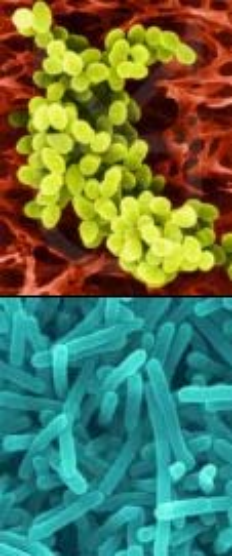


Intracellular infection and activity of antibiotics:

concepts and examples with *Staphylococcus aureus* and *Listeria monocytogenes*

Paul M. Tulkens, MD, PhD

Cellular and Molecular Pharmacology Unit

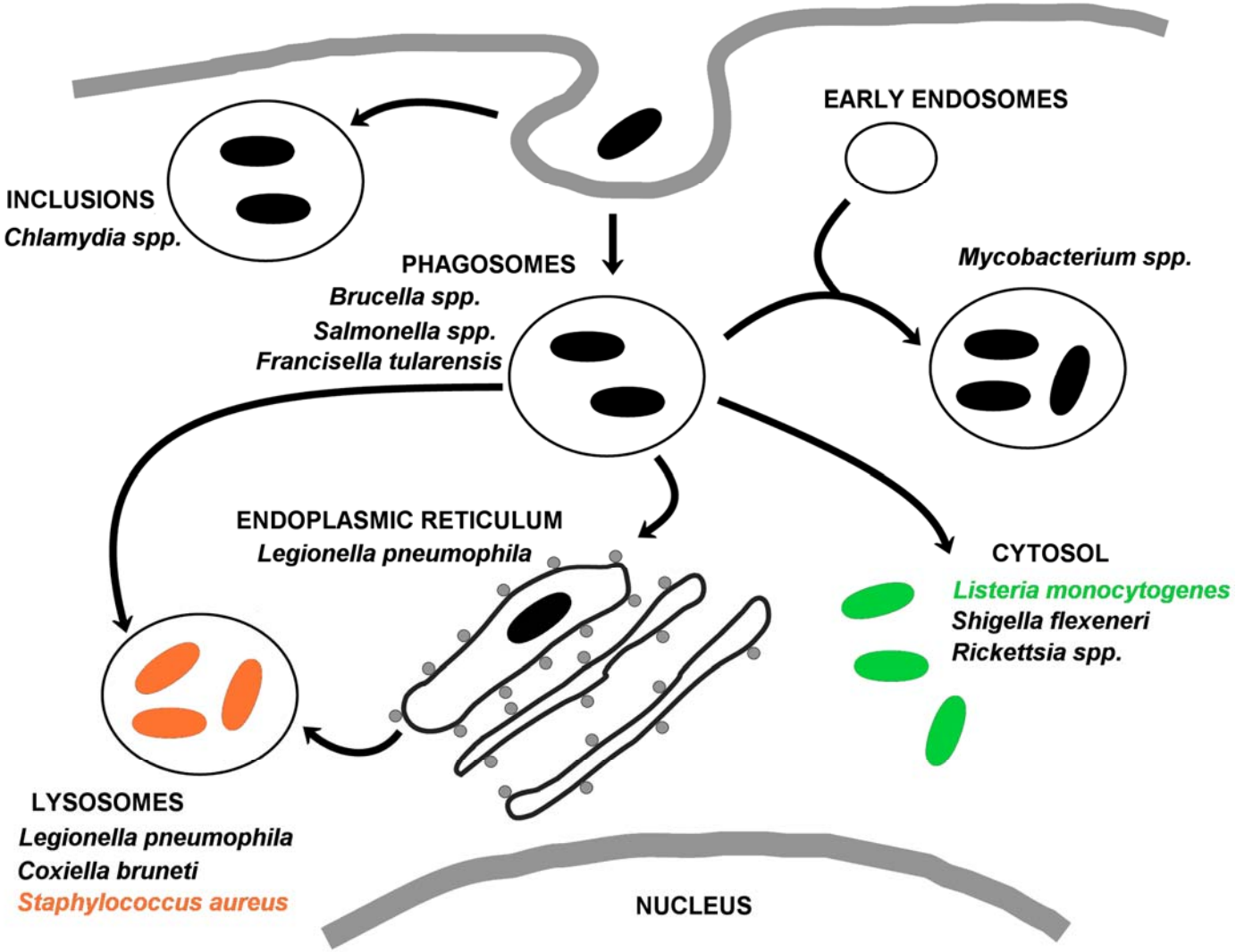
Catholic University of Louvain, Brussels, Belgium, European Union

Université catholique de Louvain
Brysselissä

Yliopistosairaala & lääketieteellinen tiedekunta

Solufarmakologian ja -toksikologian ryhmä

Intracellular infection: bacterial strategies



Carryn *et al.* Infect Dis Clin North Am. 2003;17:615-34.

S. aureus: intracellular infection and recurrence/relapses

In vivo importance assumed based on in vitro data

J Bone Joint Surg Br. 2003 Aug;85(6):918-21.

Intracellular Staphylococcus aureus. A mechanism for the indolence of osteomyelitis.

Ellington JK, Harris M, Webb L, Smith B, Smith T, Tan K, Hudson M.

Clin Infect Dis. 2001 Jun 1;32(11):1643-7. Epub 2001 Apr 30.

Intracellular persistence of Staphylococcus aureus small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease.

von Eiff C, Becker K, Metze D, Lubritz G, Hockmann J, Schwarz T, Peters G.

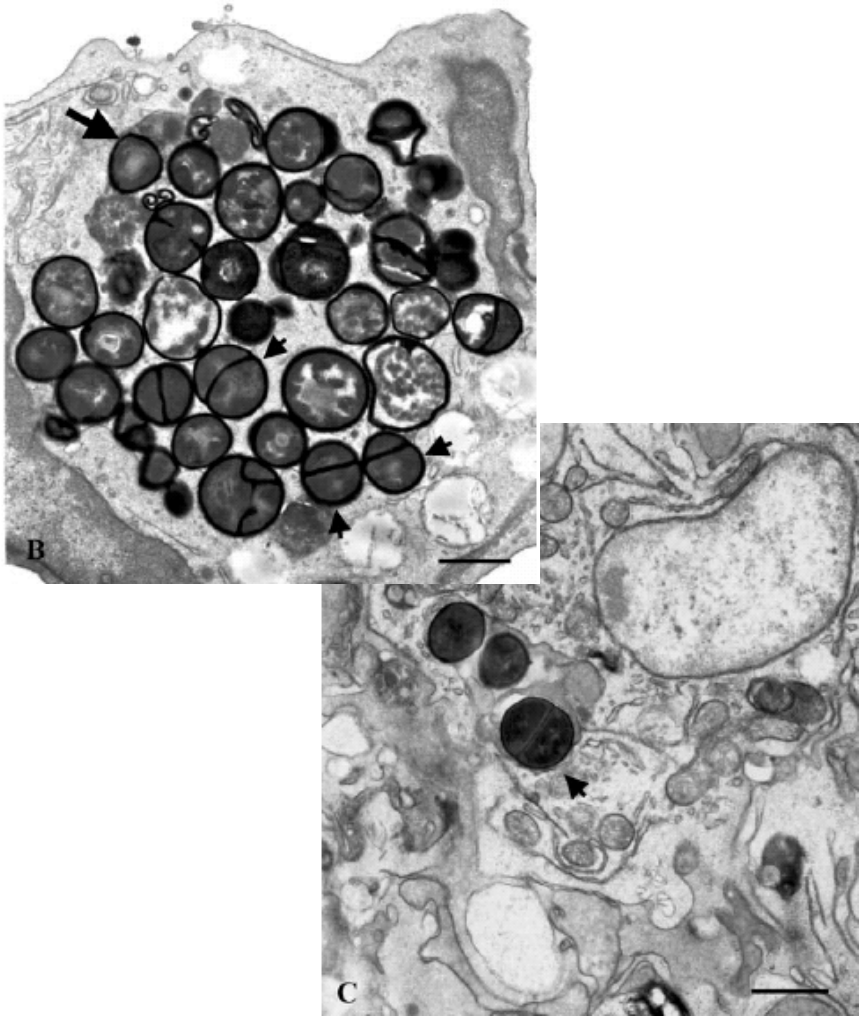
Infect Immun. 1986 Dec;54(3):833-6.

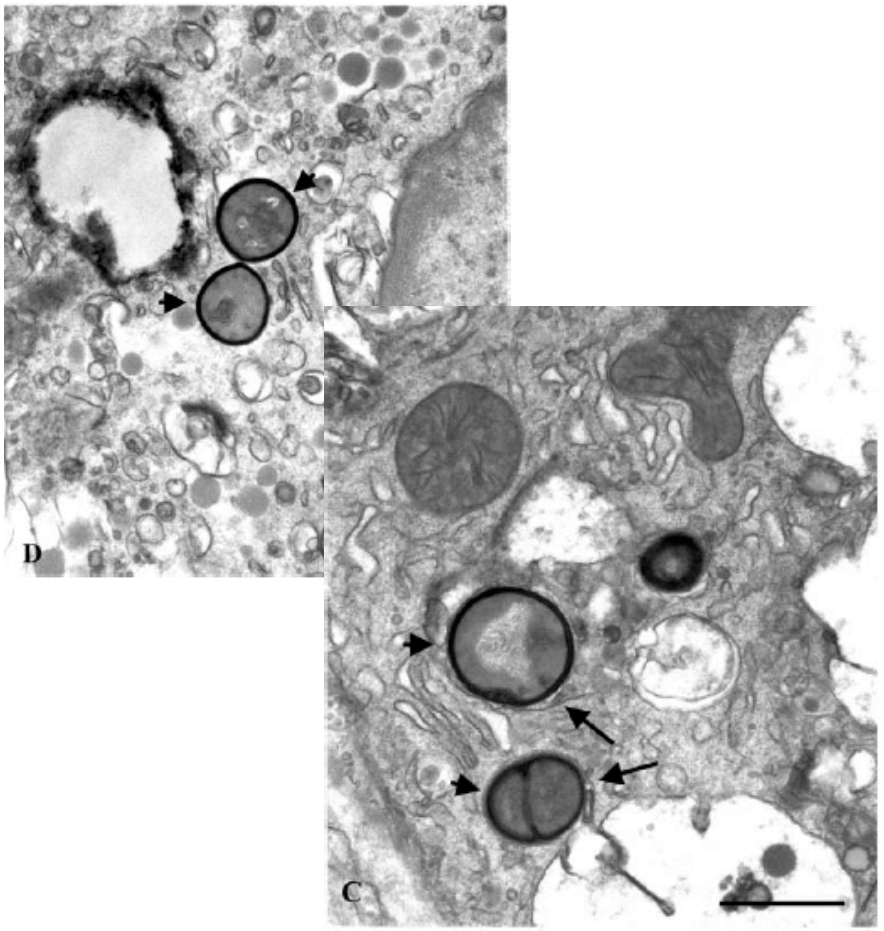
Phagocytosis of Staphylococcus aureus by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections.

Hamill RJ, Vann JM, Proctor RA.

S. aureus: subcellular localization

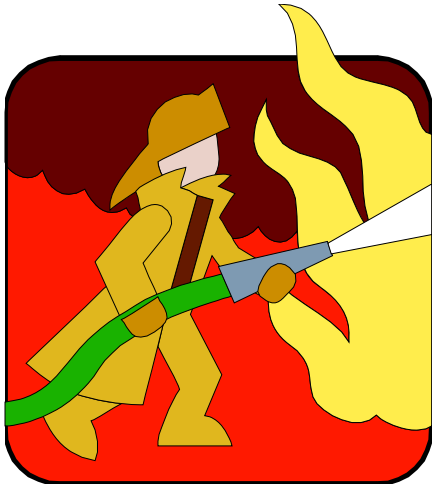
Phagocytic and non phagocytic cells in mastitis



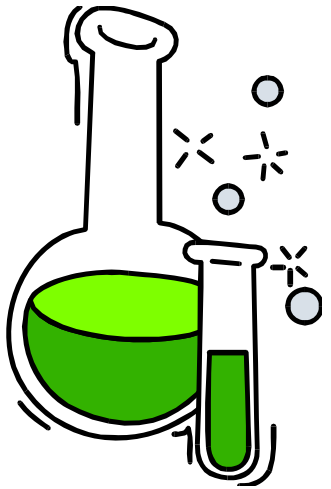


Brouillette *et al.,* Microb. Pathog. *(*2003) 35:159-68

A real need...

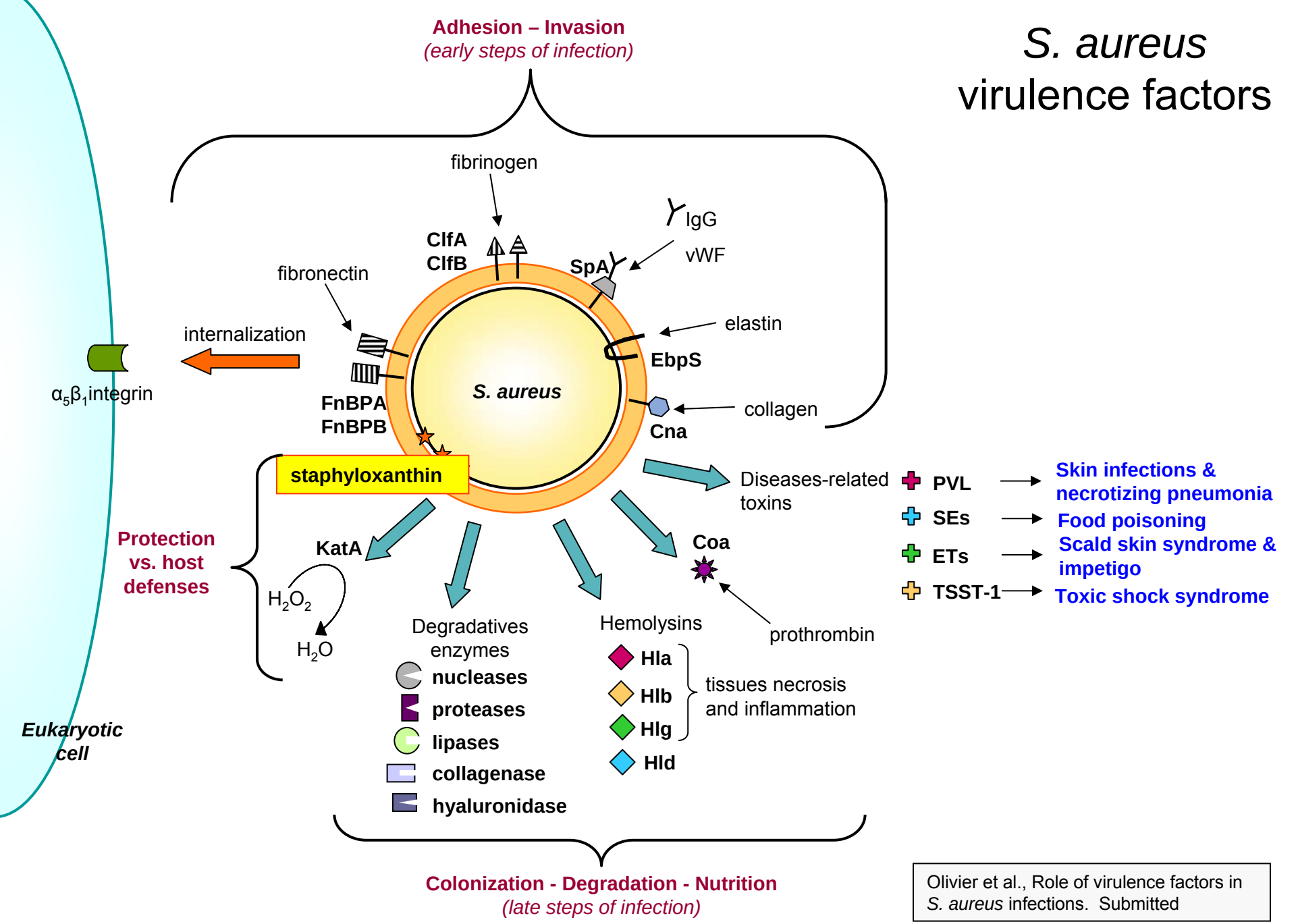


Pharmacochemistry
and Biology
to your help !



- Staphyloxanthin and intracellular survival
- Activity of availailable antibiotics
- What for the future ?

