Build up and make use of a Molecular Pharmacology Lab

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http://www.facm.ucl.ac.be/

Presented at the University of Pharmacy,
Hanoi, Vietnam

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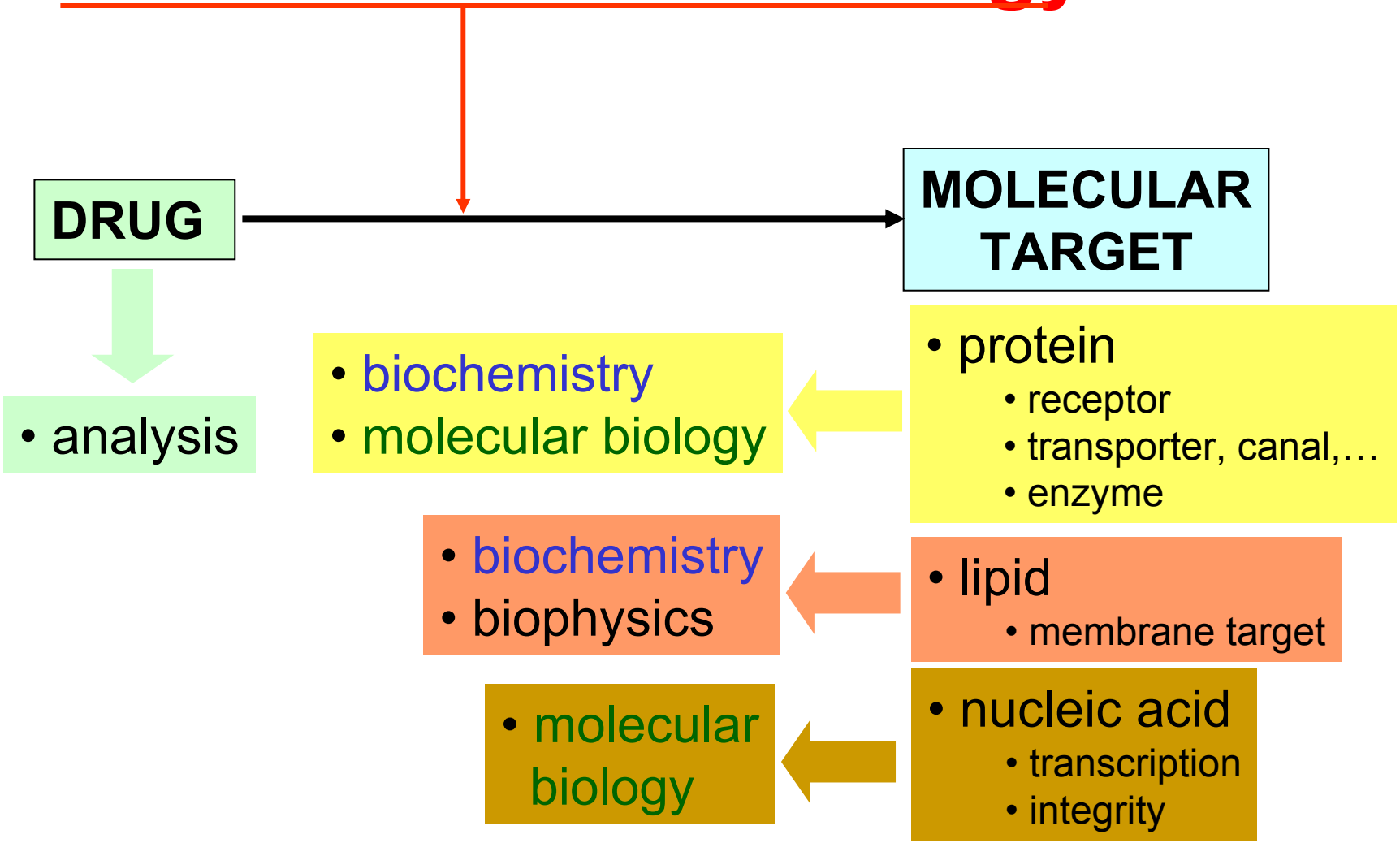
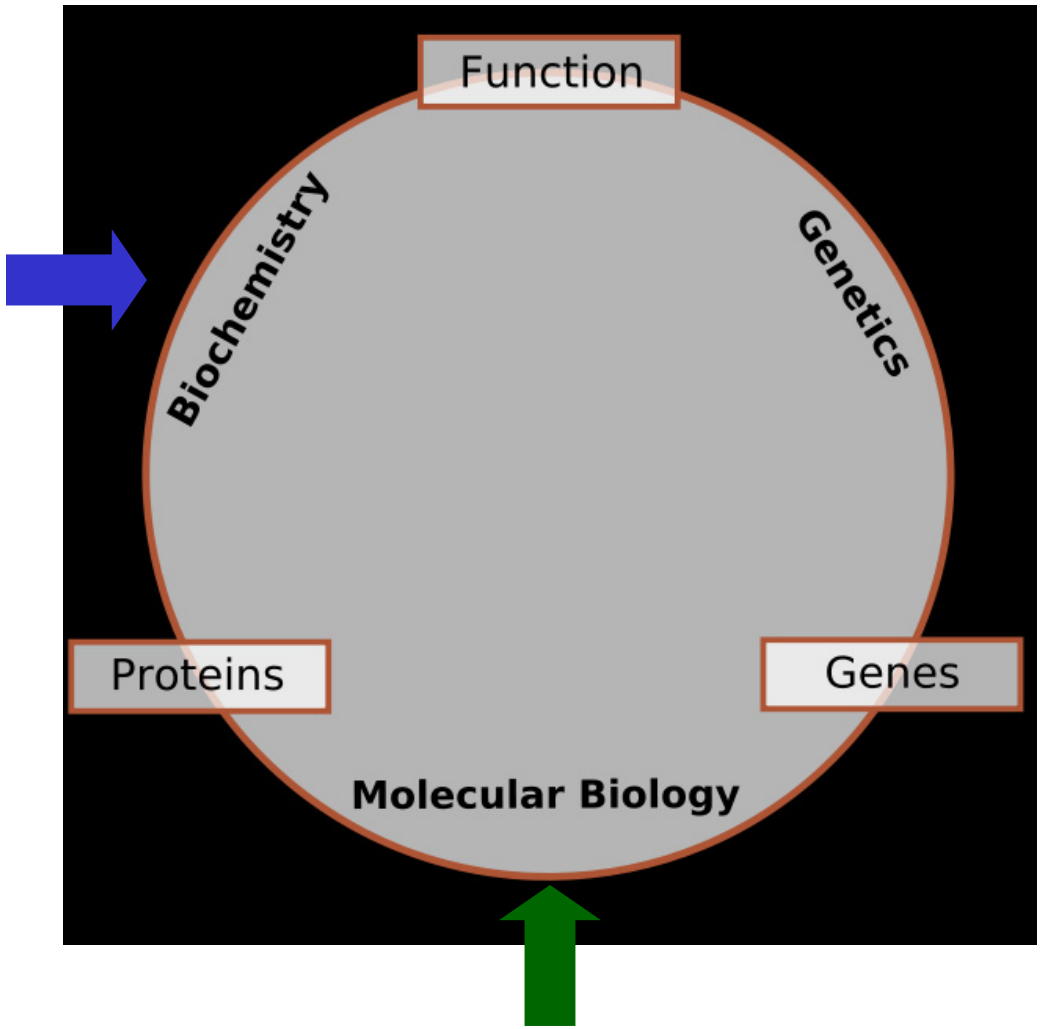
Build up and make use of a Molecular Pharmacology Lab



