Intracellular Bacterial Infections: Why Are Antibiotics Poorly Efficient and Can We Do Something About it ?

Paul M. Tulkens, MD, PhD *

Cellular and Molecular Pharmacology
Louvain Drug Research Institute
Health Science Sector
Université catholique de Louvain
Brussels, Belgium

University of Notre-Dame, Indiana, U.S.A. – 15 June 2016

* with slides borrowed from Françoise Van Bambeke and Frederic Peyrusson

Disclosures and slides availability

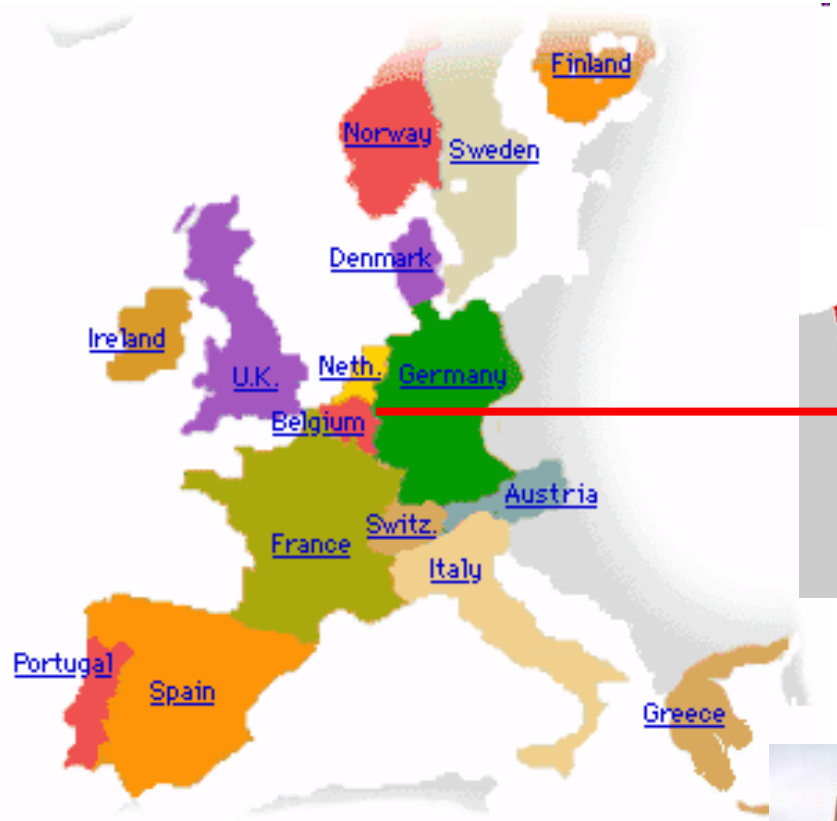
- Research grants
  - Theravance, Astellas, Targanta, Cerexa/Forest, AstraZeneca, Bayer, GSK, Trius, Rib-X, Eumedica, Debiopharm
  - Belgian Science Foundation (*F.R.S.-FNRS*), Ministry of Health (*SPF*), Walloon and Brussels Regions, European Union (*FP7 programme*)

- Speaking fees
  - Bayer, GSK, Sanofi, Johnson & Johnson, OM-Pharma

- Decision-making and consultation bodies
  - European Committee for Antimicrobial Susceptibility Testing [EUCAST] (General Assembly and steering committee (2010-2012))
  - European Medicines Agency (external ad-hoc expert)
  - US National Institutes of Health (grant reviewing)
  - **Drive-AB [*Driving reinvestment in R&D and responsible use for antibiotics*]** (governance)

Slides: http://www.facm.ucl.ac.be → Lectures

Belgium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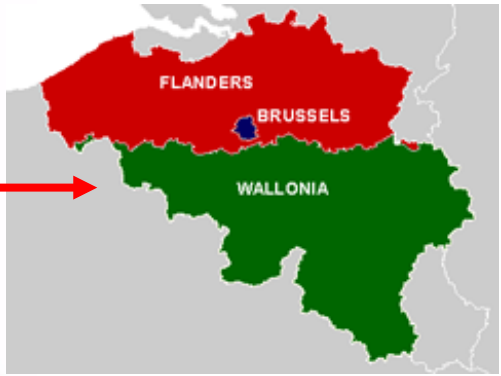
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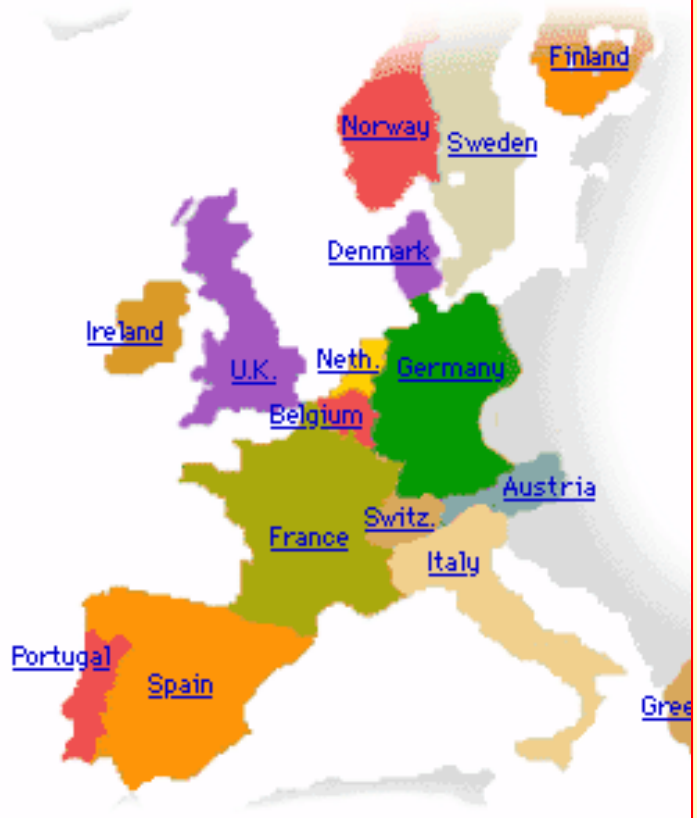


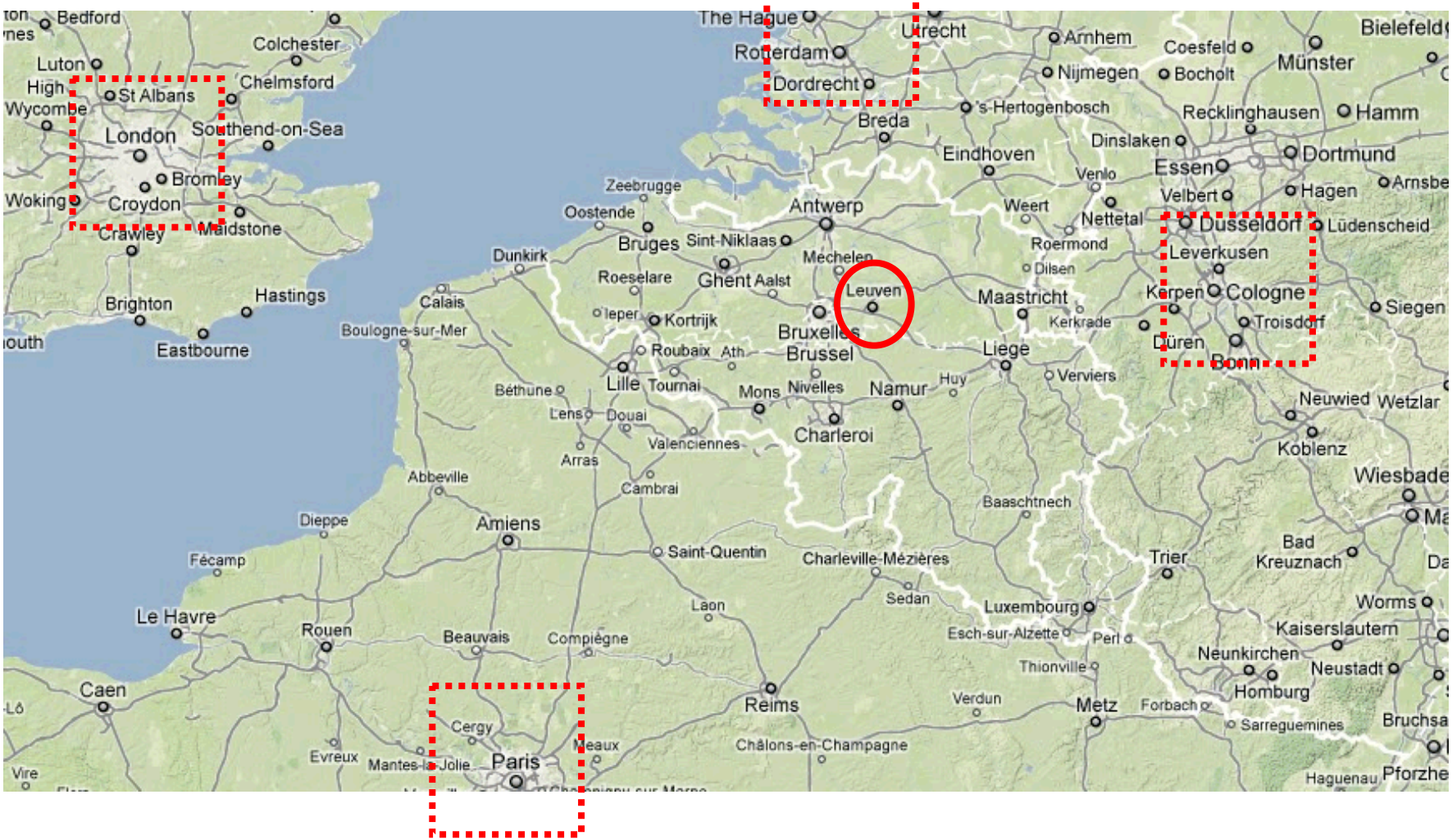
10 millions inhabitants …

10 Nobel prizes (10/850)

- **Peace**
  - Institute of International Law, Ghent (1904)
  - Auguste Beernaert (1909)
  - Henri Lafontaine (1913)
  - Father Dominique Pire (1958)
- **Literature**
  - Maurice Maeterlinck, Ghent (1911)
- **Medicine**
  - Jules Bordet, Brussels (1919)
  - Corneille Heymans, Ghent (1938)
  - Christian de Duve, Louvain (1974)
  - Albert Claude, Brussels (1974)
- **Chemistry**
  - Ilya Prigogyne, Brussels (1977)
- **Physics**
  - François Englert, Brussels (2013)

The *Catholic University of Louvain* in brief (1 of 4)

- originally founded in **1425** in the city of **Louvain** (in French and English; known as **Leuven** in Flemish)

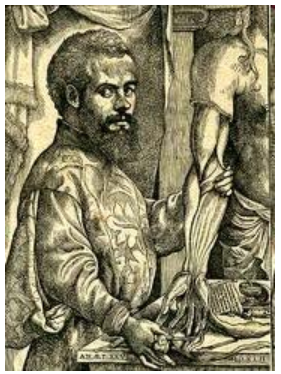


The *Catholic University of Louvain* in brief (2 of 4)

- It was one of the major University of the so-called "Low Countries" in the 1500 – 1800 period, with famous scholars and discoverers (Vesalius for anatomy, Erasmus for philosophy, …). Teaching was in Latin, Greek, and Hebrew (College of the 3 languages…)

The University in the 1500's

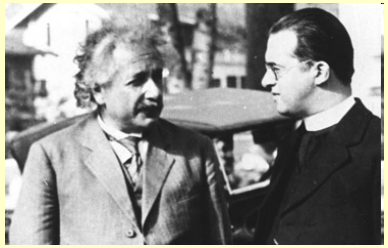
Erasmus



Vesalius

The *Catholic University of Louvain* in brief (3 of 4)

- In the 19th century, teaching was in French but in the early 1900's, a Flemish-speaking section was opened. Courses were given in both languages, attracting many students and celebrities…

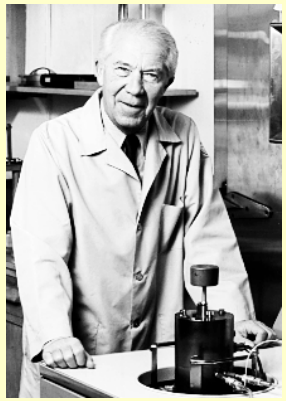


more in a minute

Prof. G. Lemaitre, professor of Physics and Mathematics at the University who, in the 1930's, made the first suggestion of the continuous expansion of the Universe ("*big bang*")
(here in conversation with A. Einstein)

Professor C. de Duve, Professor of Biochemistry, obtained the Nobel Prize (Physiology and Medicine) in 1974 for his work on intracellular organelles (lysosomes, peroxisomes…)

(here in front of a centrifuge)



- in 1968, the University was divided into
  - a French-speaking ***Université catholique de Louvain***
  - a Flemish-speaking ***Katholieke Universiteit Leuven…***

The *Catholic University of Louvain* in brief (4 of 4)

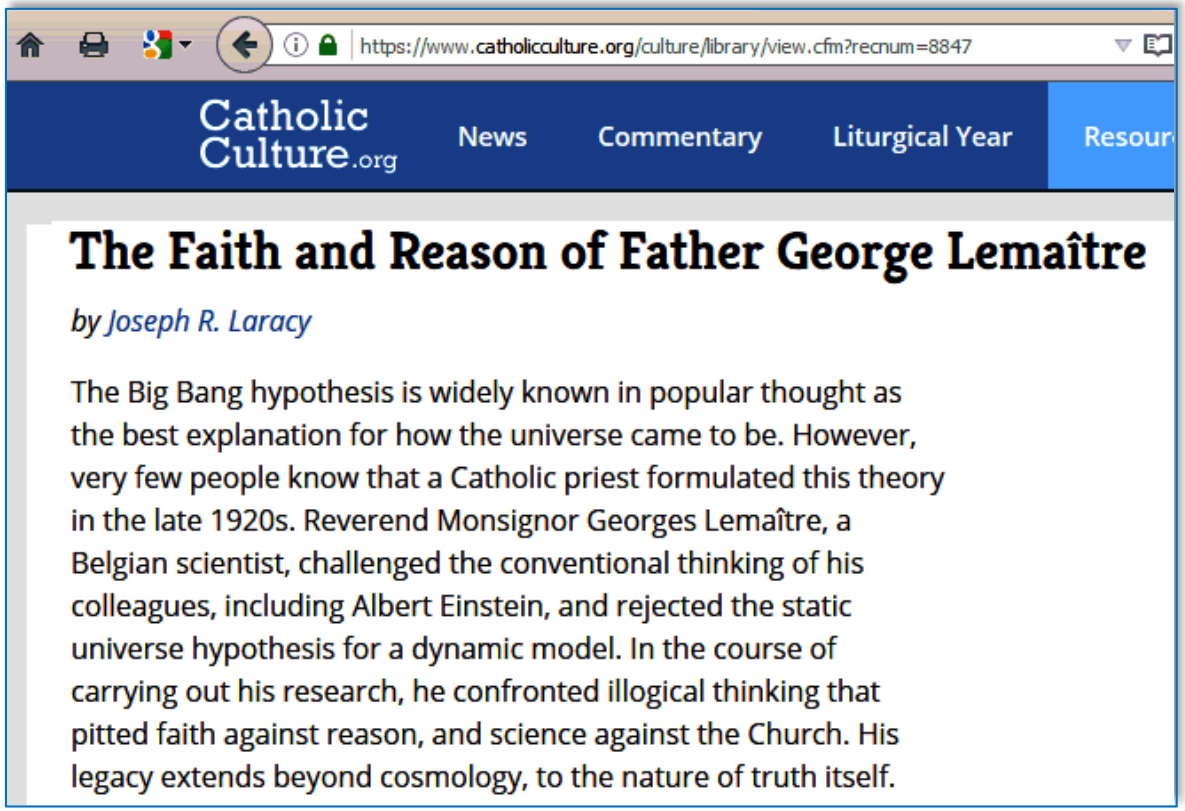
- The Flemish-speaking ***Katholieke Universiteit Leuven*** has remained in Louvain (Leuven) and is named in English "**Catholic Universiteit Leuven**".
- The French-speaking ***Université catholique de Louvain*** has moved about 25 km South in a place called "Louvain-la-Neuve, with the "Health Sciences Sector" located in Brussels (Woluwé)

Université catholique de Louvain
http://www.uclouvain.be

Katholieke Universiteit Leuven
http://www.kuleuven.be

- Together, the two Universities have about **55,000 students**

Notre Dame and the "Big Bang"



https://www.catholicculture.org/culture/library/view.cfm?recnum=8847 – last visited: 12 Jun 2016

Notre Dame and the "Big Bang"

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News Commentary Liturgical Year Resour

The Faith and Reason of Father George Lemaître

by Joseph R. Laracy

The Big Bang hypothesi
the best explanation for
very few people know th
in the late 1920s. Revere
Belgian scientist, challer
colleagues, including Alb
universe hypothesis for
carrying out his researc
pitted faith against reas
legacy extends beyond

A Homogeneous Universe of Constant Mass and Increasing Radius accounting for the Radial Velocity of Extra-galactic Nebulæ. By Abbé G. Lemaître.