S. aureus virulence factors



Olivier et al., Role of virulence factors in *S. aureus* infections. Submitted

S. aureus virulence factors

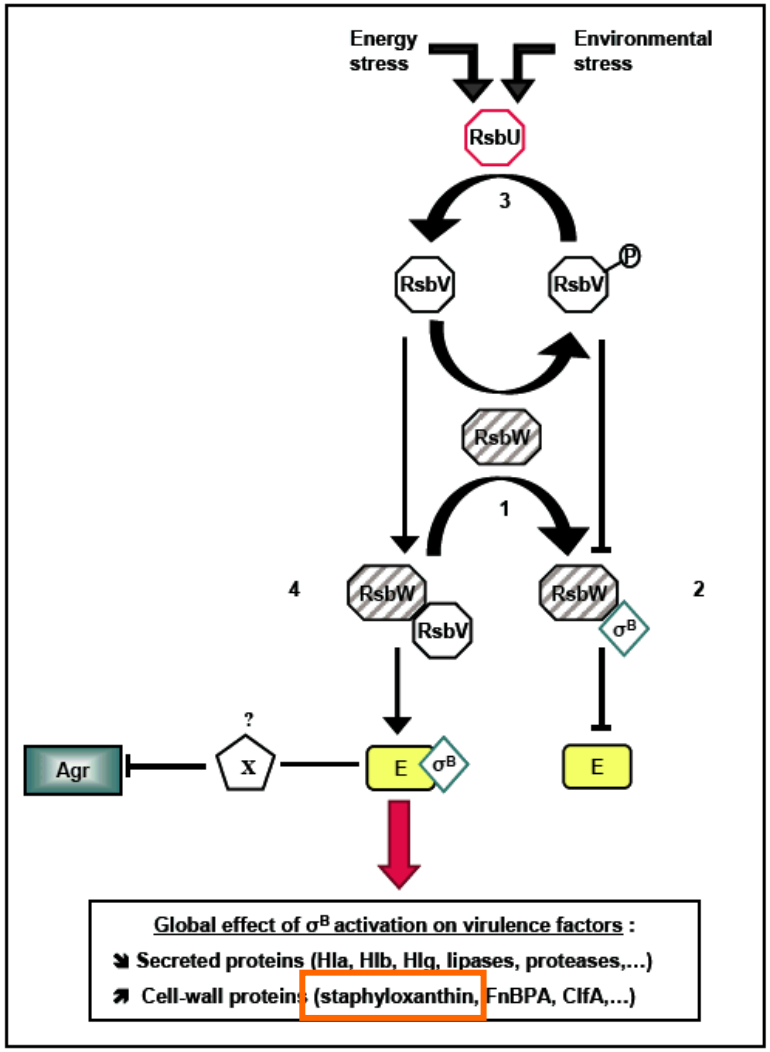


Figure 4 : Adapted from **[9,76]** Proposed model for the regulation of sigmaB in *S. aureus*. RsbW can form mutually exclusive complexes with either sigmaB or its antagonist, rsbV (step 1). RsbV is normally inactive (rsbV-P) due to phosphorylation by rsbW and is thus unable to complex with rsbW, leaving the latter free to interact with sigmaB (step 2). When bound to rsbW, sigmaB is unable to aggregate with the RNA polymerase core enzyme (E) to form an active holoenzyme (E-σ^B). Upon stress, the rsbV-P-specific phosphatase activity of rsbU, a positive activator of sigmaB, becomes activated and thus reactivates rsbV (step 3). Unphosphorylated rsbV forms a complex with rsbW (step 4), thereby releasing sigmaB. RsbW, if complexed with rsbV, is unable to bind to sigmaB, leaving the latter free to form an active sigmaB-holoenzyme (E-σ^B). SigmaB activation leads to the down-regulation of several secreted proteins and up-regulation of cell-wall proteins either directly or indirectly by the repression of agr. This repression of agr is believed to rely on an unidentified mediator.

A.C. Olivier. Intracellular fate of Staphylococcus aureus in human phagocytic and non phagocytic cells and role of virulence factors.
PhD Thesis, 2008

Staphyloxanthin synthesis

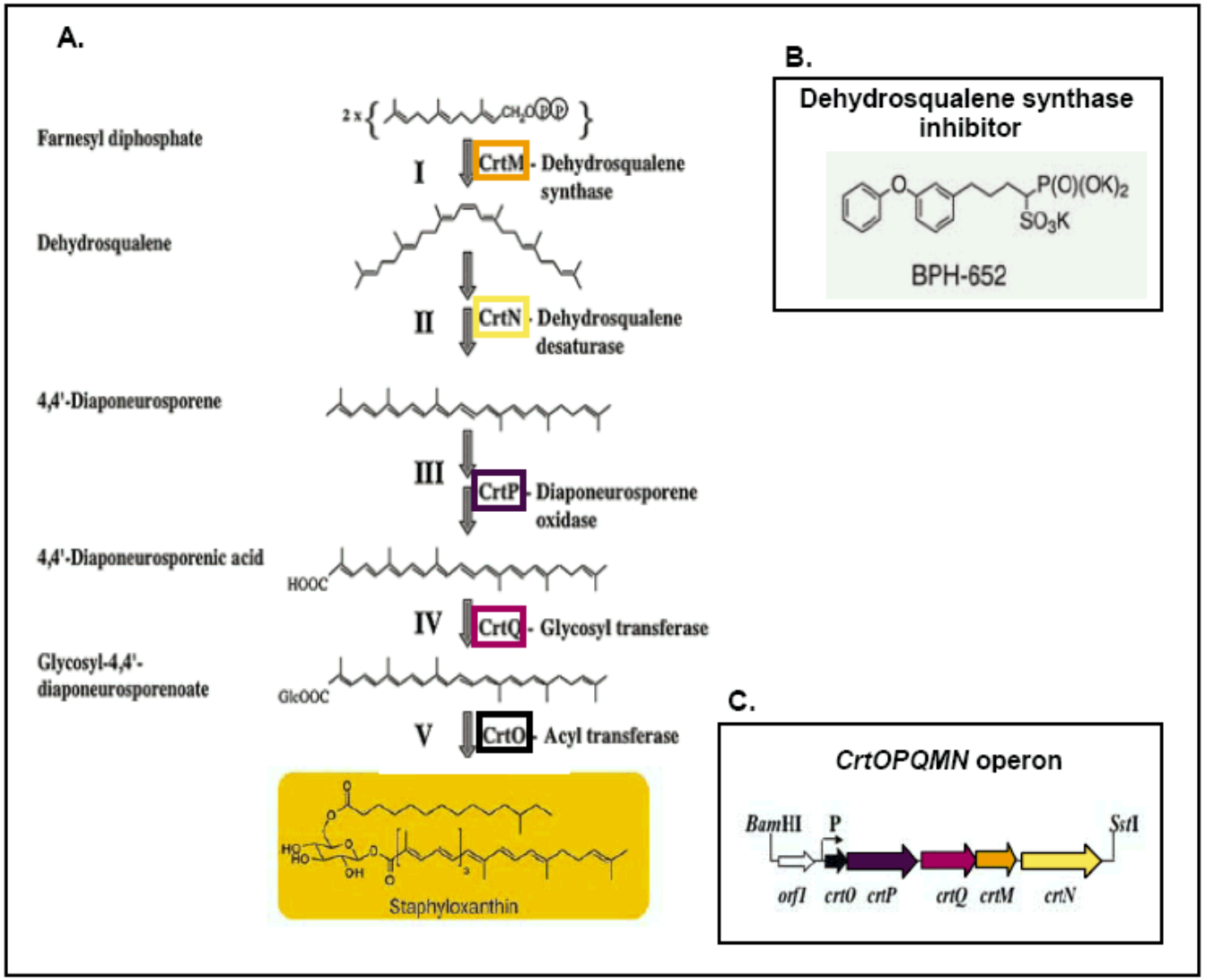
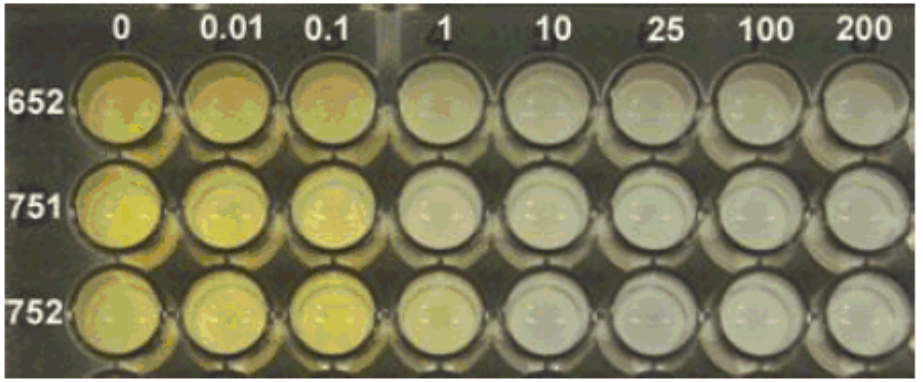


Figure 9 : Adapted from **[48,66]**. **A.** Staphyloxanthin biosynthesis pathway. **B.** Chemical structure of the dehydrosqualene synthase inhibitor BPH-652. BPH-751 is the enantiomer of BHP-652 and BPH-752 is a mixture of BPH-652 and BPH-751. **C.** organization of the *crtOPQMN* operon encoding five enzymes involved in staphyloxanthin synthesis.

Our material….

8325-4 **SH 1000**

Colonies of *S. aureus* 8325-4 (left) naturally deficient for pigment synthesis and SH1000 (right) that produces staphyloxanthin.



SH1000 culture in M-H broth incubated with increasing concentration of inhibitors (0 to 200 µM) illustrating the inhibition of pigment synthesis.

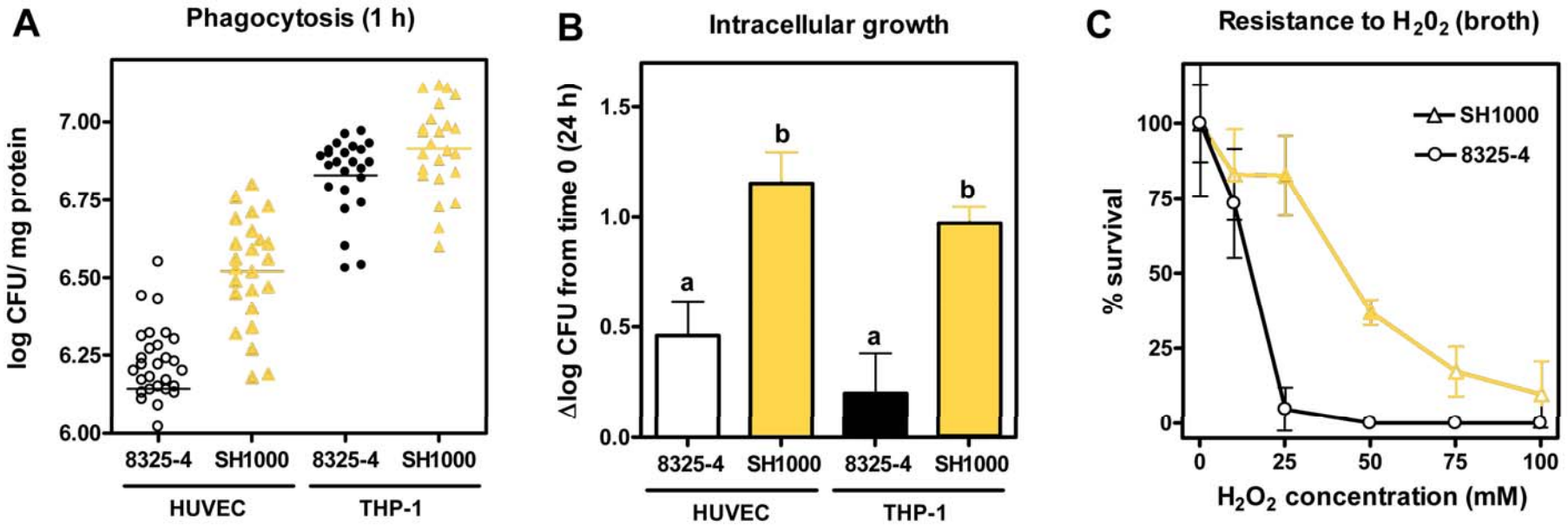
2 isogenic strains
rsbu⁻ and rsbu⁺

inhibitors of staphyloxanthin synthesis *

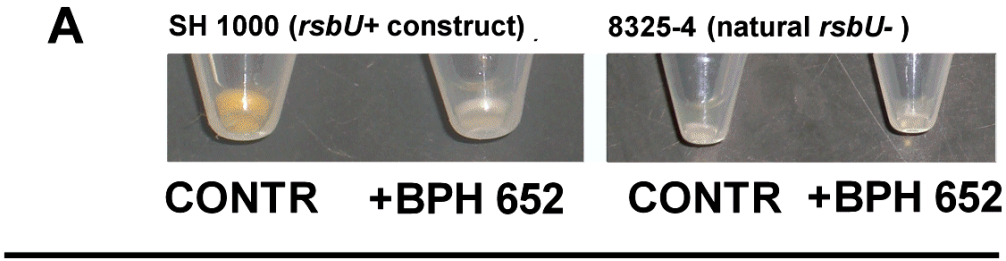
* made by E. Oldfield, Champaign, IL

Olivier *et al.* J. Infect. Dis. in press

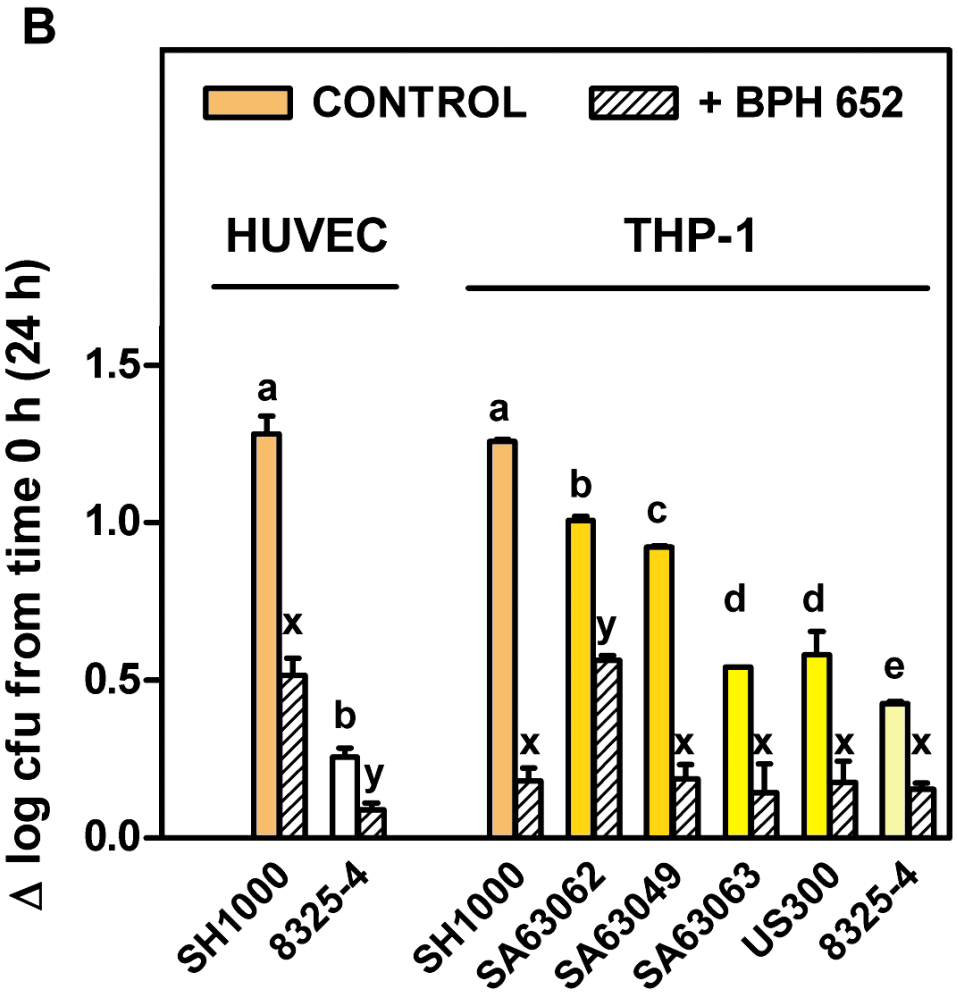
The results….



