Chemotherapy and PK/PD of intracellular infections

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Intracellular infection and recurrence/relapses: the case of *S. aureus* (as an example)

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.

Institute of Medical Microbiology, Westfalische Wilhelms-Universitat Munster, Munster, Germany. eiffc@uni-muenster.de

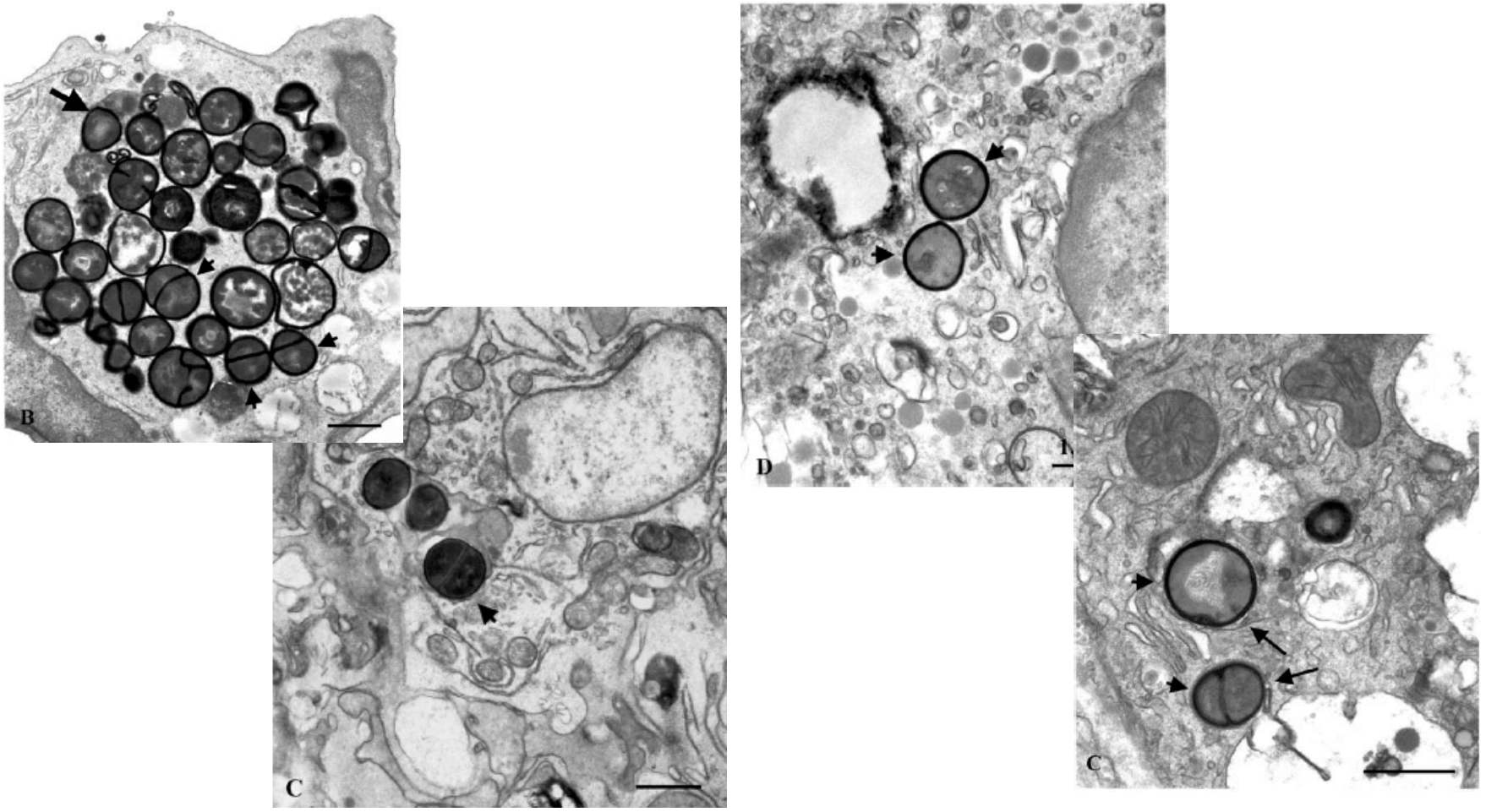
Infect Immun. 1986 Dec;54(3):833-6.

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

Hamill RJ, Vann JM, Proctor RA.

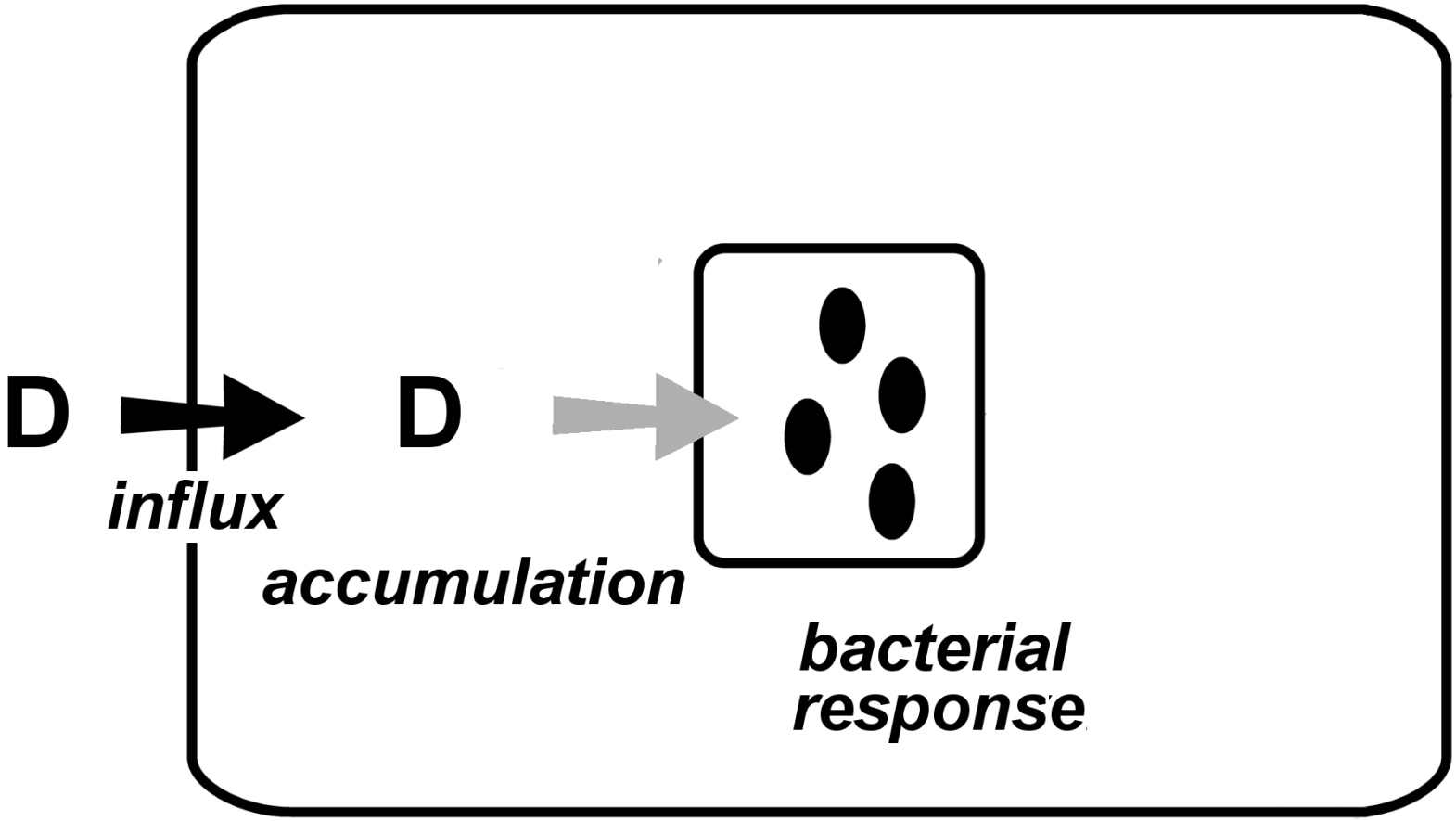
Intracellular infection and recurrence/relapses: the case of *S. aureus* (as an example)

Phagocytic and non phagocytic cells in mastitis



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

A simple scheme…



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

First (partialy wrong) statements …

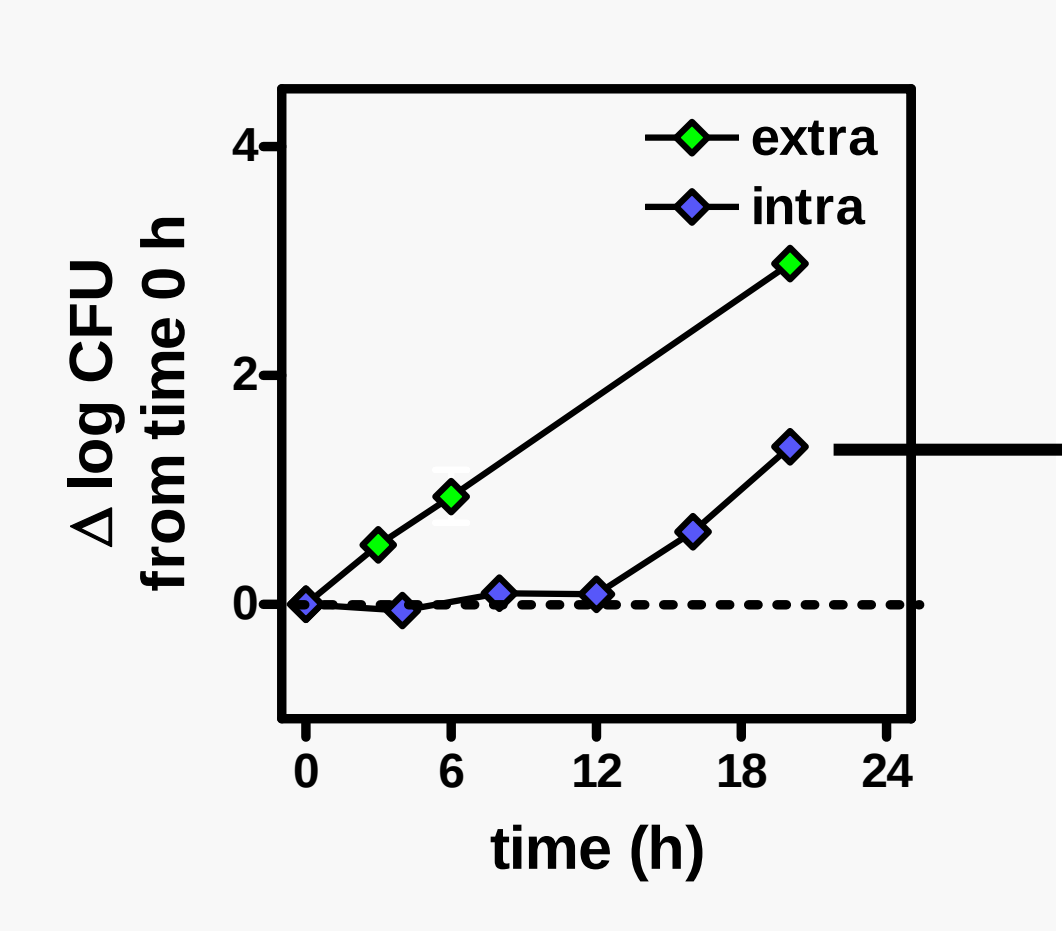
- If a drug does not *accumulate*, it cannot be active …

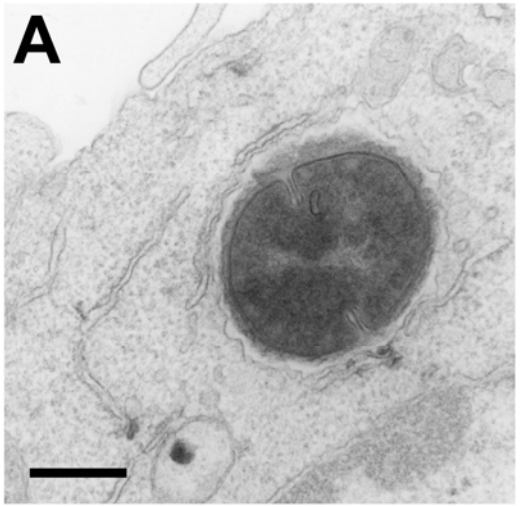
Quick answer:
this is correct if you mean "*it does not get in cells at all…*"

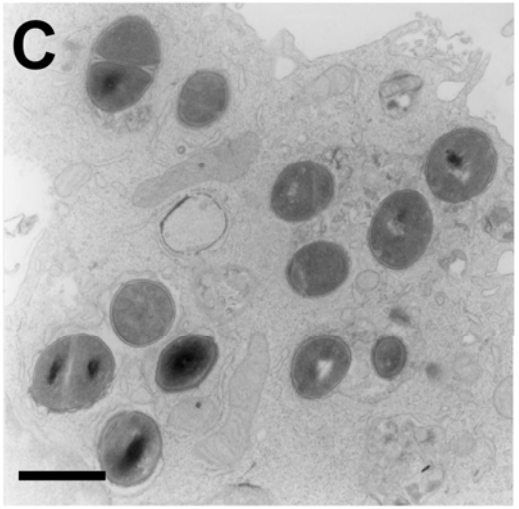
More elaborate answer:
no "accumulation" does not mean that the drug is not present, and if present, it may be active if above the critical concentration for sufficient time …

Experimental evidence:
β-lactams, known for "no accumulation" are active against intraphagocytic *L. monocytogenes* and *S. aureus* if their extracellular concentration is large enough… and if you let them enough time to act…