(*Translated by permission from "Annales de la Société scientifique de Bruxelles," Tome XLVII, série A, première partie.*)

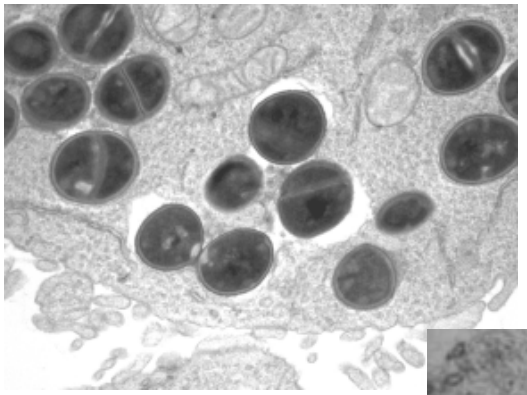
https://dx.doi.org/10.1093%2Fmnras%2F91.5.483

In 1936, Father John O'Hare, the president of the University of Notre Dame, hired Father Lemaître as a visiting professor. During that year, his course on cosmology was not only attended by graduate students, but also faculty members in the physics and mathematics departments.

https://www.catholicculture.org/culture/library/view.cfm?recnum=8847 – last visited: 12 Jun 2016

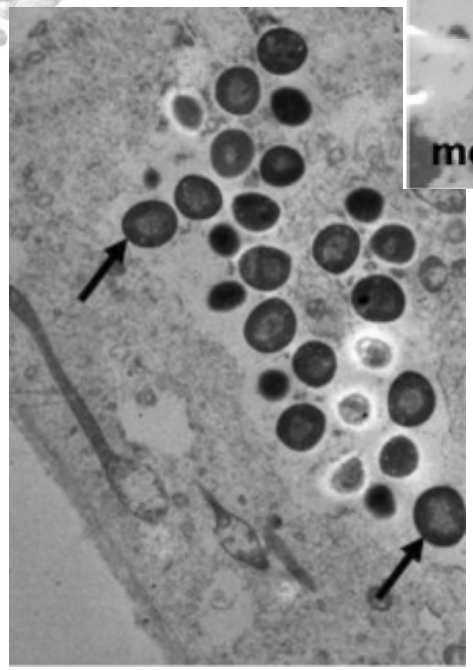
So, now, our small bang…

***S. aureus* in THP-1 macrophages**



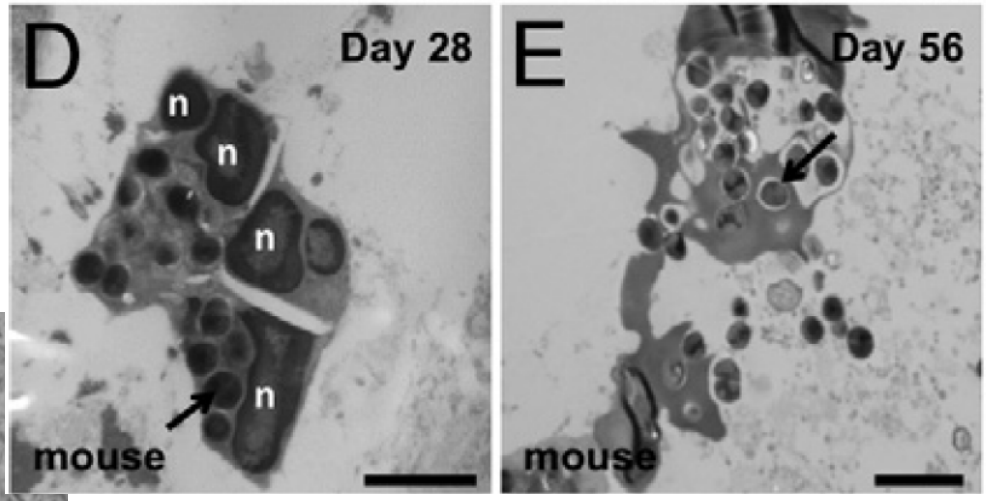
Van Bambeke & Tulkens
unpublished

***S. aureus* in human osteoblasts**



Kalinka *et al.*, Int J Med Microbiol. 2014;
304:1038-49 - PMID: 25129555

S. aureus in and released from neutrophils in a mouse osteomyelitis model



Horst *et al.* Am J Pathol 2012;181:1206–1214 - PMID: 22902429

intracellular *S. aureus*

And more bangs…

B. Löffler et al. / International Journal of Medical Microbiology 304 (2014) 170–176

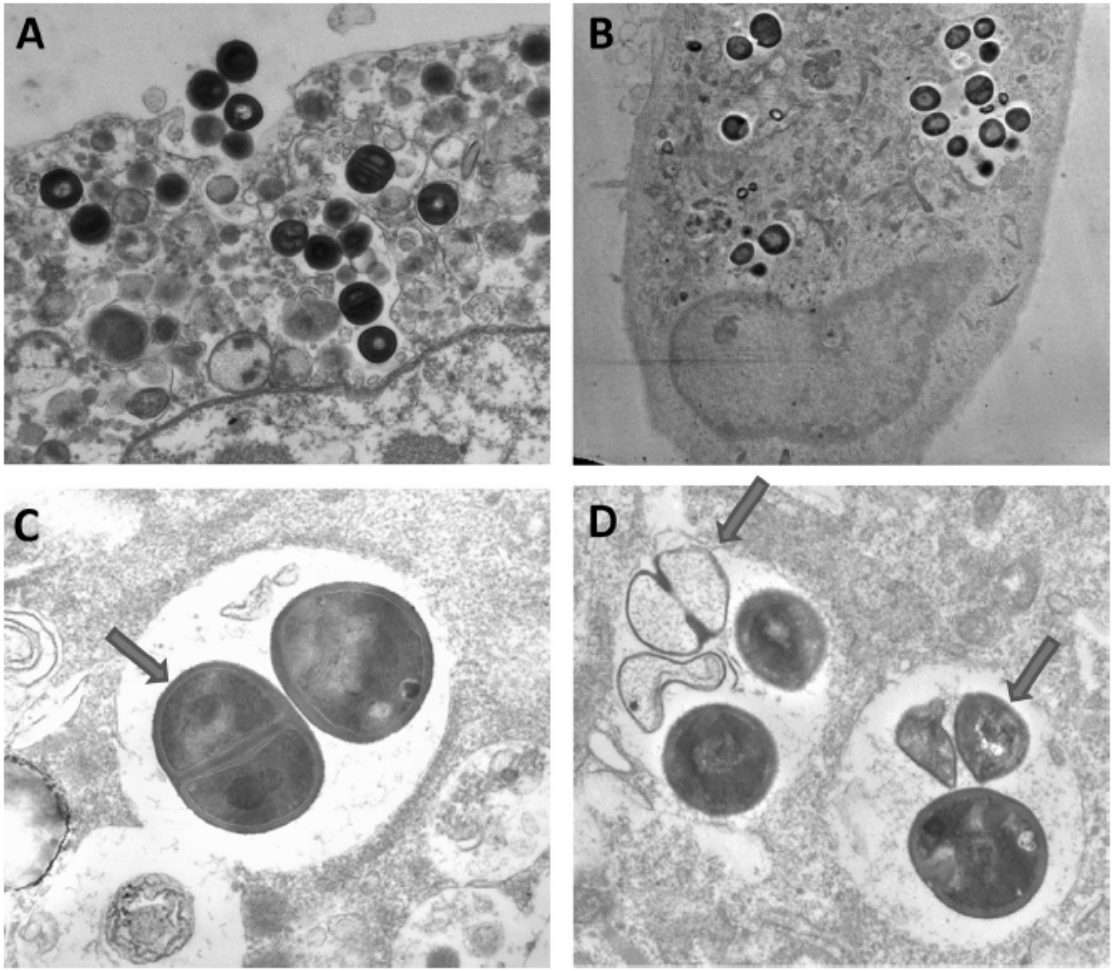


Fig. 1. Electron micrographs of different types of infected host cells. Adherence and uptake of *S. aureus* in epithelial A549 cells (A). Intracellular location of *S. aureus* after infection of primary osteoblasts (B). Dividing figure of *S. aureus* within an intracellular phagosome (C) and intracellular bacterial degradation (D) 24 h after infection of endothelial cells (HUVEC).

Why do we wish to look at intracellular activity of antibiotics ?

- Beyond truly obligate intracellular parasites (e.g., *Legionella*, *Chlamydia*, *Mycobacteriae*, …many more "common" bacteria are facultative (e.g. ***Listeria***) or occasional (e.g. ***Staphylococci***, ***Pseudomonas***…) intracellular parasites …

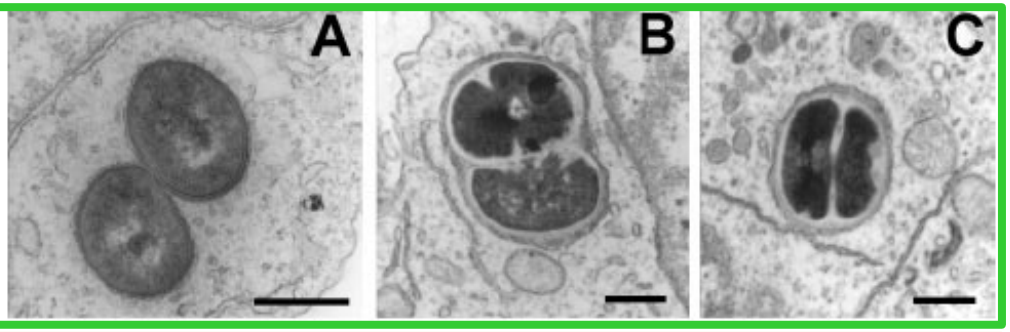
- These bacteria form a **reservoir** from where bacteria may escape causing **relapses** and **recurrences** of the infection…

- Natural defenses often restrict their growth and decrease their persistence, but not always…

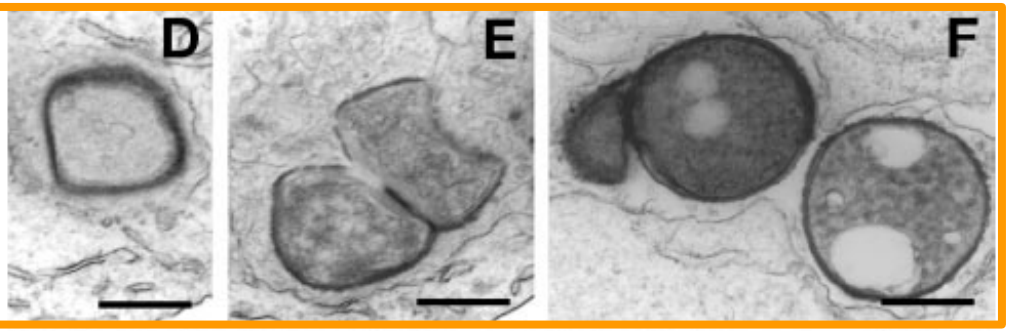
- You may need to help host defenses with **antibiotics**

Are antibiotics active at all in cells ? [1]

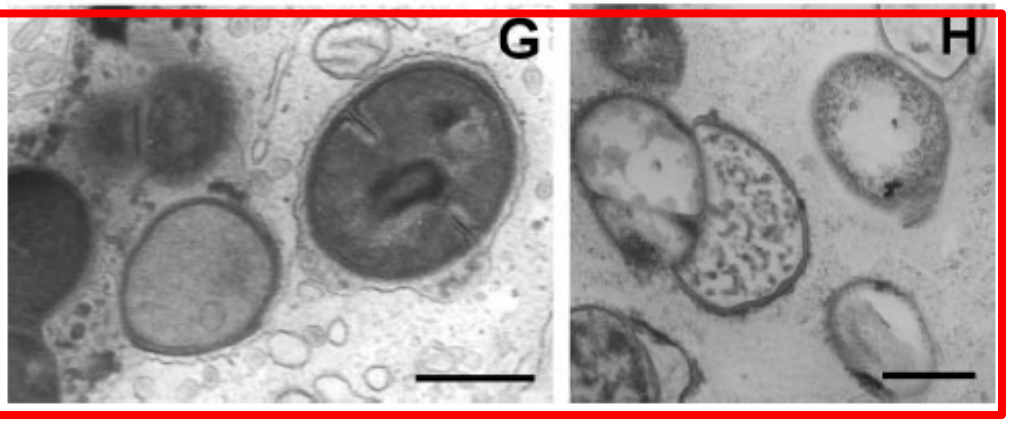
Control (no antibiotic) [2]



Oxacillin 63 mg/L
(= C_{max} and 500 x MIC)



Oritavancin 25 mg/L
(= C_{max} and 100 x MIC)



[1] THP1 monocytes model – 24 h incubation. See Barcia-Macay et al. Antimicrob Agents Chemother 2006;50:841-851 – PMID: 1649524
[2] gentamicin added at 1 x MIC to prevent extracellular growth

Intracellular activity of antibiotics

- **What has been know for long about pharmacokinetics…**
- **What has surprised us …**
- **Adding pharmacodynamics …**
- **A renewed model ?**

Intracellular activity of antibiotics

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A simple view in 1991

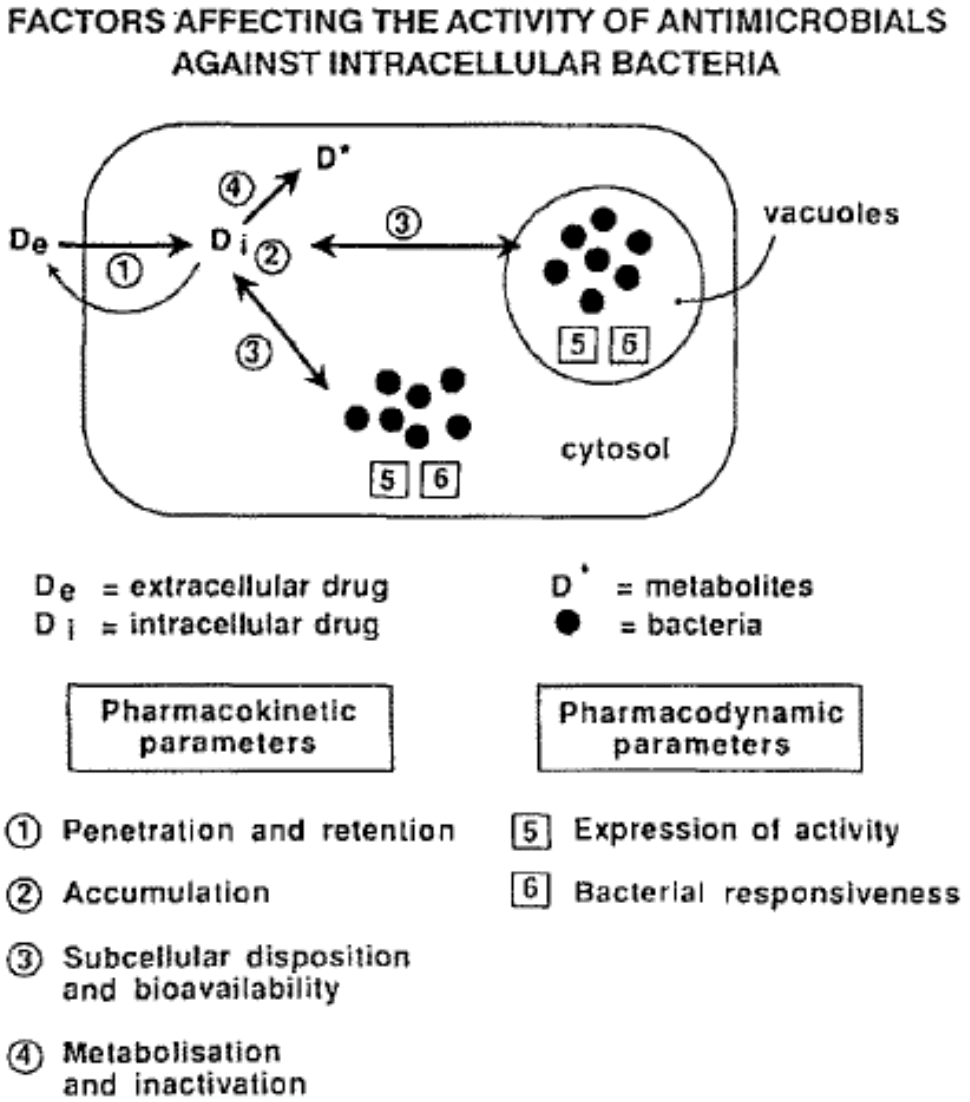


Figure 1: Pharmacokinetic and pharmacodynamic parameters involved in the activity of antimicrobial drugs against intracellular microorganisms.