From genes to function …..



What are you interested in ?

- **Proteins**

Detection of proteins
Change in expression level
Change in localization
Change in activation state; post-traductional modifications
Change in activity

- **ADN**

Amplification (for cloning, e.g.)
Detection of genes (« yes or no » reply)
Search for mutations

- **ARNm**

Expression levels
Post-transcriptional modifications

1. Build up …

ICAAC meeting; Chicago, IL

A. Plan what you need as material

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- **Proteins (activity)**

- thermostatized bath, melting ice
- spectrometer, fluorimeter, (microplate reader)
- centrifuge

- ultracentrifuge
- cell culture room / animal house (depending on your model…)
- scintillation counter, HPLC, mass spectrometry, …

A. Plan what you need as material

- **Proteins (detection)**

- western-blot :
  - gel preparation, thank, gentle agitator,
  - system for detecting signal associated to secundary antibody (luminescence, absorbance, fluorescence, …),
  - software for analysing band intensity

- cell fractionation: ultracentrifuge

- 2D-gels (proteomic analysis):
  - gel preparation, electrofocusing, thanks for running several gels in parallel
  - system for detecting signal (silver coloration, fluorophores)
  - protein identifiation (mass spectrometry)
  - appropriate sofware for gel analysis and protein identification

A. Plan what you need as material

- **DNA/RNA**

- electrophoresis thank
- PCR machine
- transluminator for band visualization + software for band analysis
- thermostatized bath (incubation over wide range of t° [4-90°C]

- real-time PCR
- fluorimeter for quantification of DNA/ARN [Qubit® for example]
- hood to prevent contaminations

B. Organize your lab in a efficient way

Contaminating is easy with DNA / RNA !