Settting up the model …

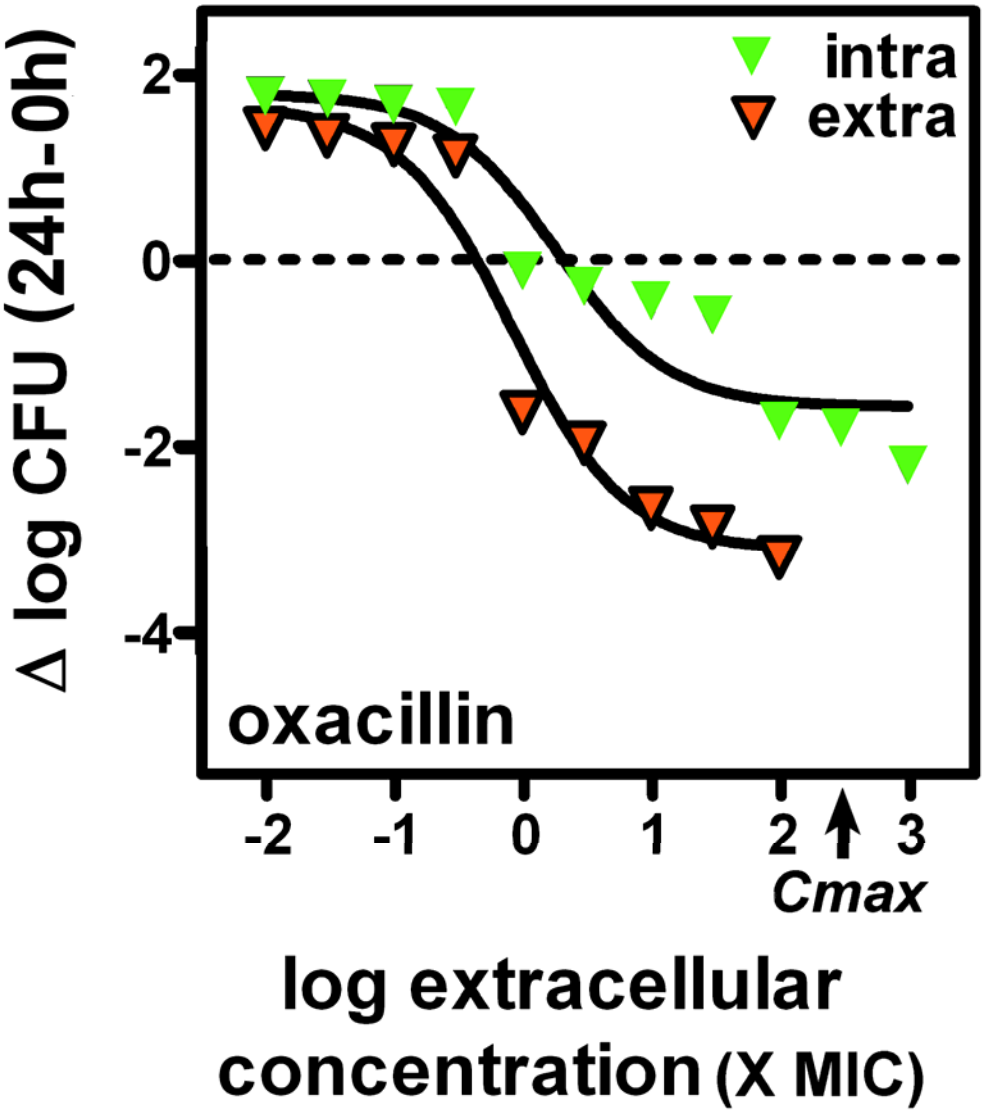






Seral *et al.* Antimicrob. Agents Chemother. 2003 47:2283-2292

Intraphagocytic *S. aureus* and β-lactams



- 24 h model
- C_{max}= 63 mg/L (total)

Barcia-Macay et al. M, Antimicrob Agents Chemother. 2006 Mar;50(3):841-51.

Observation …

- The activity of β-lactams is larger than anticipated…

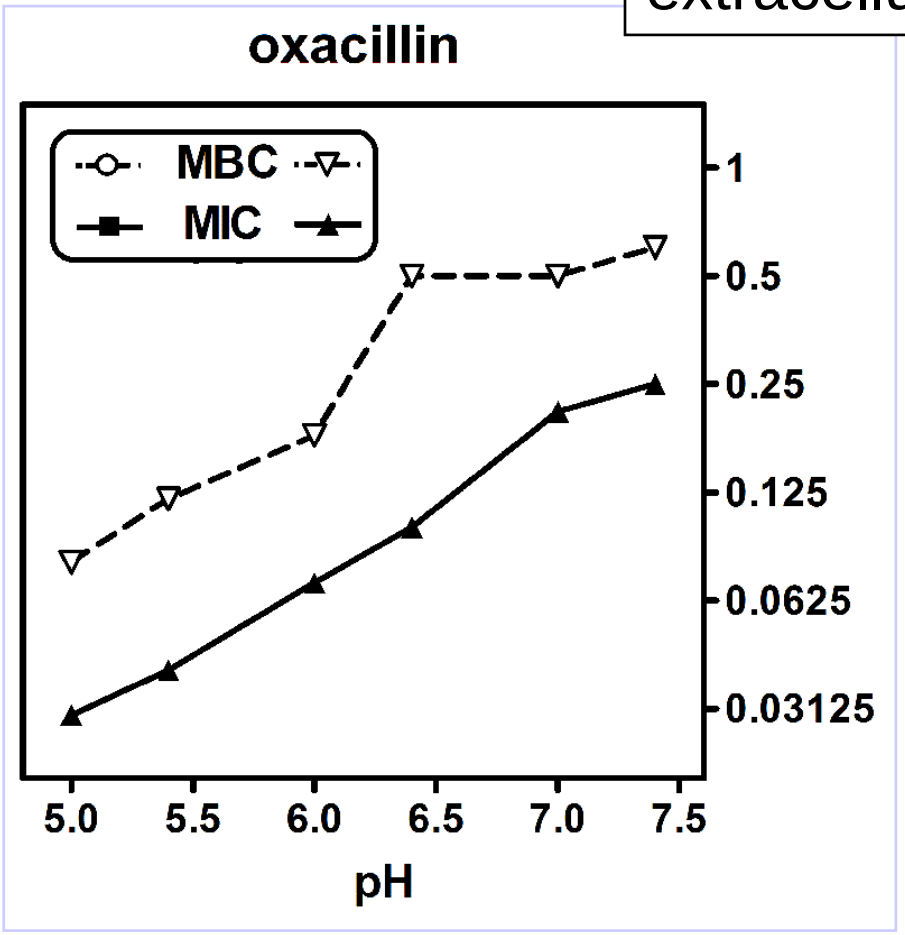
Quick answer:
You have worked at large concentration… and waited for 24h

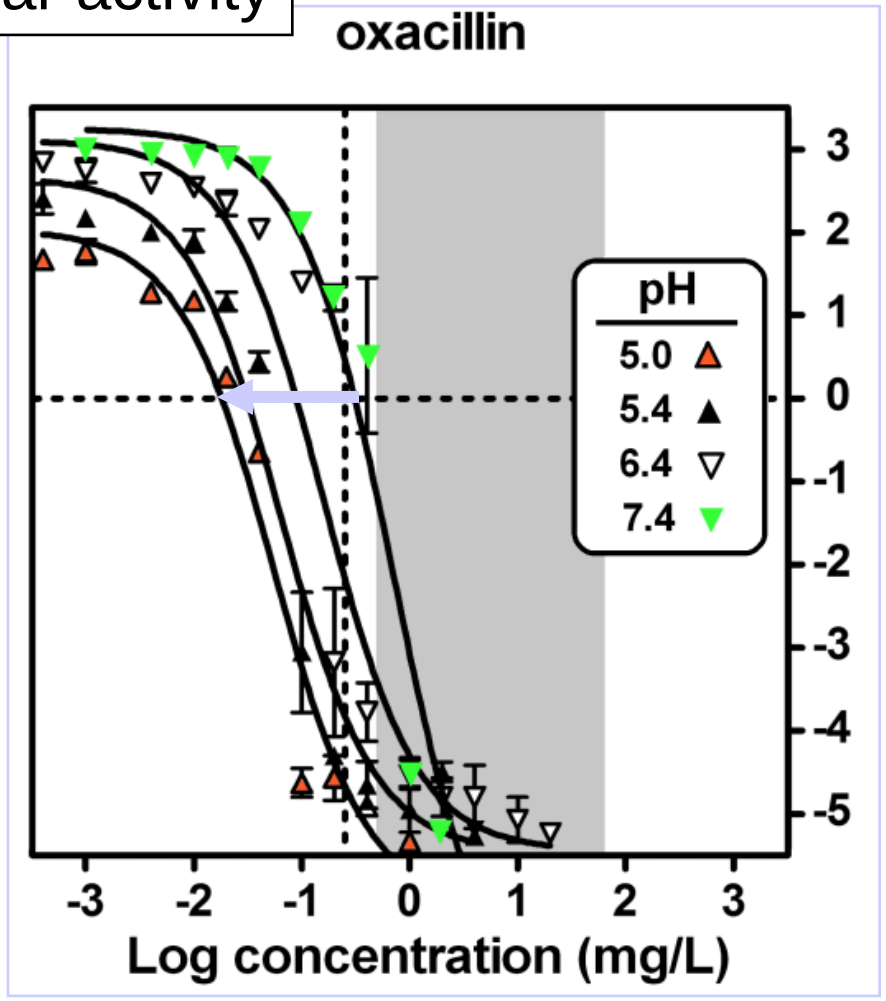
More elaborate answer:
The intracellular milieu may favor their activity …

Experimental evidence for a potential explanation:
- Acid pH increases the activity of β-lactams against intraphagocytic *S. aureus*…

Acid pH favors the activity of β-lactams …

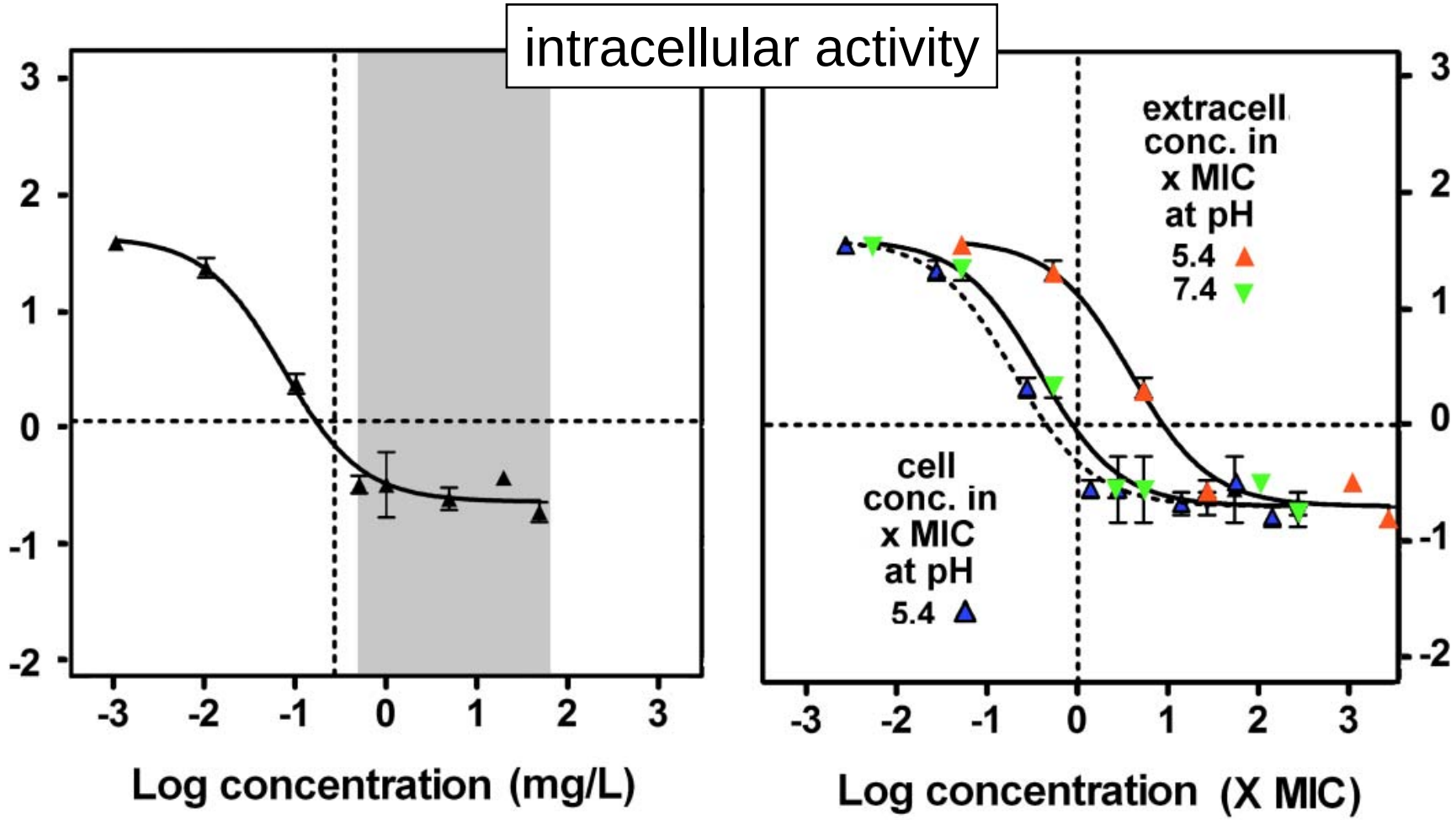
extracellular activity





Baudoux et al., J. Antimicrob. Chemother., in press.

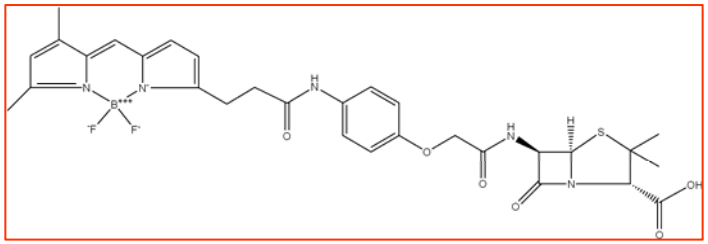
And acidity compensates for poor intracellular accumulation …



Baudoux *et al.*, J. Antimicrob. Chemother., in press.

And what about MRSA ?