Tulkens PM. Intracellular distribution and activity of antibiotics. Eur J Clin Microbiol Infect Dis. 1991 10:100-6. PubMed PMID: 1864271.

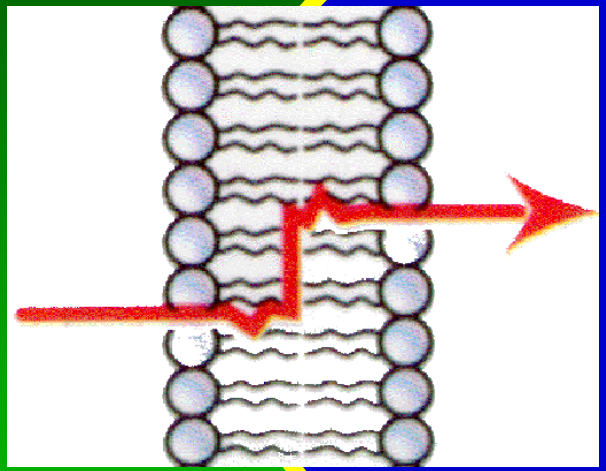
Which antibiotics accumulate in cells ?

- **beta-lactams: ≤ 1x**
- **aminoglycosides: <1 to 2 x**
- **ansamycins: 2-3 x**
- **tetracyclines: 2-4 x**
- **fluoroquinolones: 5 - 20 x**
- **macrolides: 4 to > 100 x ***
- **glycopeptides: 1 to 400 x !! ****

*** azithromycin, ketolides**
**** oritavancin**

How do antibiotics penetrate in cells ?

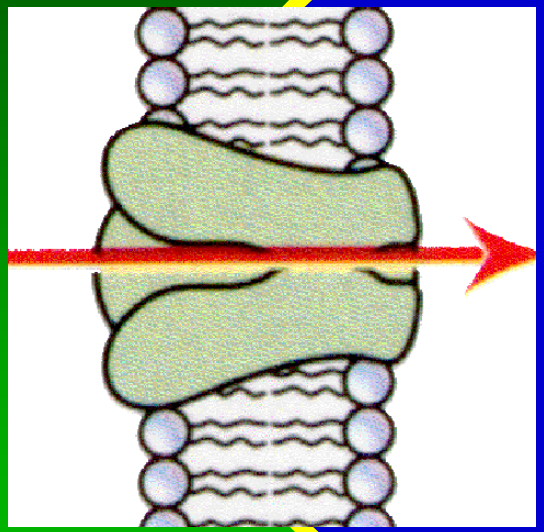
1. diffusion



- macrolides
- fluoroquinolones
- tetracyclines
- ansamycines
- β-lactams,
- ...

How do antibiotics penetrate in cells ?

2. carrier-mediated influx

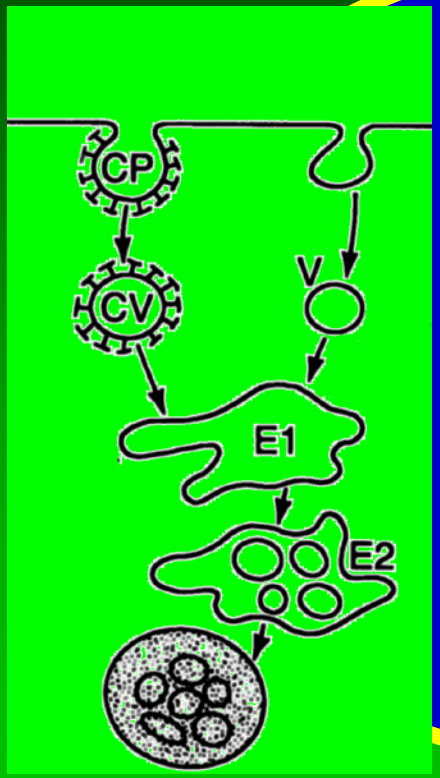


- specific structure
- (some energy-dependent)
- saturable
- competition by analogues

highly variable rom on cell type to another

How do antibiotics penetrate in cells ?

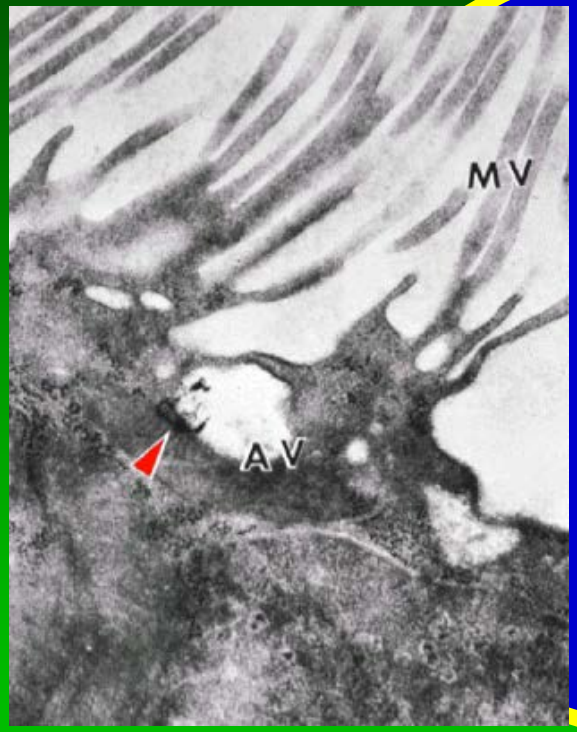
3. pinocytosis



- aminoglycosides
- glycopeptides

How do antibiotics penetrate in cells ?

receptor-mediated pinocytosis in kidney cortex

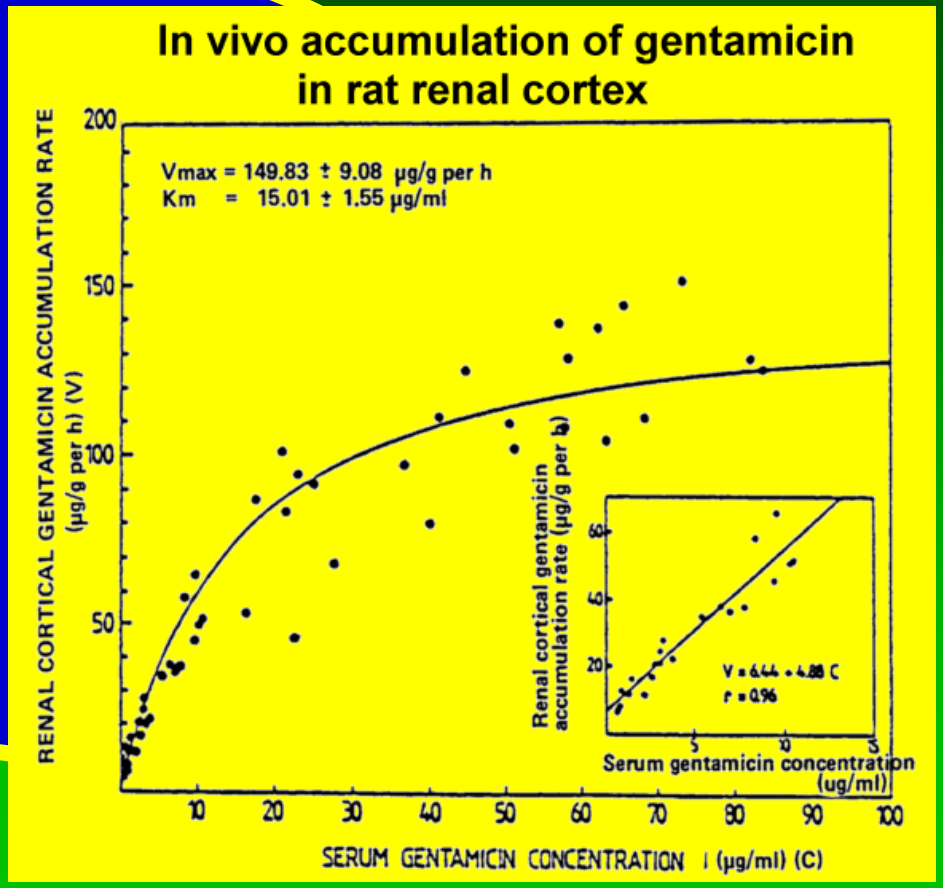


Binding to megalin and acidic phospholipids

Silverblatt & Kuehn C. Kidney Int. 1979;15:335-45 - PMID: 513493

Moestrup *et al*. J Clin Invest. 1995;96:1404-13 – PMID: 7544804

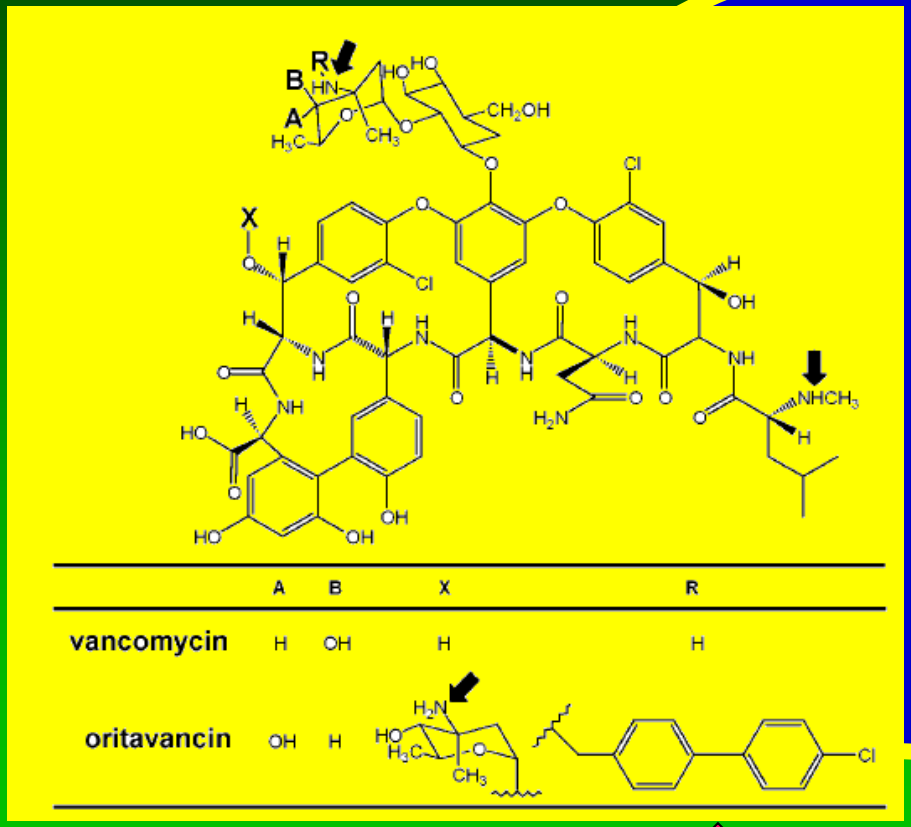
Sastrasinh et al. J Pharmacol Exp Ther 1982;222:350-8 - PMID: 7097565



Giuliano *et al*. J Pharmacol Exp Ther 1986;236:470-5 - PMID: 3944768

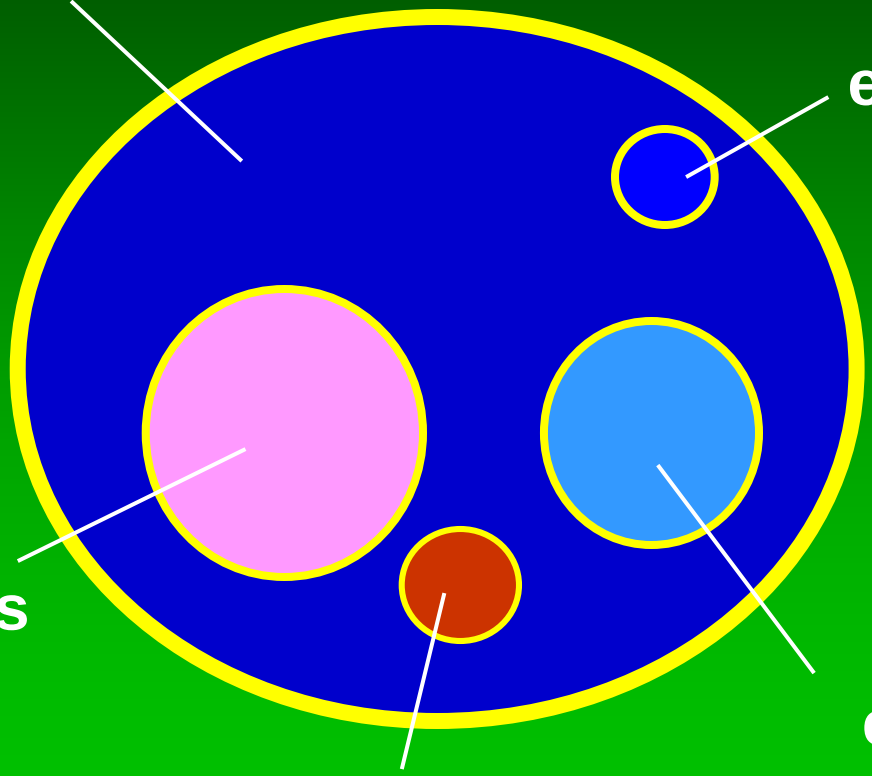
How do antibiotics penetrate in cells ?

membrane binding and uptake of lipoglycopeptides

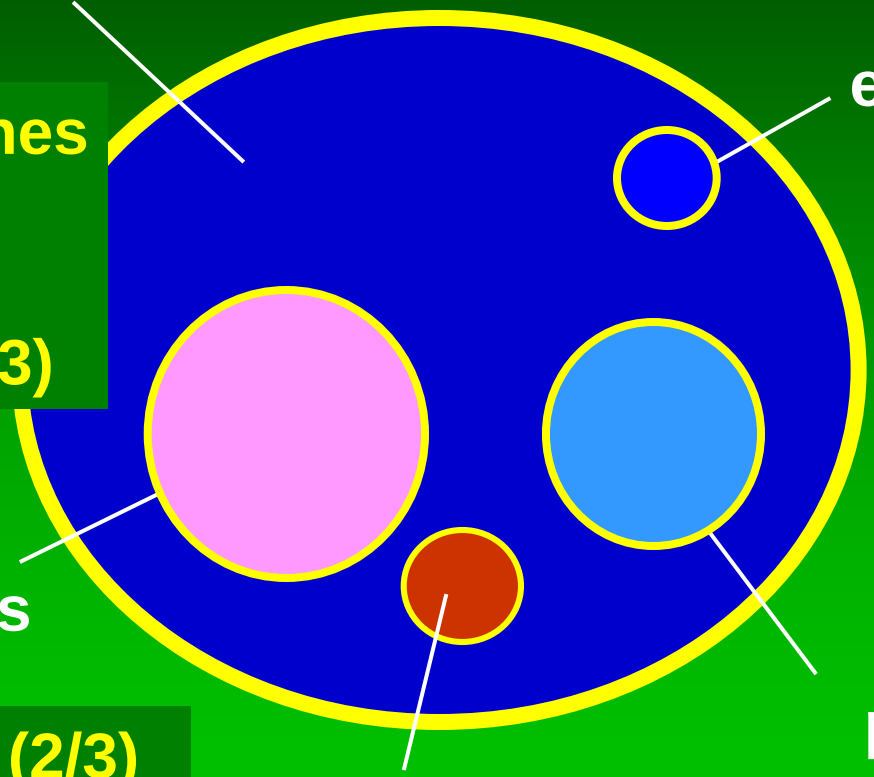


Van Bambeke *et al*. Antimicrob Agents Chemother 2004;48:2853-2860 – PMID: 15273091

But once in cells, where are the drugs ?