Olivier et al. J. Infect. Dis. in press



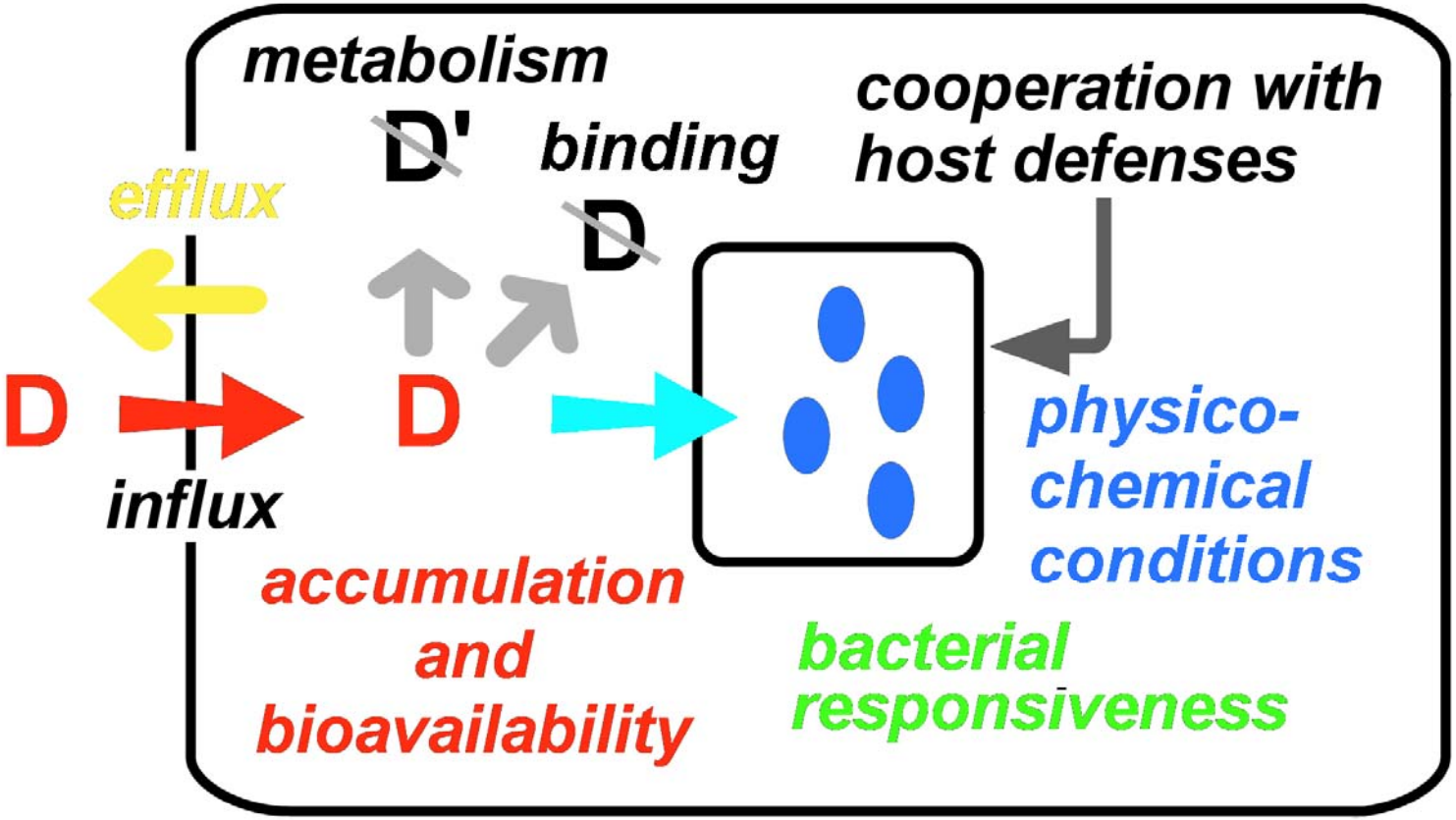
And with other strains….



- the more staphyloxanthin is produced, the more successful is the strain for intracellular growth …
- blocking staphyloxanthin synthesis makes all strain equally ill-successful…

Olivier *et al.* J. Infect. Dis. in press

Antibiotics and intracellular infection…



Carryn *et al.* Infect Dis Clin North Am. 2003 Sep;17(3):615-34.

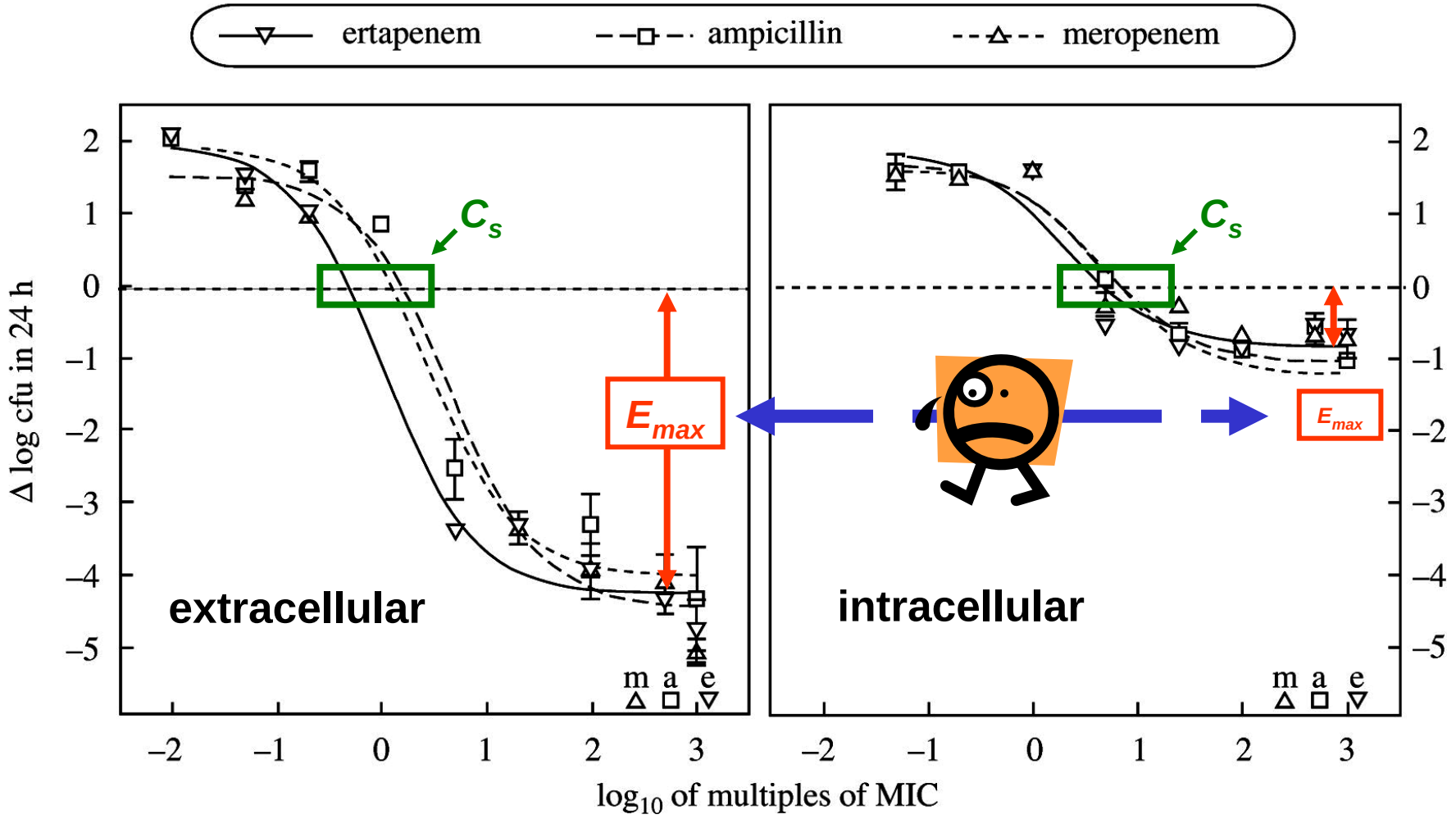
β-lactams: a **pharmacokinetic** paradox

Accumulation of antibiotics in macrophages

- **beta-lactams: ≤ 1x**
- **aminoglycosides: <1 to 2 x**
- **ansamycins: 2-3 x**
- **tetracyclines: 2-4 x**
- **fluoroquinolones: 10-20 x**
- **macrolides: 4 to > 100 x**

Tulkens PM, Intracellular distribution and activity of antibiotics. Eur J Clin Microbiol Infect Dis. 1991; 10:100-6

β-lactams and *S. aureus*... (MSSA)



Lemaire et al., JAC 55:897-904, 2005

The intracellular **pharmacodynamic** paradox of β-lactams…

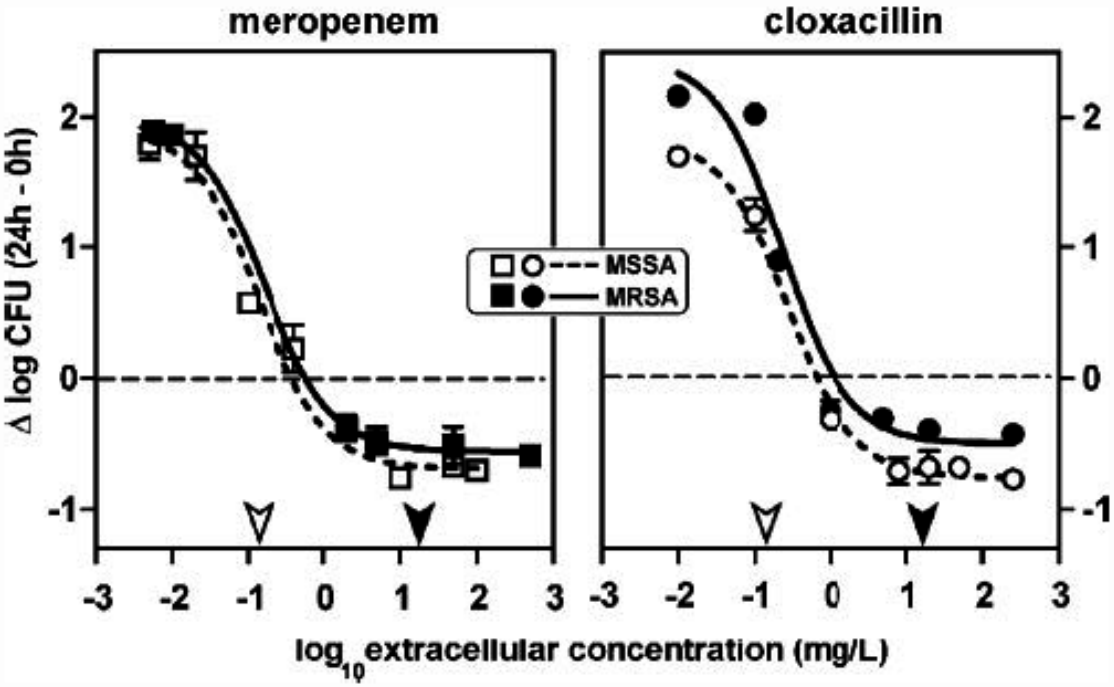
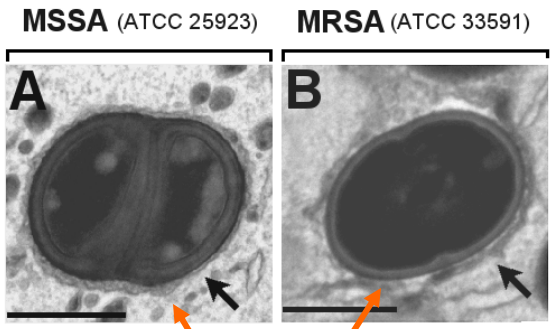


FIG. 1. Concentration killing effects of meropenem (squares; left panel) and cloxacillin (circles; right panel) toward MSSA strain ATCC 25923 (open symbols and dotted line) and MRSA strain ATCC 33591 (closed symbols and continuous line) after phagocytosis by THP-1 macrophages. Cells were incubated with the antibiotics for 24 h at the concentrations (total drug) indicated on the abscissa. All values are the means ± standard deviations of three independent determinations (standard deviation bars that are not visible are smaller than the size of the symbols). The arrows along the abscissa point to the MIC of the organisms determined in broth at pH 7.4 (open arrows, MSSA strain ATCC 25923; closed arrows, MRSA ATCC 33591).



Hint:
pH in vacuoles is acidic

Lemaire et al., AAC (2007) 51:1627-1632

Back to the future…

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1972, p. 350–355

Vol. 2, No. 5
Printed in U.S.A.

Suppression of Intrinsic Resistance to Methicillin and Other Penicillins in *Staphylococcus aureus*

L. D. SABATH, STEVEN J. WALLACE, AND DEBORAH A. GERSTEIN

Channing Laboratory and Thorndike Memorial Laboratory, Harvard Medical Unit, Boston City Hospital, and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02118

Received for publication 5 September 1972

TABLE 1. *Effect of pH of medium (BHI) on methicillin resistance of Staphylococcus aureus*

Antibiotic	*S. aureus* strain[a]	MIC (μg/ml) when tested at		Ratio[b]
		*p*H 7.4	*p*H 5.2	
Methicillin	M-S 1^{P+}	6.2	3.1	2
	M-S Oxford^{P-}	3.1	1.6	2
	M-R 1^{P+}	1,600	12.5	128
	M-R 1^{P-}	1,600	12.5	128
	M-R Col^{P-}	1,600	12.5	128
Cloxacillin	M-S 1^{P+}	1.6	0.2	8
	M-S Oxford^{P-}	0.4	0.1	4
	M-R 1^{P+}	1,600	0.4	4,000
	M-R 1^{P-}	1,600	0.4	>4,000
	M-R Col^{P-}	1,600	0.4	>4,000

Biochemistry comes to your help…

Supplemental Material can be found at:
http://www.jbc.org/cgi/content/full/M800079200/DC1

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 19, pp. 12769–12776, May 9, 2008

Restoration of Susceptibility of Methicillin-resistant *Staphylococcus aureus* to β-Lactam Antibiotics by Acidic pH

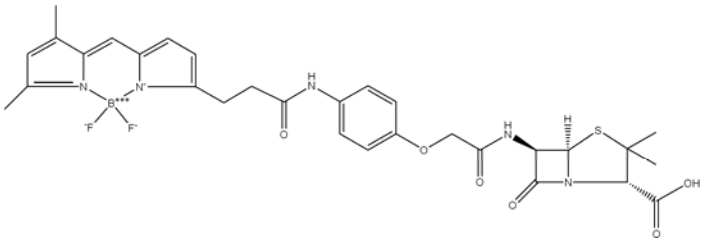
ROLE OF PENICILLIN-BINDING PROTEIN PBP 2a[*S]

Received for publication, January 4, 2008, and in revised form, March 11, 2008 Published, JBC Papers in Press, March 12, 2008, DOI 10.1074/jbc.M800079200

Sandrine Lemaire[‡1], Cosimo Fuda[§], Françoise Van Bambeke[‡2], Paul M. Tulkens[‡], and Shahriar Mobashery[§]

From the [‡]Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, B-1200 Brussels, Belgium and the [§]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

Acid pH favors penicillin binding to PBP2a



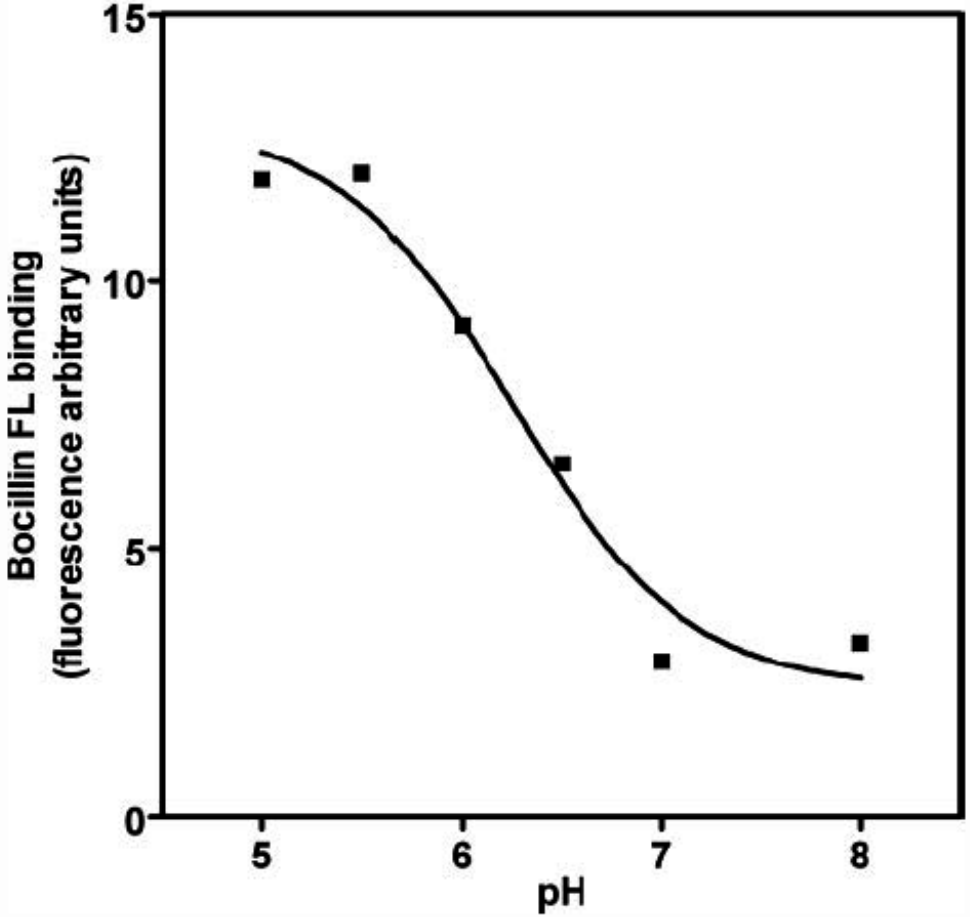


FIGURE 2. **Influence of pH on the binding of Bocillin FL to whole cells and to purified PBP 2a.** *Upper panel*, growing bacteria were incubated in broth at 37 °C with Bocillin FL for 30 min at the pH indicated in the abscissa, and the samples were prepared for fluorescence measurement. *White bars*, MSSA ATCC 25923; *gray bars*, MRSA COL. The values are the means ± S.D. ($n = 3$). *Bars* with different letters are significantly different from all others ($p < 0.01$). *Lower panel*, Bocillin FL (0.2 μg) was mixed for 20 min with 3 μM purified PBP 2a in 50 mM phosphate buffers adjusted to different pH values before being applied to gel for electrophoretic separation (the value recorded in HEPES buffer at pH 7.0 was not significantly different from that shown for the corresponding phosphate buffer here).