Acid pH favors penicillin binding to PBP2a



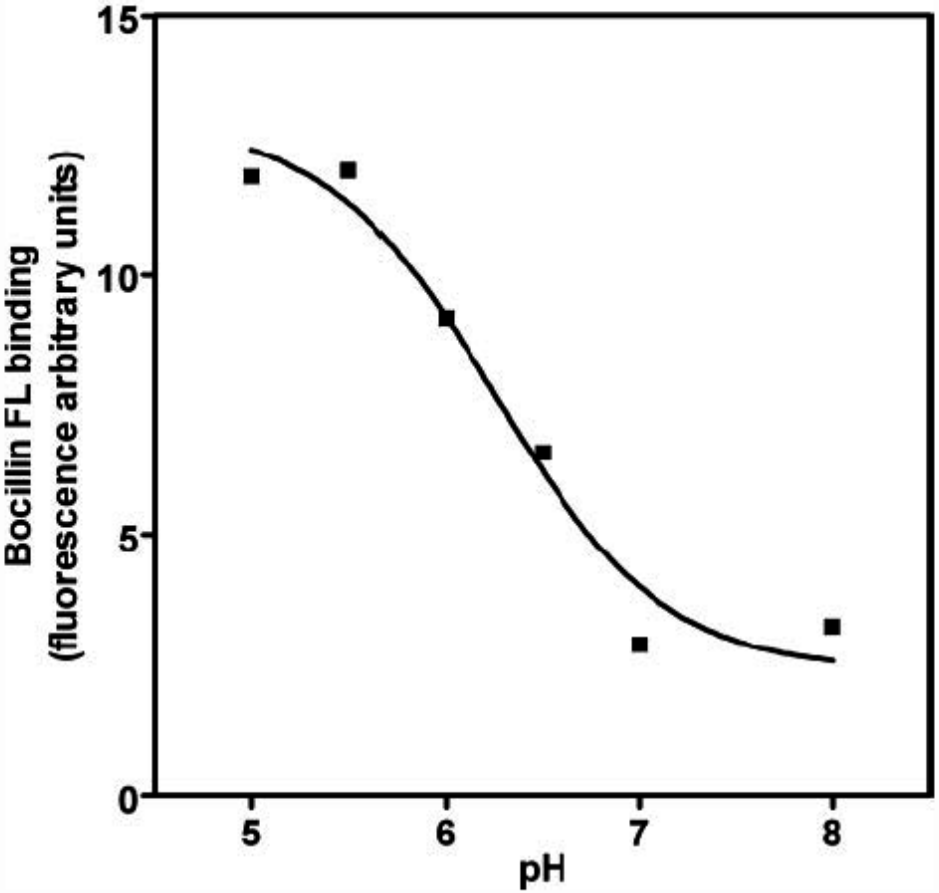


FIGURE 2. **Influence of pH on the binding of Bocillin FL to whole cells and to purified PBP 2a.** *Upper panel*, growing bacteria were incubated in broth at 37 °C with Bocillin FL for 30 min at the pH indicated in the abscissa, and the samples were prepared for fluorescence measurement. *White bars*, 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

The future is (perhaps) here…

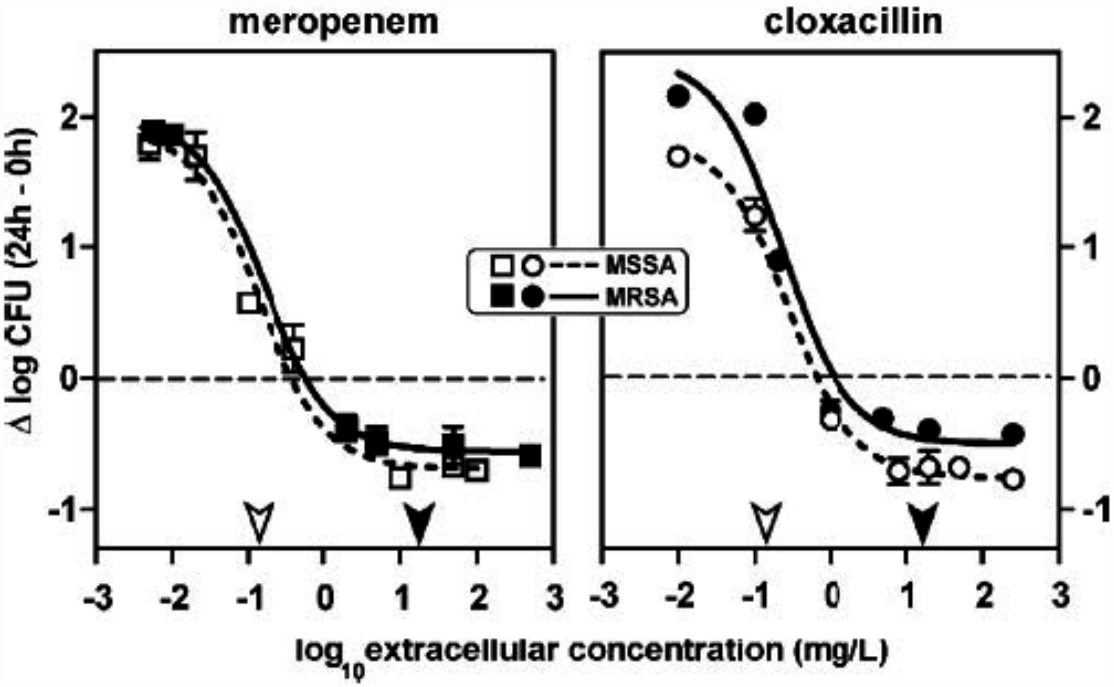
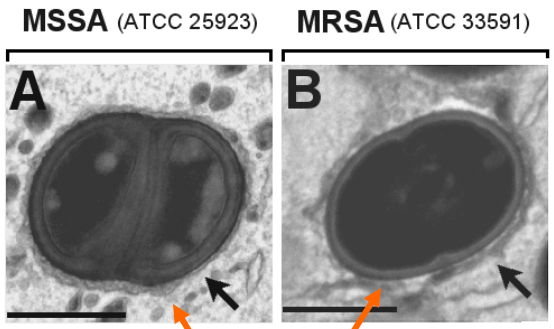


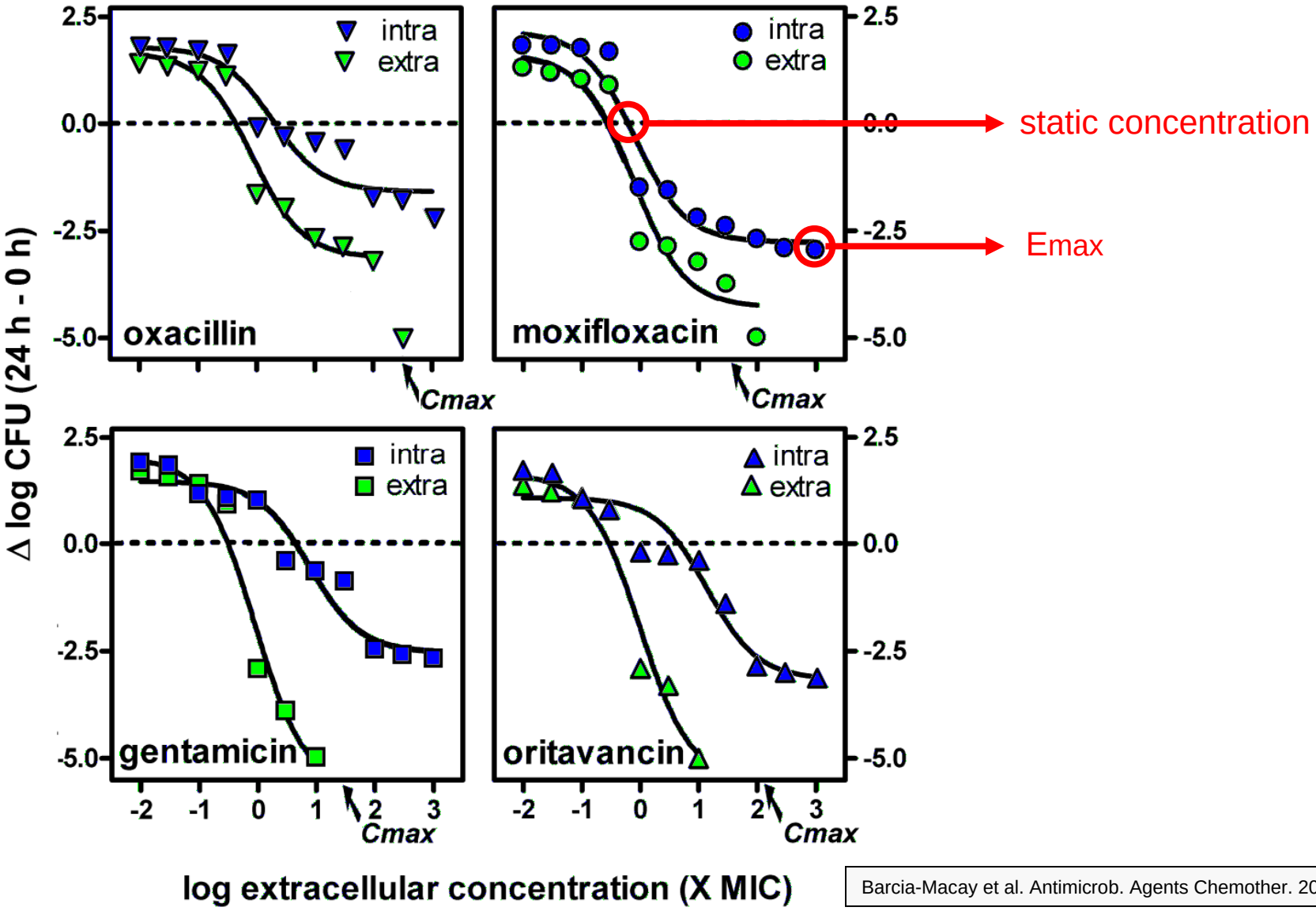
FIG. 1. Concentration killing effects of meropenem (squares; left panel) and cloxacillin (circles; right panel) toward MSSA strain ATCC 25923 (open symbols and dotted line) and MRSA strain ATCC 33591 (closed symbols and continuous line) after phagocytosis by THP-1 macrophages. Cells were incubated with 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

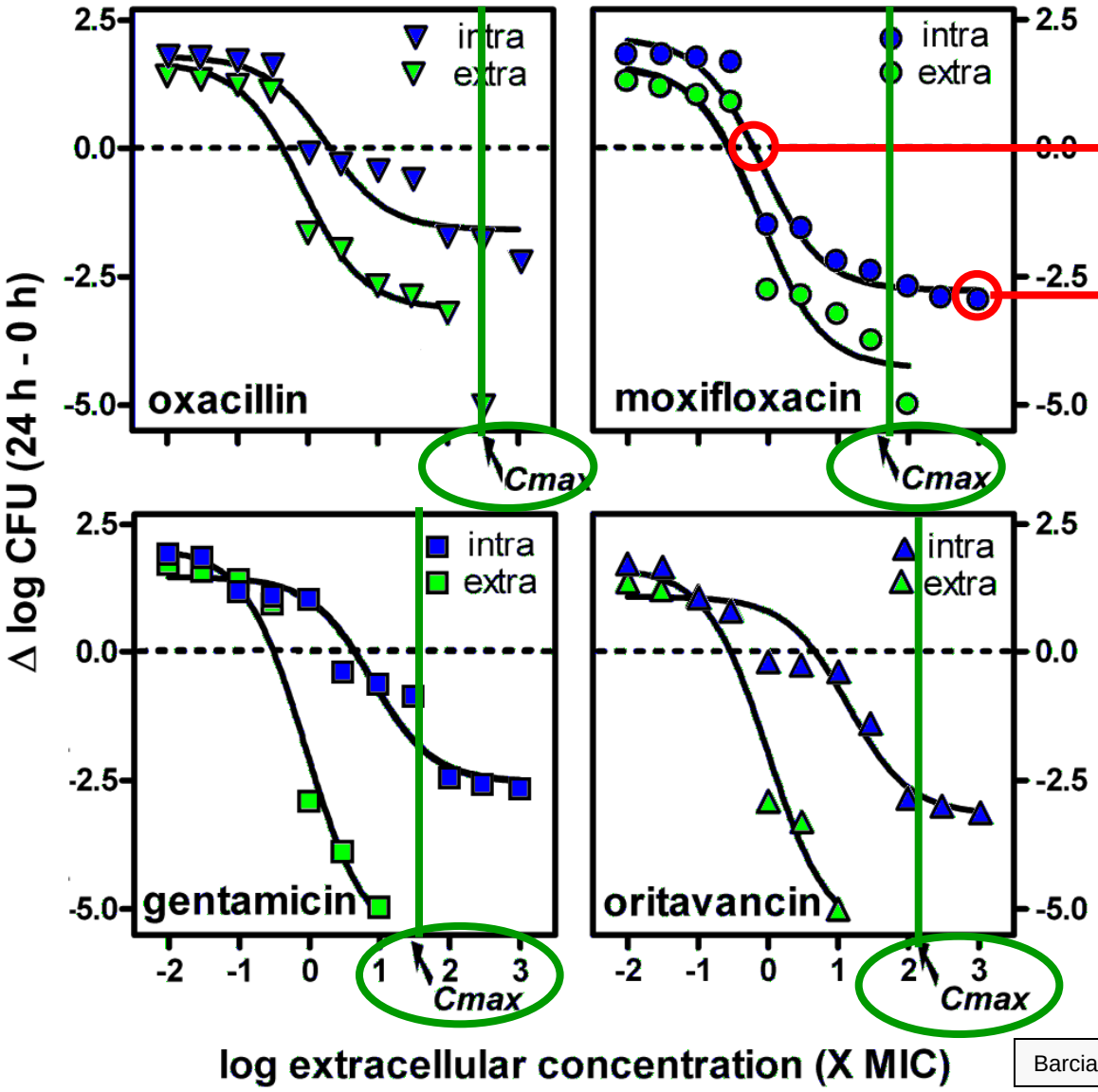
Lemaire et al., AAC (2007) 51:1627-1632

Using the model for other antibiotics …



Barcia-Macay et al. Antimicrob. Agents Chemother. 2006, in press

Using the model for other antibiotics …



static concentration

Emax

And adding PK…

Max. clinically-meaningful concentration

Barcia-Macay et al. Antimicrob. Agents Chemother. 2006, in press

This is what you the may find…

- **Aminoglycosides** accumulate (slowly) in phagolysosomes and their activity is defeated by the acid pH…
  ➔ Activity will be poor (but not nil)
- **Macrolides** accumulate … but their activity is severely defeated by the acid pH * … and they are only bacteriostatic…
  ➔ Activity will not exceed a zero growth effect *
- **Quinolones** accumulate modestly, but their activity is maintained at acid pH … and they have access to most intracellular compartments…
  ➔They tend to be the most active intracellular drugs …
  but their intracellular activity is at most similar to their extracellular activity …