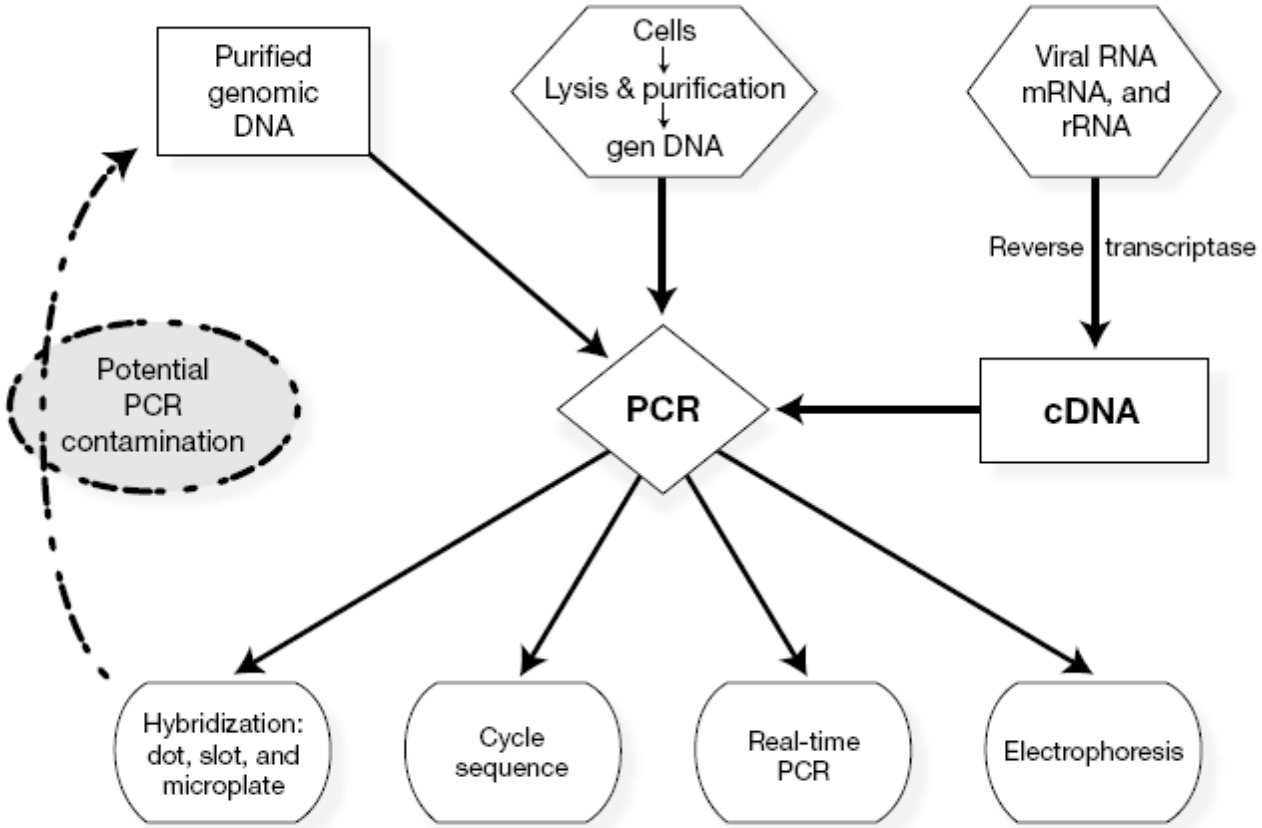


FIGURE 1. Outline of sample processing and analysis in a PCR laboratory.

www.biosupplynet.com/pdf/01_PCR_Primer_p.5_14.pdf

How to prevent contamination ?