Subcellular localization: a quick answer ?



- fluoroquinolones
- beta-lactams
- ansamycins
- macrolides (1/3)

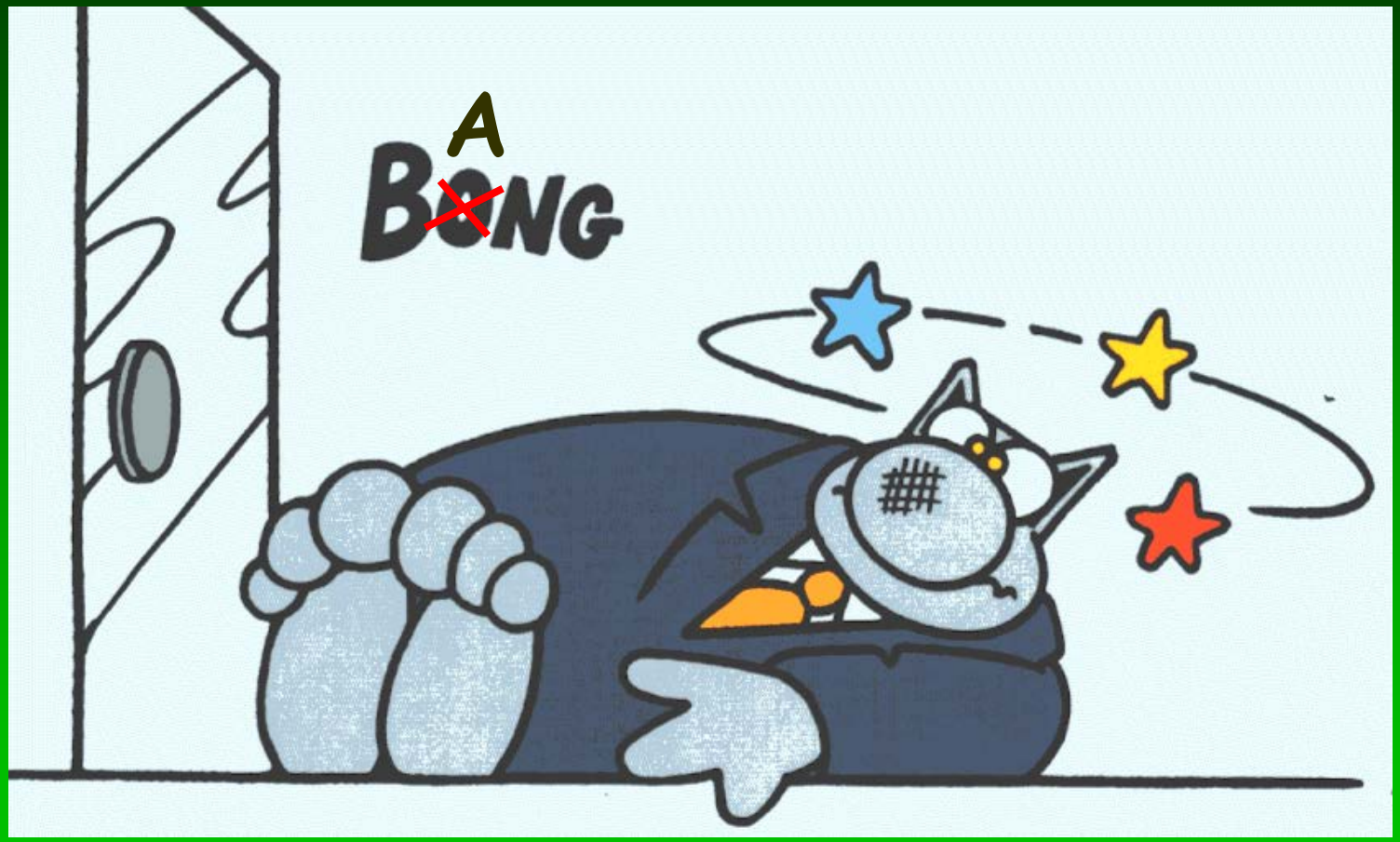
- macrolides (2/3)
- aminoglycosides

So, what we know in a nutshell ...

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400		
	Azithromycin	40 to 300	~ 16 to 120		
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown
	Lincomycin	1 to 4	~ 15 to 60		
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown	
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000		Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500		Lysosomes
	Telavancin	50 (after 24 h)	~ 4500		Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown

Adapted from Van Bambeke *et al.*, Curr Opin Drug Discov Devel 2006;9:218-230 – PMID: 16566292

But where does this lead us for activity ?



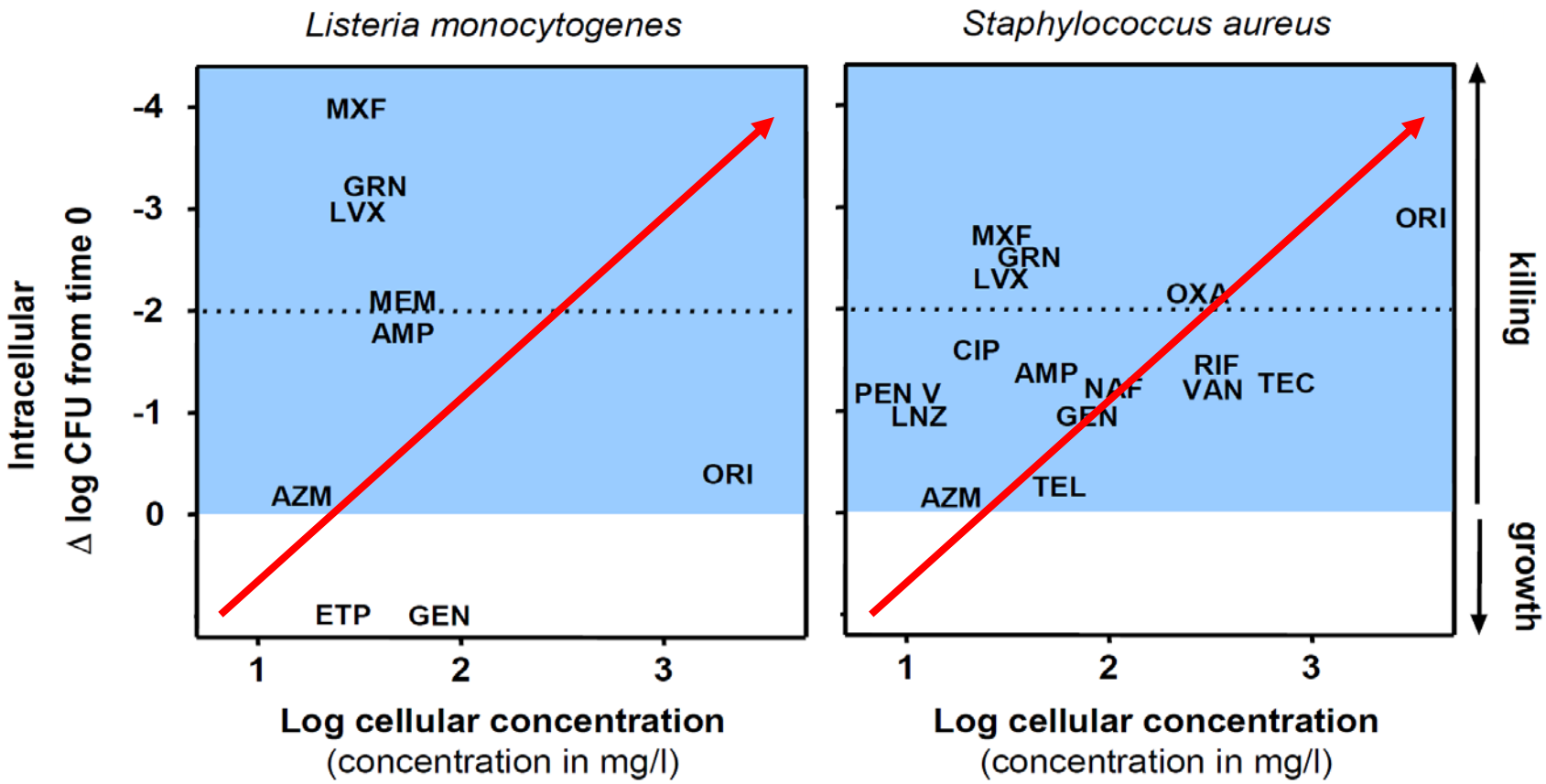
Ph. Geluck, with permission

* taken from a slide presented at ECCMID in 2002

Intracellular activity of antibiotics

- What has been know for long about pharmacokinetics…
- **What has surprised us …**
- Adding pharmacodynamics …
- A renewed model ?

Intracellular activity is not directly correlated to accumulation



AMP=ampicillin; **AZM**=azithromycin; **CIP**=ciprofloxacin; **ETP**=ertapenem; **GEN**=gentamicin; **GRN**=garenoxacin; **LNZ**=linezolid; **LVX**=levofloxacin; **MEM**=meropenem; **MXF**=moxifloxacin; **NAF**=nafcillin; **ORI**=oritavancin; **OXA**=oxacillin; **PEN V**=penicillin V; **RIF**=rifampicin; **TEC**=teicoplanin; **TEL**=telithromycin; **VAN**=vancomycin

Adapted from Van Bambeke *et al.*, Curr Opin Drug Discov Devel 2006;9:218-230 – PMID: 16566292

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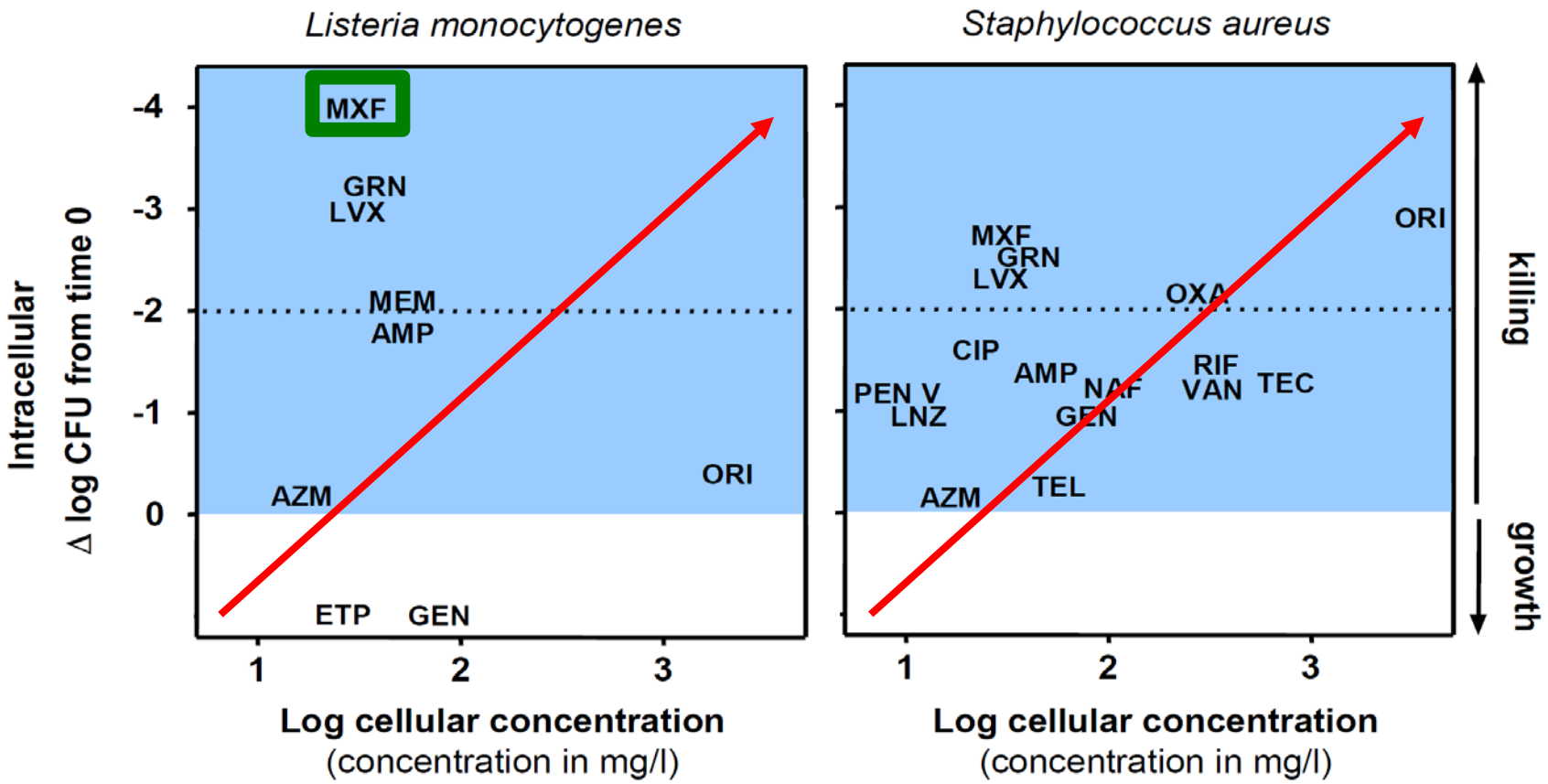