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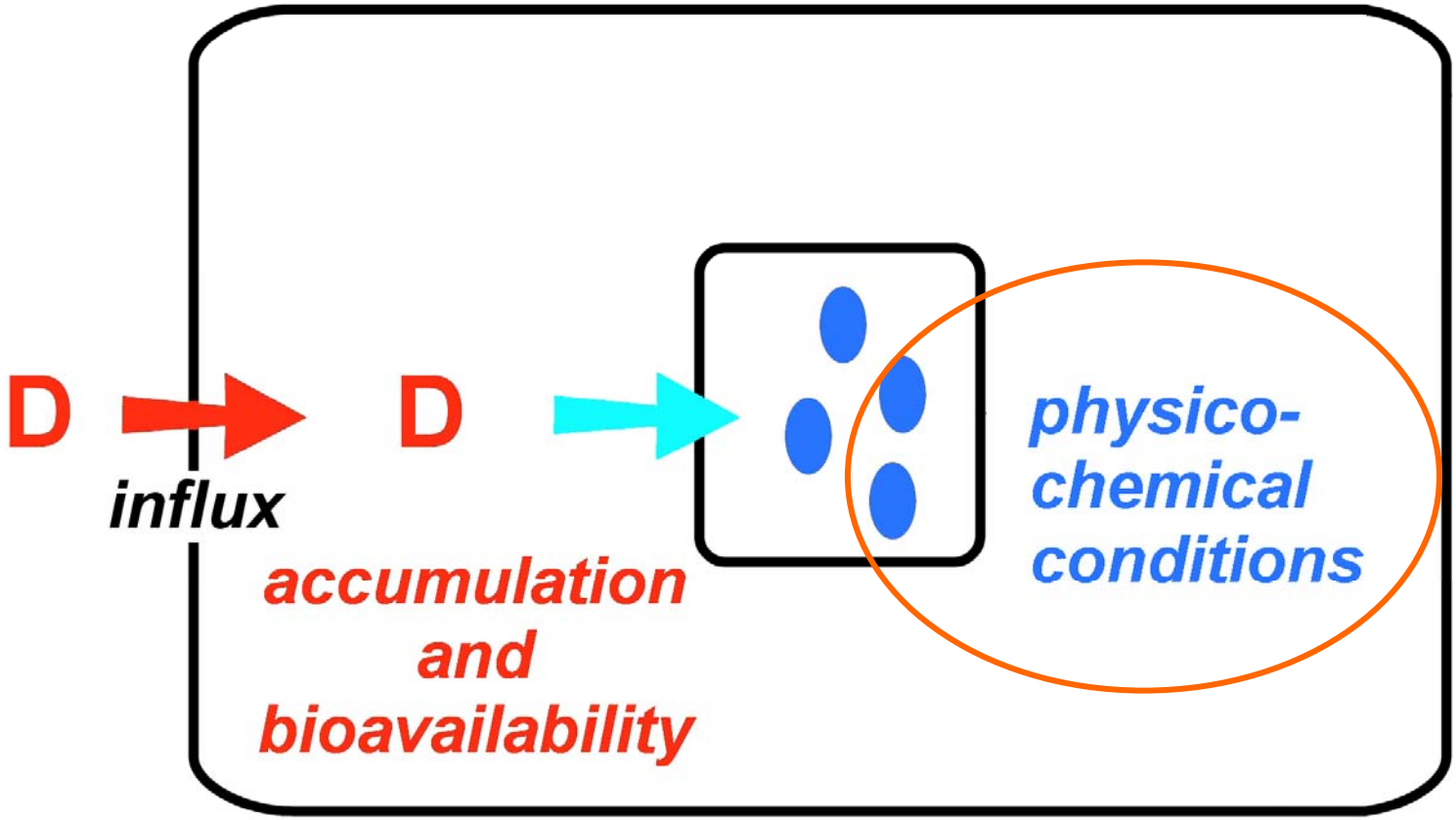
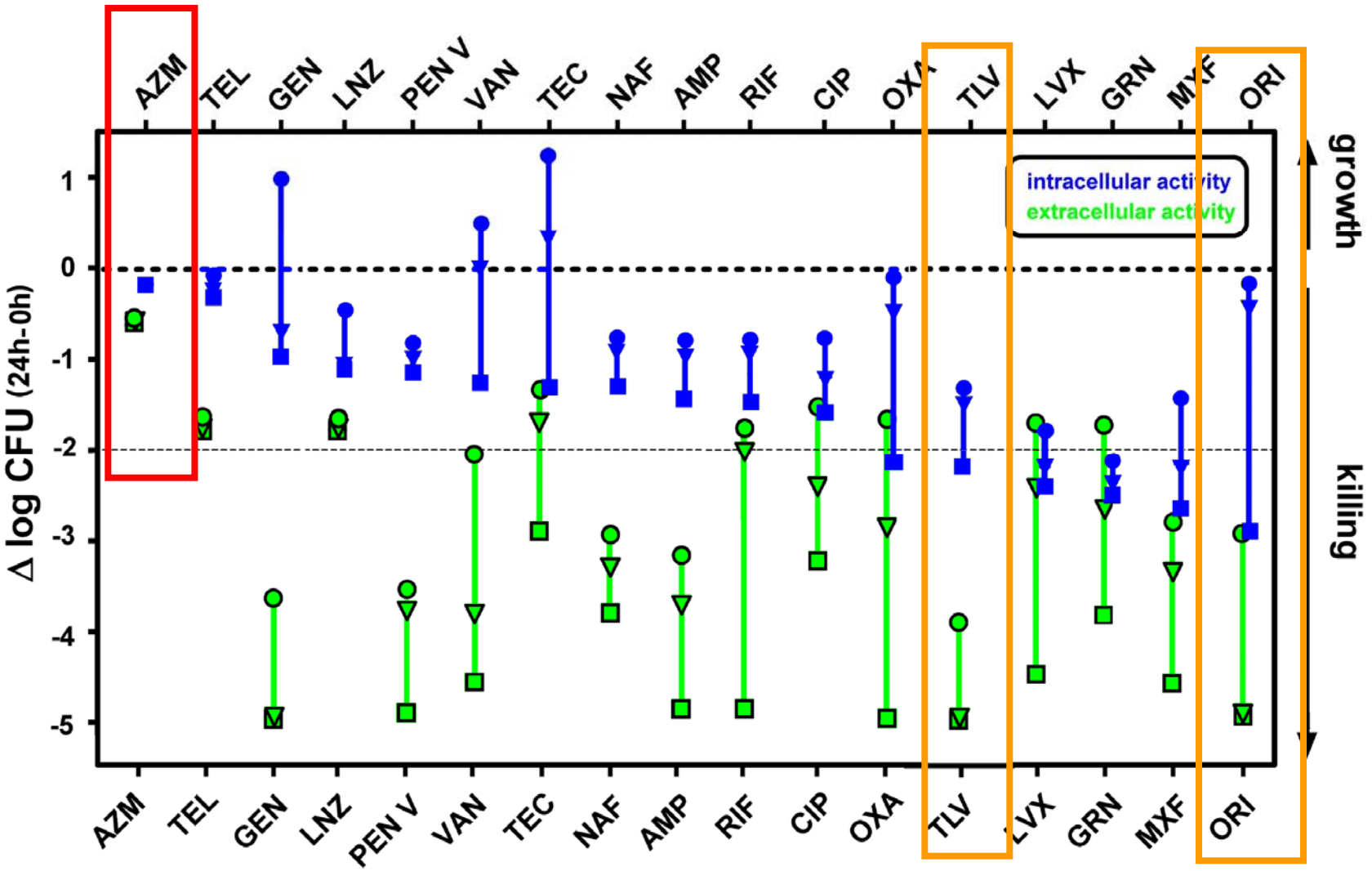
* May not be true for most recent ones
(will be talked more about at ICAAC)

The scheme gets a bit more complex



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

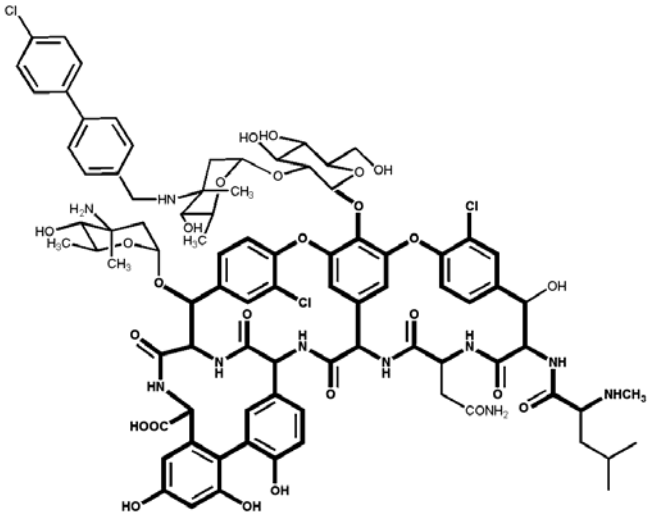
Screening available antibiotics



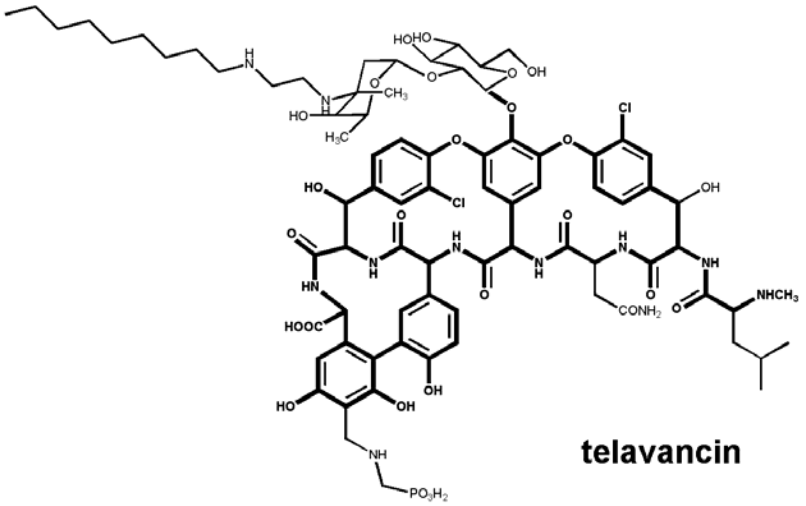
Barcia-Macay et al. Antimicrob. Agents Chemother. 2006, in press

New lipoglycopeptides (oritavancin,telavancin)

Hemi-synthetic derivatives derived from vancomycin

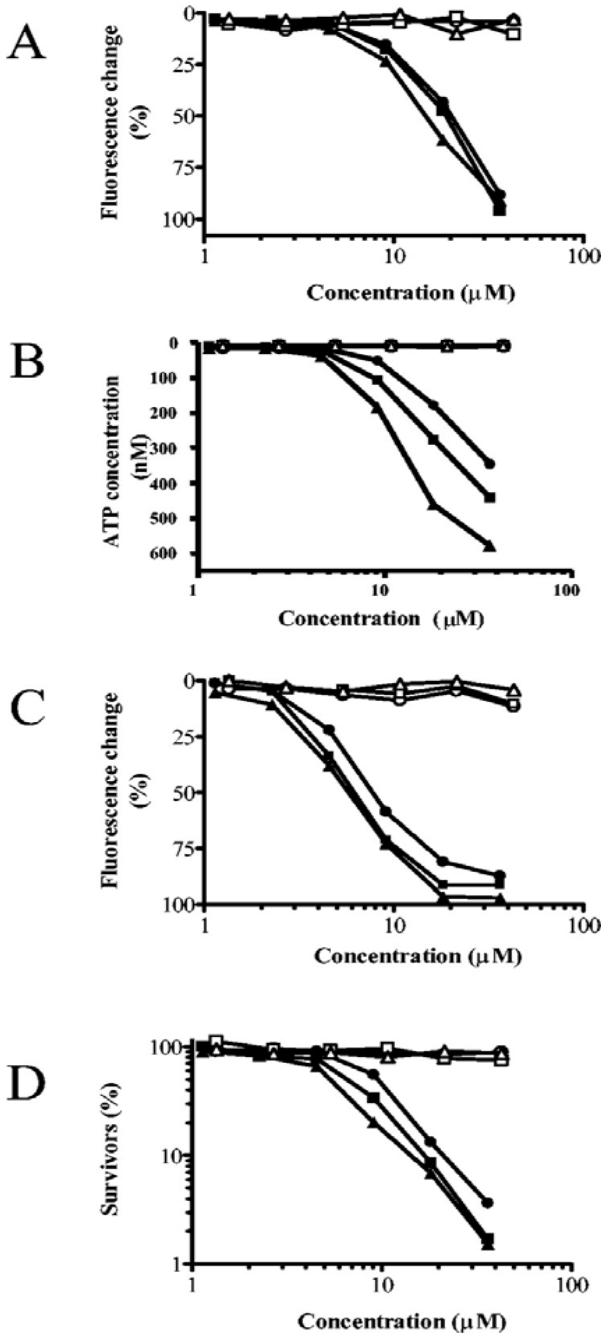


oritavancin



telavancin

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



Telavancin causes simultaneous membrane alterations and loss of viability

Correlation between change in

- membrane potential (A),
- ATP leakage (B),
- permeability (C), and
- cell viability (D)

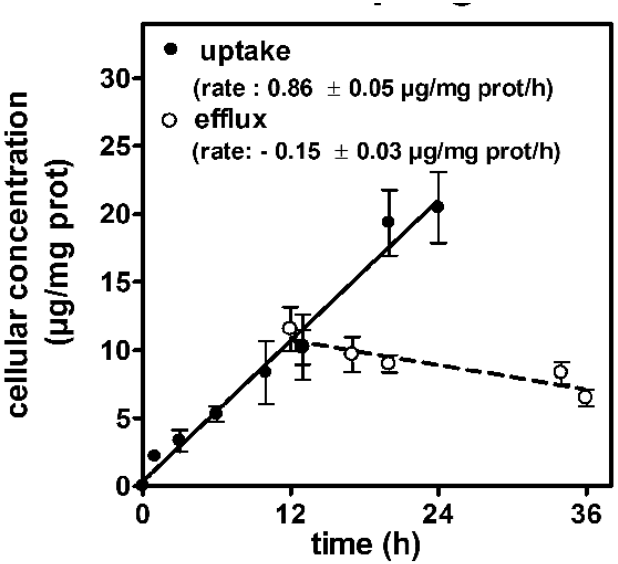
at various times after antibiotic addition (15-60 min)

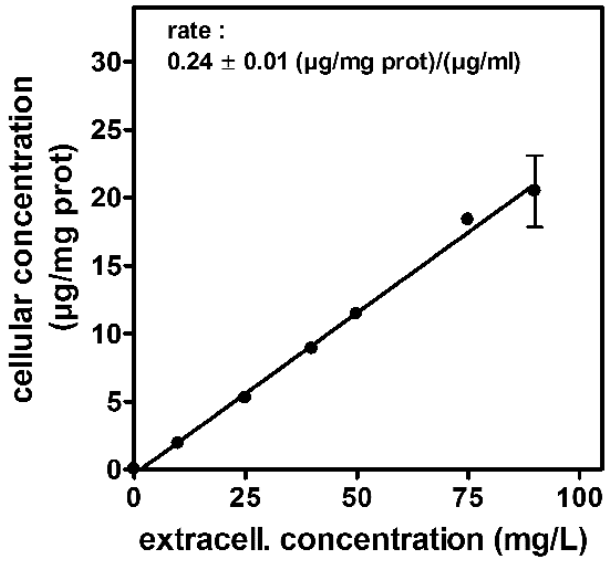
closed symbols, telavancin;

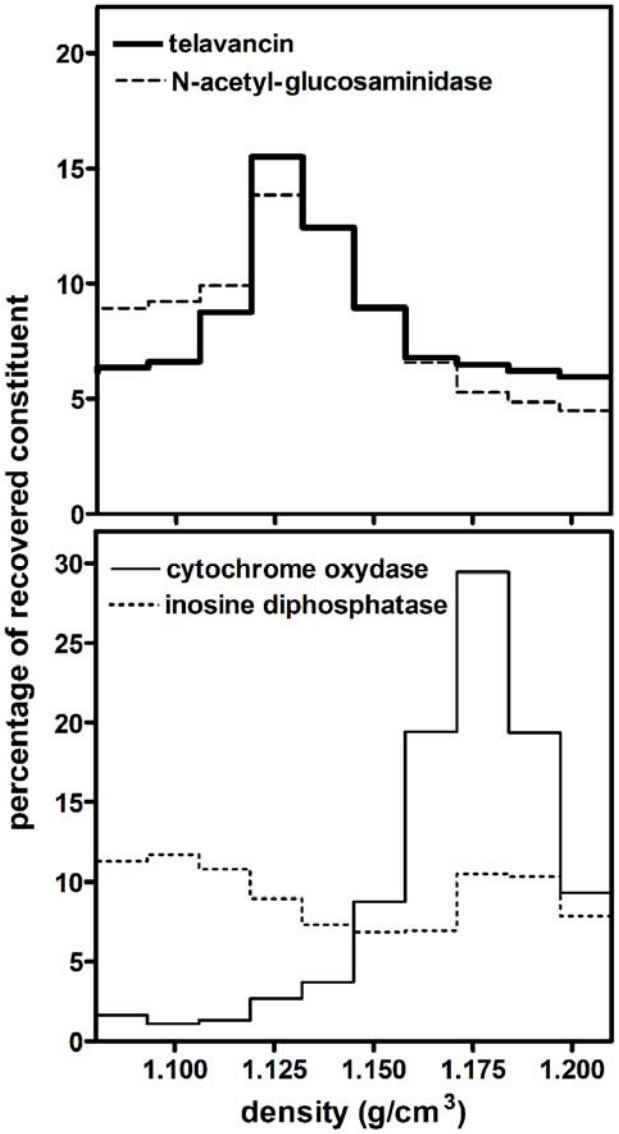
open symbols, vancomycin.

