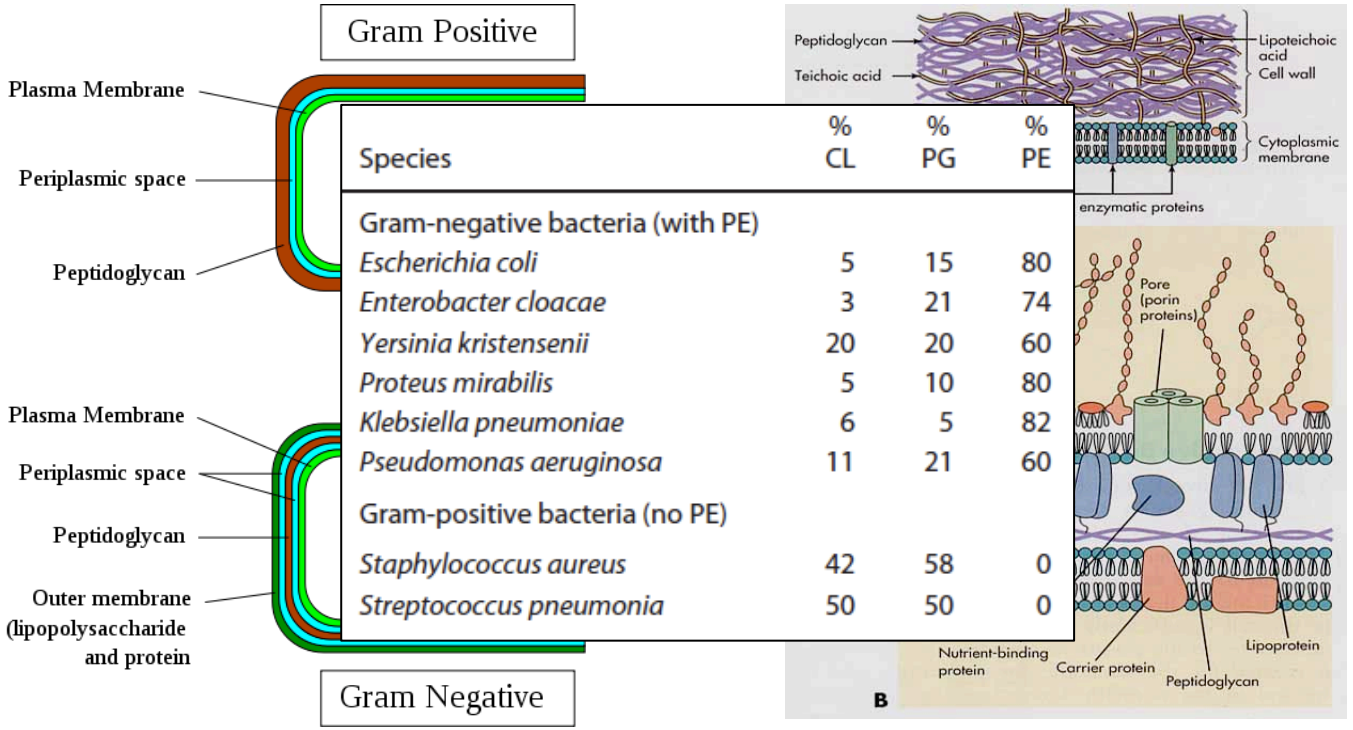
- Biophysics
  - Model membranes



Leonard T. Nguyen, Evan F. Haney and Hans J. Vogel, *Trends in Biotechnology*, **2011**, Vol. 29, No. 9

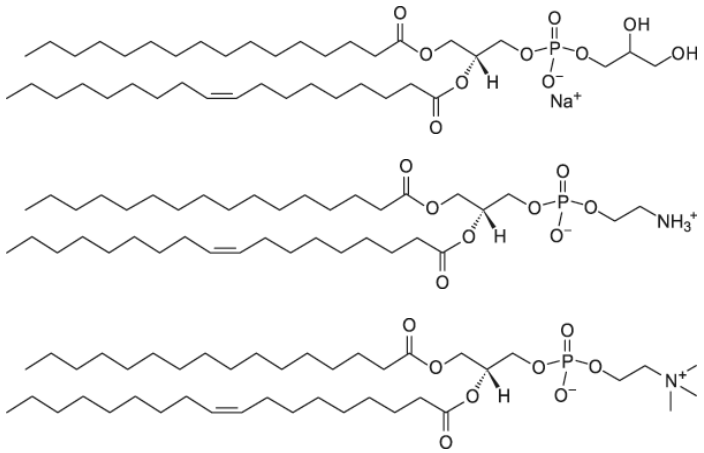
Mechanism of action

- Biophysics
  - Model membranes
  - Bacterial membrane vs Eukaryotic membrane



Species	% CL	% PG	% PE
Gram-negative bacteria (with PE)			
Escherichia coli	5	15	80
Enterobacter cloacae	3	21	74
Yersinia kristensenii	20	20	60
Proteus mirabilis	5	10	80
Klebsiella pneumoniae	6	5	82
Pseudomonas aeruginosa	11	21	60
Gram-positive bacteria (no PE)			
Staphylococcus aureus	42	58	0
Streptococcus pneumonia	50	50	0