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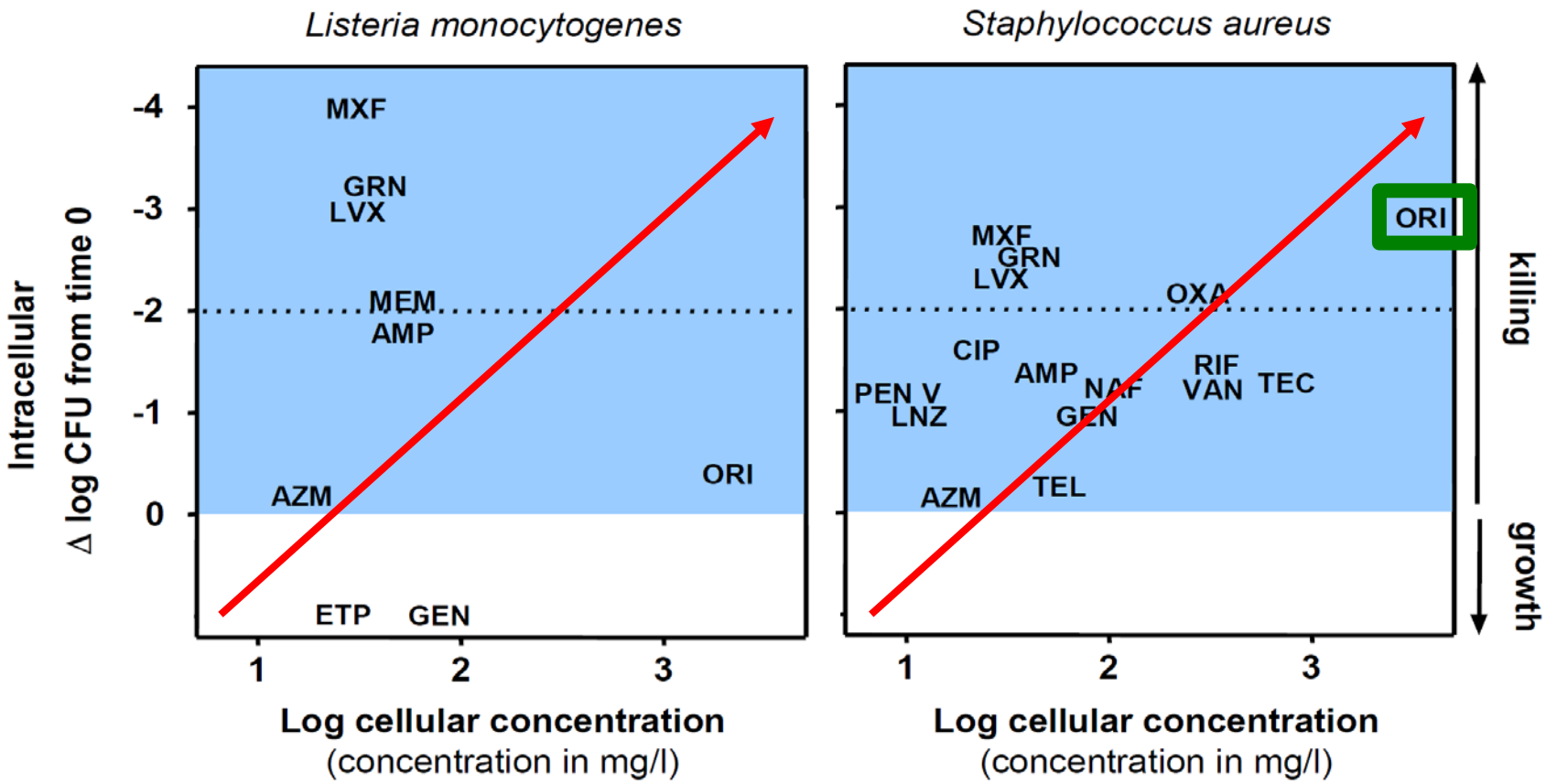

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Adapted from Van Bambeke *et al.*, Curr Opin Drug Discov Devel 2006;9:218-230 – PMID: 16566292

Thus, there is now an obvious conclusion

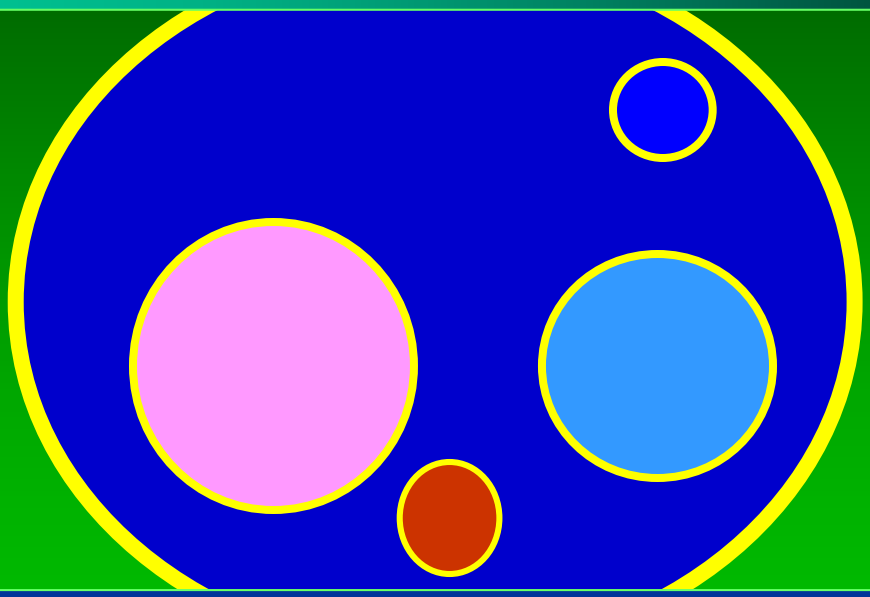
"Accumulation only" may not be the key property

One size does not fill all

Each class of antibiotic / bacteria combination may need to be examined separately

Subcellular bioavailability of antibiotics ?

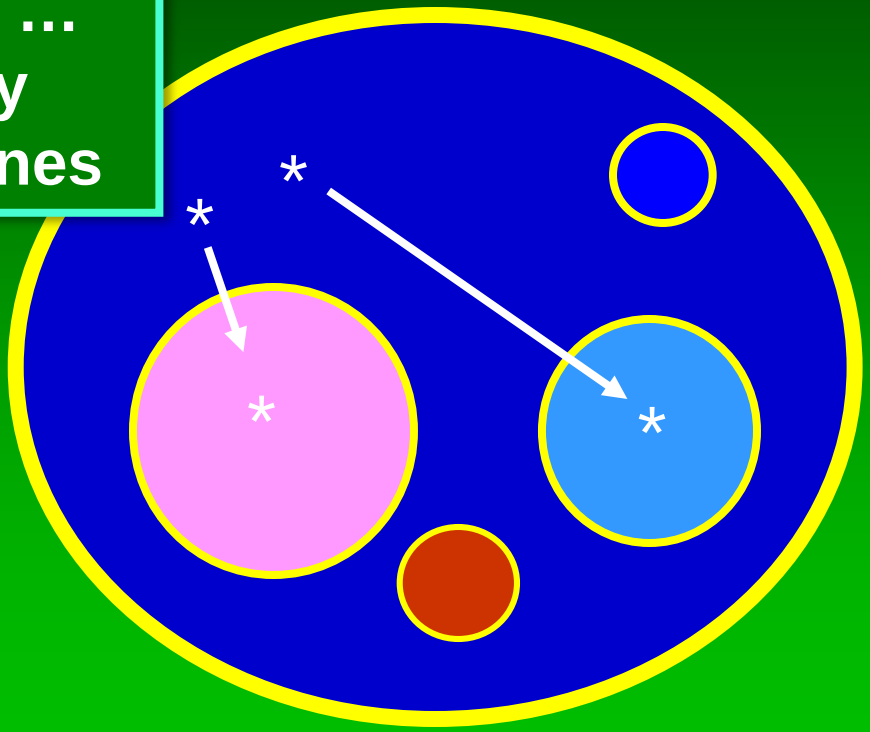
High **Fair** **Nil**



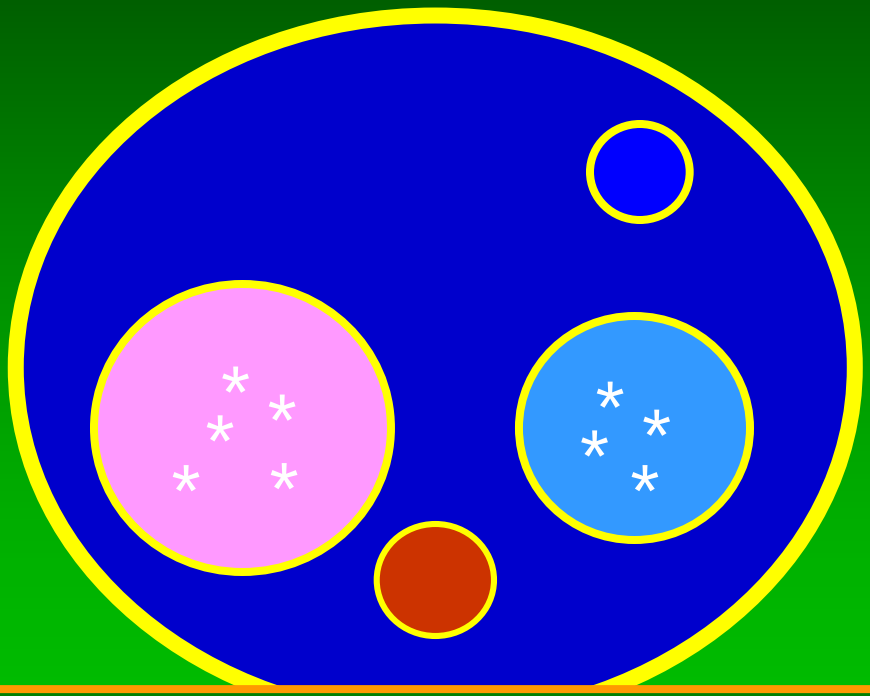
FQ / oxazolidinones / β-lactams **ML / AG**

Subcellular bioavailability of antibiotics ?

Fluoroquinolones, β-lactams, oxazolidinones, … may move easily across membranes



Subcellular bioavailability of antibiotics ?



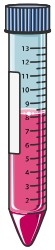
Conversely, poorly diffusible antibiotics (aminoglycosides, oritavancin, e.g.) or subjected to proton-trapping sequestration (macrolides, e.g.), may remained confined where they are ...

Intracellular activity of antibiotics

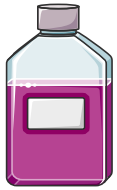
- What has been know for long about pharmacokinetics…
- What has surprised us …
- **Adding pharmacodynamics …**
- A renewed model ?

24h pharmacodynamic dose-effect model

1. Cell exposure to a a wide range of extracellular concentrations of the antibiotic



Opsonization (45', 37°c)
9 mL RPMI +
1 mL human serum



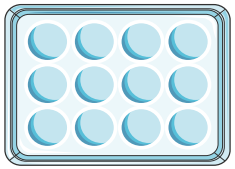
500,000 THP-1 cells/mL

4 cfu/cell (MOI = 4)

Phagocytosis (1 h)

Extracellular Wash
GEN 50 µg/mL
(45 min)

Typical post-phagocytosis inoculum:
5 to $7x10^5$ CFU/mg prot.



Incubation (with ATB)
(T0, T24 h)

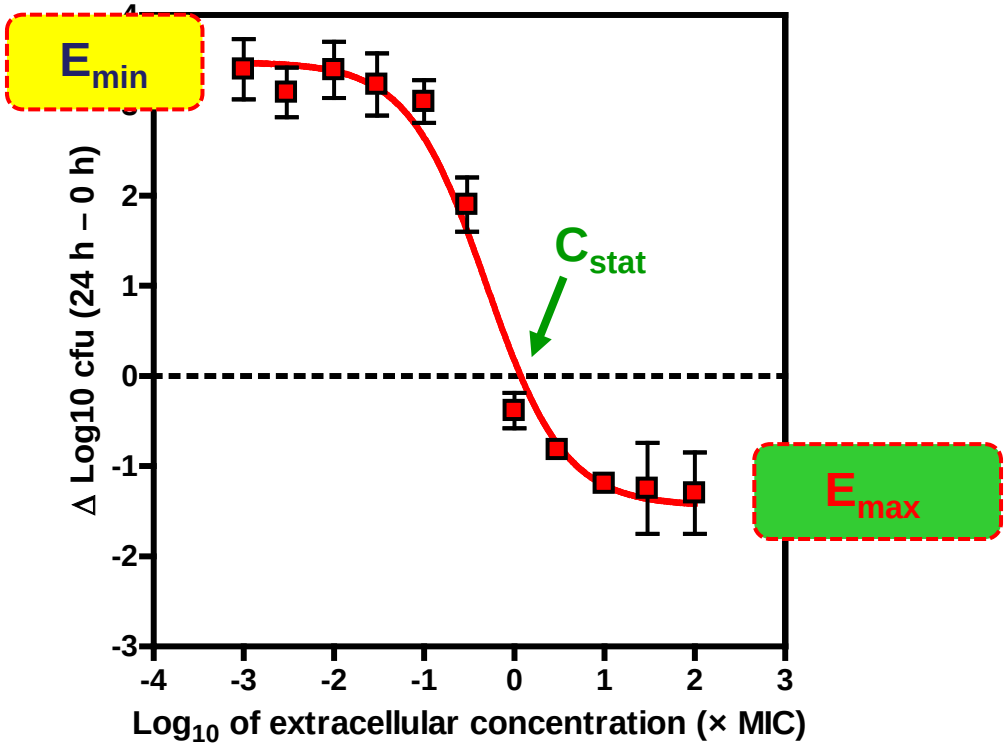
- Cell washing, collection, and lysis
- Cell-associated CFUs counting
- Cell Protein content determination

This example is for *S. aureus*.
Similar design for other bacteria

Buyck et al. In vitro Models for the Study of the Intracellular Activity of Antibiotics; In "Bacterial Persistence", Molecular Biology Laboratory Protocols Series, J. Michiels and M. Fauvart, editors, 2016, p 147-157 - DOI: 10.1007/978-1-4939-2854-5

Interpretation of the results of the 24h dose-effect model

2. Analysis of the response



E_{min}: cfu increase (in log_{10} units) at 24 h from the corresponding initial inoculum as extrapolated for an infinitely low antibiotic concentration

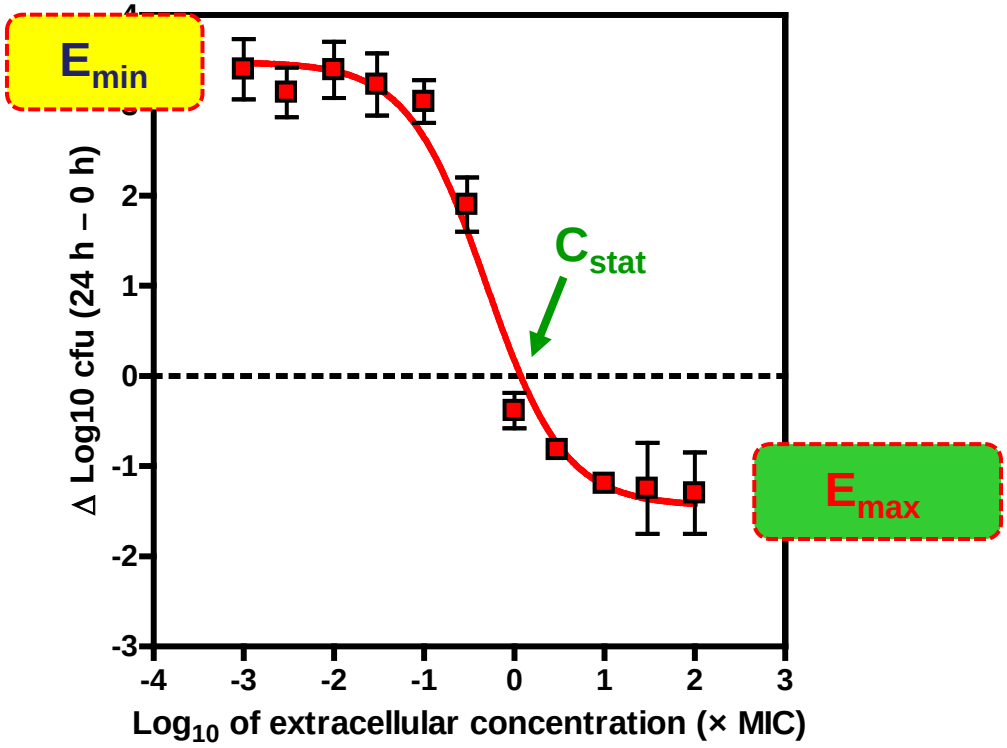
Static concentration (C_{stat}): extracellular concentration resulting in no apparent bacterial growth (number of cfu identical to the initial inoculum)

E_{max}: cfu decrease (in log_{10} units) at 24 h from the corresponding initial inoculum as extrapolated from infinitely large antibiotic concentration

Reference: Barcia-Macay M, Seral C, Mingeot-Leclercq MP, Tulkens PM, Van Bambeke F. Pharmacodynamic evaluation of the intracellular activity of antibiotics against *Staphylococcus aureus* in a model of THP-1 macrophages. Antimicrobial Agents and Chemotherapy (2006) 50:841-851 – PMID: 16495241

Interpretation of the results of the 24h dose-effect model

2. the analysis of the response



E_{min}: at 24 h f al inocu infinitely low antibiotic concentration

Bacteria !

Static concentration (C_{stat}): extrac ing in no (tial (numb inoculum)

Potency !