1. Organize your lab!

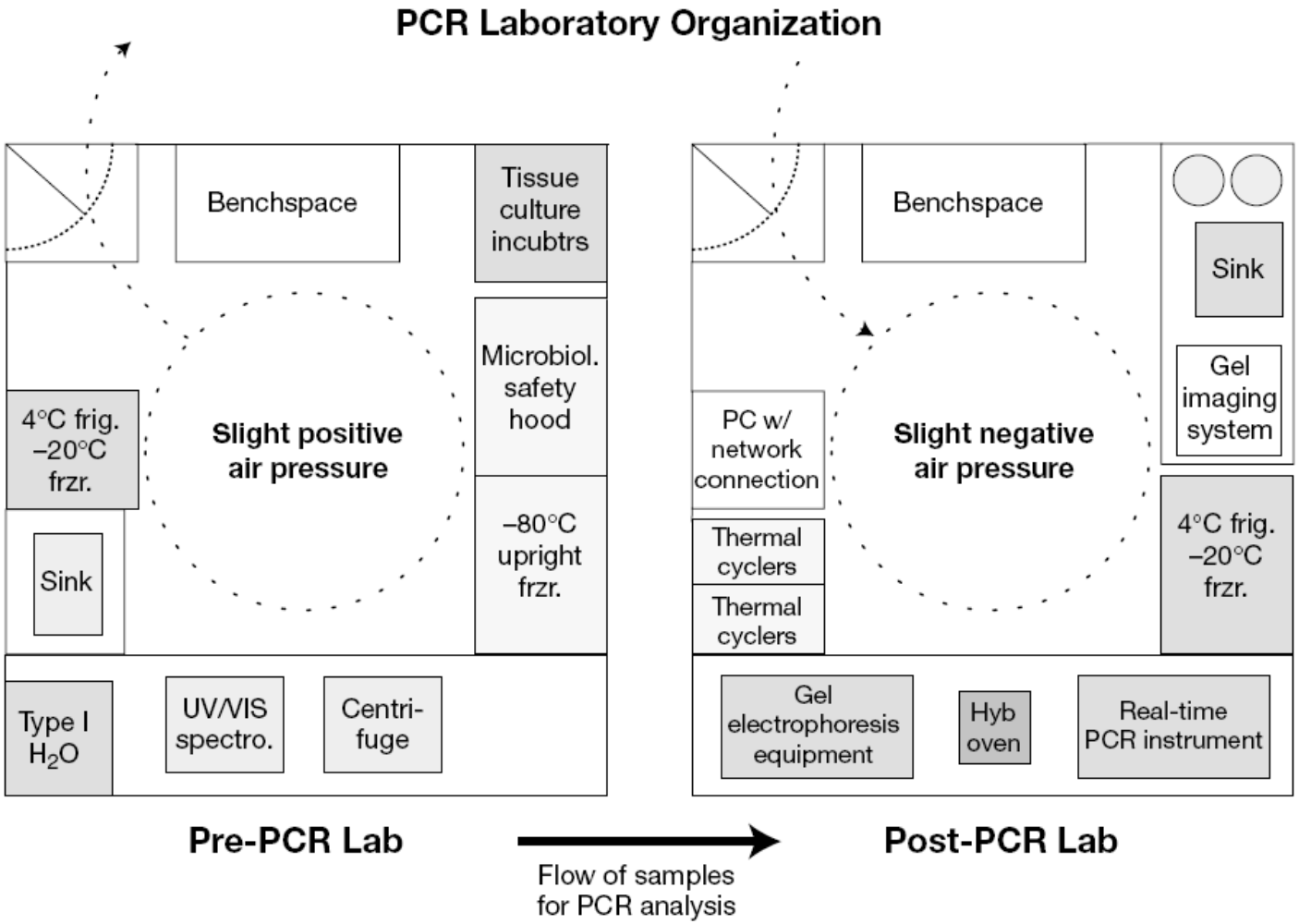
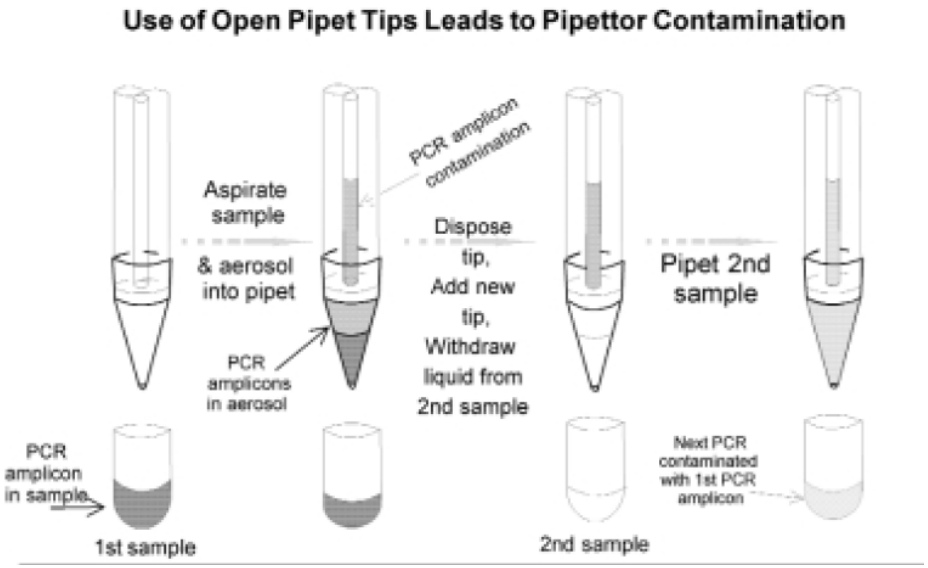