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

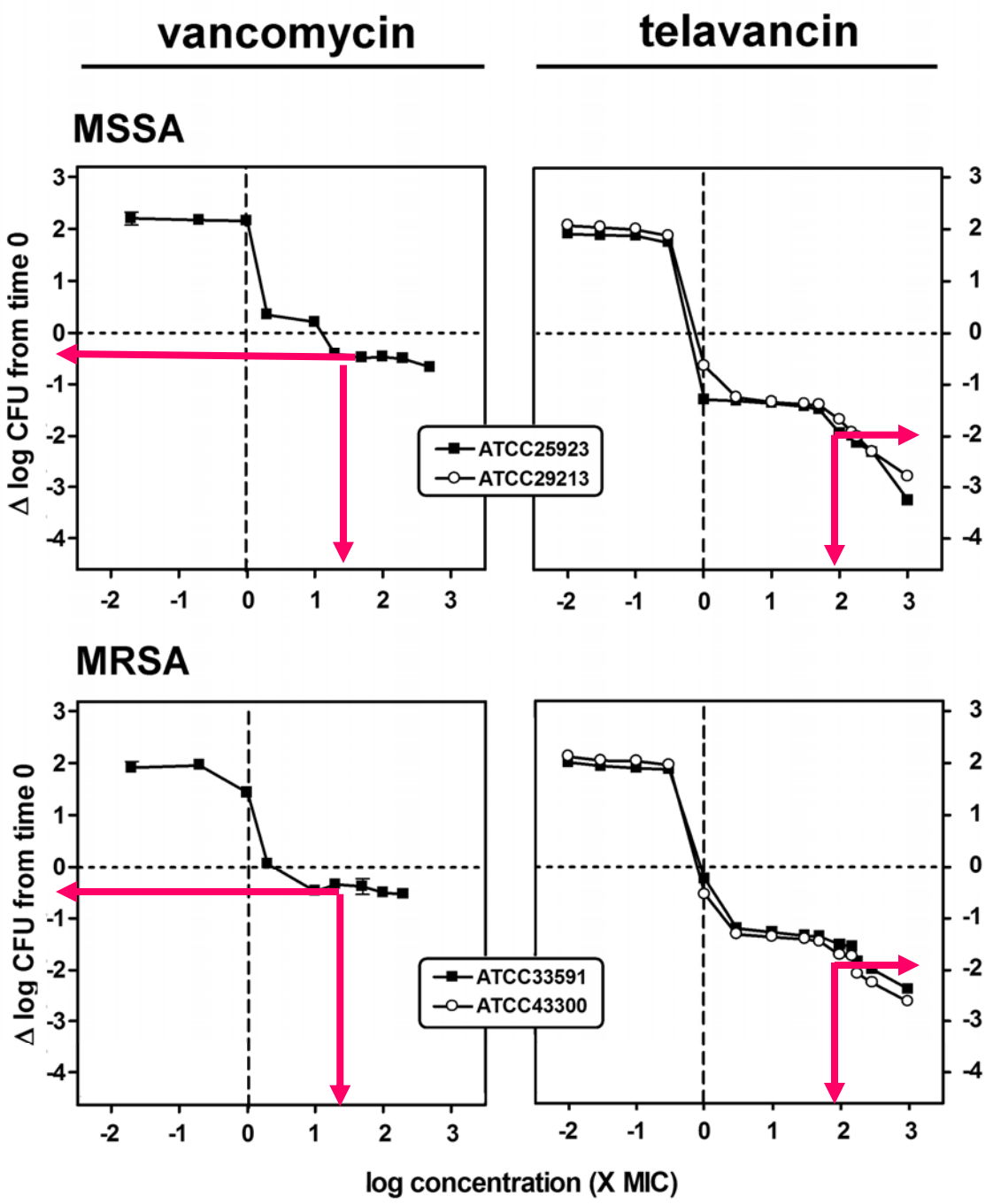
Telavancin intracellular accumulation and subcellular disposition







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



Intracellular activity of telavancin *vs.* vancomycin:

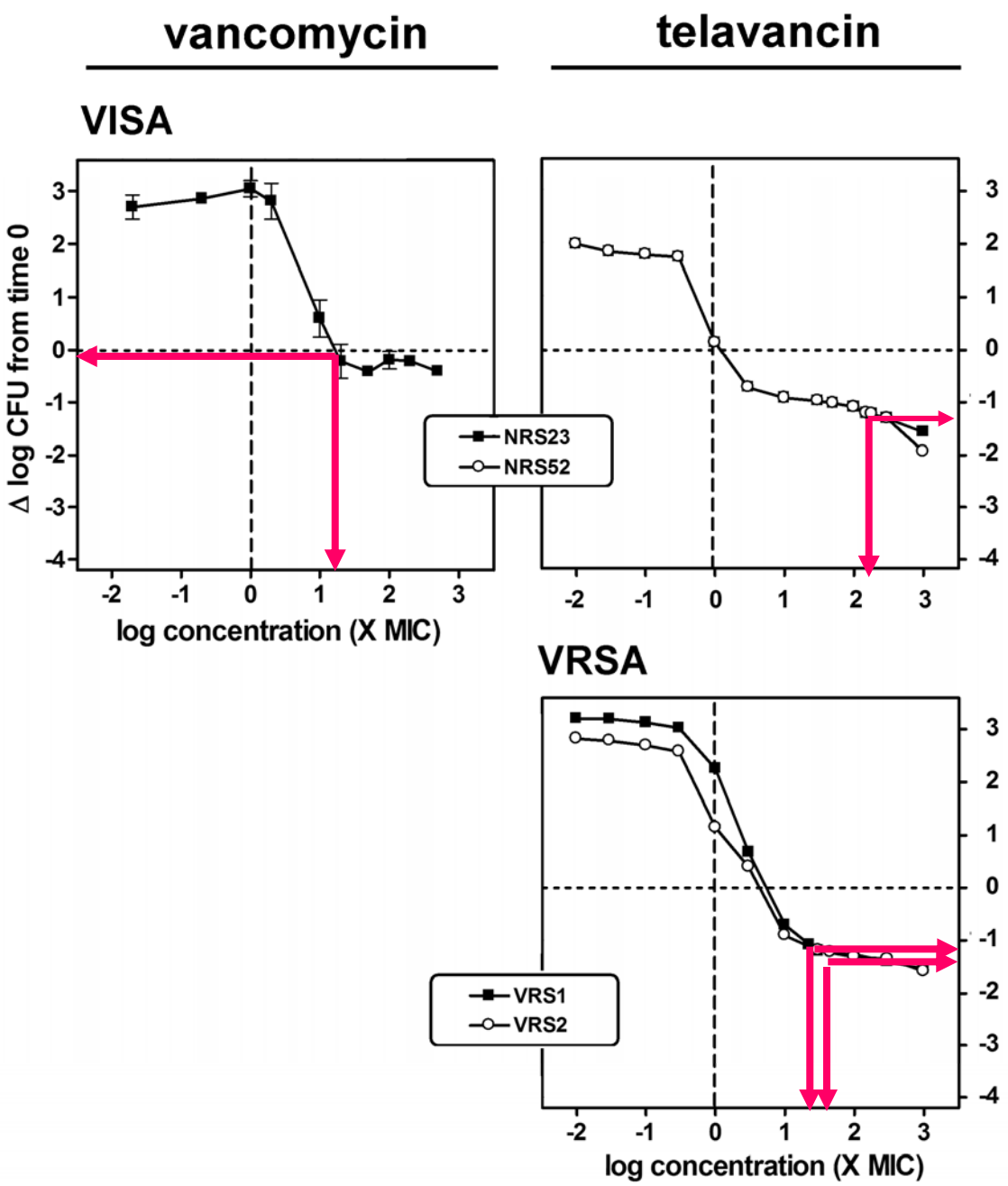
➔ MSSA

➔ MRSA

24h CFU ↘ at C_{max}:

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

Barcia-Macay *et al.*, JAC2006; 58:1177-1184.



Intracellular activity of telavancin *vs.* vancomycin:

➔ VISA

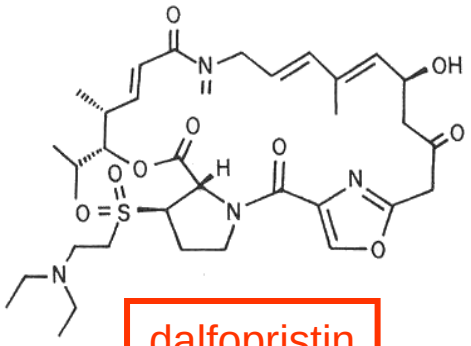
➔ VRSA

24h CFU ↘ at C_{max}:

- vanco: static
- TLV: ~ 1.2 log

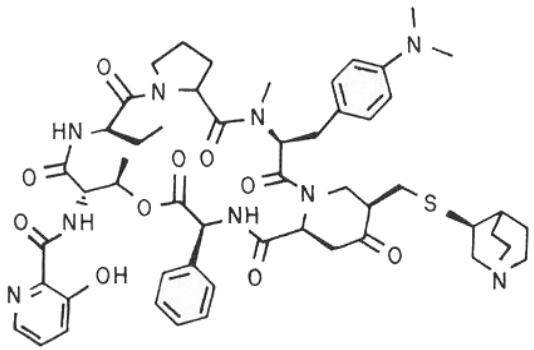
Barcia-Macay *et al.*, JAC2006; 58:1177-1184.