Mechanism of action



- Biophysics
  - Model membranes
  - Bacterial membrane vs Eukaryotic membrane

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Gram-negative bacteria (with PE)			
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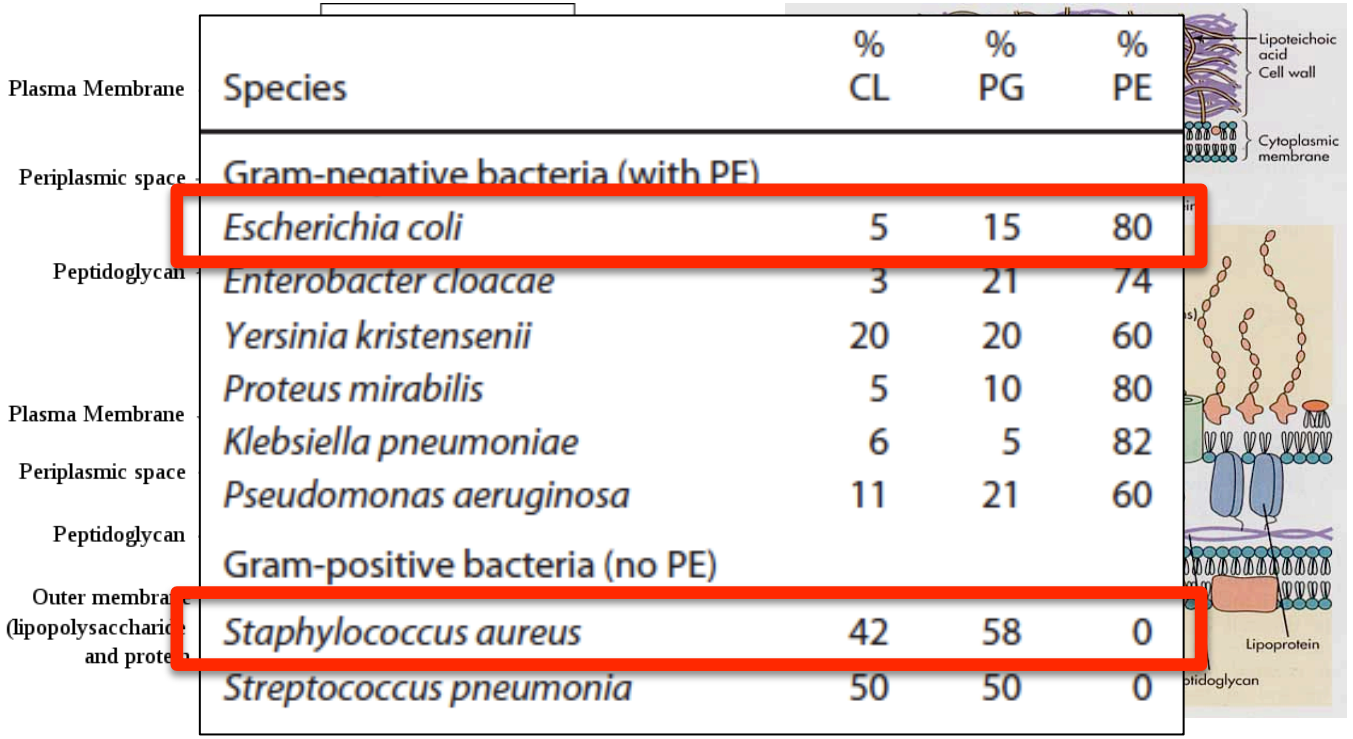
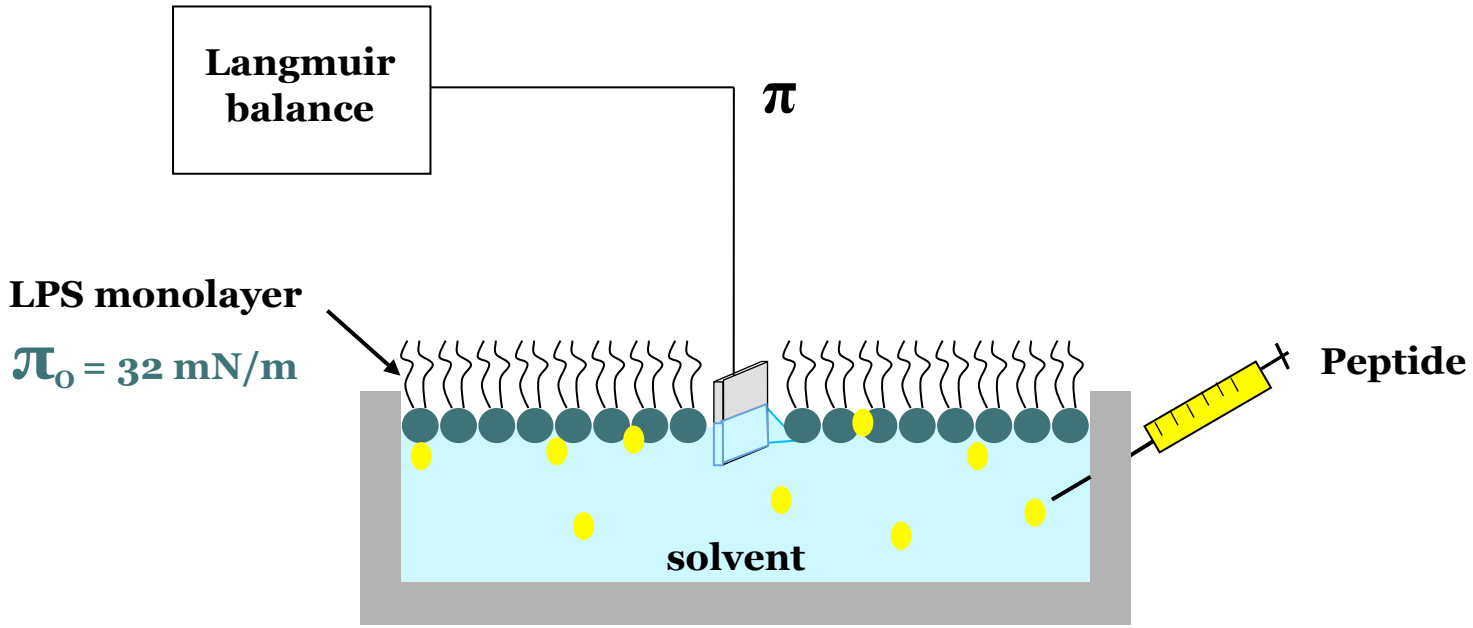
Gram-positive bacteria (no PE)			
Staphylococcus aureus	42	58	0
Streptococcus pneumonia	50	50	0