Lemaire et al., JBC (2008) 283:12769-12776

Acid pH favors oxacillin-induced conformational change in PBP2a

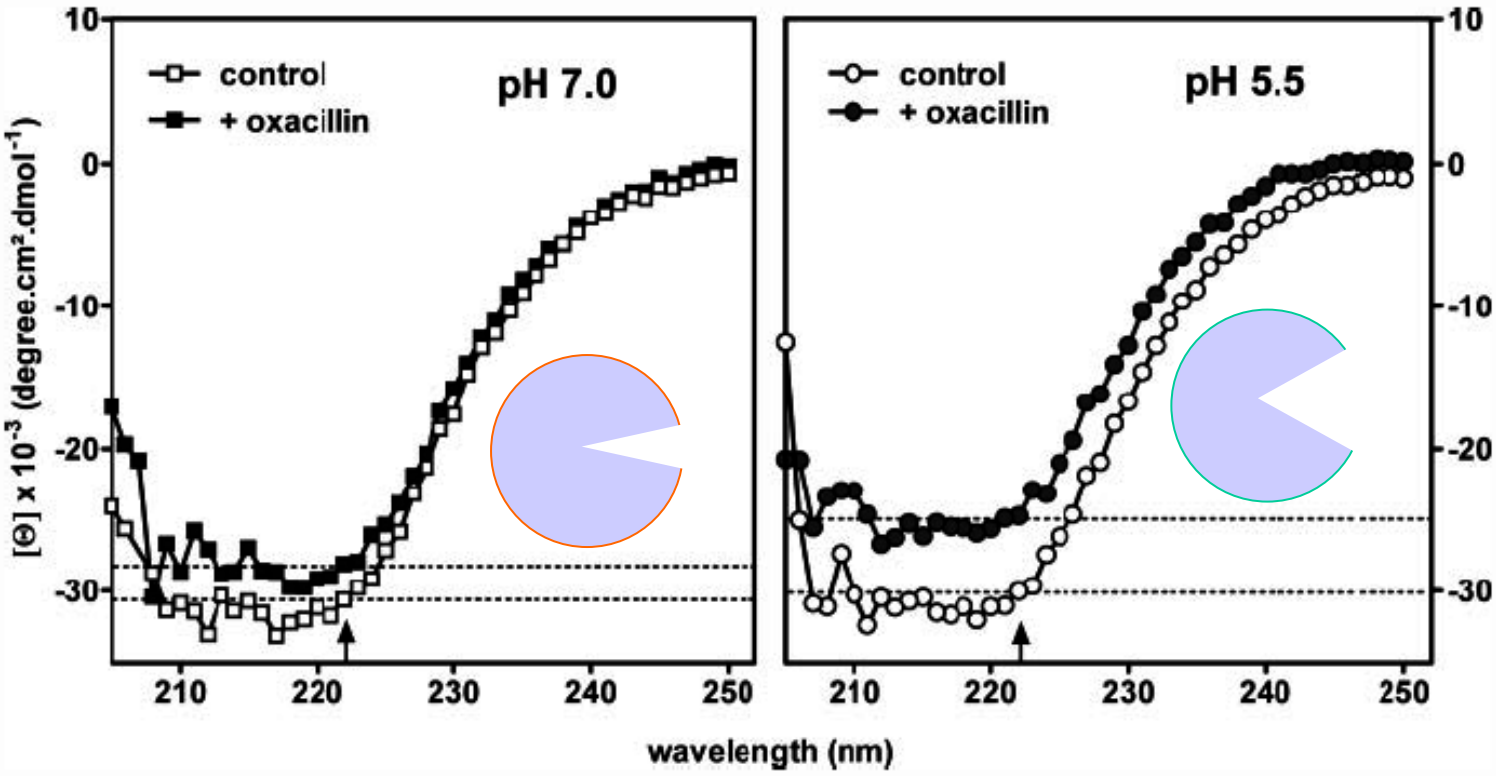
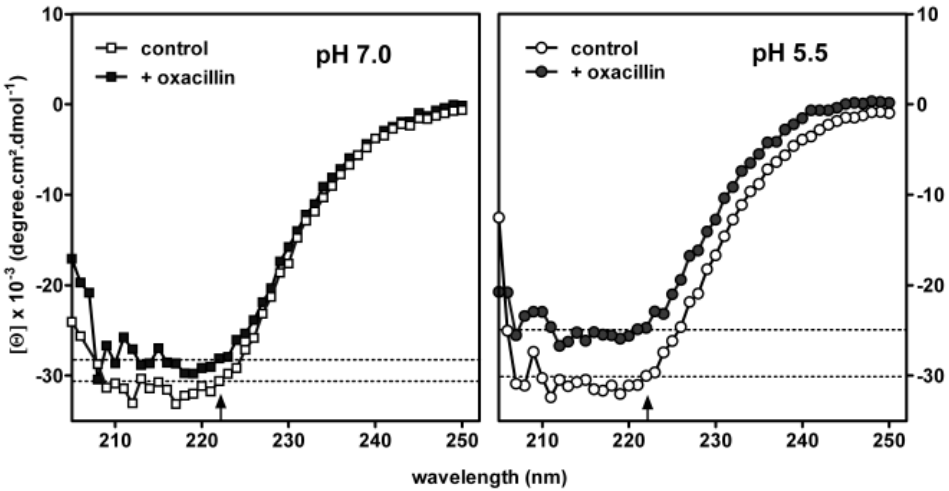


FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7.0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at 222 nm (*vertical arrow* on the *abscissa*) for each condition. The spectrum of oxacillin has been subtracted from all data points.

Lemaire et al., JBC (2008) 283:12769-12776

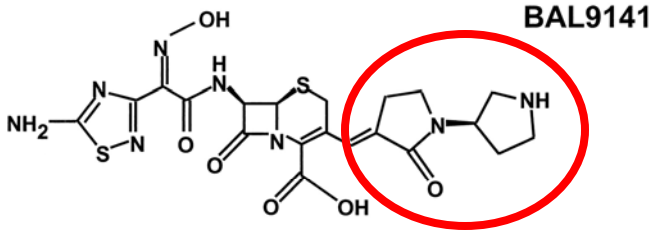
PBP2a: acid pH *vs.* ceftobiprole

Figure 4



acid pH and PBP2a

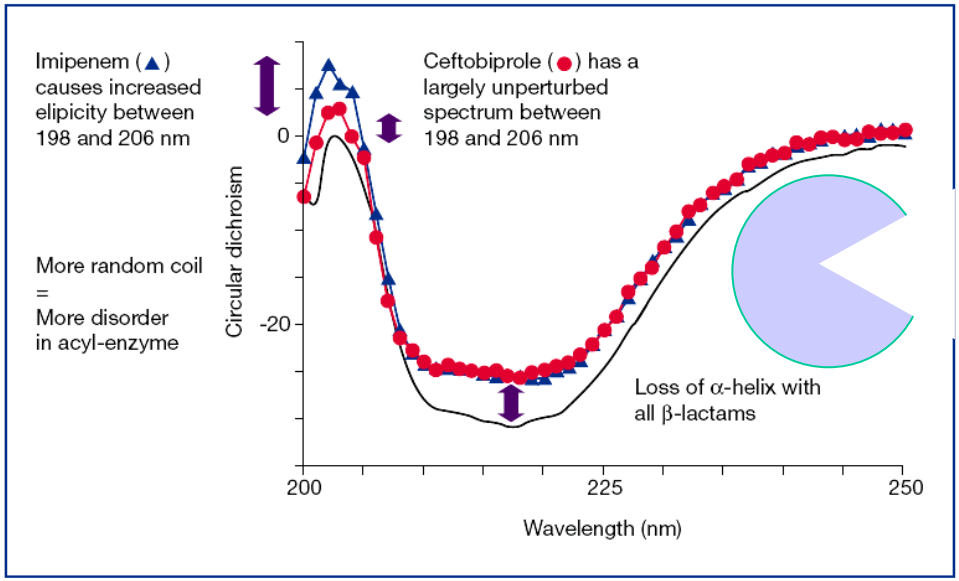
Lemaire et al., J Biol Chem. 2008;283(19):12769-76.



ceftobiprole and PBP2a

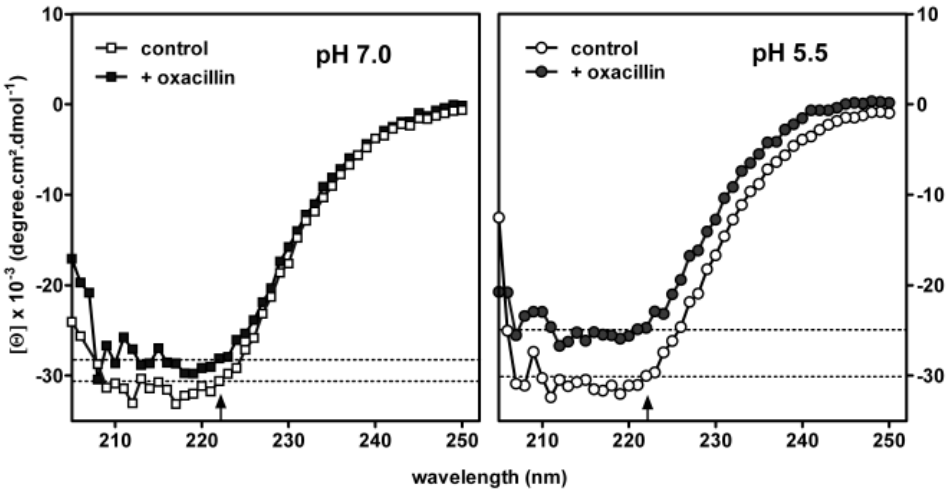
Lovering et al. ECCMID 2006, Abstract P1586.

Figure 5. Loss of secondary structure accompanies acylation.



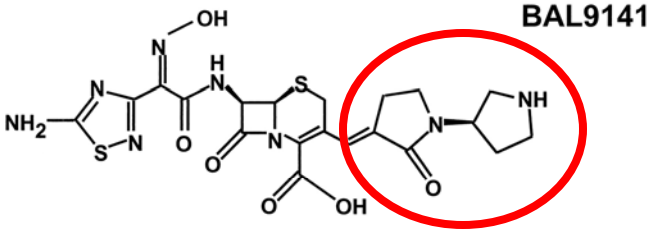
PBP2a: acid pH *vs.* ceftobiprole

Figure 4



acid pH and PBP2a

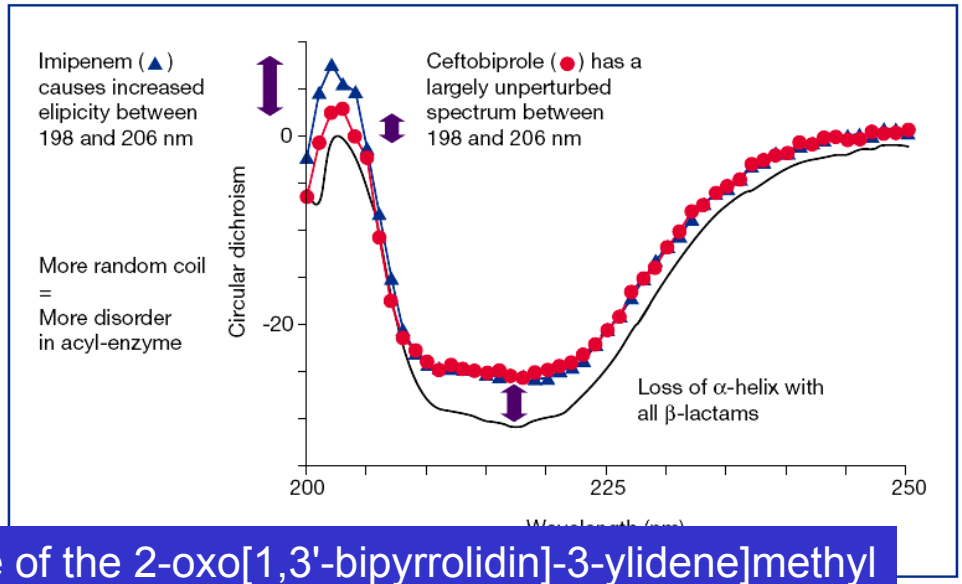
Lemaire et al., J Biol Chem. 2008;283(19):12769-76.



ceftobiprole and PBP2a

Lovering et al. ECCMID 2006, Abstract P1586.