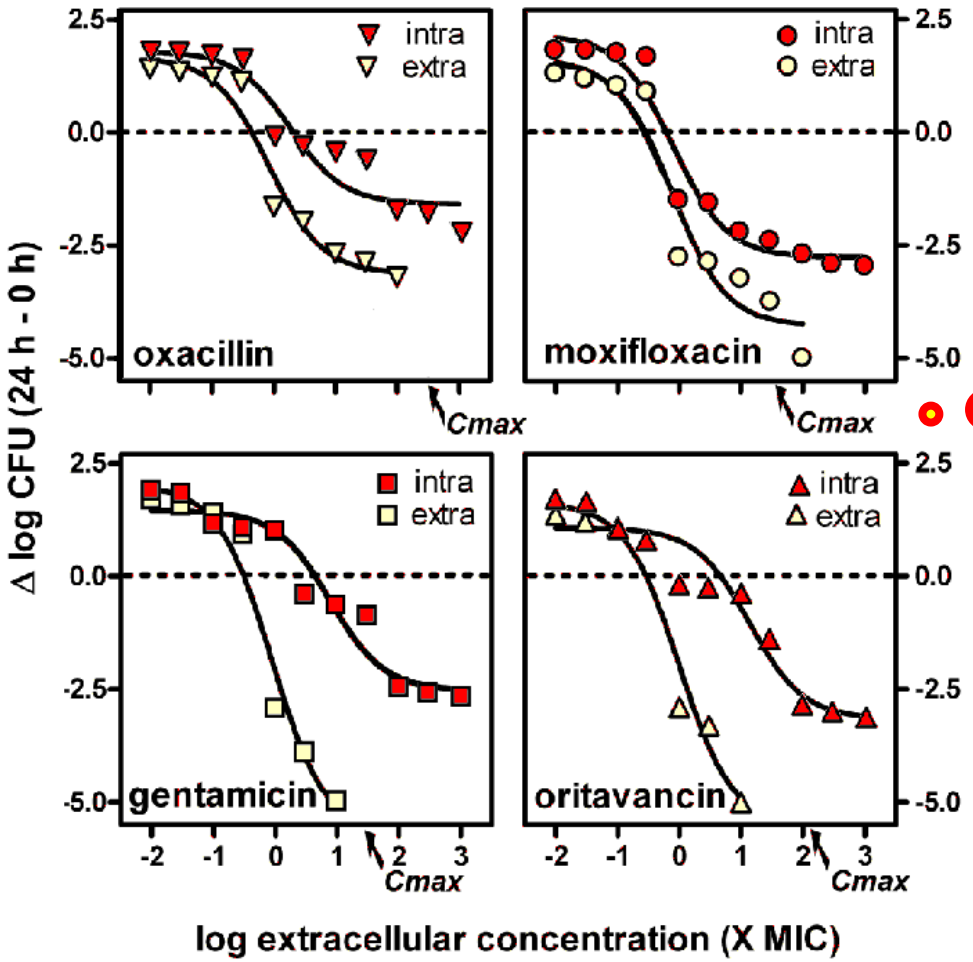
E_{max}: cfu decrease (in log_{10} units) at 24 h from the corresponding initial concentration

Maximal effect

Reference: Barcia-Macay M, Seral C, Mingeot-Leclercq MP, Tulkens PM, Van Bambeke F. Pharmacodynamic evaluation of the intracellular activity of antibiotics against *Staphylococcus aureus* in a model of THP-1 macrophages. Antimicrobial Agents and Chemotherapy (2006) 50:841-851 – PMID: 16495241

Are intracellular and extracellular activities equal ?

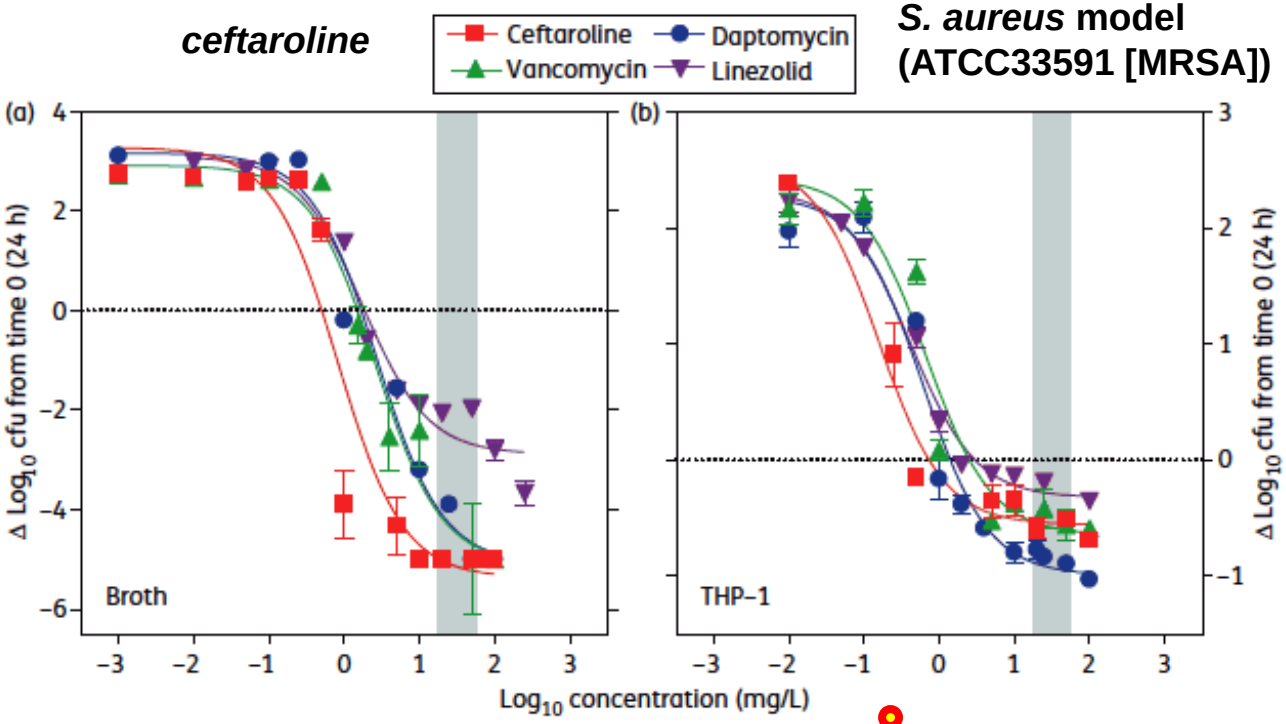
***S. aureus* model (ATCC25223)**



compare the extracellular and the intracellular E_{max}

Barcia-Macay *et al*. Antimicrob Agents Chemother (2006) 50:841-851 – PMID: 16495241

Antibiotics have a much lower intracellular E_{max}…



- 5 log !

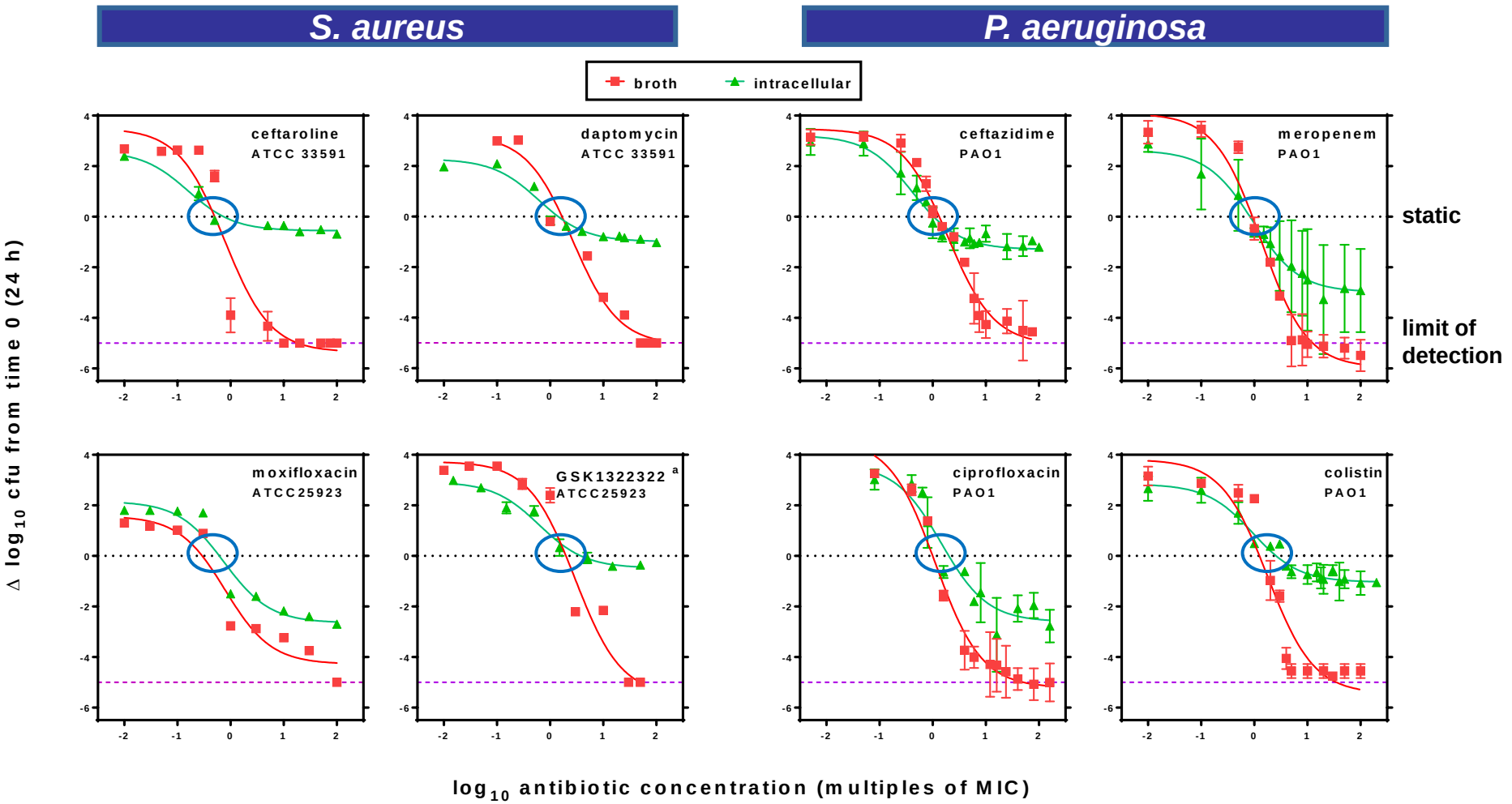
- 1 log !

Figure 1. Concentration-dependent activities of four antistaphylococcal antibiotics against extracellular [MHB broth pH 7.4 (a)] and intracellular [THP-1 monocytes (b)] forms of *S. aureus* strain ATCC 33591 (MRSA). For these experiments, broths or inf cells were incubated presence of increasing concentrations of antibiotic (total drug; abscissa). The ordinates show the change in the medium (broth) or per mg or cell protein (THP-1). Note that because of the marked difference in the amplitud broth versus bacteria in THP-1 cells, the scale extends from −6 to 4 in panel (a) and from −1 to 3 in pa showing the zero value (no apparent change from the initial, post-phagocytosis inoculum). All values are the SD bars are smaller than the size of the symbols). The lowest limit of detection corresponds to a cfu the original inoculum. The grey zone shows the range of maximal serum concentrations observed in h based on the following reported C_{max} values: ceftaroline, 21 mg/L; vancomycin, 20–50 mg/L; daptomycin, footnote c in Table 2).

compare the extracellular and the intracellular E_{max}

Melard *et al.* J Antimicrob Chemother (2013) 68: 648–658 – PMID: 23188792

Antibiotics have a much lower intracellular E_{max} … but also often a similar C_s than in broth



Van Bambeke & Tulkens, ASM Microbe 2016 – poster SARTURDAY 571 – Session 188

Numerical values…

antibiotic	strain	E_{max} (log_{10} CFU decr.		C_s (multiple of MIC)	
		broth	intracellular	broth	intracellular
S. aureus					
ceftaroline	ATCC33591	-5.3	-0.56	0.5	0.8
	multiple strains	-5.1	-0.58	0.7	3.7
daptomycin	ATCC33591	-5.1	-0.99	0.15	1.4
GSK1322322 [a]	ATCC25923	-5.5	-0.48	1.9	3.8
moxifloxacin	ATCC25923	-4.3	-2.7	0.3	0.6
P. aeruginosa					
ceftazidime	PAO1	-5.1	-1.3	1.3	1.0
meropenem	PAO1	-6.0	-3.0	0.9	0.9
colistin	PAO1	-5.4	-1.0	1.2	2.5
ciprofloxacin	PAO1	-5.2	-2.6	1.0	2.0
RX-P853 [b]	multiple strains	-5.1	-2.4	0.6	1.8

[a] a novel peptide deformylase inhibitor with activity against multi-resistant *S. aureus*
[b] a novel inhibitor of bacterial protein synthesis acting a the translation step with broad spectrum activity

Van Bambeke & Tulkens, ASM Microbe 2016 – poster SARTURDAY 571 – Session 188

A few more comparisons of E_{max}… [a]

Antibiotic Class	Molecule *	Emax (Δlog_{10}CFU at 24h)	
		Extracellular **	intracellular
beta-lactams	oxacillin [1]	-3.1	-1.6
	ceftaroline [2]	-5.4	-0.6
lipopeptides	daptomycin [2]	-5.1	-1.0
fluoroquinolones	moxifloxacin [4]	-4.8	-2.0
	ciprofloxacin [5]	-4.9	-1.6
pyrrolocytosines	RX-P873 [6]	-4.2	-0.7
peptides (defensins)	NZ2114 [7]	-4.1	-1.5
deformylase inhibitors	GSK1322322 [3]	-4.8	-0.4
glycopeptides	vancomycin [2]	-5.1	-0.6
lipoglycopeptides	oritavancin [1]	-5.5	-3.1
oxazolidinones	linezolid [2]	-2.9	-0.3

* all molecules but linezolid are highly bactericidal by conventional MBC/MIC measurements

** limit of detection: -5.5 log_{10} units

[a] Reminder: E_{max} is the maximal reduction of the initial inoculum for an infinitely large drug concentration

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lipoglycopeptides	oritava		-3.1
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once transferred back to broth, intracellular surviving bacteria have the same MIC as the extracellular ones and are NOT "small colony variants" (SCVs)

* all molecules but linezolid are highly bactericidal by conventi BC/MIC m urements

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References: [1] AAC (2006) 50:841-851; [2] JAC (2013) 68: 648–658; [3] AA (2015) 59:5747-5760; [4] JAC (2011) 66:596-607; [5] IJAA (2011) 38:52-59; [6] AAAC (2015) 59:4750-4758; [7] JAC (2010) 65:1720-1724

[a] Reminder: E_{max} is the maximal reduction of the initial inoculum for an infinitely large drug concentration

Some antibiotics are better… [a]