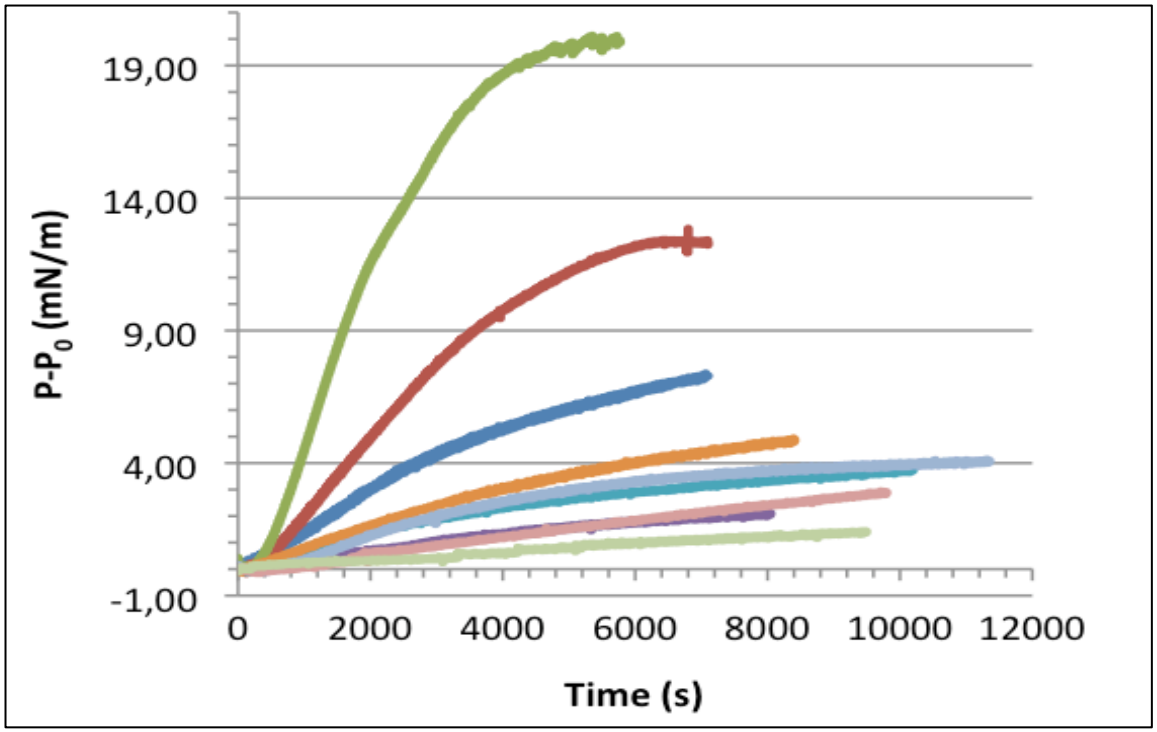
Mechanism of action

- Biophysics
  - Interaction with LPS (*S.minnesota*)
    - Monolayers



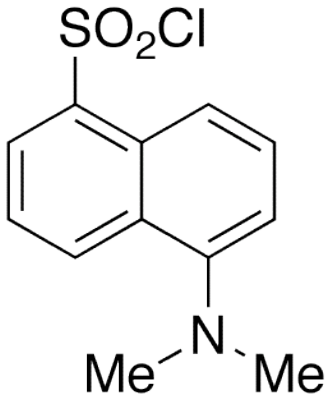
Mechanism of action

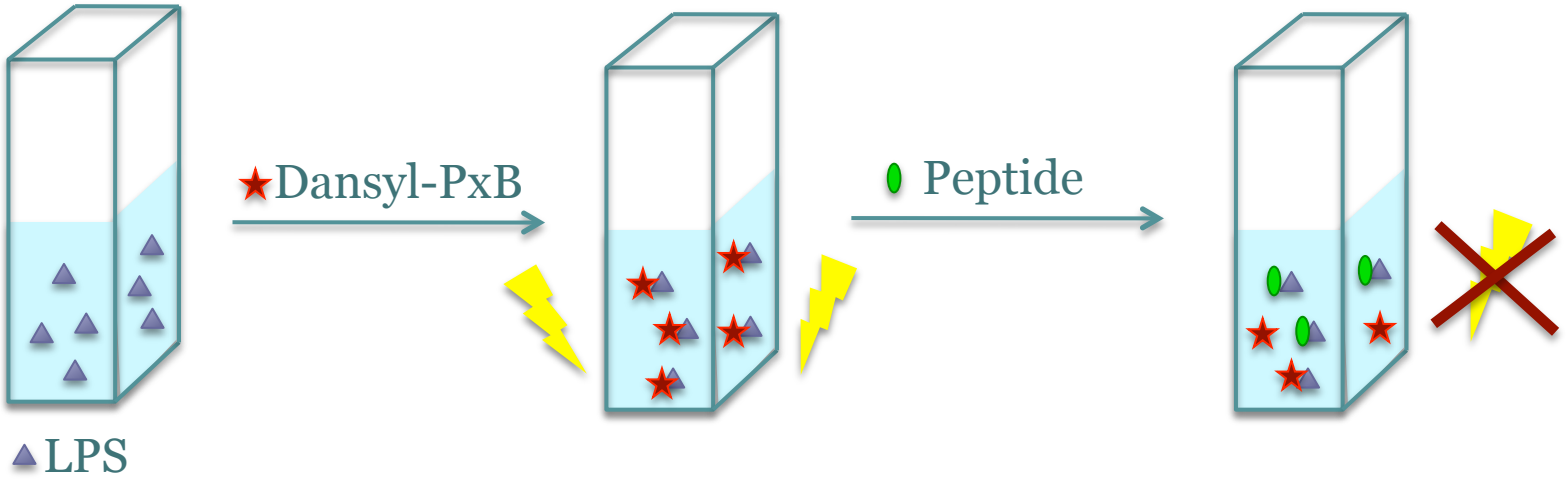
- Biophysics
  - Interaction with LPS (*S.minnesota*)
    - Monolayers



Mechanism of action

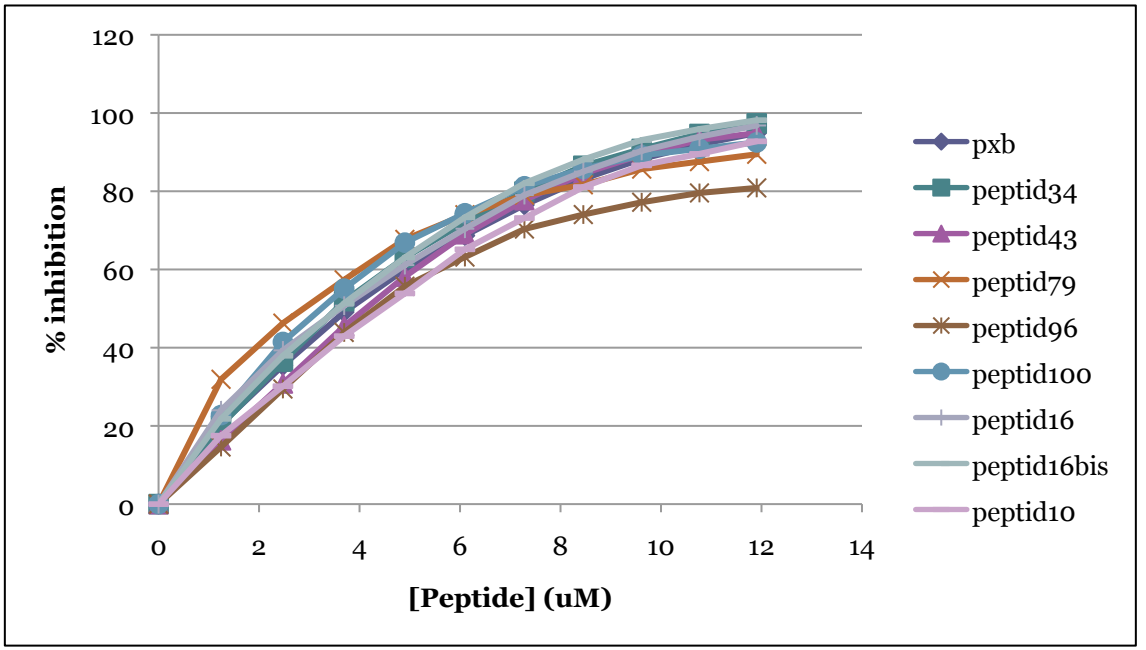
- Biophysics
  - Interaction with LPS (*S.minnesota*)
    - Monolayers
    - Dansyl-PxB liposomes: Affinity assay