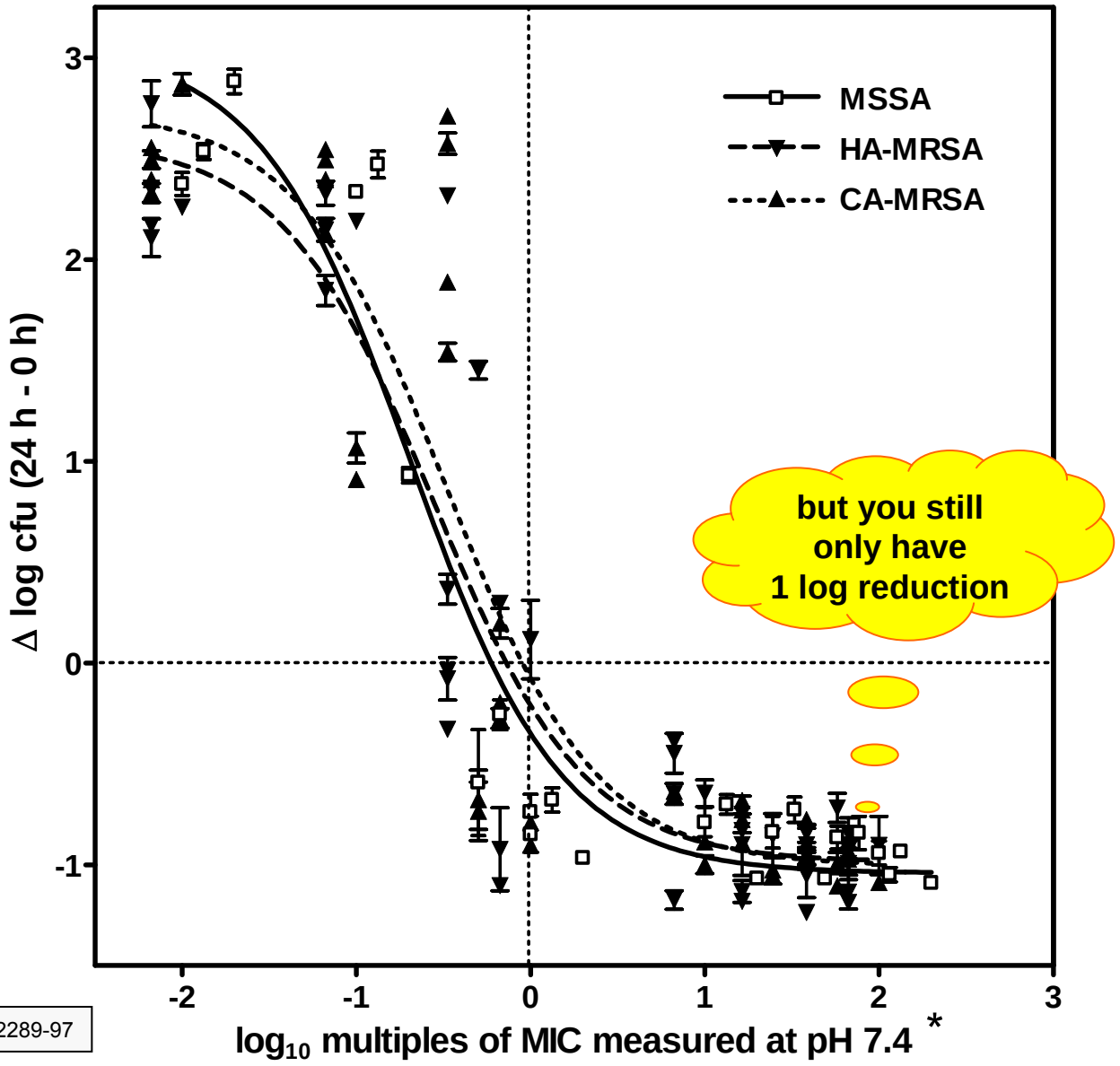
Figure 5. Loss of secondary structure accompanies acylation.



Conclusion: acid pH **or** the presence of the 2-oxo[1,3'-bipyrrolidin]-3-ylidene]methyl moiety (ceftobiprole) induces PBP2a conformational change facilitating acylation

Ceftobiprole is equally active against intracellular (THP-1 macrophages)

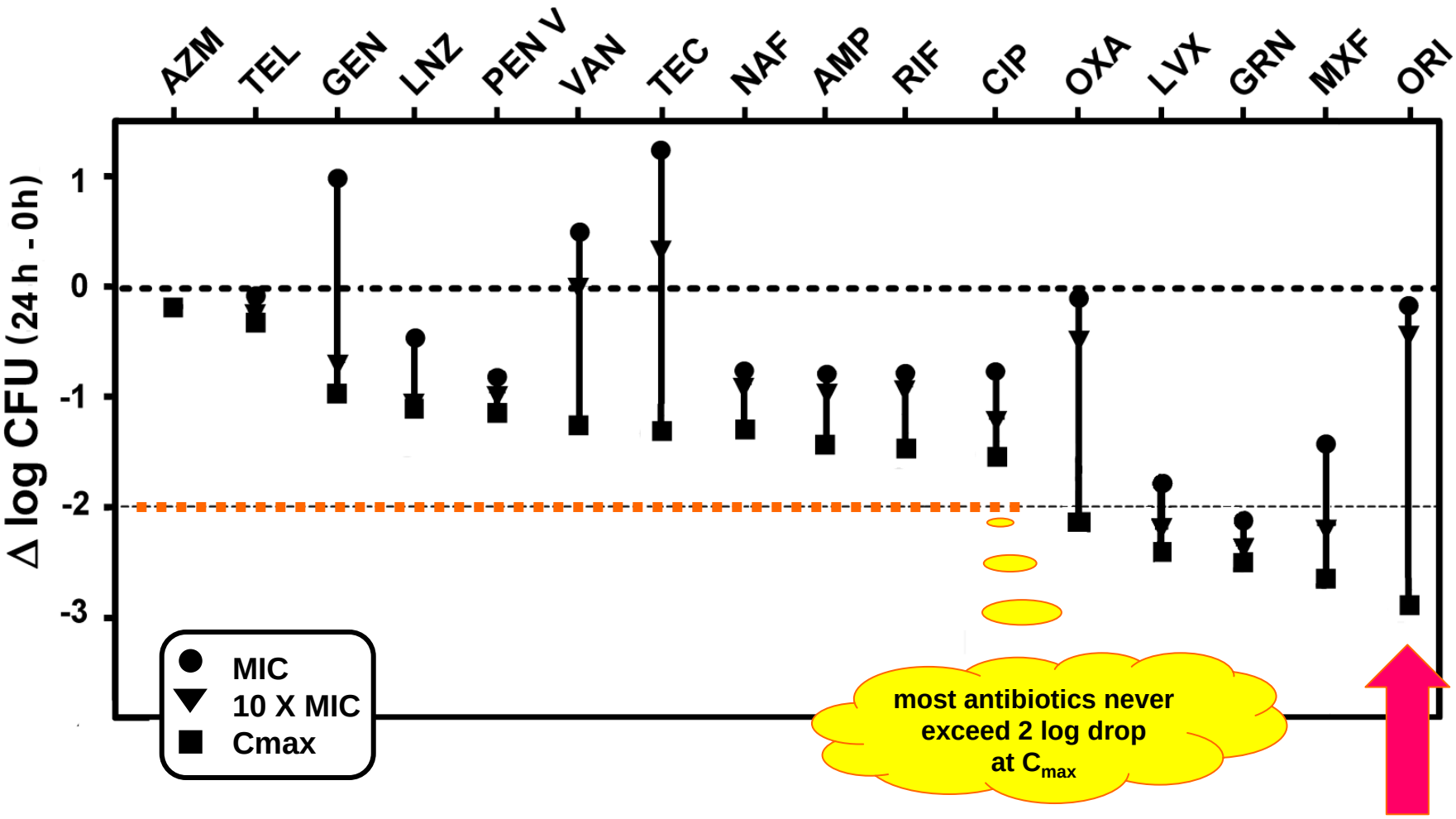
- MSSA,
- HA-MRSA, or
- CA-MRSA



Lemaire et al., Antimicrob Agents Chemother. 2009; 53:2289-97

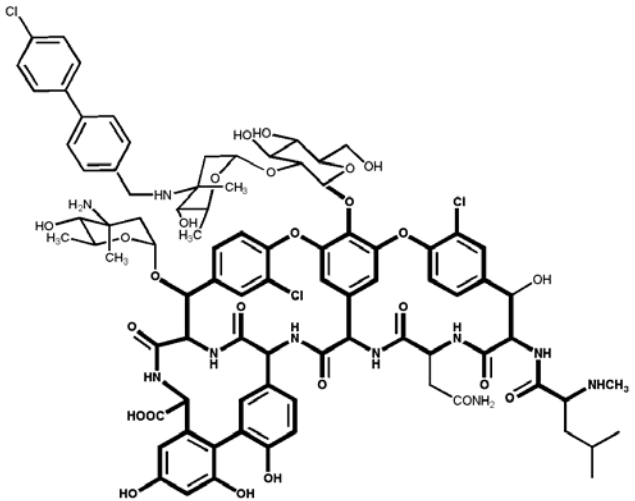
* all MICs were 0.5-2 mg/L

A summary PK/PD paradox for other antibiotics

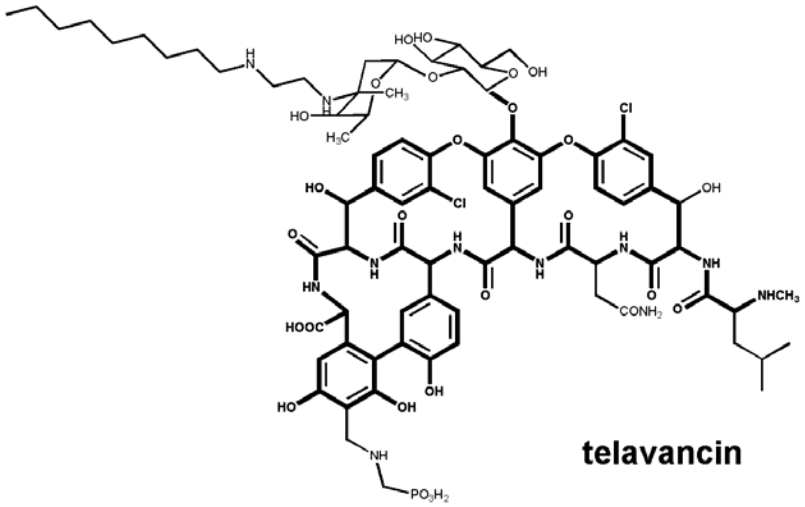


Barcia-Macay *et al.* Antimicrob Agents Chemother. 2006; 50:841-51.

New glycopeptides (oritavancin,telavancin)



oritavancin



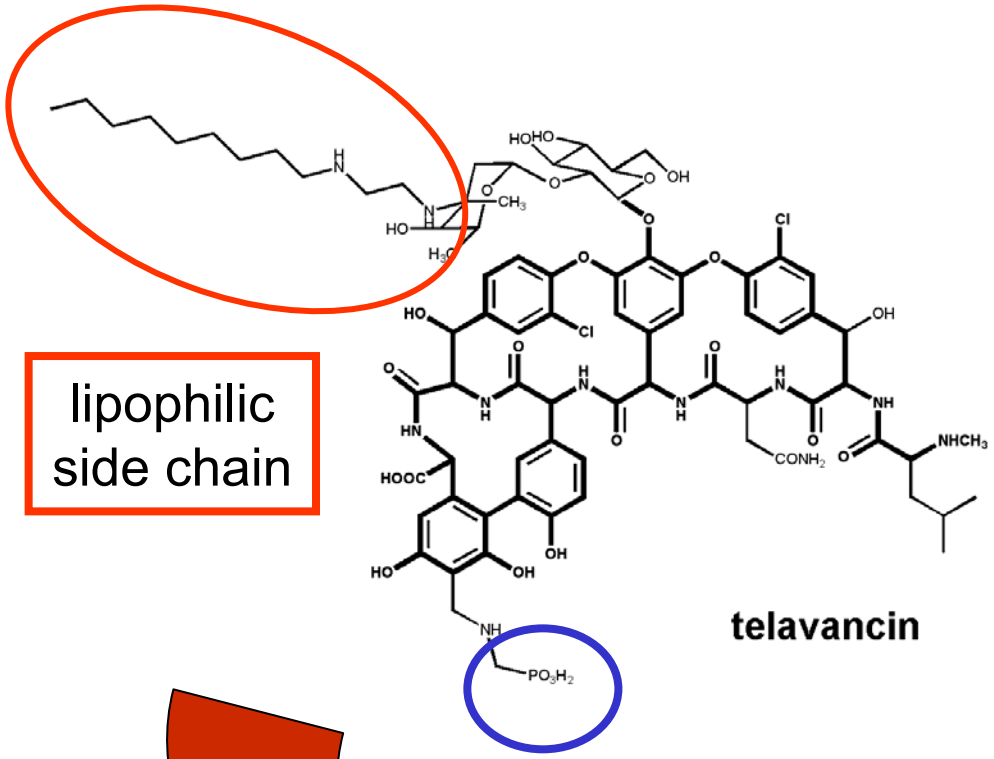
telavancin

Van Bambeke, Current Opinion in Pharmacology (2004) 4:471-478

Telavancin (and oritavancin) new modes of action …

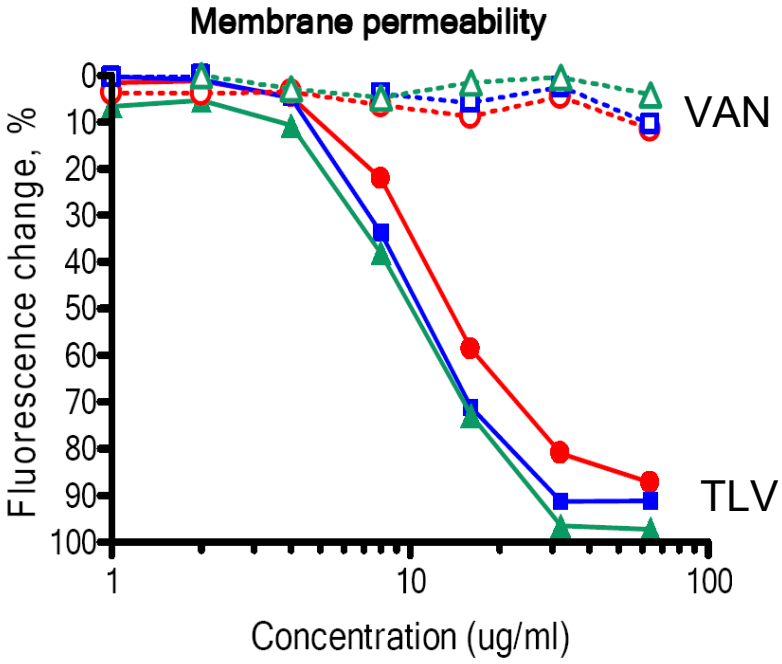
- Possibility of dimerization
  - ➔ potential increase of intrinsic activity against D-Ala-D-Ala displaying organisms (MSSA, MRSA, VISA)
- Membrane destabilization effects…
  - ➔ strong concentration-dependent bactericidal effect (all strains ...) *

Telavancin …



lipophilic side chain

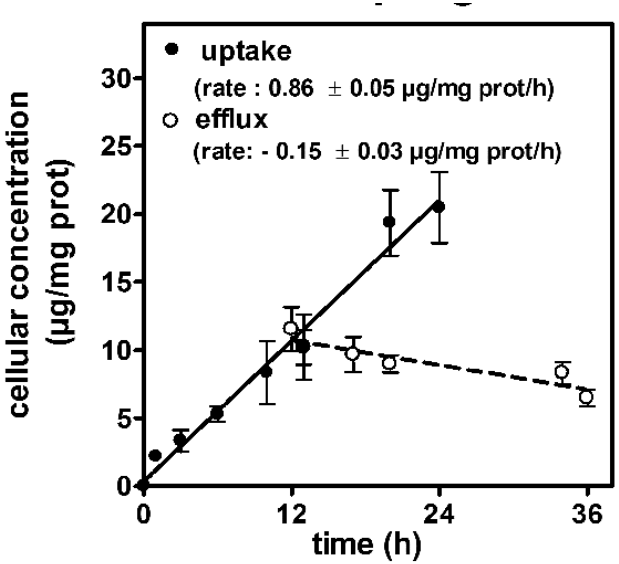
polar group

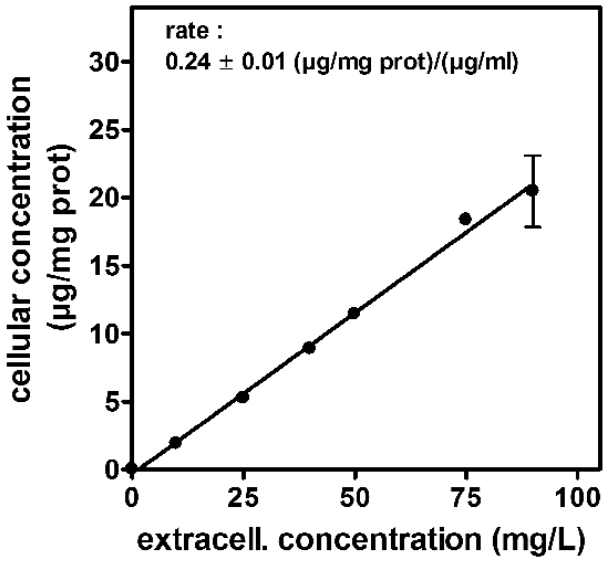


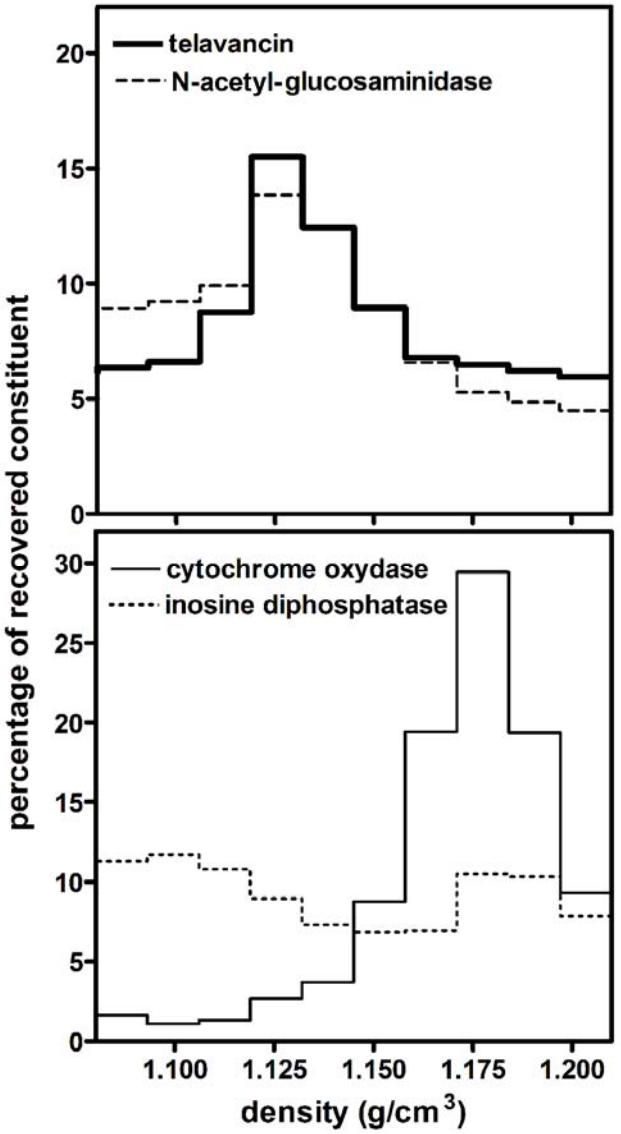
this causes increase in bacterial membrane permeability