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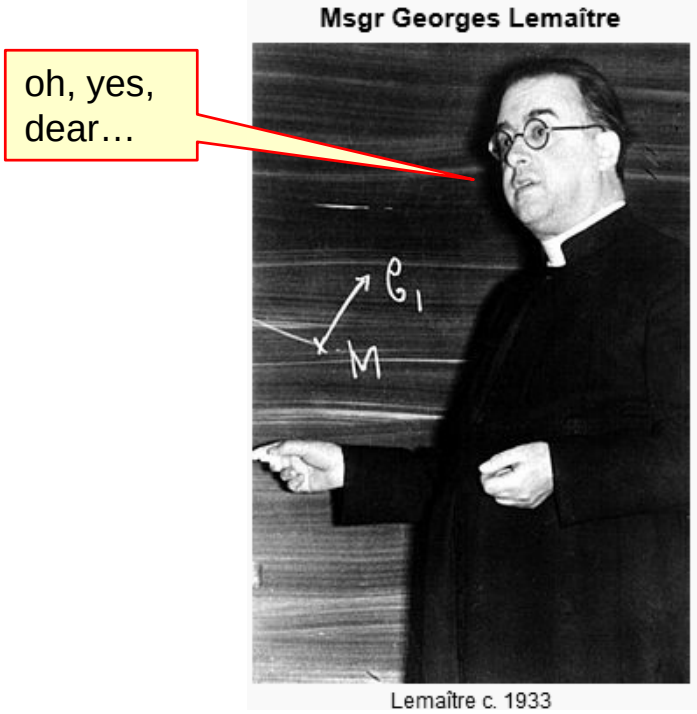
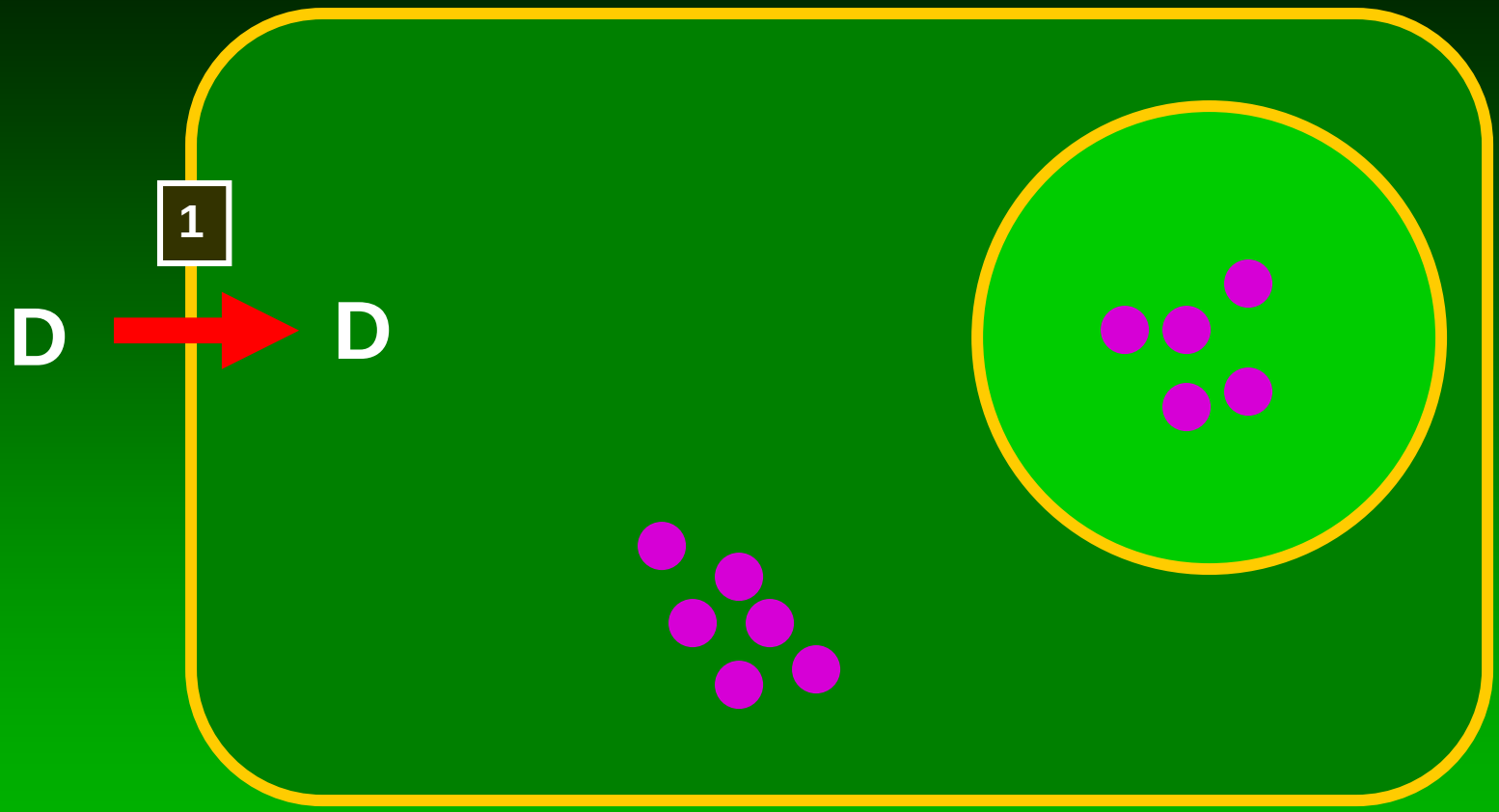
Intracellular activity of antibiotics

- What has been know for long about pharmacokinetics…
- What has surprised us …
- Adding pharmacodynamics …
- **A renewed model ?**



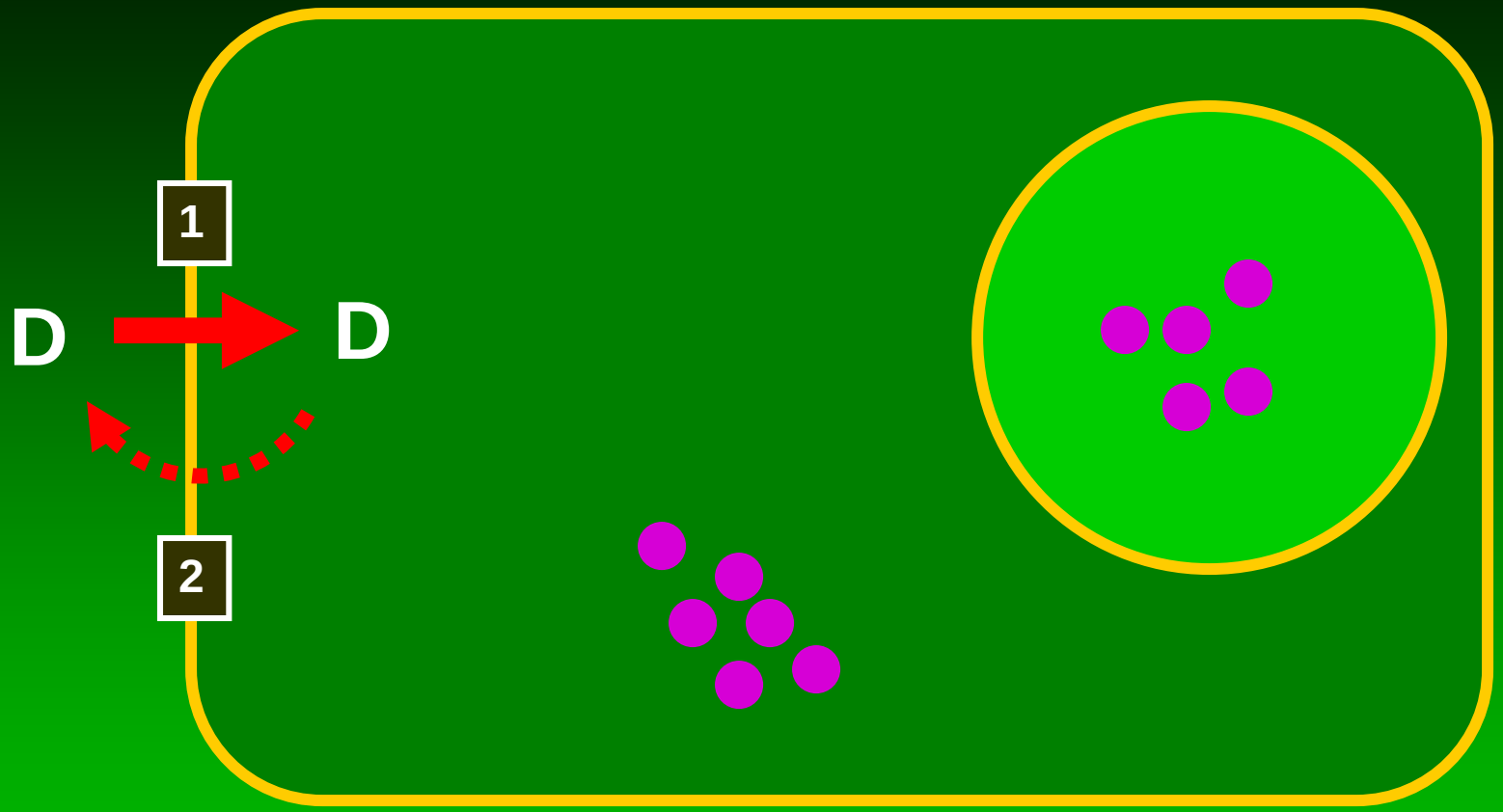
The seven pillars of intracellular activity ?



1. Penetration

This is obvious:
no penetration = no activity
ex.: aminoglycosides in short term exposures

The seven pillars of intracellular activity ?

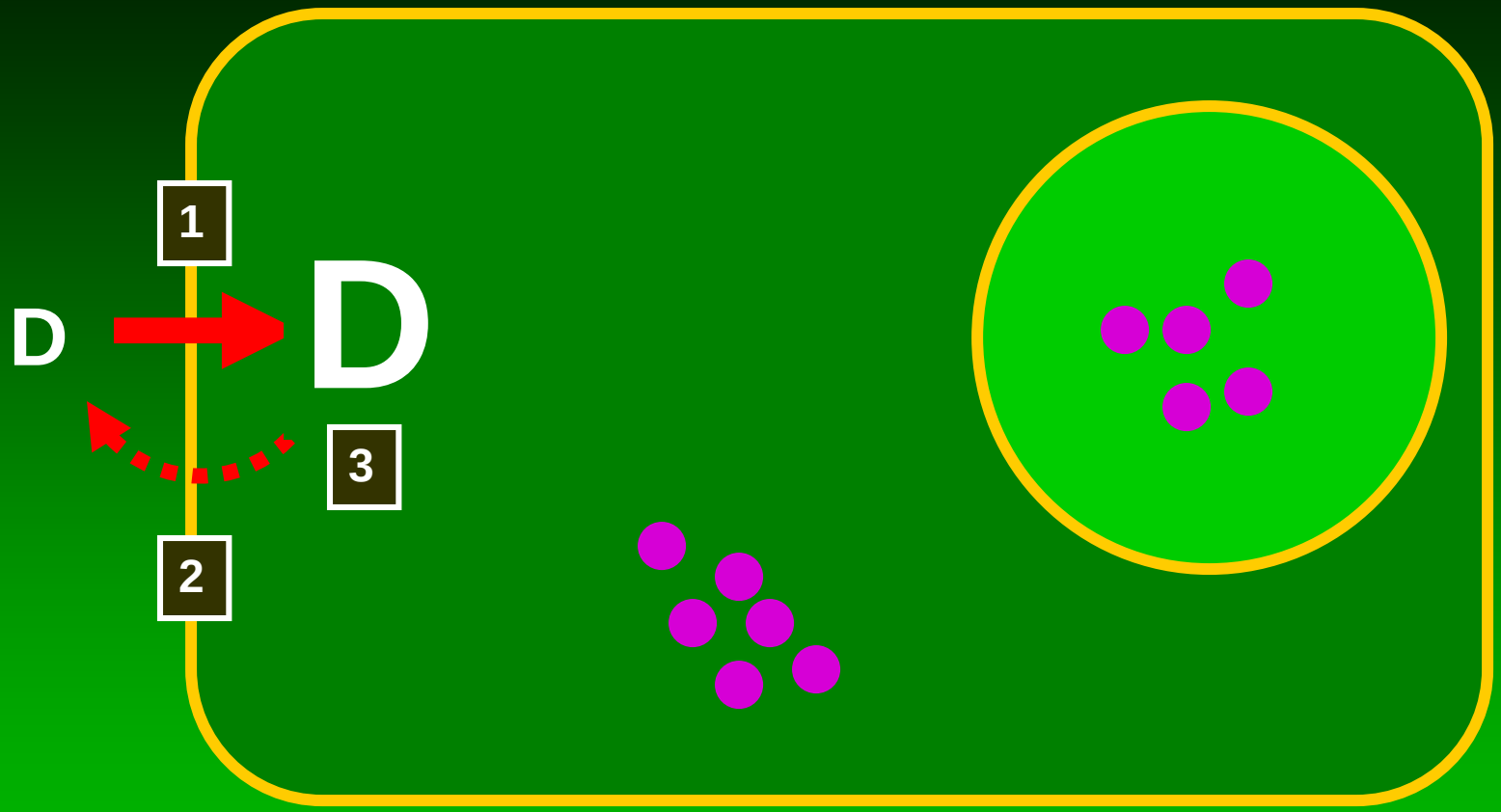


1. Penetration

2. No efflux

Also obvious:
efflux decreases the intracellular concentration
ex.: fluoroquinolones (MRP4), macrolides (Pg-p)

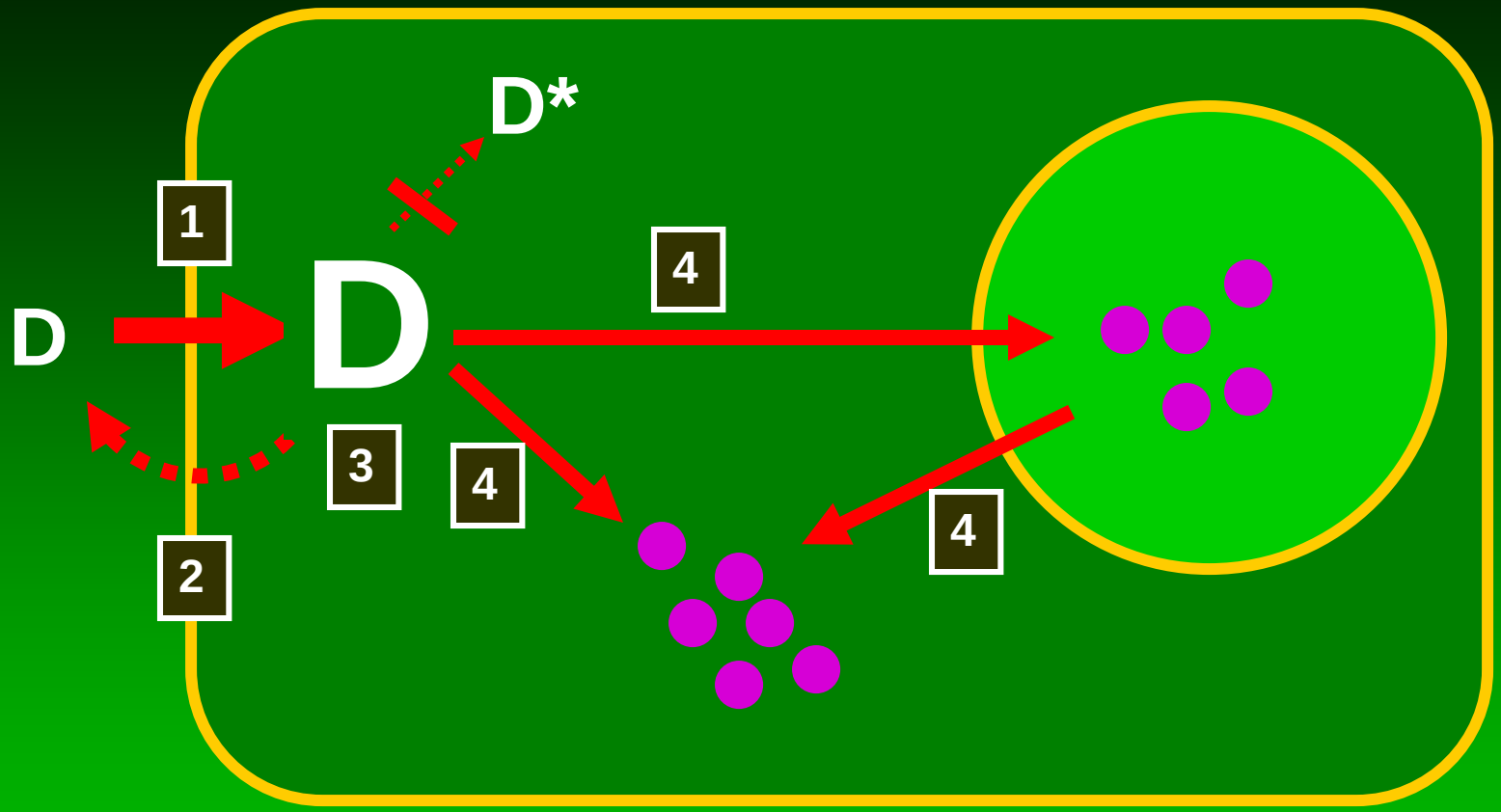
The seven pillars of intracellular activity ?