Mechanism of action

- Biophysics
  - Interaction with LPS (*S.minnesota*)
    - Monolayers
    - Dansyl-PxB liposomes: Affinity assay

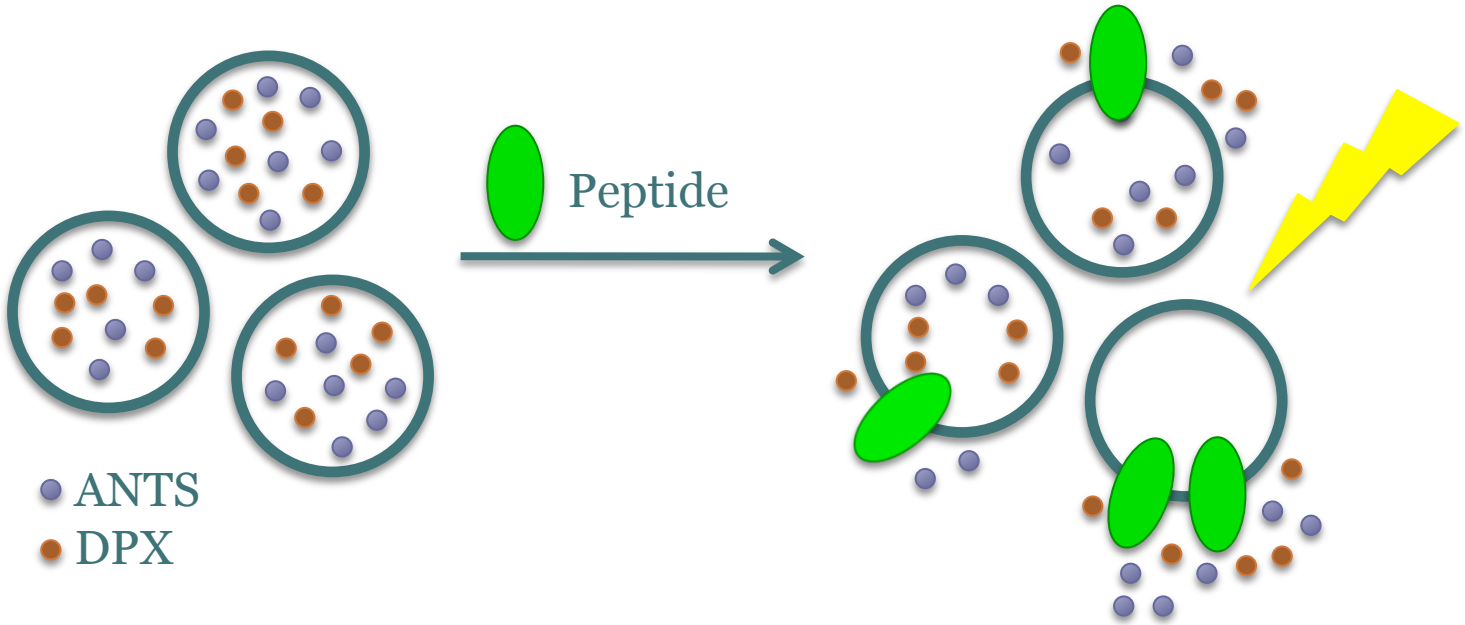


Mechanism of action

- Biophysics
  - Interaction with LPS (*S.minnesota*)
  - Effect on the membrane

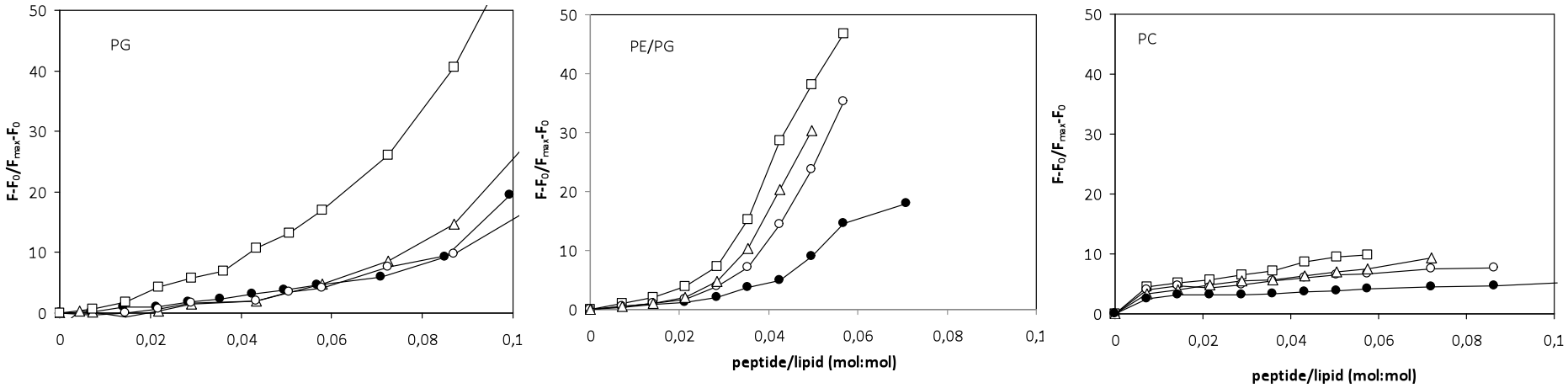
Mechanism of action

- Biophysics
  - Interaction with LPS (*S.minnesota*)
  - Effect on the membrane
    - Leakage