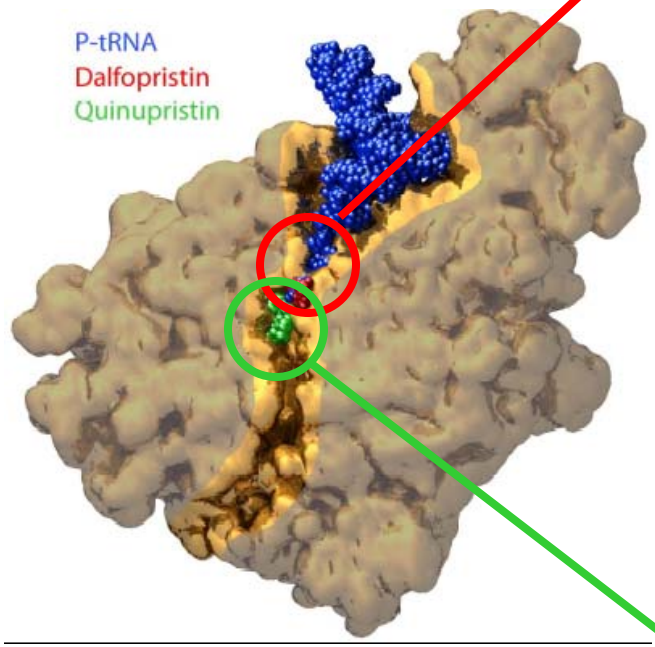
SYNERCID® = quinupristin + dalfopristin



dalfopristin

quinupristin





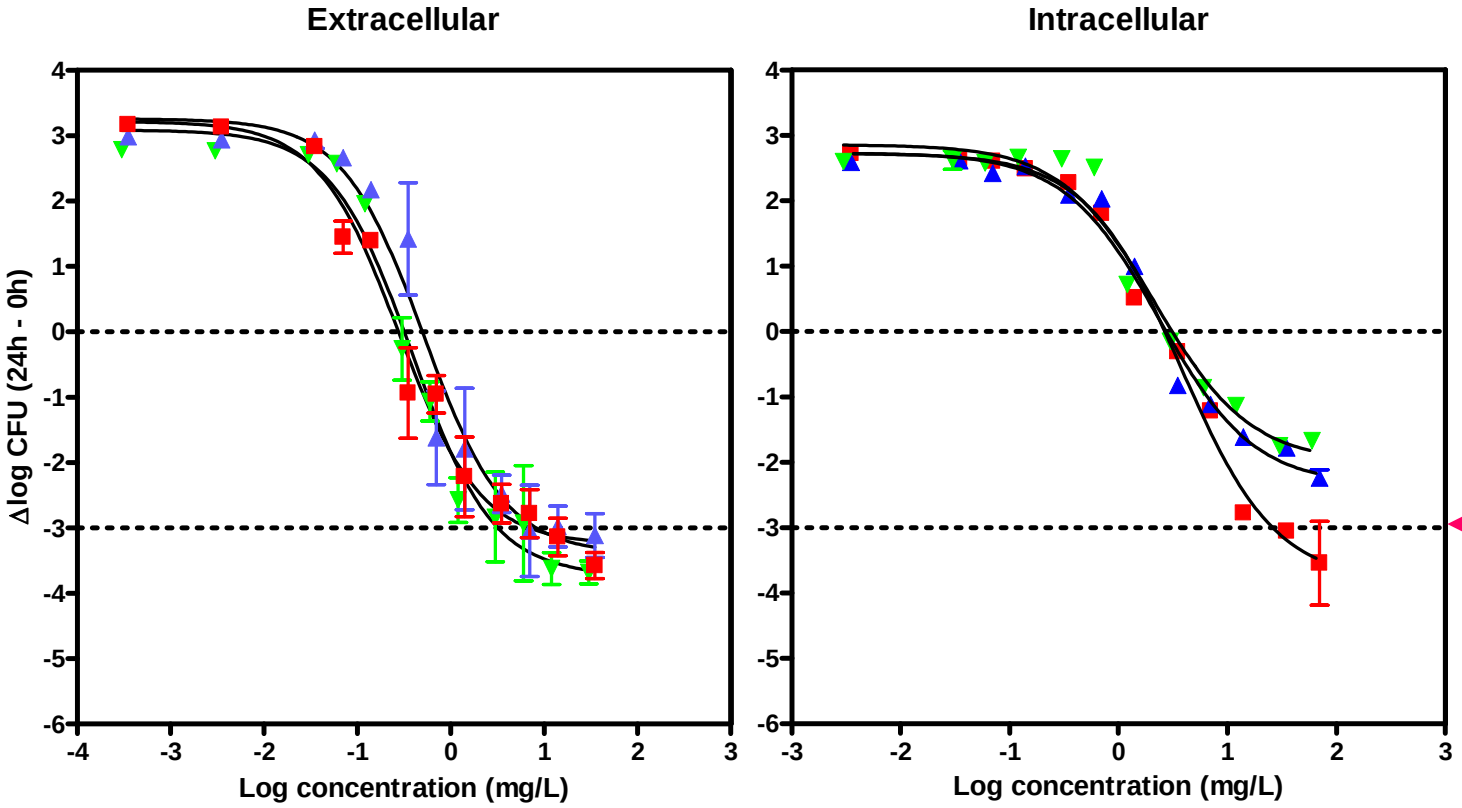
S_A blocks peptide bound formation

SYNERGY

S_B blocks the path of the nascent peptide

Harms *et al,* BMB Biol (2004) 2:4

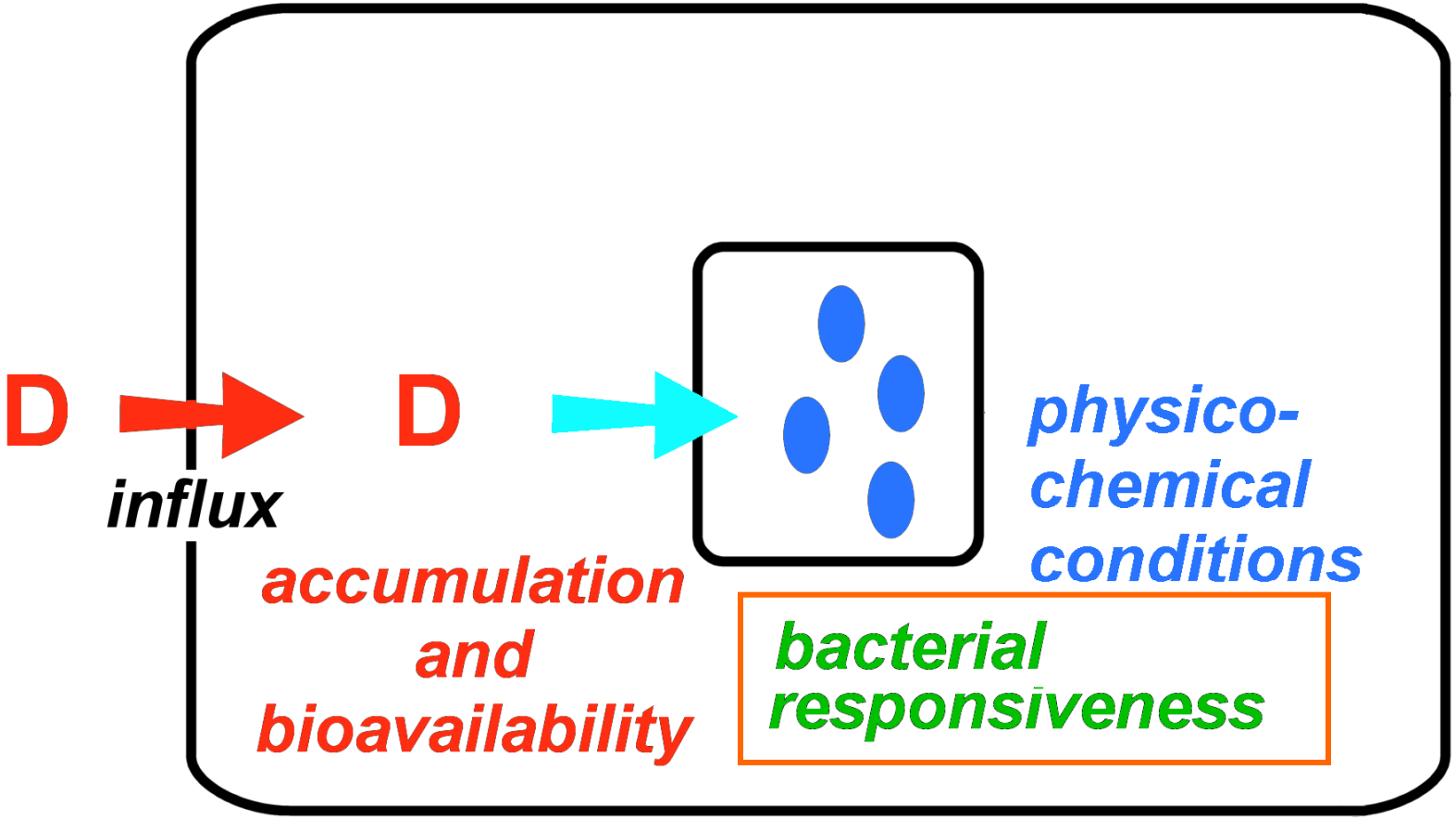
SYNERCID® is also quite active against intracellular *S. aureus*



3 log cfu decrease = CLSI criterion for bactericidal effect

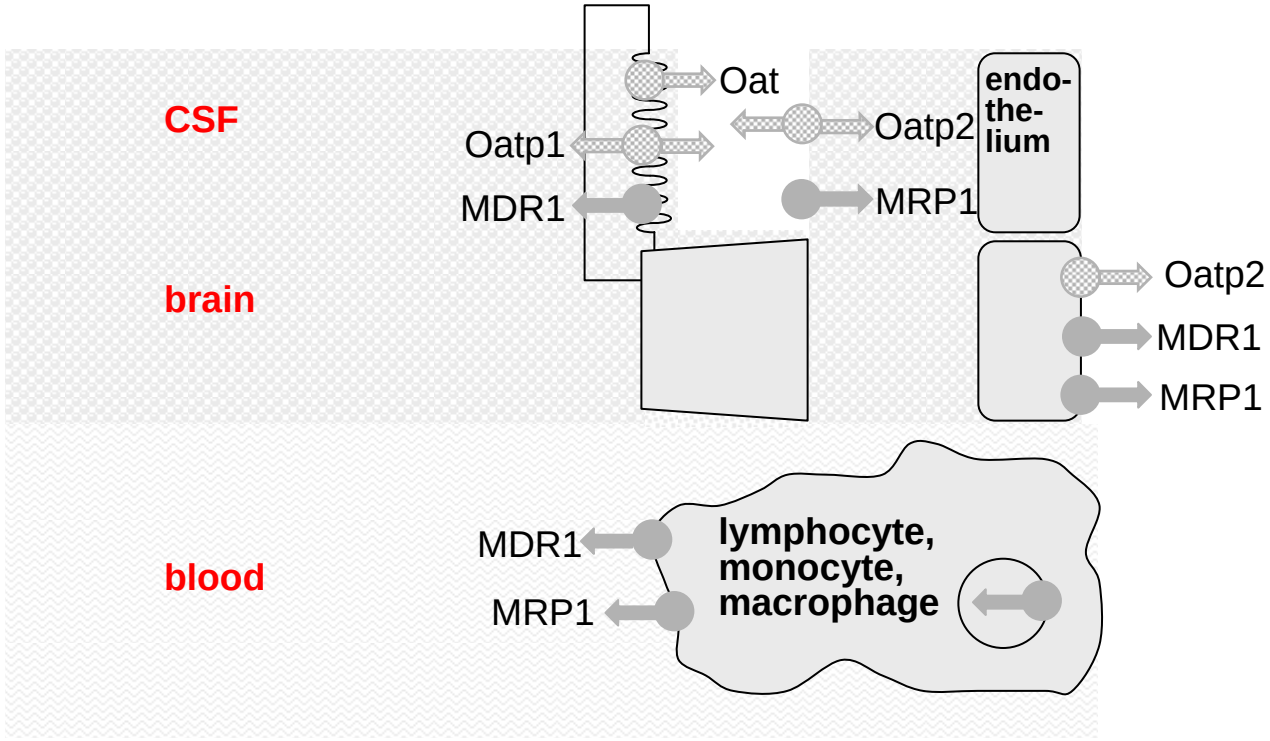
Baudoux et al., unpublished

The scheme gets again a bit more complex



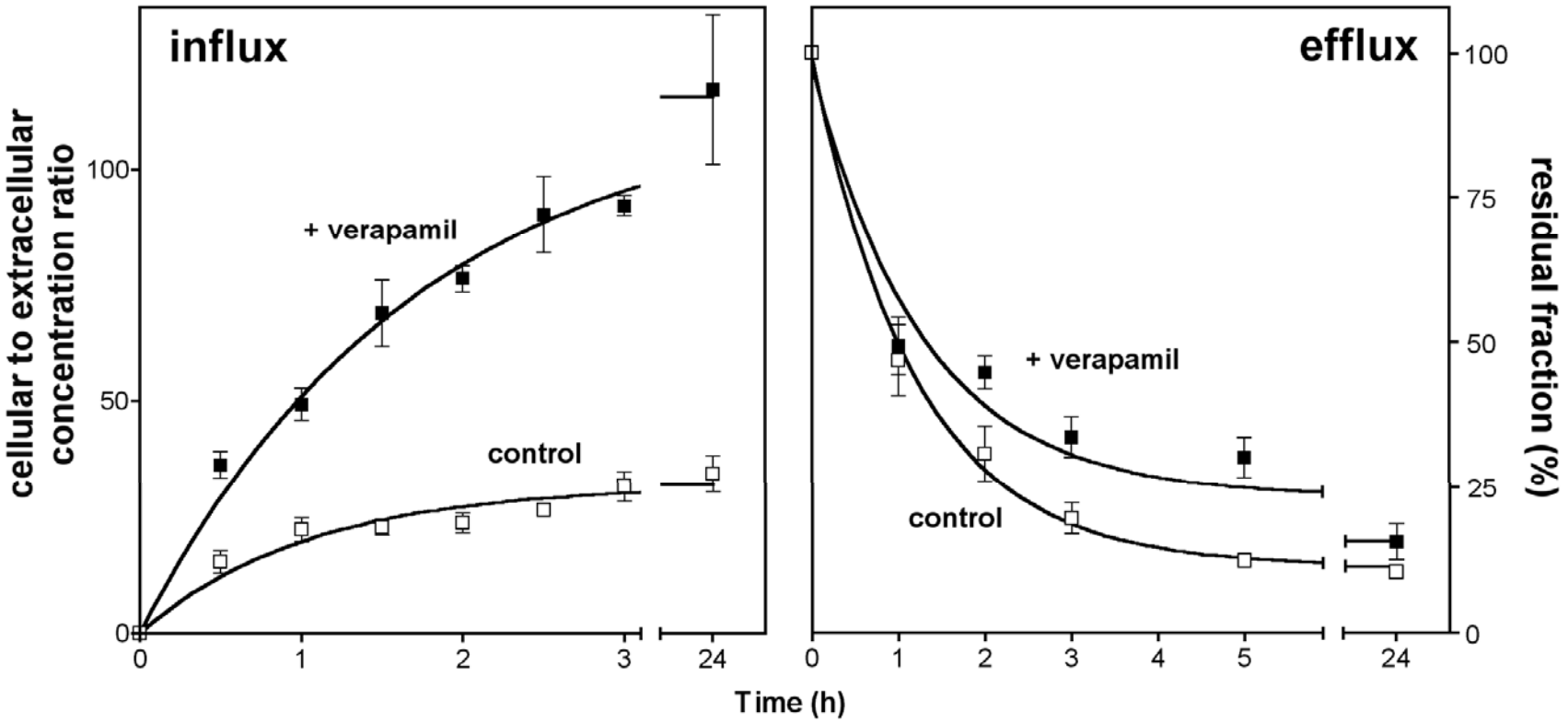
Exceprt from Carryn *et al.* Infect Dis Clin North Am. 2003 Sep;17(3):615-34.

Efflux and transport of antibiotics in eucaryotic cells: trans-barrier passage and intracellular accumulation



Van Bambeke et al. J. Antimicrob. Chemother. (2003) 51:1067-1077

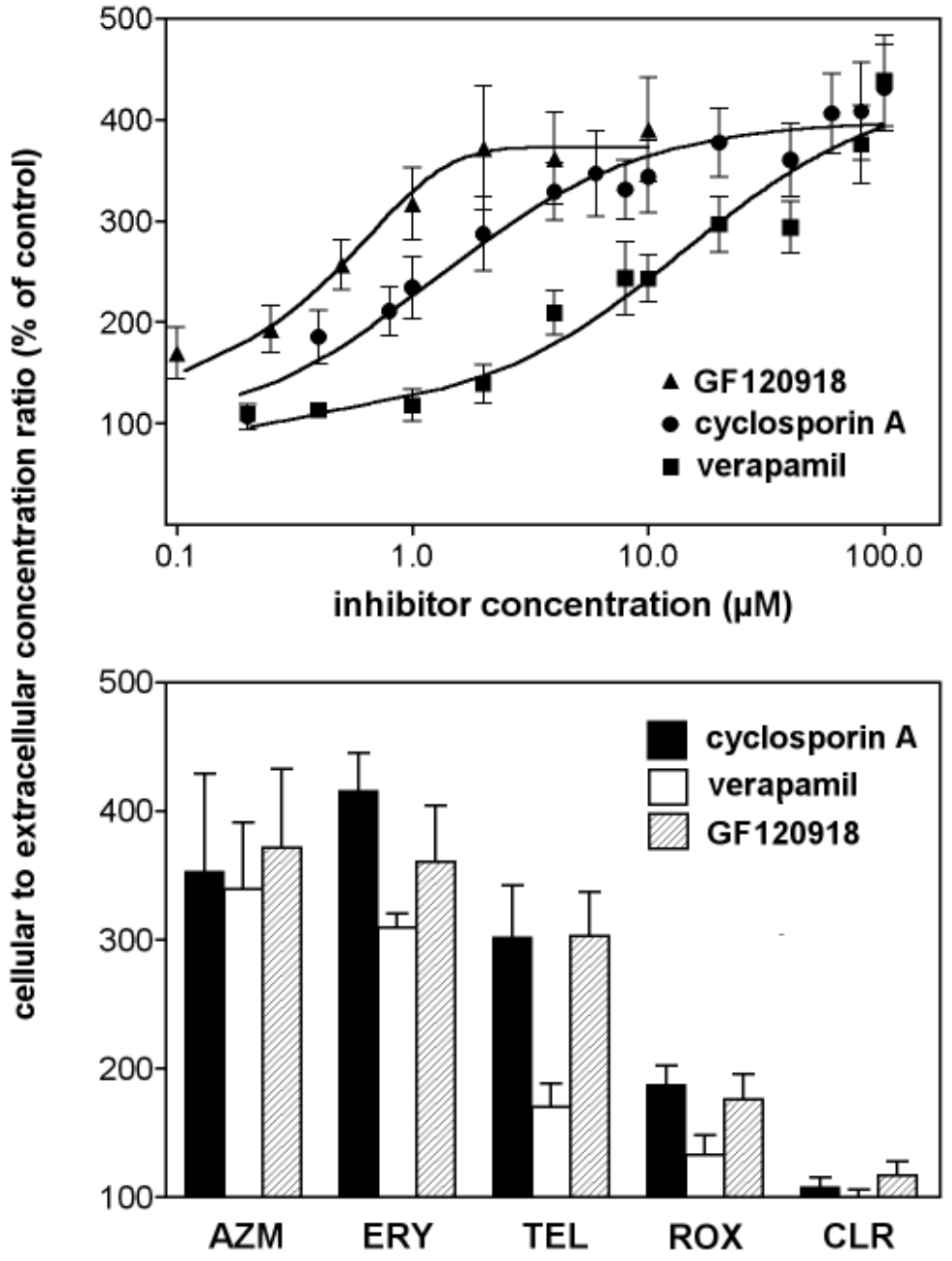
Azithromycin accumulation in macrophages is sub-optimal because of effflux through P-glycoprotein



Kinetics of uptake (A) and release (B) of azithromycin in J774 murine macrophages with (open squares) or without (closed squares) 20 µM verapamil.