Higgins *et al*., Antimicrob Agents Chemother. (2005) 49:1127-34

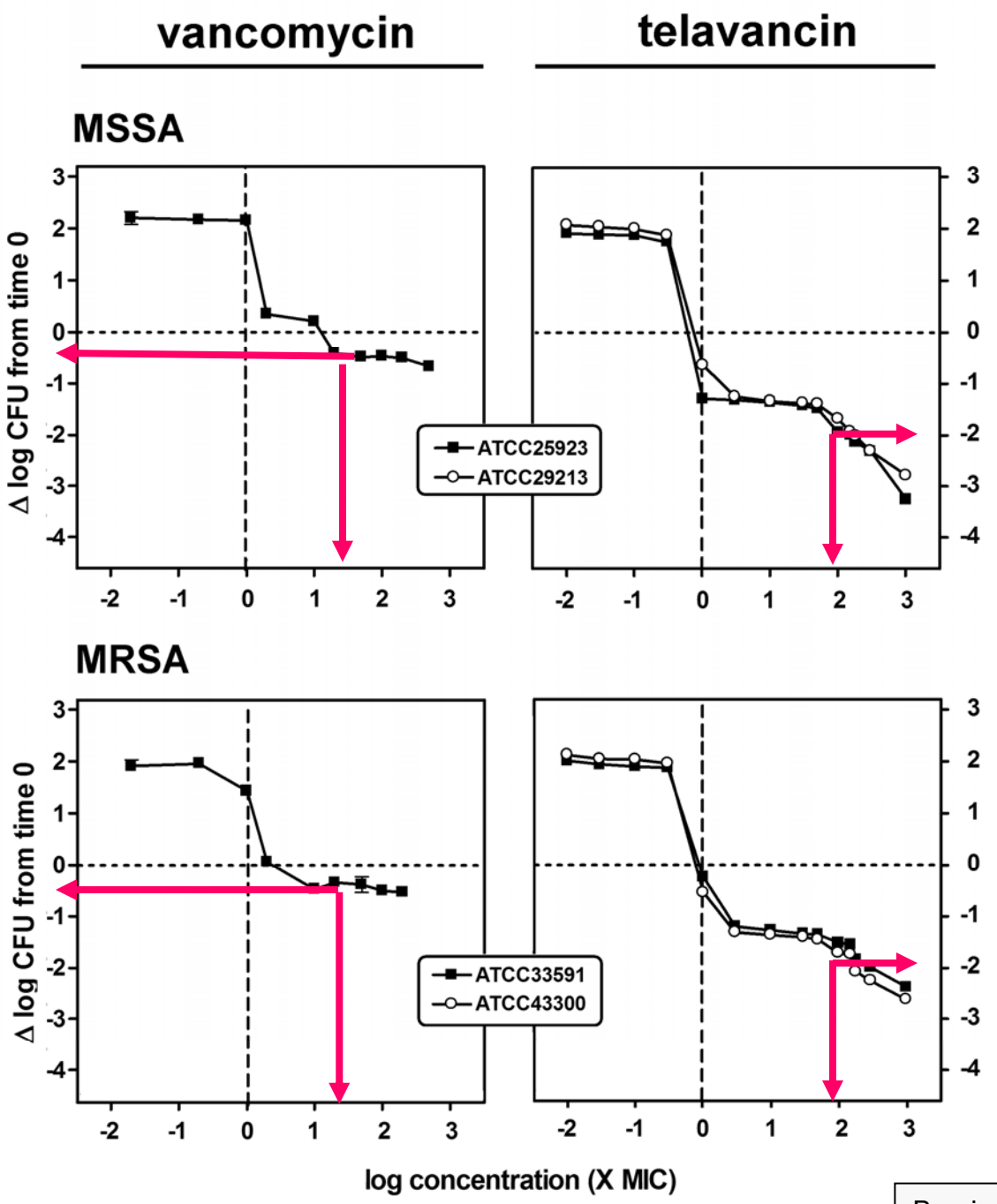
Telavancin intracellular accumulation and subcellular disposition







Barcia-Macay et al? JAC 2008; 61:1288-1294



Intracellular activity of telavancin *vs.* vancomycin:

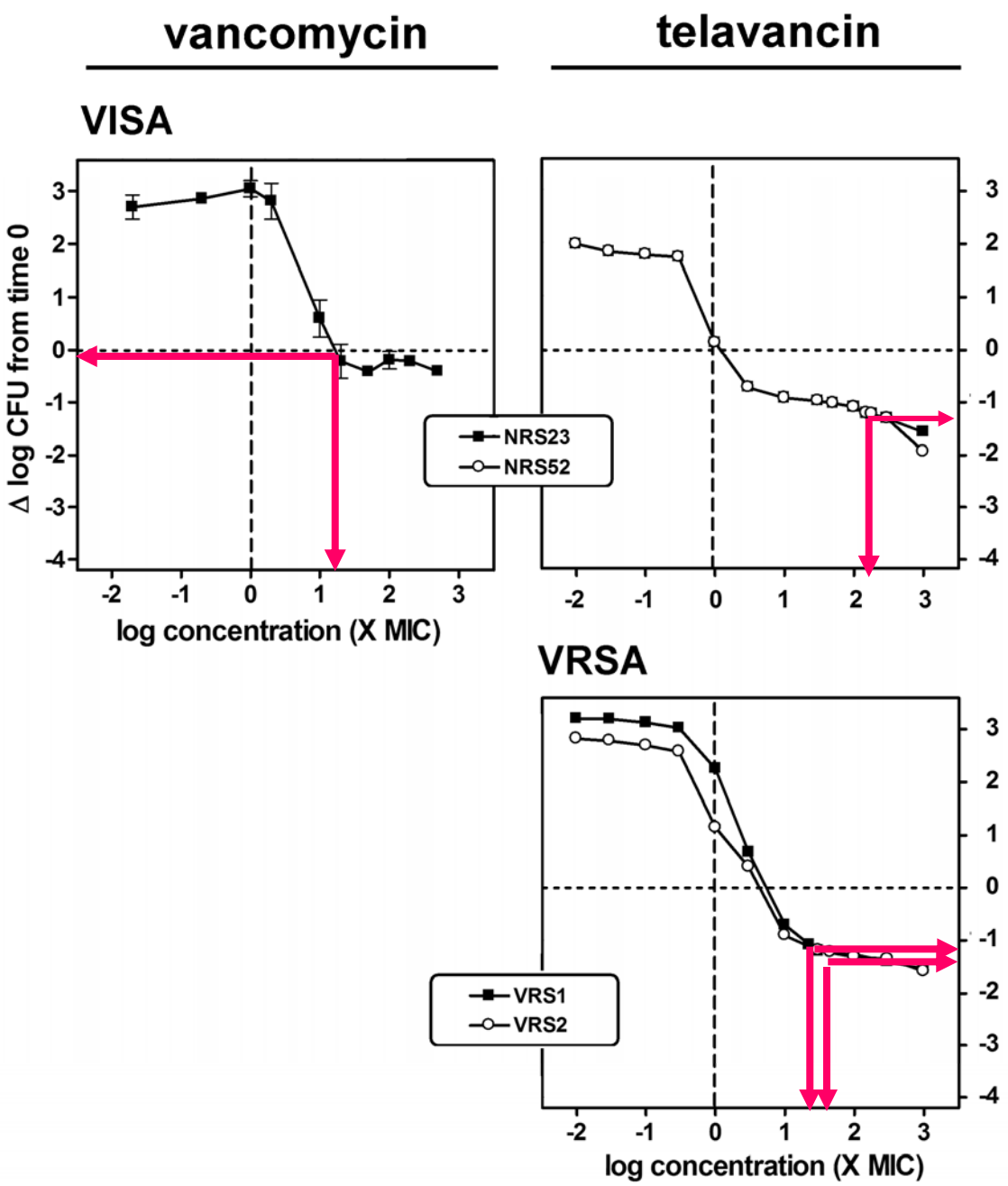
➔ MSSA

➔ MRSA

24h CFU ↘ at C_{max}:

- vanco: ~ 0.5 log
- TLV: ~ 2 log

Barcia-Macay *et al.,* JAC 2006; 58:1177–1184



Intracellular activity of telavancin *vs.* vancomycin:

➔ VISA

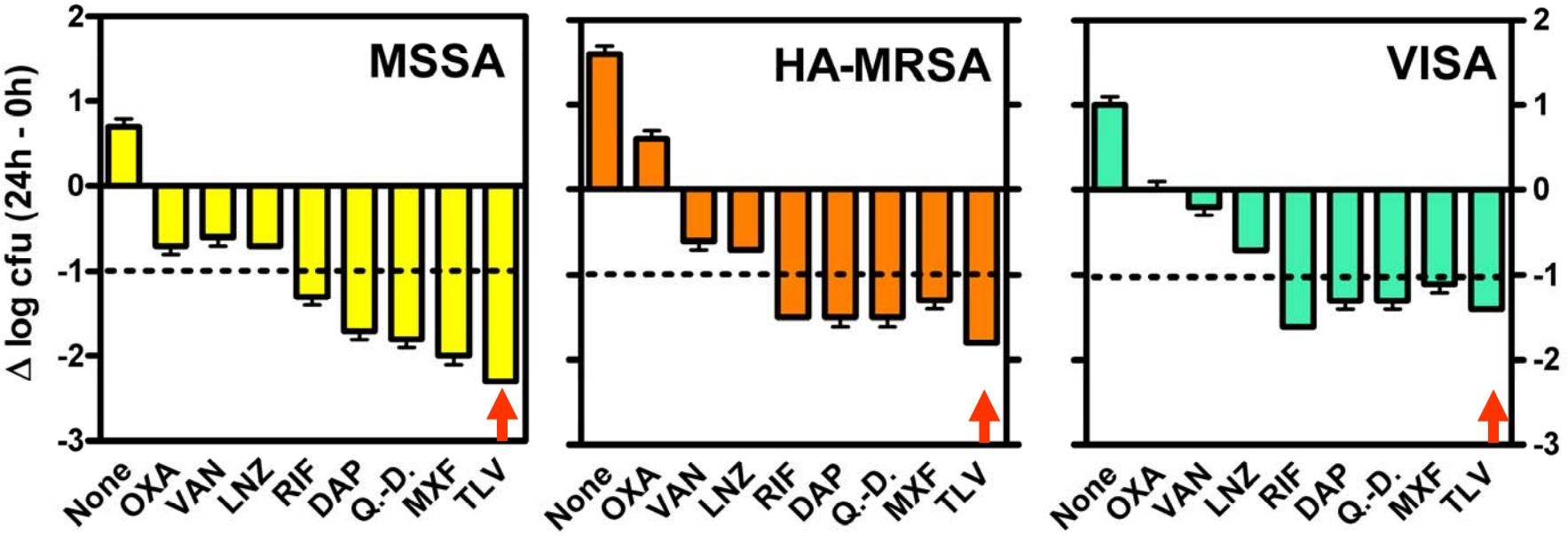
➔ VRSA

24h CFU ↘ at C_{max}:

- vanco: static
- TLV: ~ 1.2 log

Barcia-Macay *et al.*, JAC 2006; 58:1177–1184

Telavancin intracellular activity in comparison with other drugs…



Lemaire et al., ISSSI 2006 – Sept. 3-6, 2006

SCVs of *S. aureus* as a cause of persistent foreign body infection

Rev Infect Dis. 1987 Nov-Dec;9(6):1168-74.

Prosthetic valve endocarditis due to small-colony staphylococcal variants.

Baddour LM, Christensen GD.

Clin Infect Dis. 1999 Oct;29(4):932-4.

Bloodstream infections caused by small-colony variants of coagulase-negative staphylococci following pacemaker implantation.

von Eiff C, Vaudaux P, Kahl BC, Lew D, Emler S, Schmidt A, Peters G, Proctor RA.

Emerg Infect Dis. 2003 Oct;9(10):1316-8.

Small colony variants of Staphylococcus aureus and pacemaker-related infection.

Seifert H, Wisplinghoff H, Schnabel P, von Eiff C.

Int J Artif Organs. 2006 Apr;29(4):360-7.

Emerging Staphylococcus species as new pathogens in implant infections.

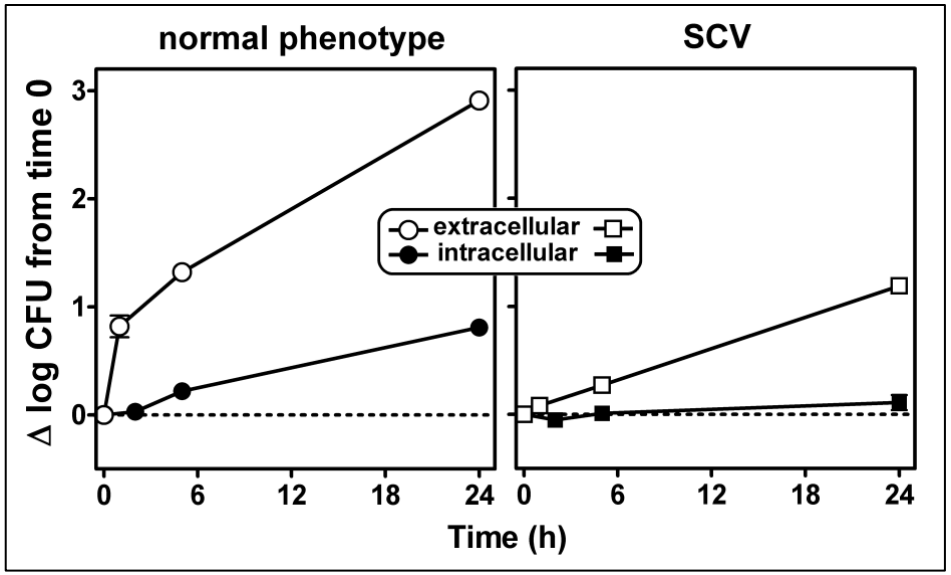
von Eiff C, Arciola CR, Montanaro L, Becker K, Campoccia D.