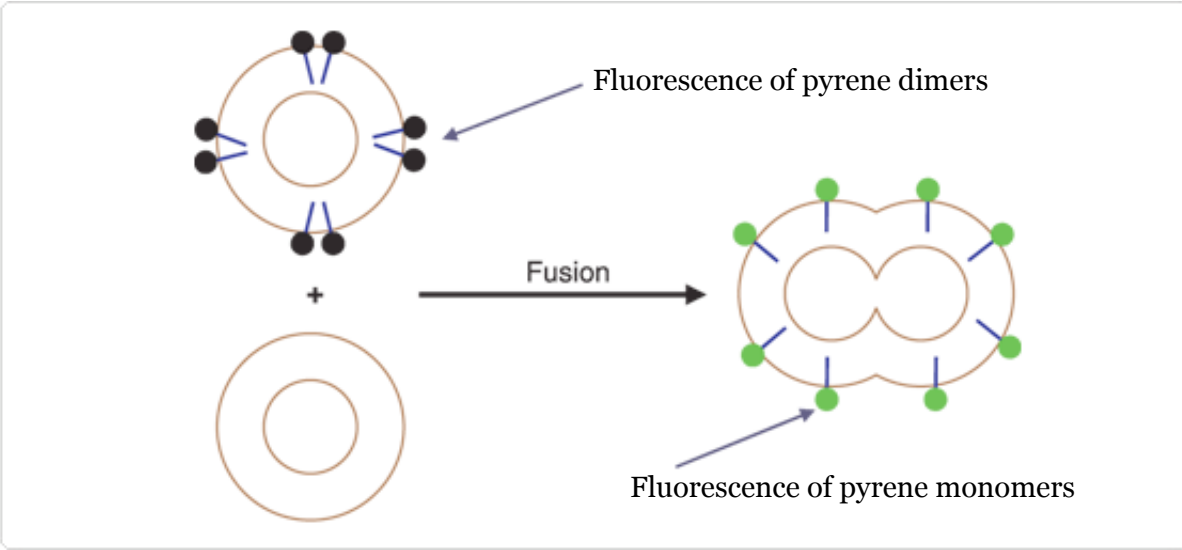
Mechanism of action

- Biophysics
  - Interaction with LPS (*S.minnesota*)
  - Effect on the membrane
    - Leakage

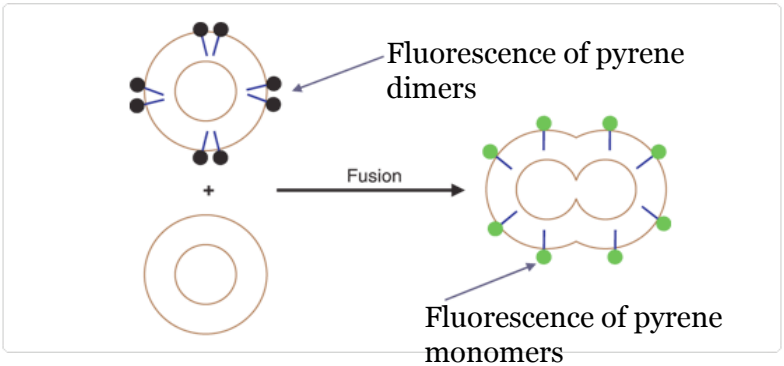


Mechanism of action

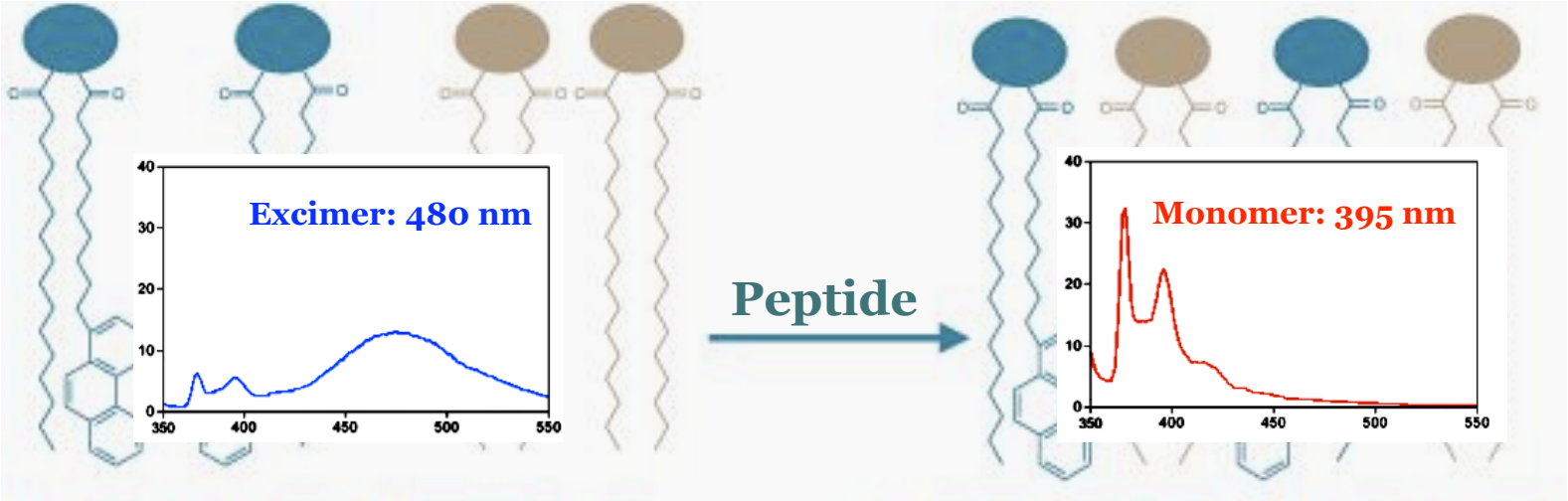
- Biophysics
  - Interaction with LPS (*S.minnesota*)
  - Effect on the membrane
    - Leakage
    - Fusion