Seral et al. Antimicrob. Agents Chemother. (2003) 47:1047-1051

Characterizing P-gp-mediated efflux and ranking macrolides



Seral et al. Antimicrob. Agents Chemother. (2003) 47:1047-1051

A bit more about the P-gp… with daptomycin …

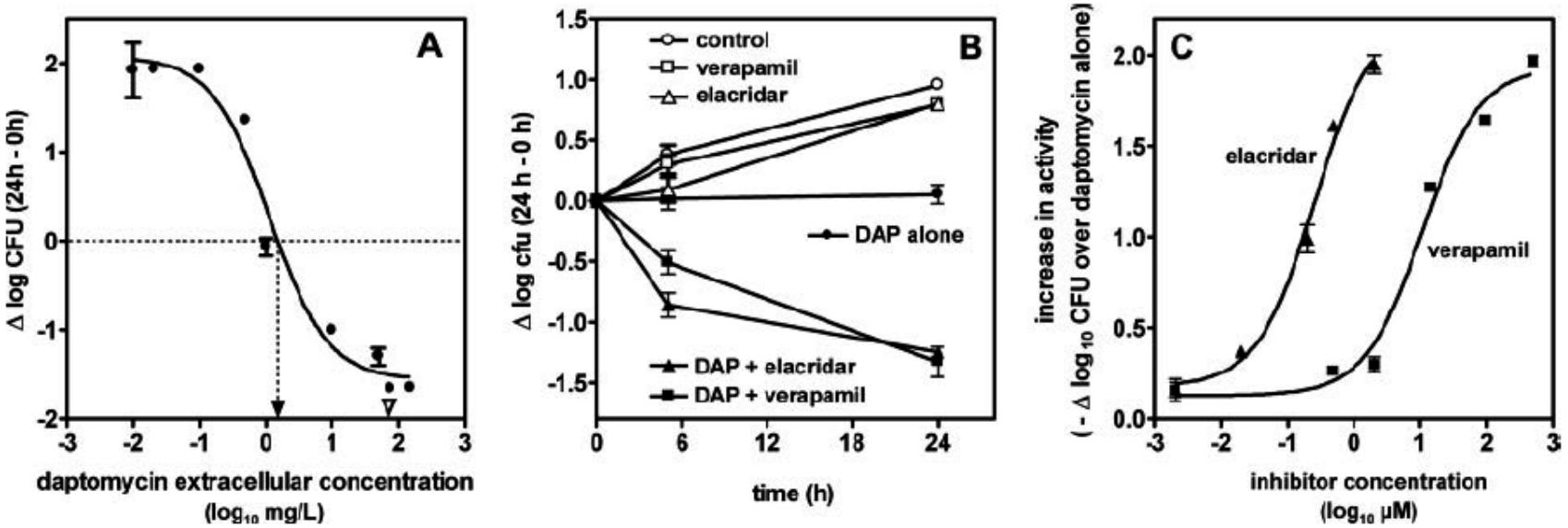
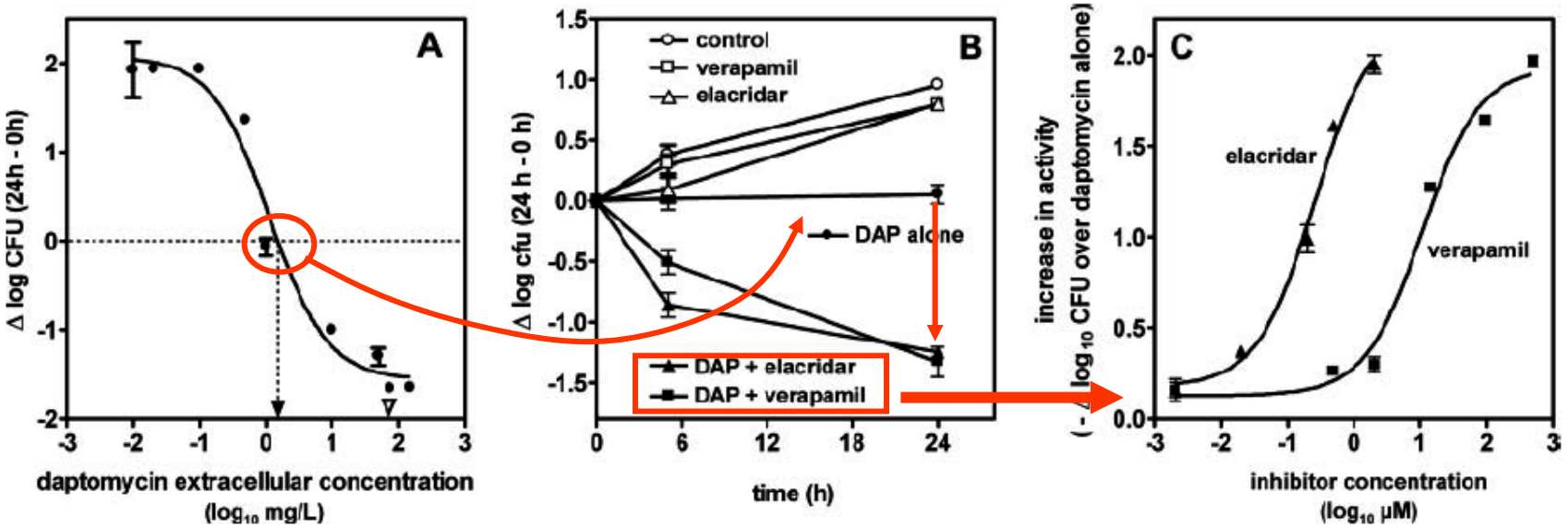


FIG. 1. Intracellular activity of daptomycin towards *S. aureus* ATCC 25923 in THP-1 macrophages. (A) Dose-response curves over a wide range of extracellular concentrations. The ordinate shows the change in the number of CFU (Δ log CFU) per milligram of cell protein at 24 h compared to the postphagocytosis inoculum. A sigmoidal (slope factor, 1) function was used for regression (see Table 1 for goodness-of-fit and regression parameters). The dotted horizontal line indicates a static effect, which was reached for the extracellular concentration shown by the vertical dotted line. For reference, the open triangle on the abscissa indicates the serum C_{max} (total drug) observed in volunteers receiving the clinically recommended dose of 4 mg/kg of body weight daptomycin (77 mg/liter) (67). (B) Influence of time and of the presence of efflux transporter inhibitors on the rate and the extent of the activity of daptomycin at a fixed extracellular concentration. The ordinate is as in panel A. Control, no treatment; DAP, daptomycin (1 mg/liter); verapamil (100 μM); elacridar (GF 120918; 0.5 μM). (C) Influence of the concentration of verapamil or elacridar on the activity of daptomycin (1 mg/liter) measured at 24 h. The ordinate shows the increase in activity defined as the difference between the change in CFU observed in the presence of the inhibitors minus what is observed with daptomycin alone (the graph shows the negative value of this difference to avoid describing increases in activity by decrements in the ordinate). All values are means ± standard deviations (n = 3; when not visible, the standard deviation bars are smaller than the symbols).

Lemaire et al. Antimicrob. Agents Chemother. 2008; 51:2748-2757

A bit more about the P-gp… with daptomycin …



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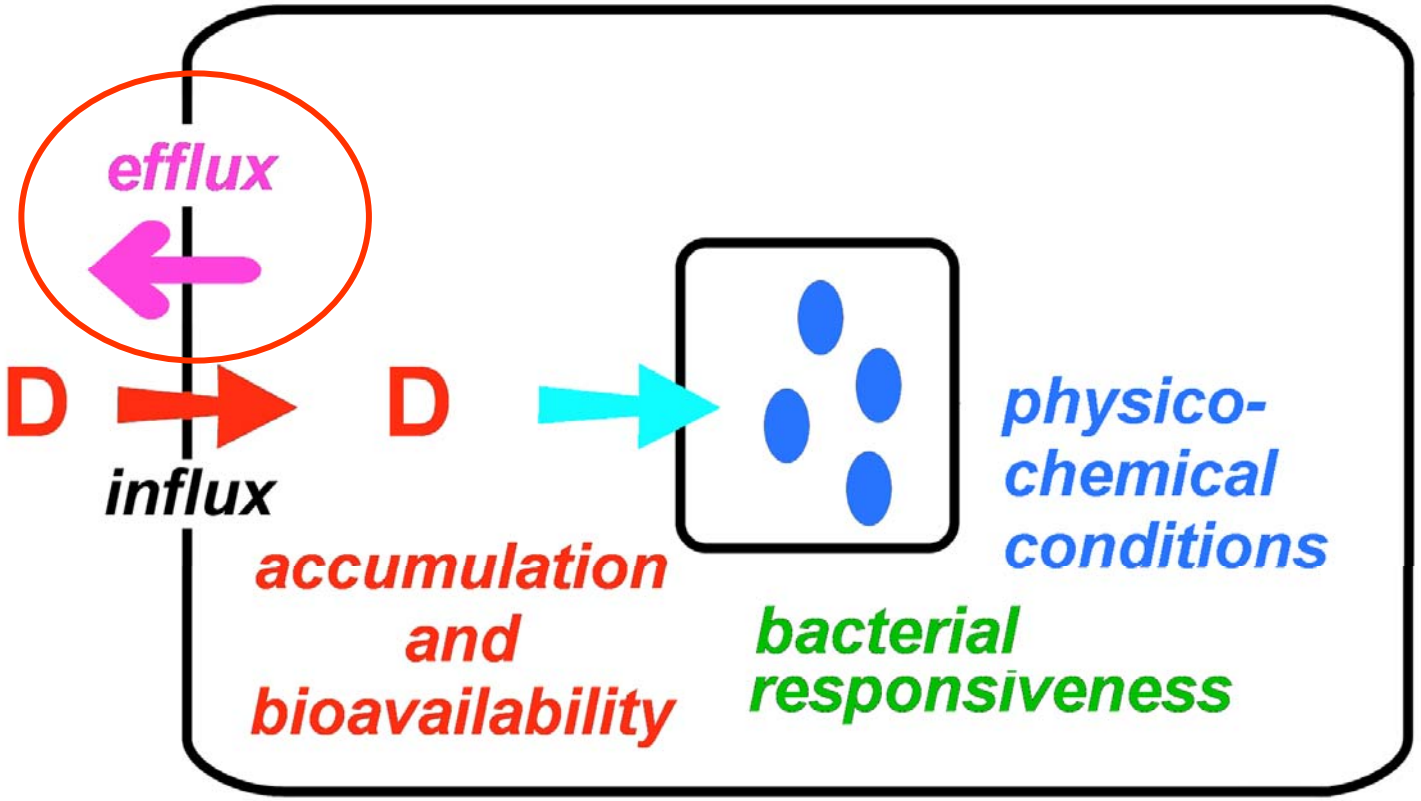
Lemaire et al. Antimicrob. Agents Chemother. 2008; 51:2748-2757

The scheme gets again and again a bit more complex

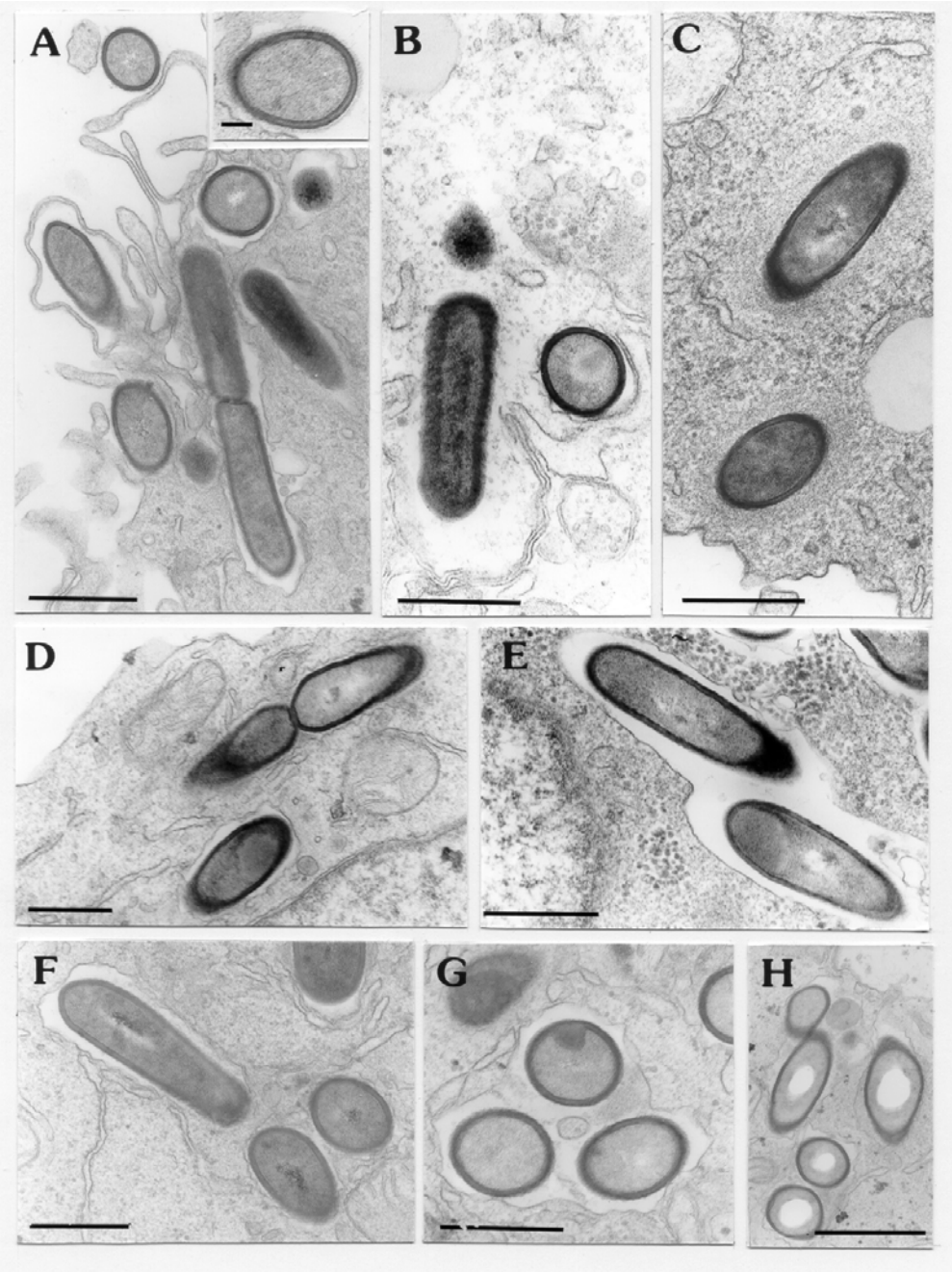


Exceprt from Carryn *et al.* Infect Dis Clin North Am. 2003 Sep;17(3):615-34.

Cooperation with host defenses: a story with Listeria monocytogenes ...

The intracellular pathway of Listeria monocytogenes ...