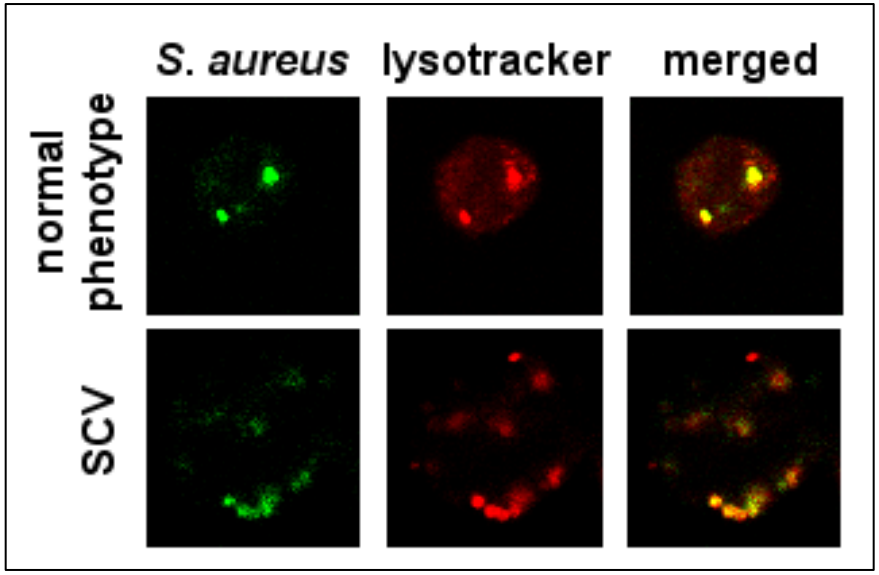
Int J Artif Organs. 2007 Sep;30(9):778-85.

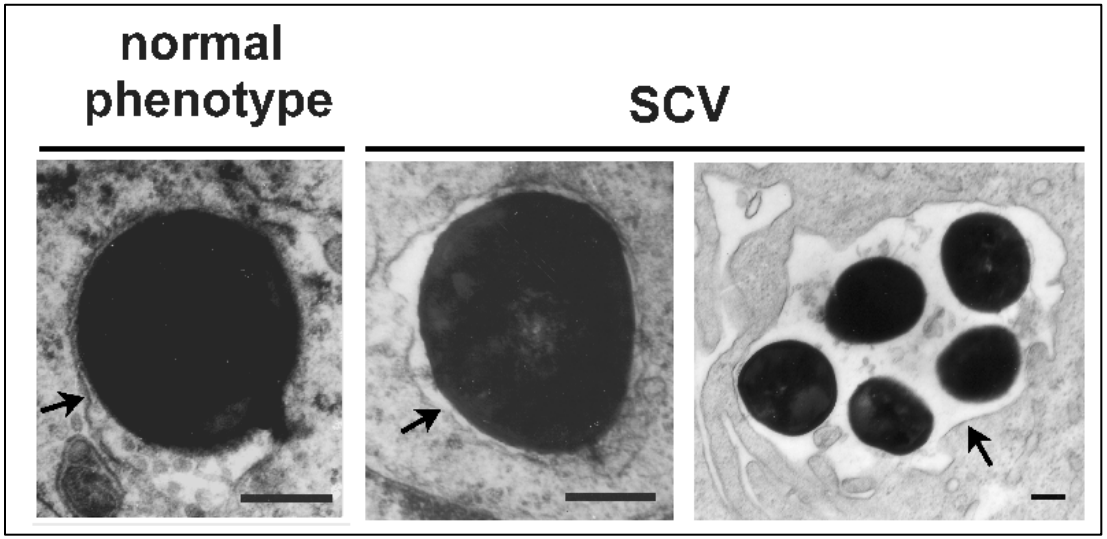
Small-colony variants (SCVs) of staphylococci: a role in foreign body-associated infections.

von Eiff C, Becker K.

Intracellular SCVs…

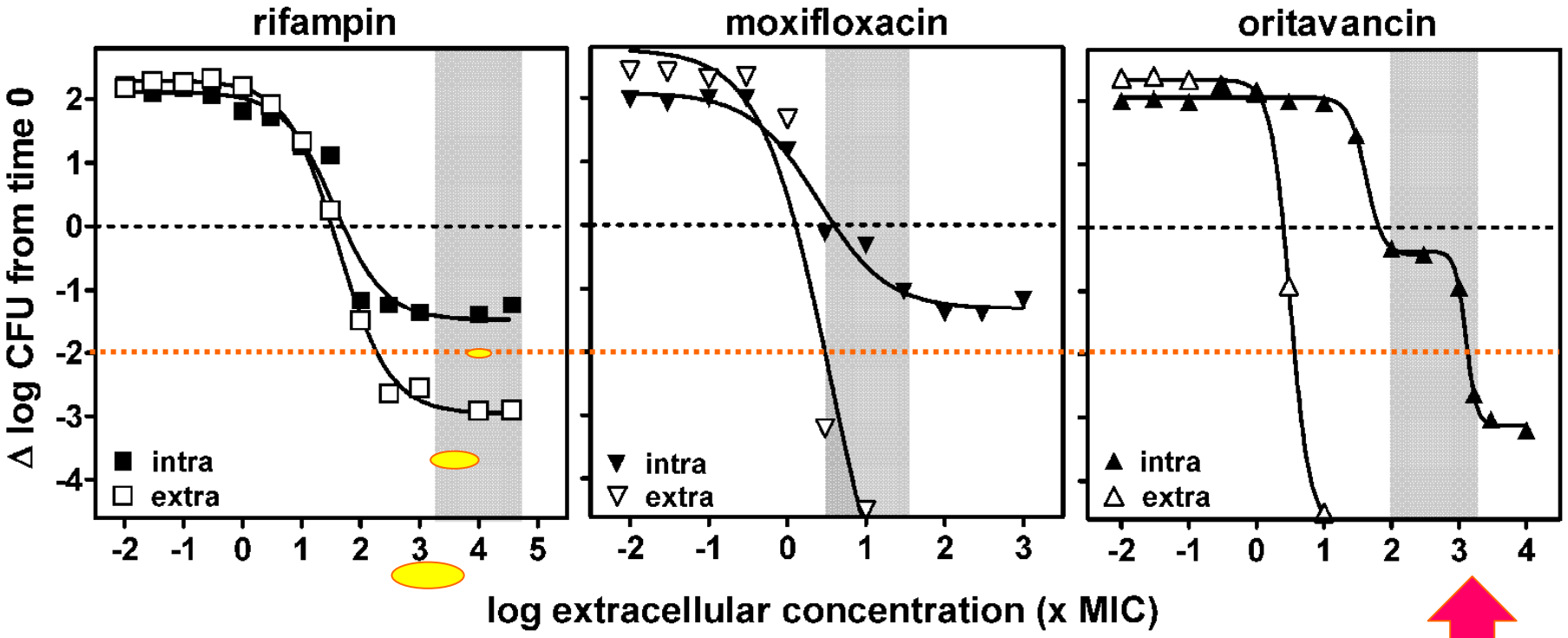






Nguyen et al., AAC 2009; 53:1434–1442

Intracellular SCVs and antibiotics …

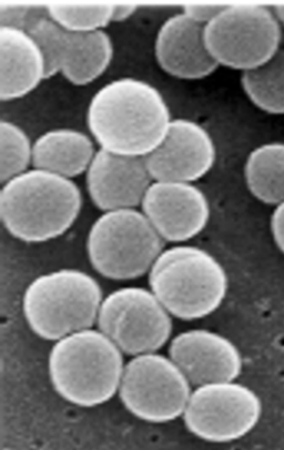


Nguyen et al., AAC 2009; 53:1434–1442

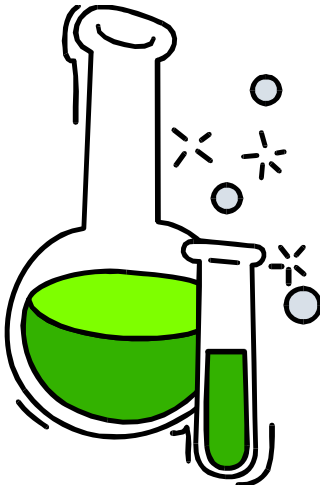
it's hard to exceed 2 log drop…

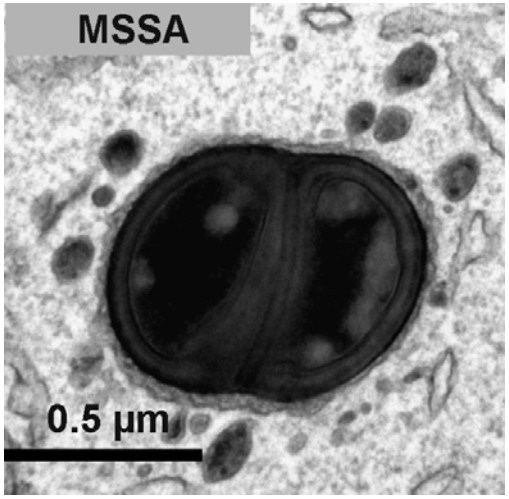
Perhaps due to membrane-detabilization effect …

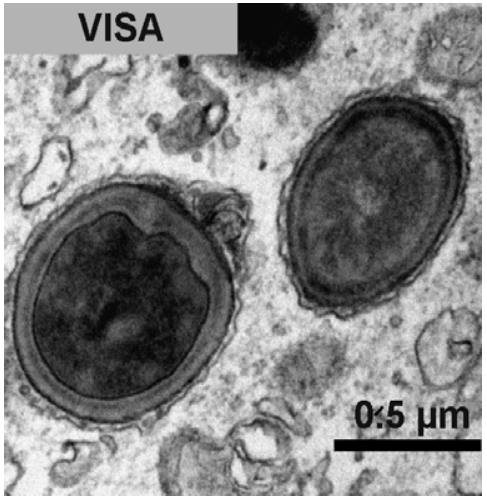
- Domenech et al. Biochim Biophys Acta. 2009 May 18. [Epub]
- Baudoux et al. ICAAC 2009 [C1-1354]

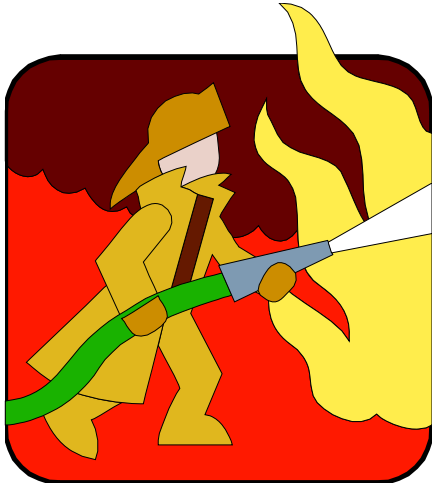


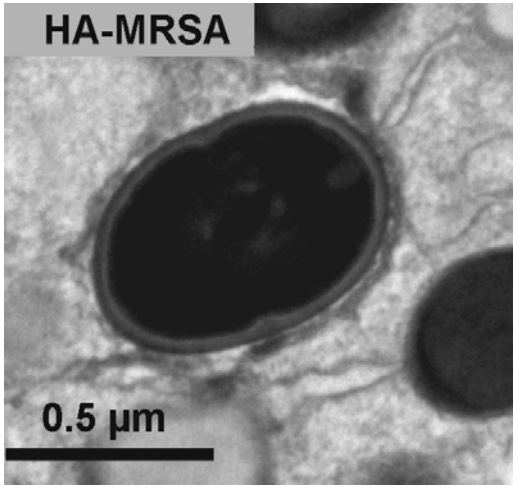
Will this be successful ?