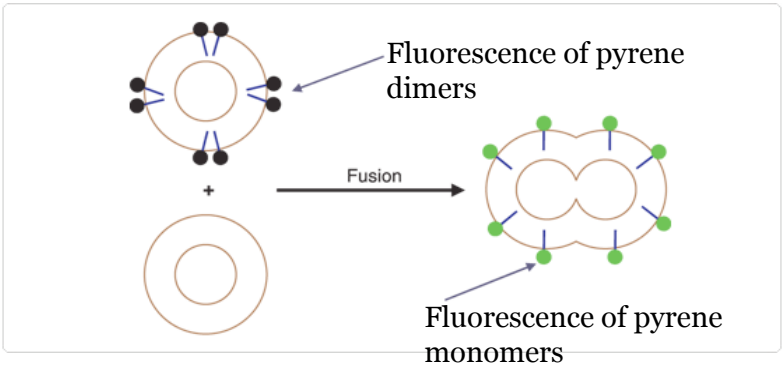
Mechanism of action



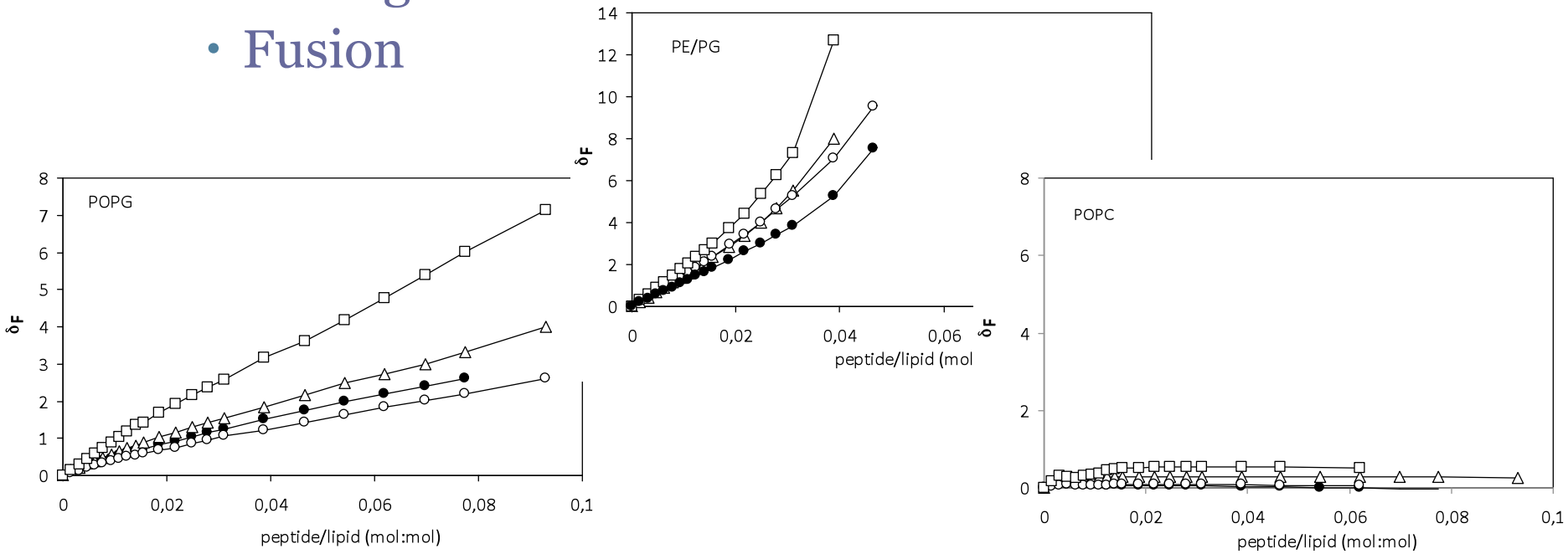
- Biophysics
  - Interaction with LPS (*S.minnesota*)
  - Effect on the membrane
    - Leakage
    - Fusion



Mechanism of action



- Biophysics
  - Interaction with LPS (*S.minnesota*)
  - Effect on the membrane
    - Leakage
    - Fusion

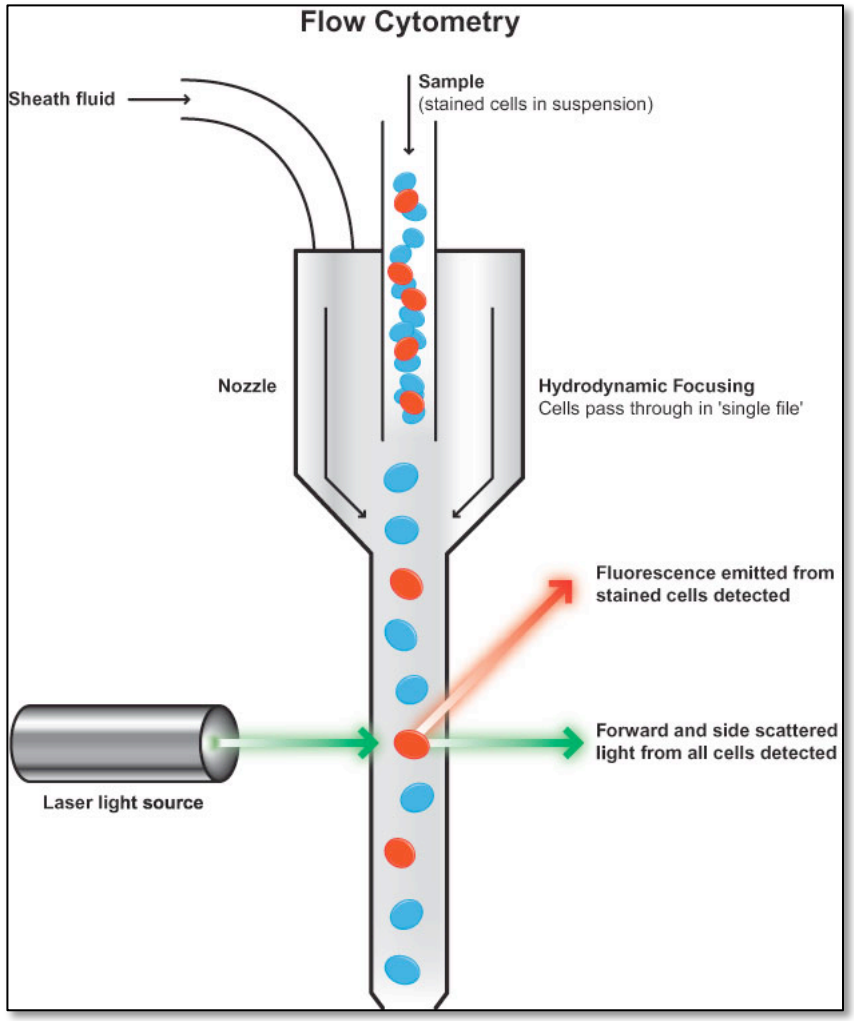


Mechanism of action

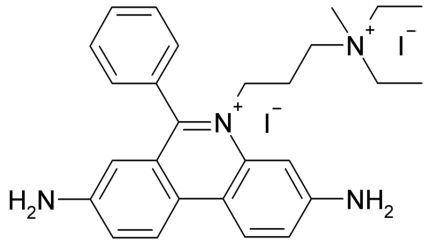
- Flow cytometry

http://probes.invitrogen.com/resources/education/tutorials/4Intro_Flow/player.html