A-C: control
D-E: with gamma-interferon



Ouadrhiri et al. Antimicrob. Agents Chemother. (1999) 43:1242-1251

L. monocytogenes, gamma-interferon, and antibiotics

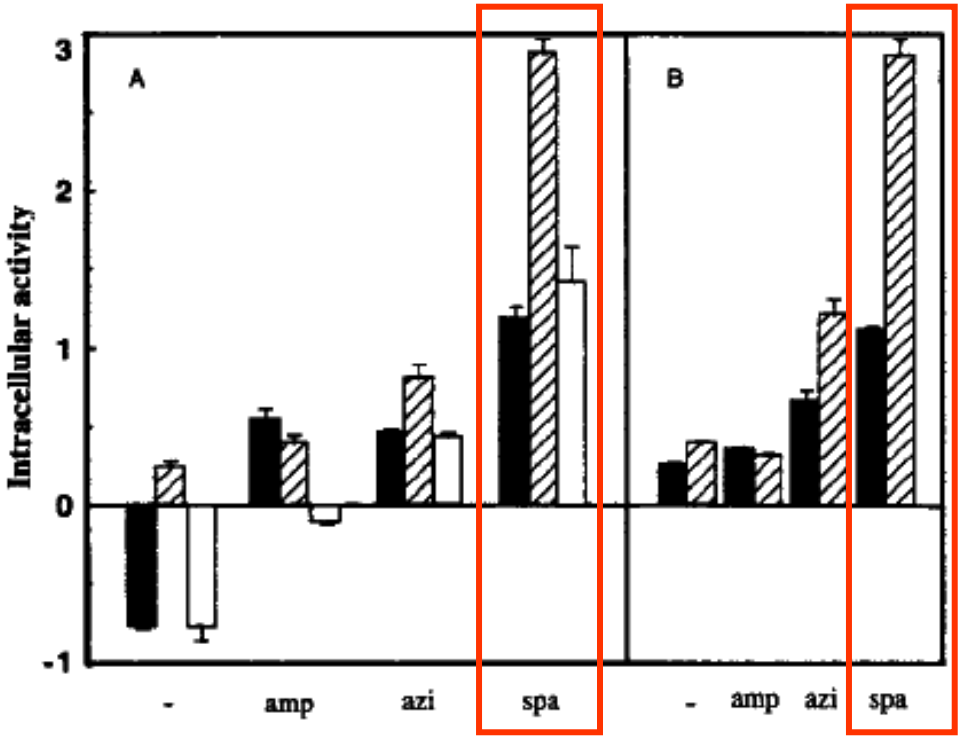
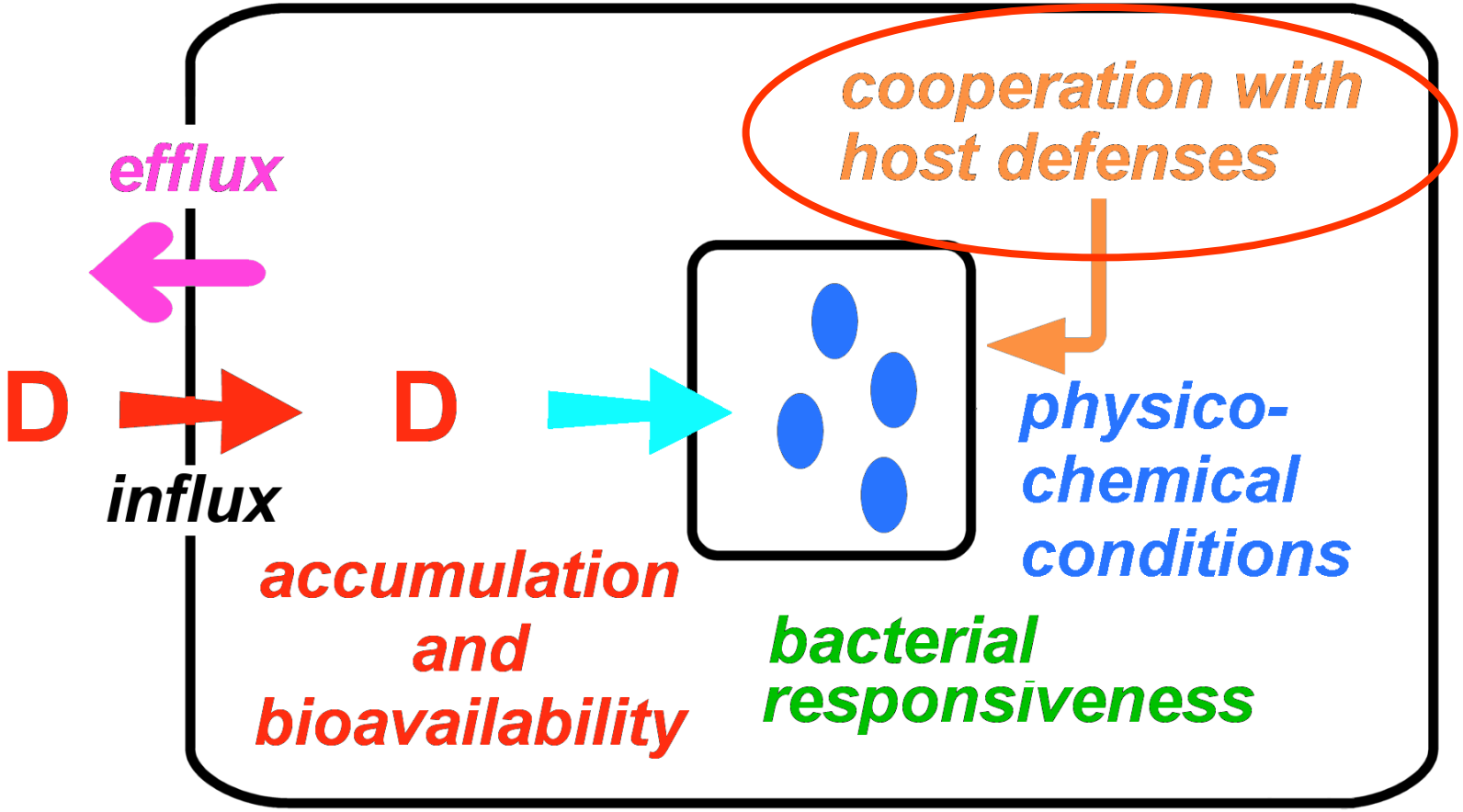


FIG. 7. Influence of the exposure of THP-1 macrophages to IFN-γ (100 U/ml), catalase, and L-MMA on the intrinsic activity of antibiotics towards intracellular *L. monocytogenes*. −, no antibiotic; amp, ampicillin; azi, azithromycin; spa, sparfloxacin. (A) Infection performed with the virulent variant Hly^+ in control (closed bars), in IFN-γ-treated cells (hatched bars), or in IFN-γ-treated THP-1 cells exposed to L-MMA and catalase (open bars). (B) Infection performed with the nonvirulent variant Hly^- in control (closed bars) and in IFN-γ-treated cells (hatched bars). Activity is defined as the $\log_{10}$ of the ratio of the number of CFU observed immediately after phagocytosis and washing to that after 5 h of incubation with the antibiotics (a negative value therefore means bacterial growth). Data are shown as means ± SD ($n = 3$). A statistical analysis of the differences seen between pertinent experimental groups of panel A is presented in Tables 1 and 2. For panel B, the difference between the data obtained for cells incubated with azithromycin or sparfloxacin alone and cells incubated with the same antibiotics but preexposed to IFN-γ is significant ($P < 0.005$ for azithromycin; $P < 0.001$ for sparfloxacin).

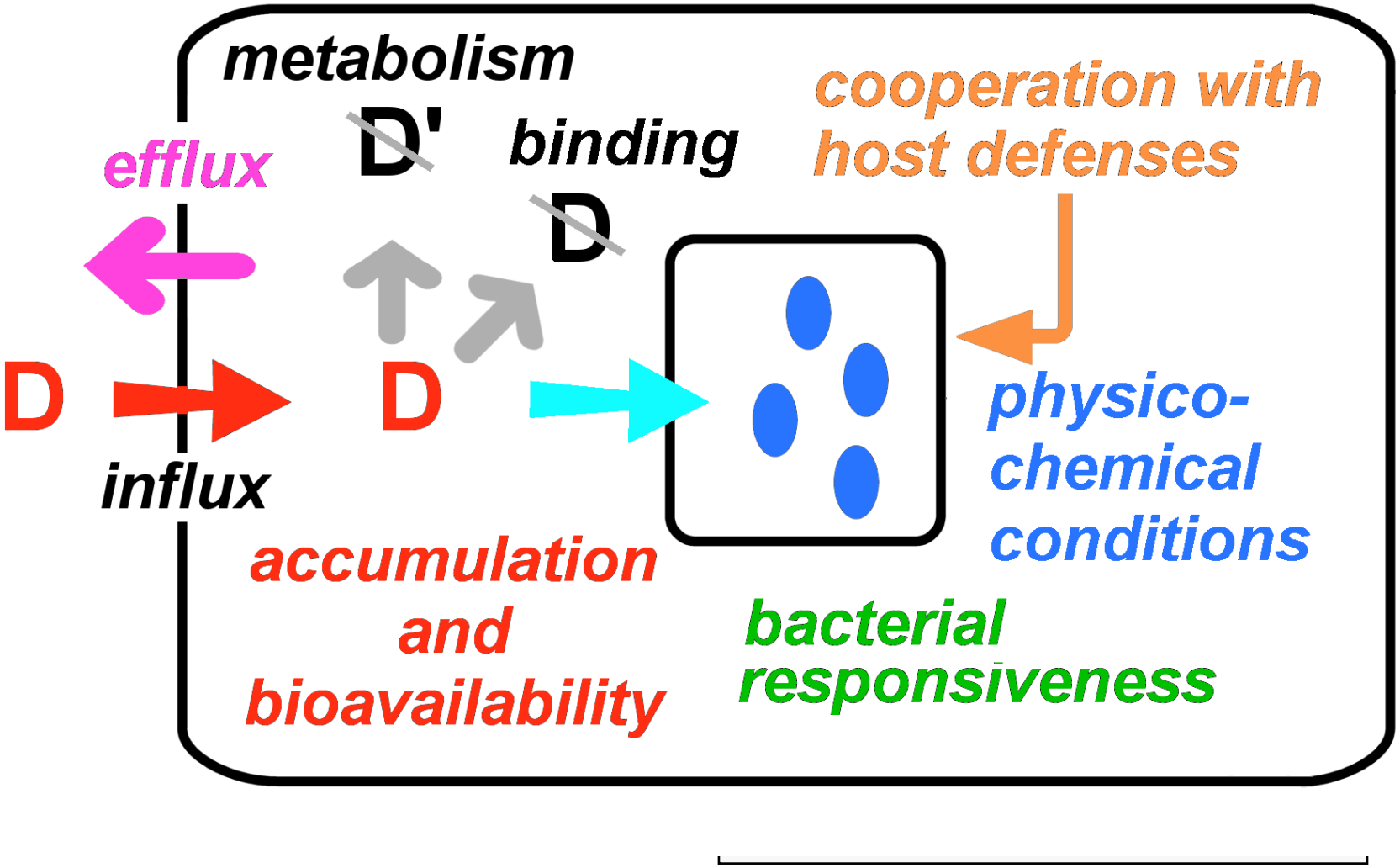
Ouadrhiri et al., Antimicrob. Agents Chemother. 1999; 43:1242-1251

At the end the scheme is not that simple at all …



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

And I still forgot a few things …



So, the magic balls have to go through many screens before they eventually reach and act on their targets…

How many will hit their target(s) ?

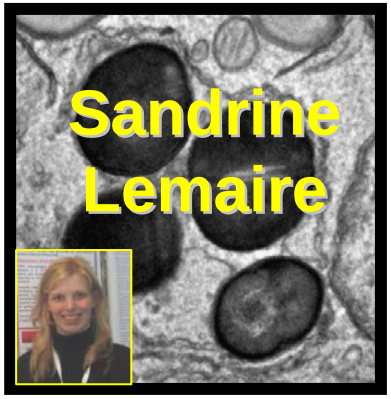
It all depends who you are…

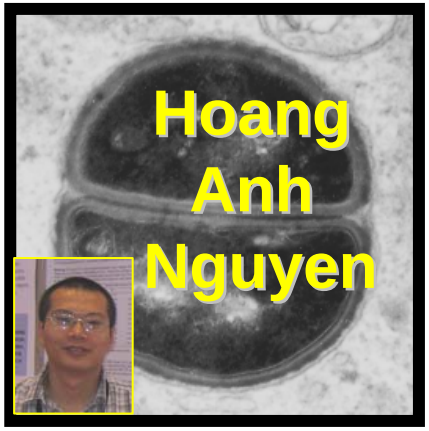
Paul Ehrlich (1854-1915)

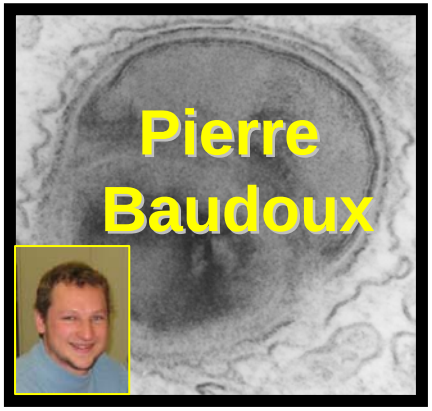
He devoted himself to chemotherapy, basing his work on the idea that the chemical constitution of drugs must be studied in relation to their mode of action and their affinity for the cells of the organisms against which they were directed. These would be, as Ehrlich expressed it, «magic bullets» which would go straight to the organisms at which they were aimed.

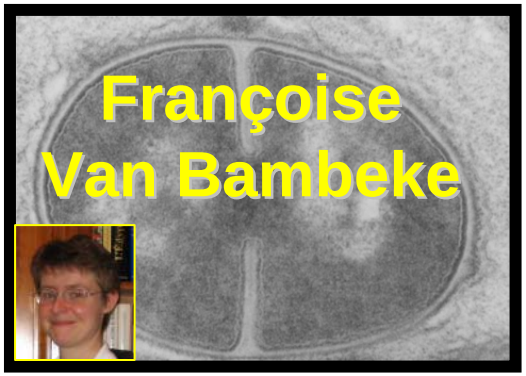
From *Nobel Lectures, Physiology or Medicine 1901-1921*, Elsevier Publishing Company, Amsterdam, 1967

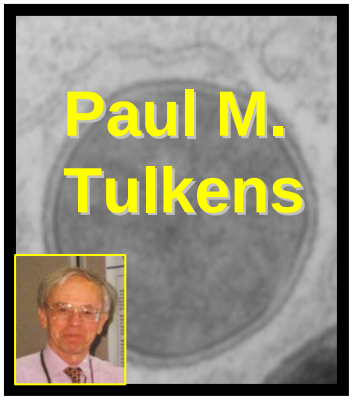
Anyway, the Staph kept us busy and happy …

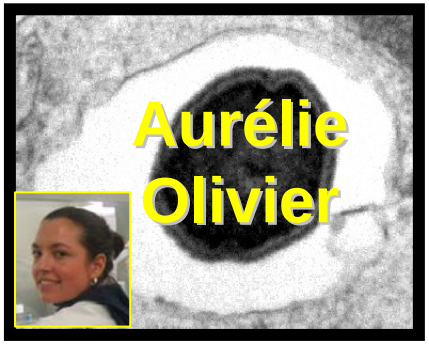












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- P. Appelbaum, Hershey Medical Center, Hershey, PA, USA
- and many others…