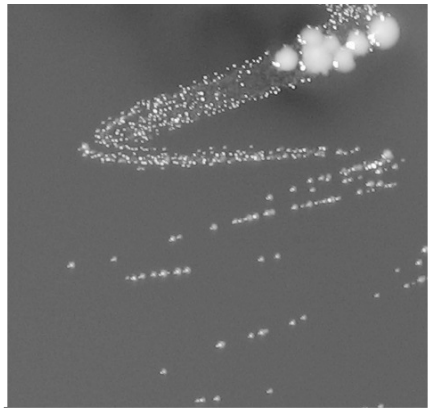




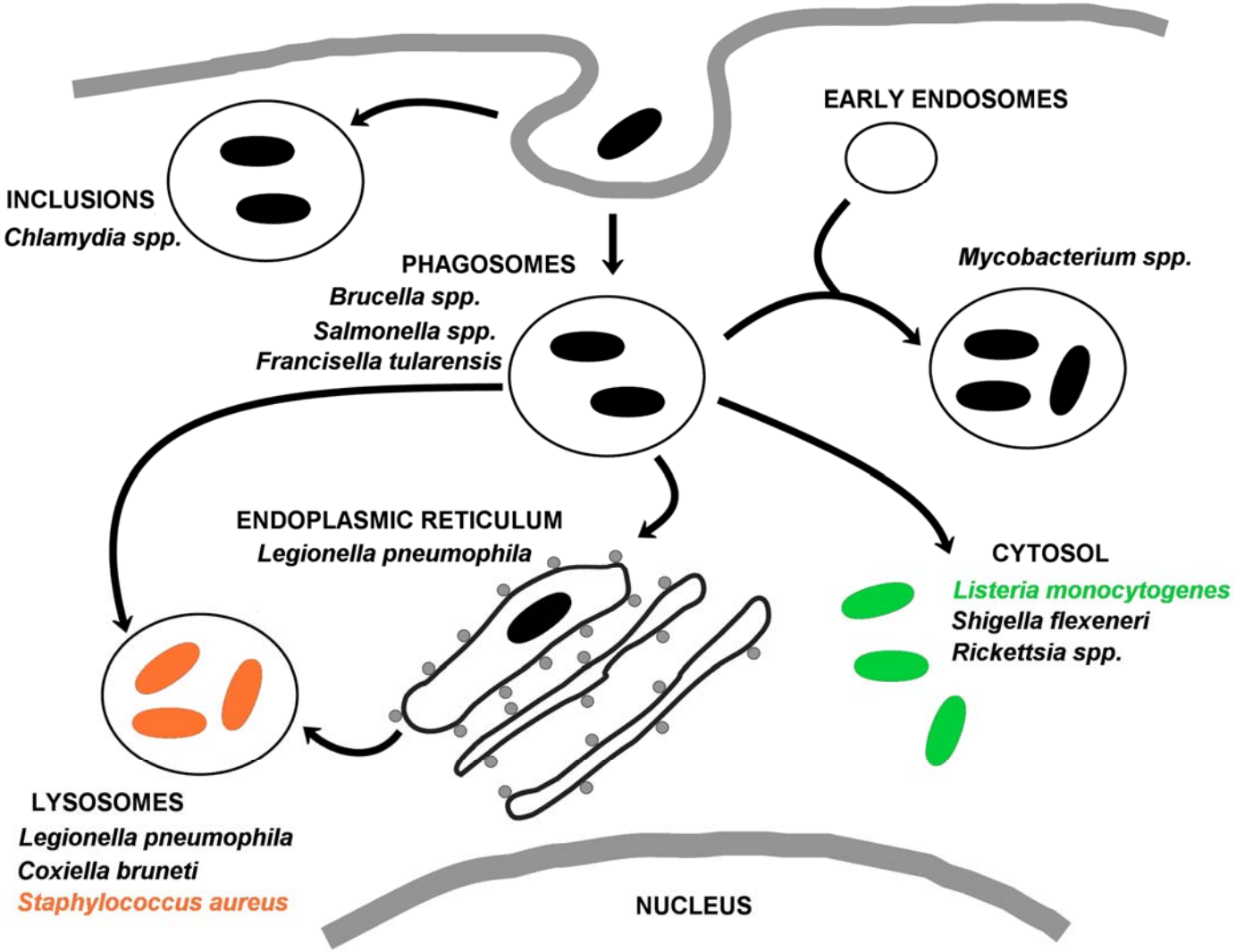




SCV

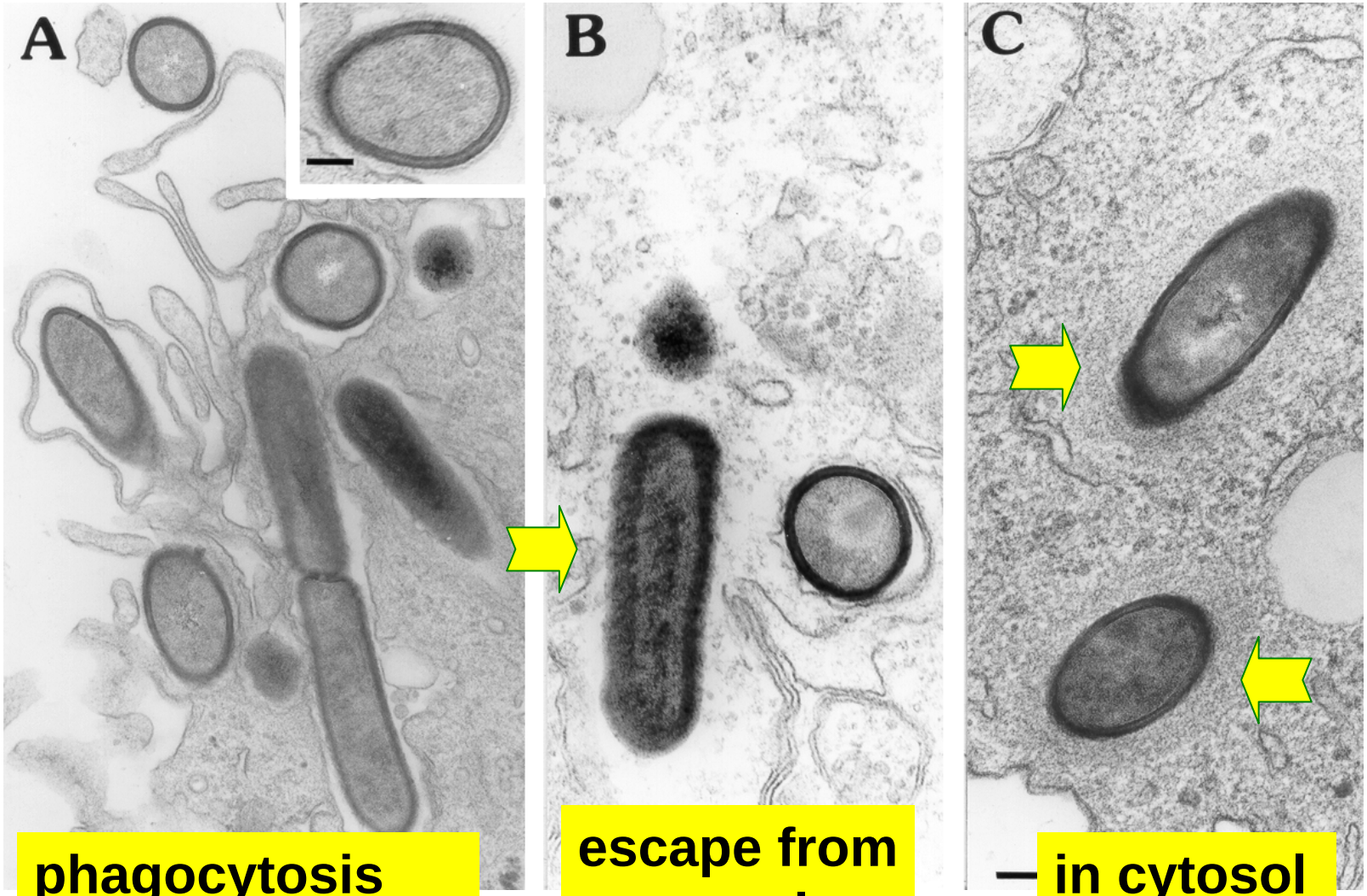


A few words about *Listeria*…



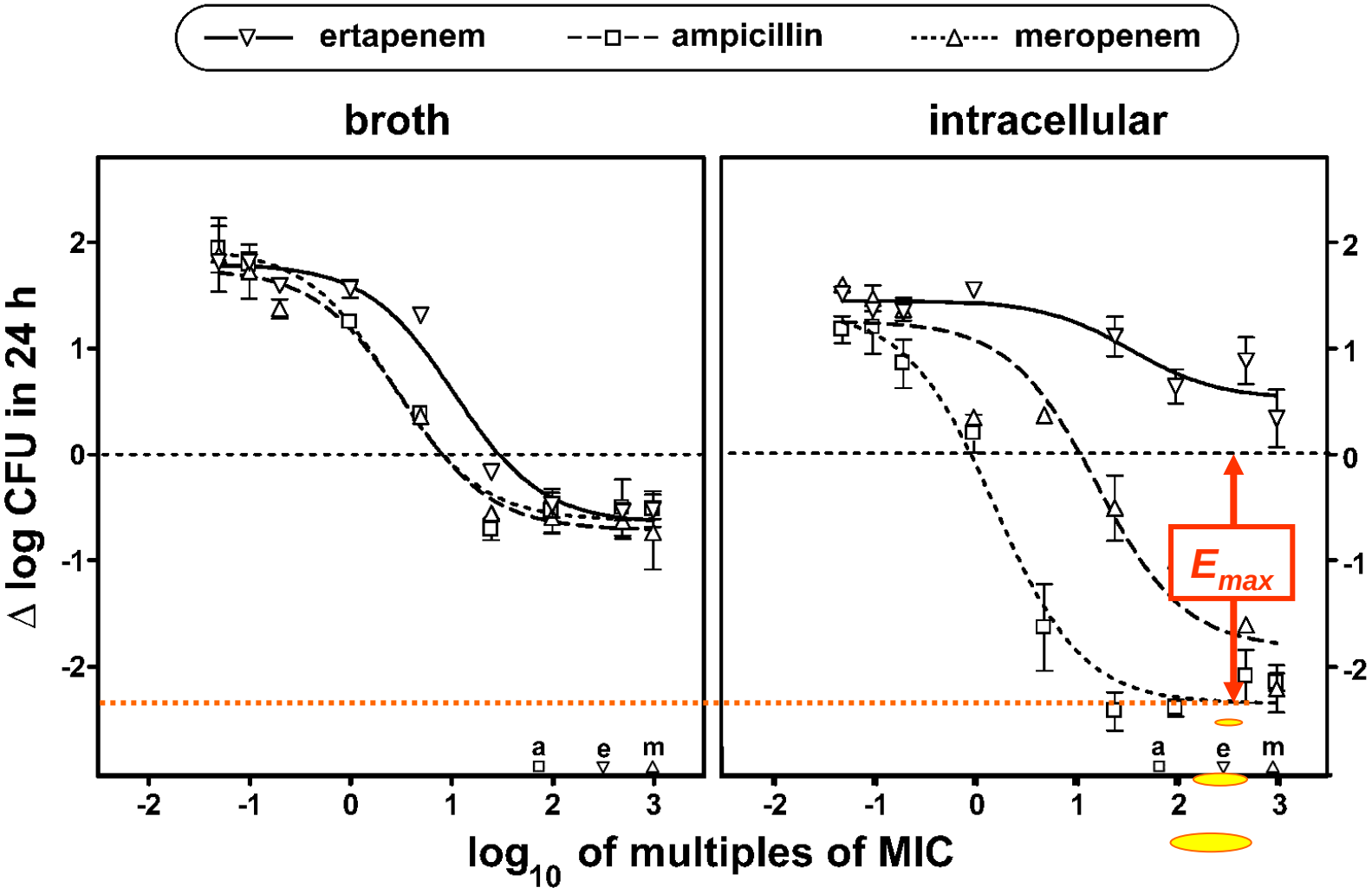
Carryn *et al.* Infect Dis Clin North Am. 2003;17:615-34.

As if you were there …



Ouadrhiri et al. Antimicrob Agents Chemother. 1999; 43:1242-51.

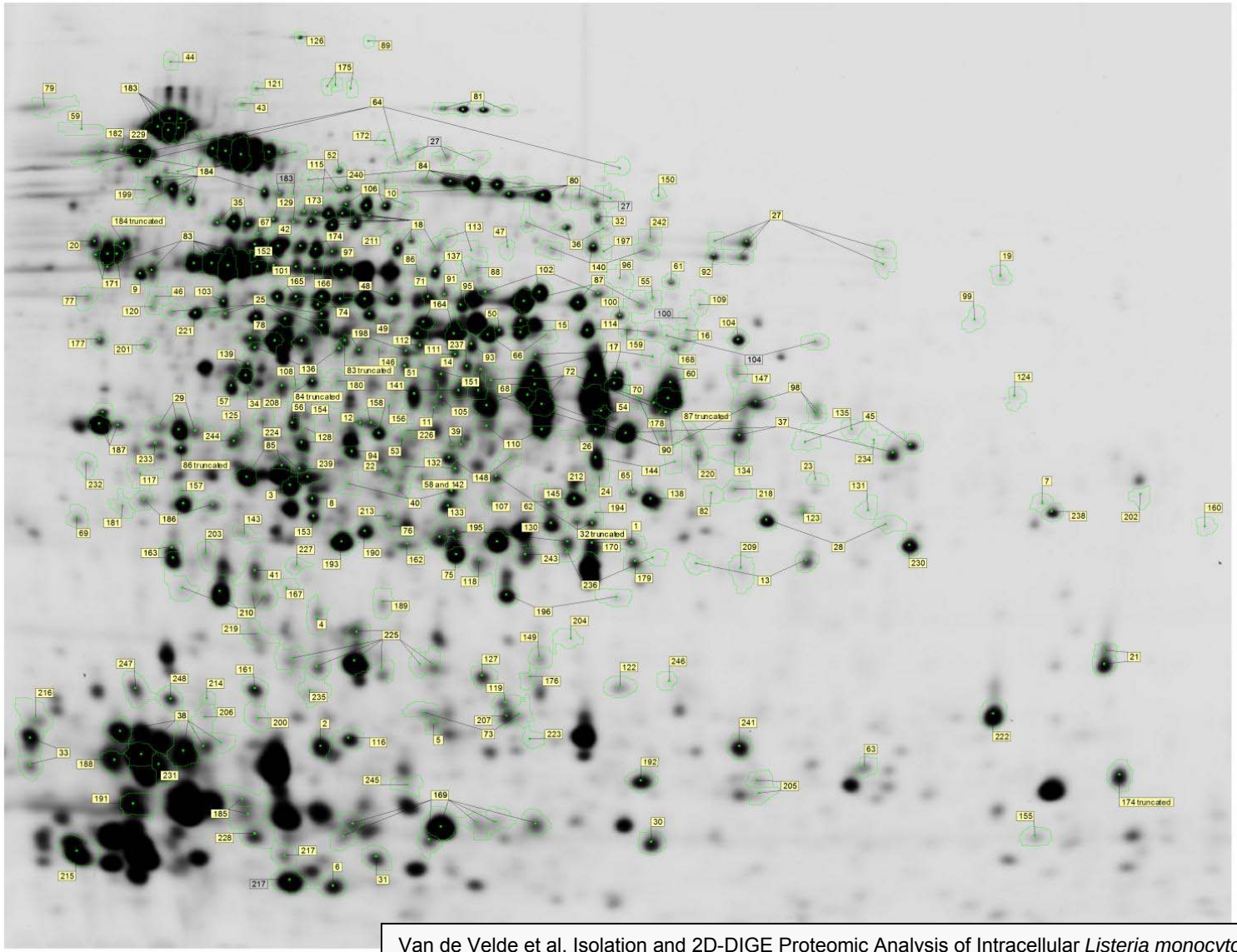
A surprise …



Lemaire et al. J Antimicrob Chemother. 2005; 55:897-904.

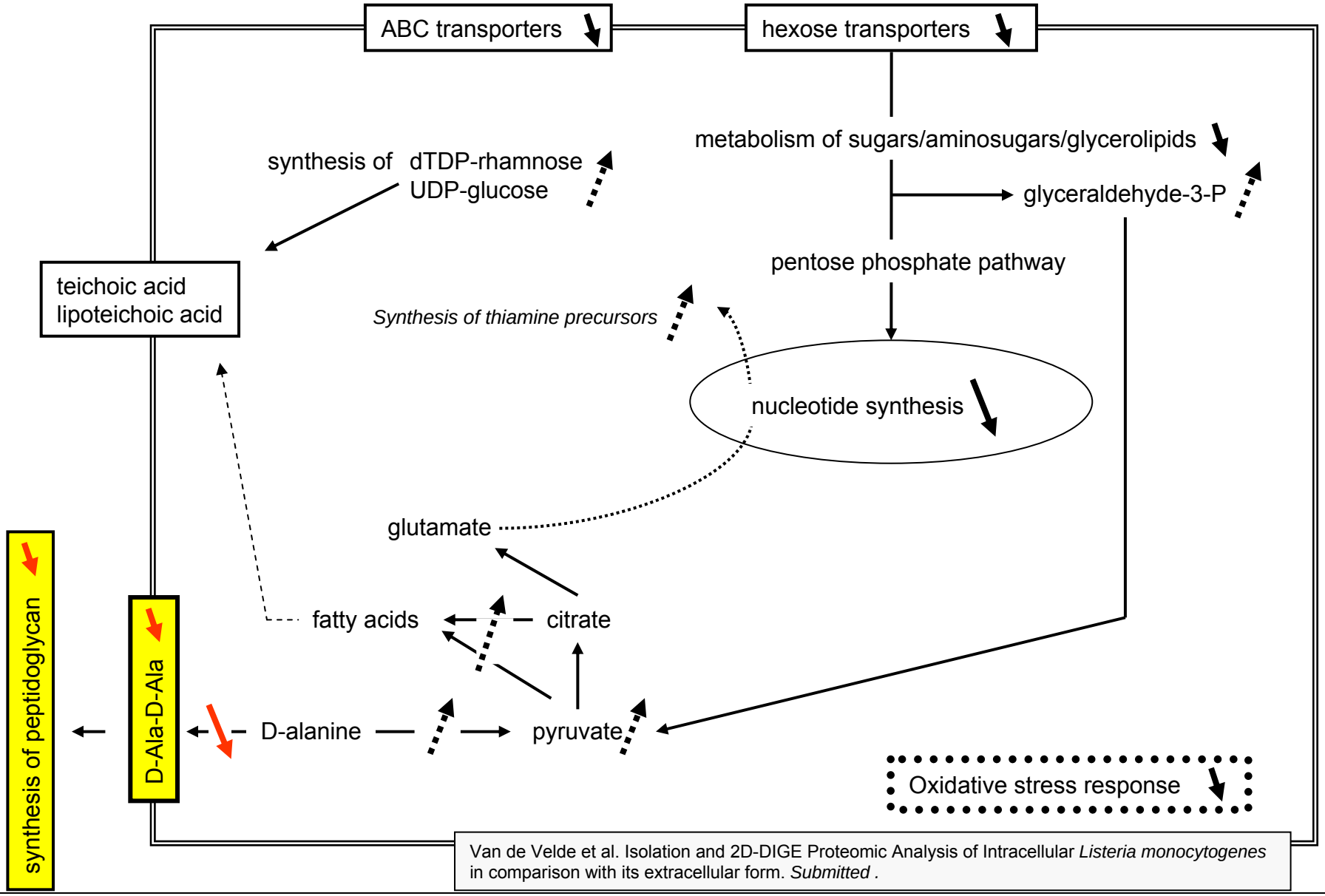
ampicillin is MORE active intracellularly

A potential explanation … through proteomics



Van de Velde et al. Isolation and 2D-DIGE Proteomic Analysis of Intracellular *Listeria monocytogenes* in comparison with its extracellular form. *Submitted* .

What may change in intracellular *Listeria*…



Van de Velde et al. Isolation and 2D-DIGE Proteomic Analysis of Intracellular *Listeria monocytogenes* in comparison with its extracellular form. *Submitted* .

Pähkinänkuoressa *

- solunsisäinen bakteeri on todellisuutta …
- Antivirulence (*I don't know how to translate "antivirulence"! antiviraalinen?*) terapia on hyvä projekti …
- suurin osa käytössä olevista antibiooteista toimii huonosti solunsisäisiä bakteereja vastaan, …
  ja jos ne toimivat, niin niiden vaikutus on paljon heikompi kuin solunulkoisia bakteereja vastaan
  (**valmistaudu pahimpaan**)
- meillä voi olla hyviä
  (mutta harvinaisia) **yllätyksiä**…

* Kindly translated by Miika Vikkula…

But let us hope…

A few review papers to help …

- Van Bambeke et al.
  The bacterial envelope as a target for novel anti-MRSA antibiotics.
  Trends Pharmacol Sci. 2008 Mar;29(3):124-34. PubMed PMID: 18262289.
- Mouton et al.
  Tissue concentrations: do we ever learn?
  J Antimicrob Chemother. 2008 Feb;61(2):235-7.
  PubMed PMID: 18065413.
- Van Bambeke et al.
  Cellular pharmacodynamics and pharmacokinetics of antibiotics: current views and perspectives.
  Curr Opin Drug Discov Devel. 2006 Mar;9(2):218-30.
  PubMed PMID: 16566292.
- Van Bambeke et al.
  Glycopeptide antibiotics: from conventional molecules to new derivatives.
  Drugs. 2004;64(9):913-36.
  PubMed PMID: 15101783.
- Carryn et al.
  Intracellular pharmacodynamics of antibiotics.
  Infect Dis Clin North Am. 2003 Sep;17(3):615-34.
  PubMed PMID: 14711080.

papers and slides available on **www.facm.ucl.ac.be**