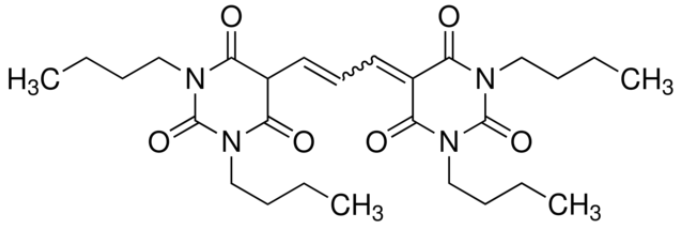
Mechanism of action



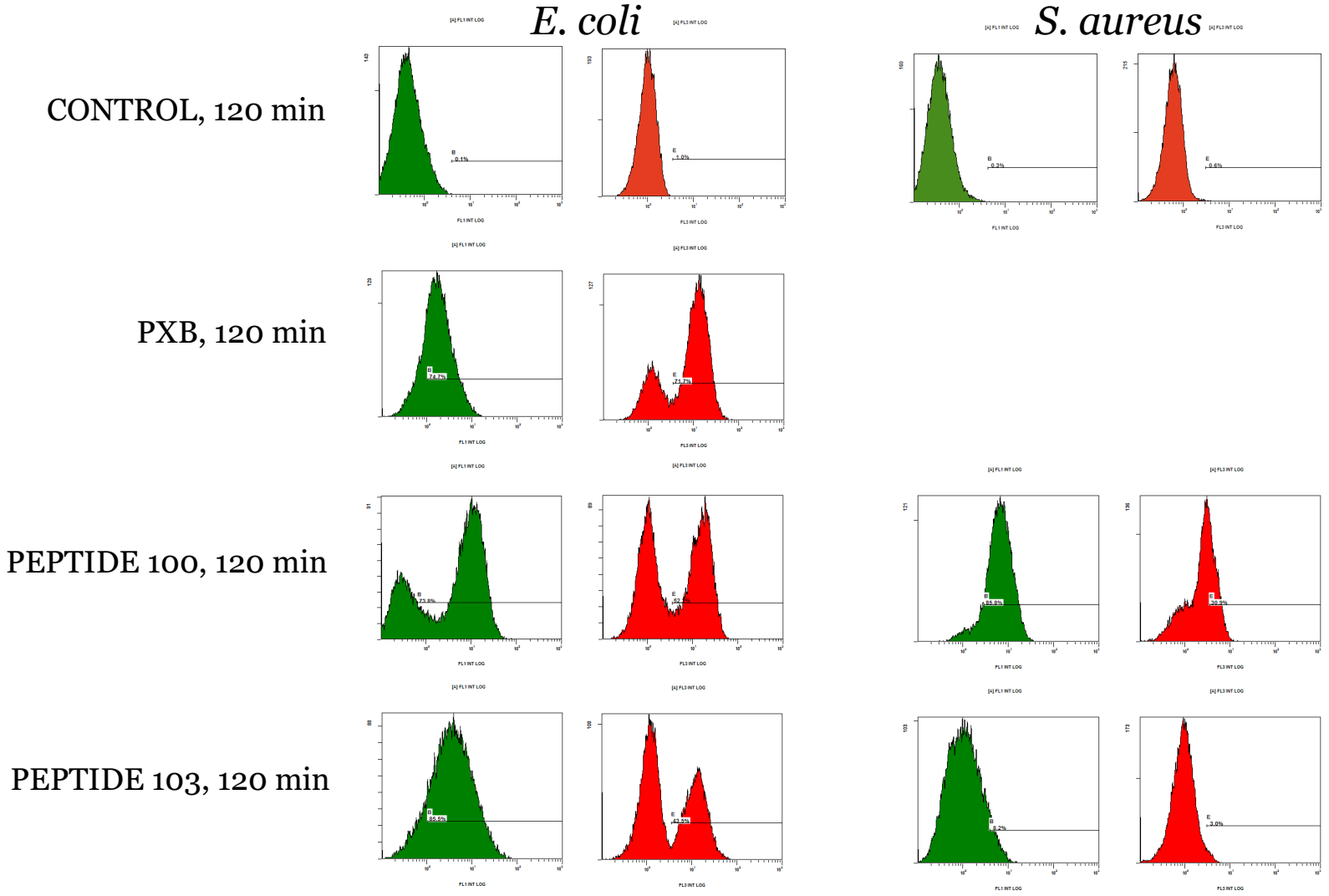
Propidium iodide



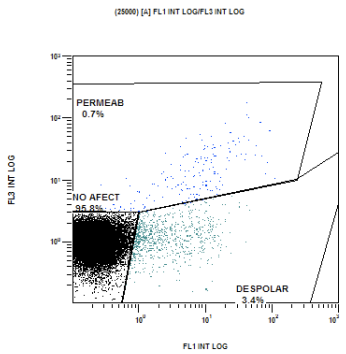
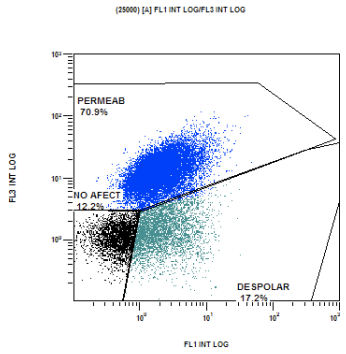
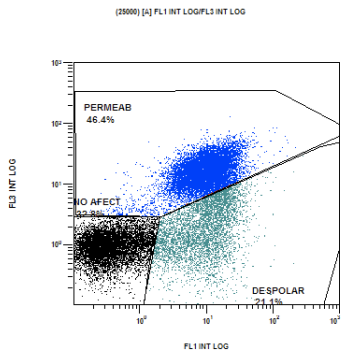
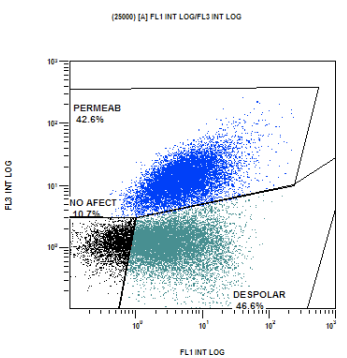
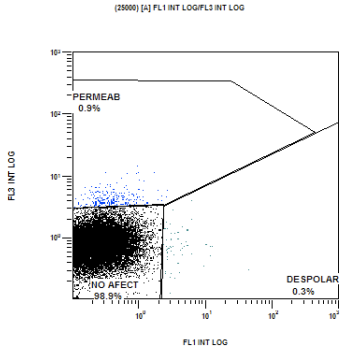
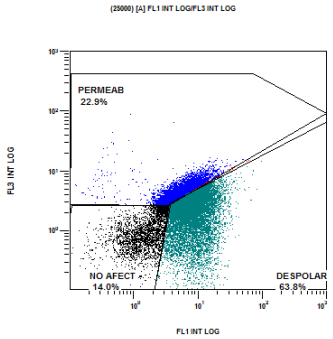
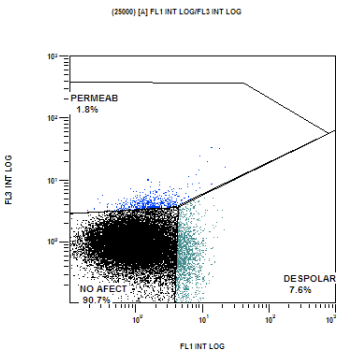
Bis-(1,3-Dibutylbarbituric Acid)Trimethine Oxonol
$DIBAC_4(3)$



Mechanism of action



Mechanism of action

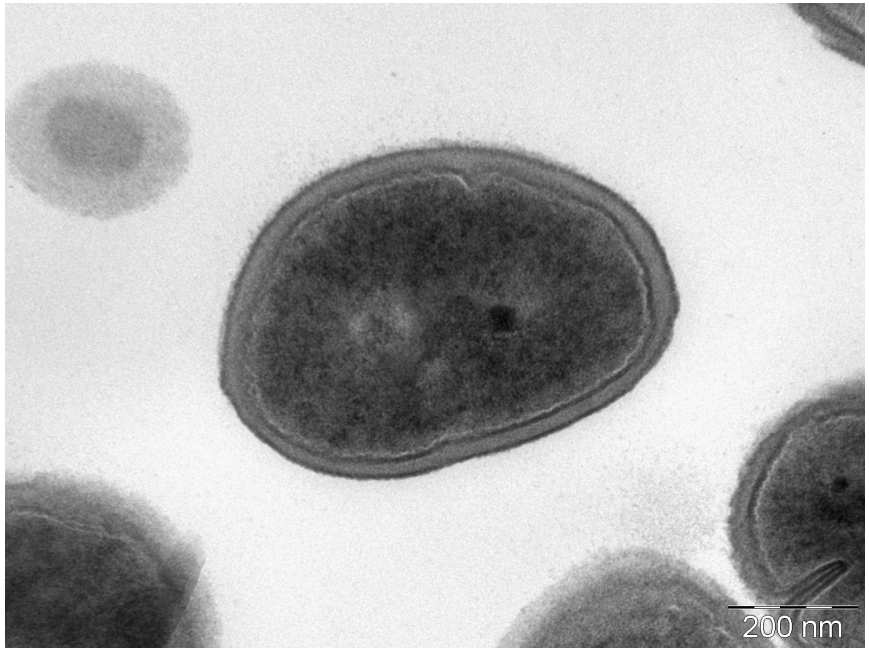
	CONTROL, 120 min	PXB, 120 min	PEPTIDE 100, 120 min	PEPTIDE 103, 120 min
E. coli				
S. aureus				

The flow-cytometric tests show a different behaviour of the peptides front Gram+ and Gram- bacteria

Mechanism of action

- Transmission Electron Microscopy (TEM)