1. Penetration
2. No efflux
3. Accumulation

Much less obvious …
no simple correlation accumulation-activity
ex.: fluoroquinolones, macrolides, β-lactams…

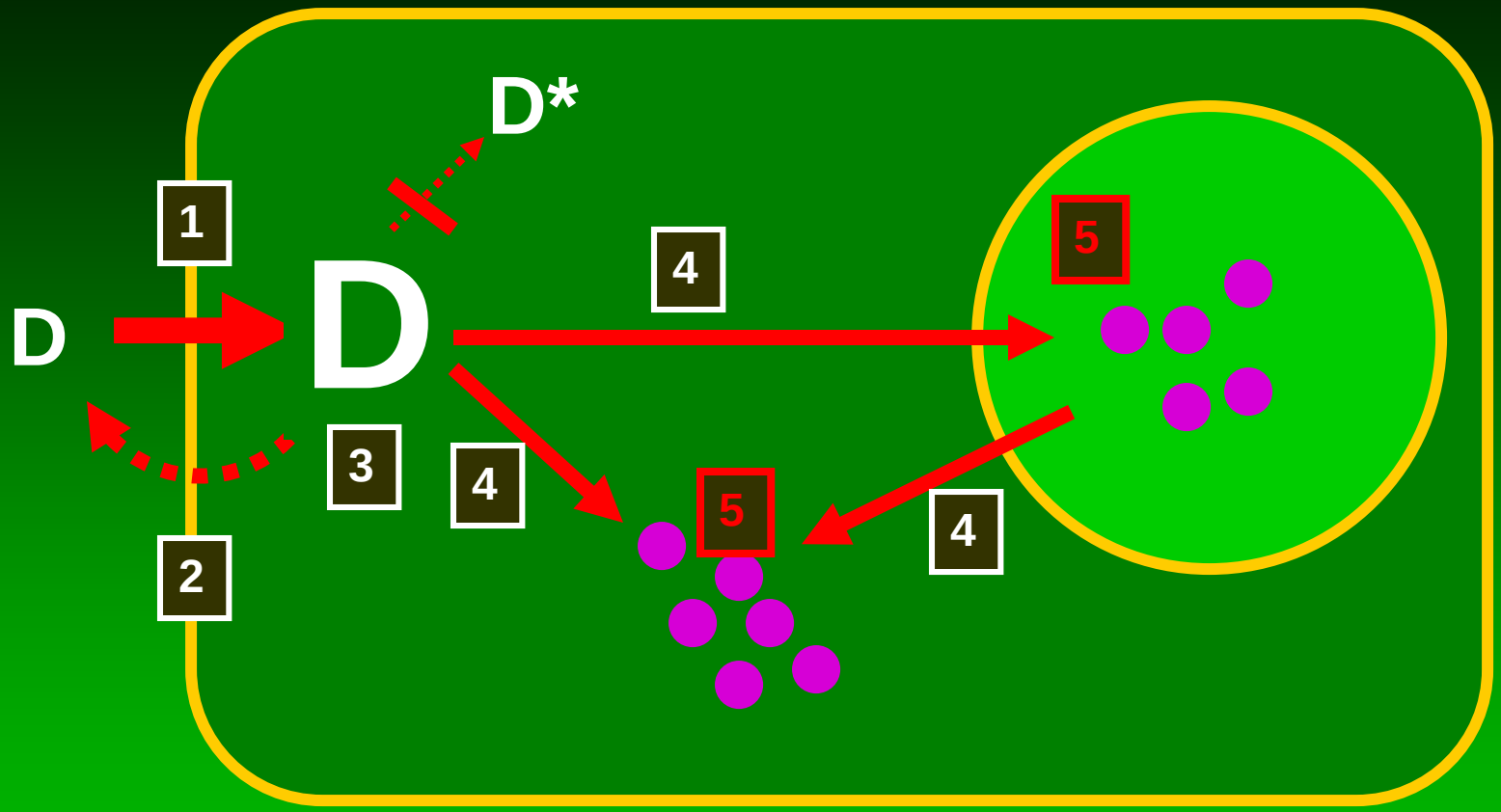
The seven pillars of intracellular activity ?



1. Penetration
2. No efflux
3. Accumulation
4. Subcell. bioavailability

This is probably the most critical property
ex.: fluoroquinolones, oxazolidinones
vs macrolides and aminoglycosides

The seven pillars of intracellular activity ?



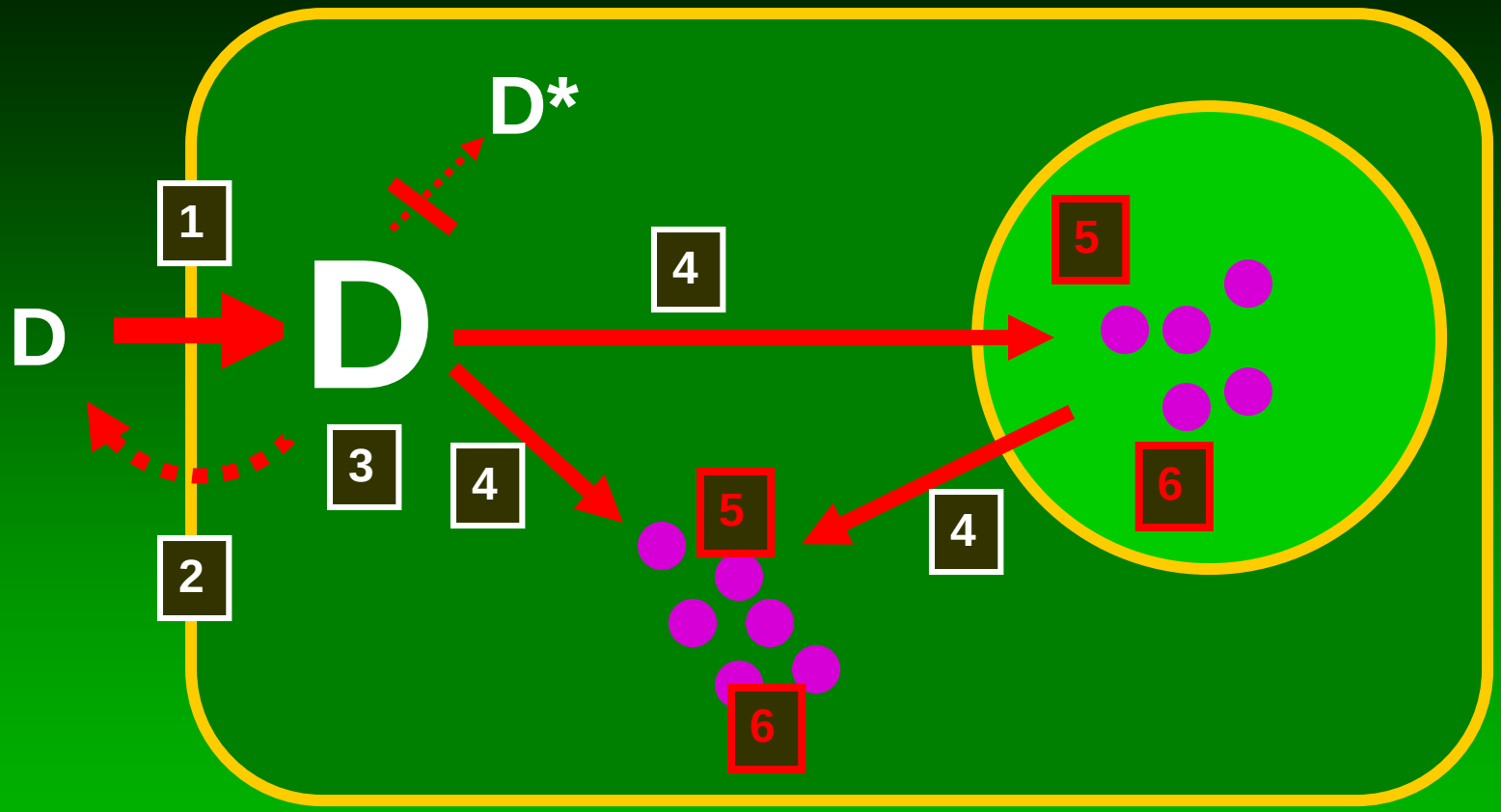
Interesting aspect but could vary for drugs and bugs …

- one + example: intracellular MRSA and conventional β-lactams… (not shown in this lecture)

4. Subcell. bioavailability

5. Expression of activity

The seven pillars of intracellular activity ?



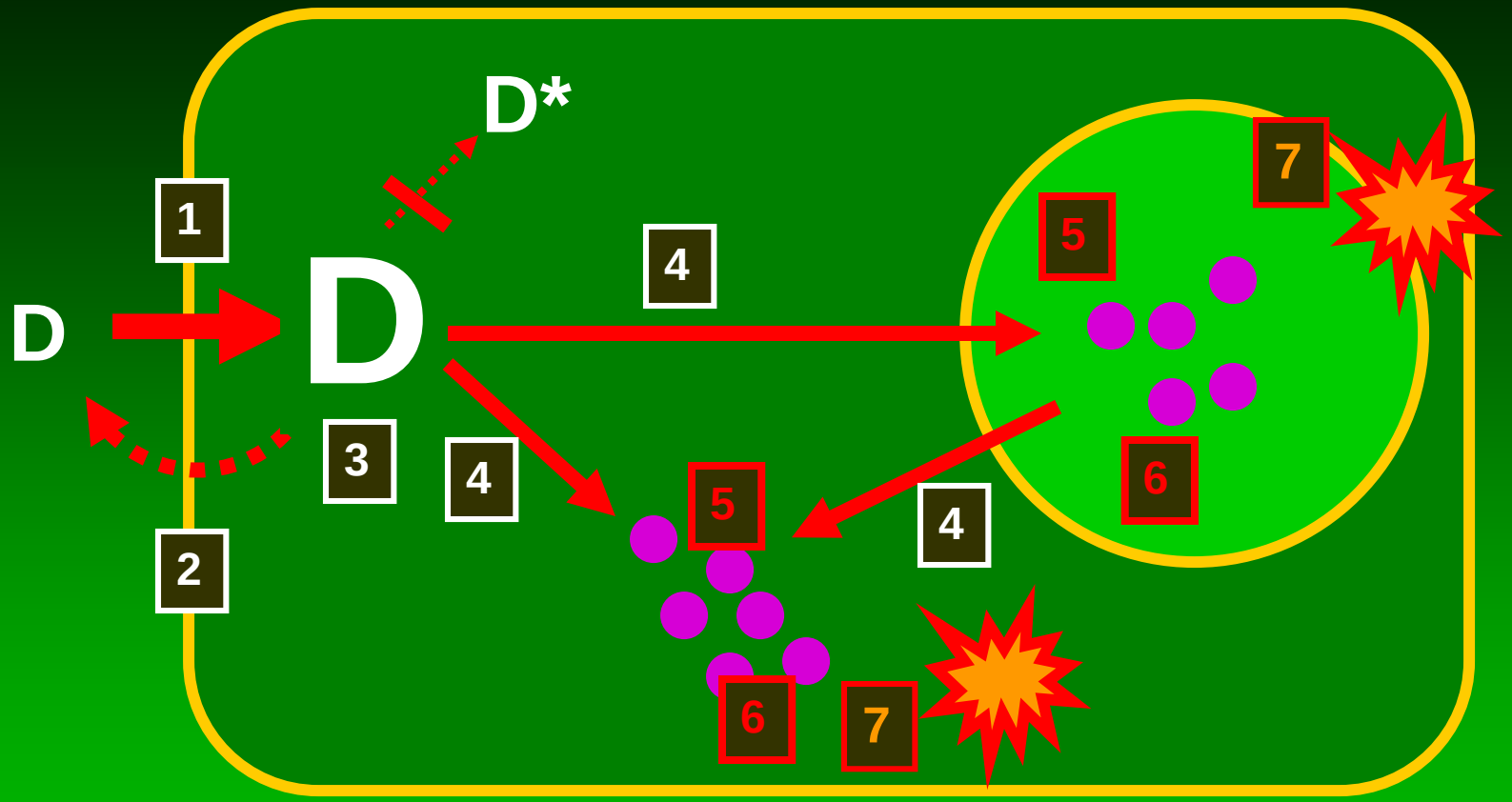
Probably critical to explain the non-eradication or part of the intracellular inoculum…
→ future therapeutic targets ?

4. Subcell. bioavailability

5. Expression of activity

6. Bacterial responsiveness (population)

The seven pillars of intracellular activity ?



1. Penetration
2. No efflux
3. Accumula
4. Subcell.

Not addressed here but probably very important

5. Expression of activity
6. Bacterial responsiveness and pharmacodynamics
7. Cooper. with host def.

But what can we do NOW ?

- All tested antibiotics fail to eradicate intracellular *S. aureus* (and many other bacteria) in the THP-1 model (and in other models)…
- Some antibiotics, however, fare better (moxifloxacin, oritavancin, e.g.) and could be our drugs of (desperate) choice…
- We must now try to understand the reasons for this global failure … and/or screen for better compounds (follow us…)
- In the meantime, intracellular organisms will remain a cause of concern and may (unfortunately) justify large doses and prolonged treatments… which is what we most often do…

But this work would not have been possible without

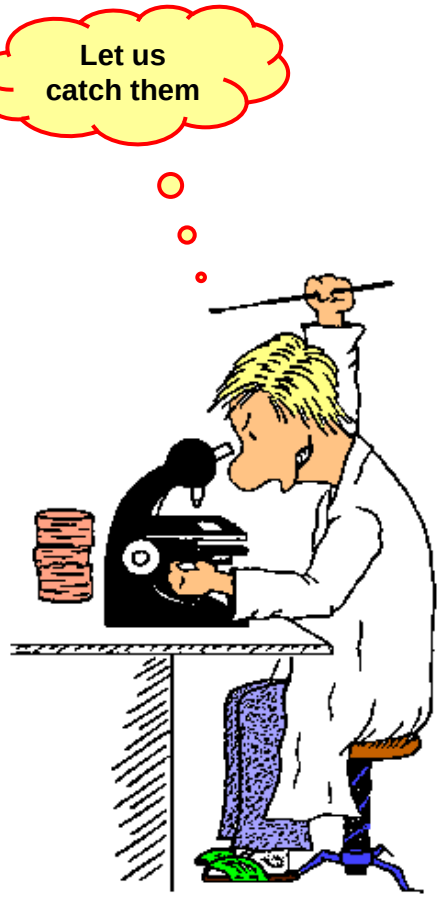
The drugs…

- **β-lactams**: penicillin V, oxacillin, cloxacililn, ceftaroline*, ceftobiprole* (+ avibactam*)
- **aminoglycosides:** gentamicin, amikacin
- **lincosamides:** clindamycin, pirlimycin
- **fluoroquinolones:** ciprofloxacin, pefloxacin, lomefloxacin, sparfloxacin, moxifloxacin,, garenoxacin*, gemifloxacin, finafloxacin*, delafloxacin*
- **oxazolidinones**: linezolid, radezolid*, tedizolid*
- **glycopeptides**: vancomycin, telavancin*, oritavancin*,
- **macrolides:** clarithromycin, azithromycin, solithromycin*,
- **other classes**: daptomycin, GSK 1322322*, gepoditacin*, Debio1452*
- etc…

* new molecules studied at preclinical level

The people…

- M.B. Carlier *,**
- A. Zenebergh **
- B. Scorneaux *
- Y. Ouadrhiri *
- S. Caryn *,**
- C. Seral **
- M. Barcia-Macay *
- H.A. Nguyen **
- J.M. Michot *
- B. Marquez **
- C. Vallet *
- S. Lemaire *,**
- A. Melard
- J. Buyck **
- D. Das **
- F. Peyrusson *
- **F. Van Bambeke (current head of the group)**
- …



* doctoral fellow; ** post-doctoral fellow