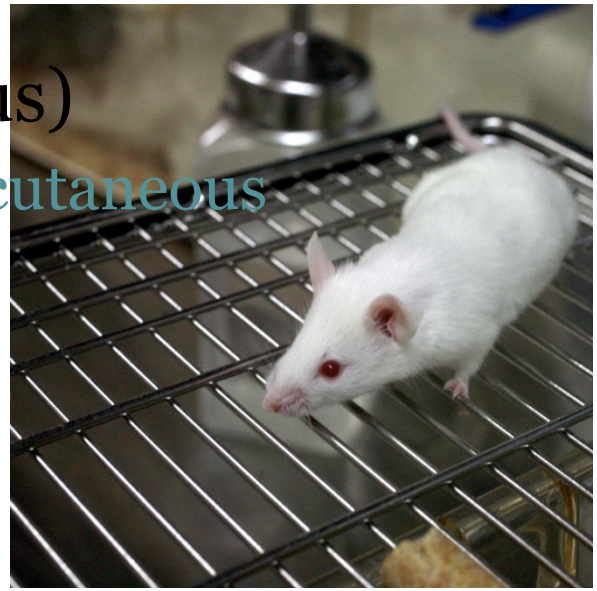
CONTROL



PÈPTID (MIC)

In-Vivo Toxicity

- Acute toxicity test in mice for peptide 103
- LD_{50} = 283 mg/Kg (subcutaneous)
  - Polymyxin LD_{50} = 59,5 mg/Kg, subcutaneous



5 reversions in 6 consecutive animals (OECD 425). Animals (3) administered at 200 mg/kg survived after 14 days; Necropsy indicated no visual damage in vital organs. Mice (3) administered at 400 mg/Kg died

Conclusions

- Activity in Gram-
- Activity in Gram+ (S.aureus)
- Different mechanism of action
- Low toxicity
- Easy synthesis

Acknowledgments

- Funding has been provided by:
  - Ministerio de Ciencia e Innovación (CTQ2008-06200)
  - Generalitat de Catalunya (VAL-TEC 08-1-0016, ACC10)
  - Fundació Bosch i Gimpera (UB).

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THANK YOU FOR YOUR ATTENTION!