FIGURE 2. Organization of a PCR laboratory with separate pre- and post-PCR rooms.

www.biosupplynet.com/pdf/01_PCR_Primer_p.5_14.pdf

How to prevent contamination

2. Use appropriate material !



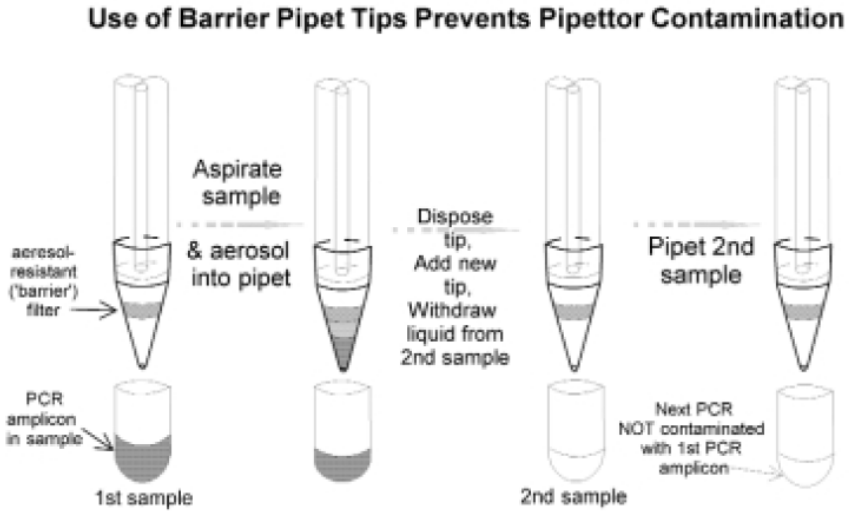


FIGURE 3. Use of barrier tips to prevent amplicon contamination in the PCR laboratory.

www.biosupplynet.com/pdf/01_PCR_Primer_p.5_14.pdf

How to prevent contamination

3. Perform quality control !

→ **positive control :**
- matrix ~ gene of interest
- PCR mixture
- enzyme (polymerase)

shows that PCR conditions are appropriate to detect the gene of interest

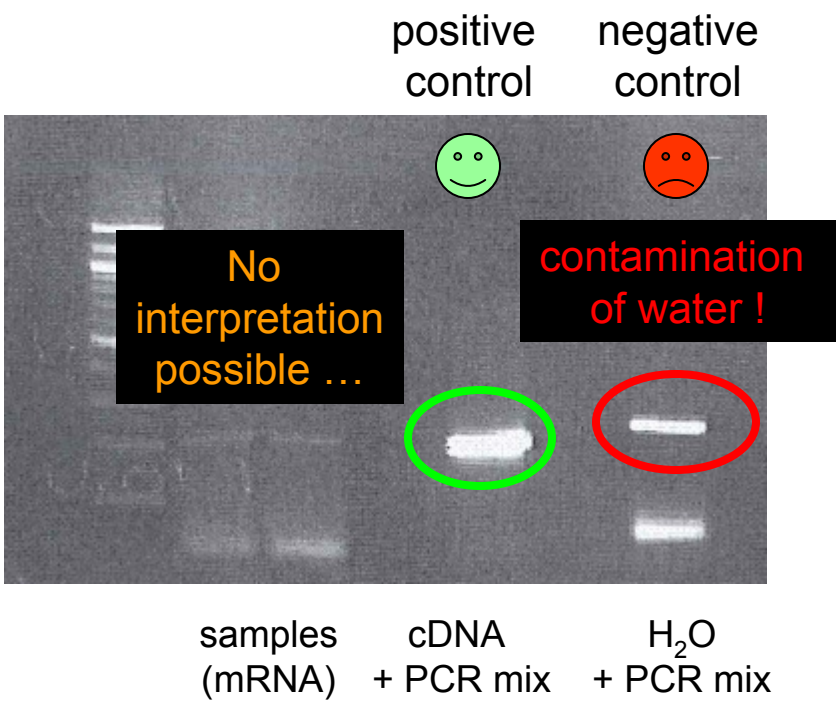
→ **negative control :**
- water used for sample dilution
- PCR mixture
- enzyme (polymerase)

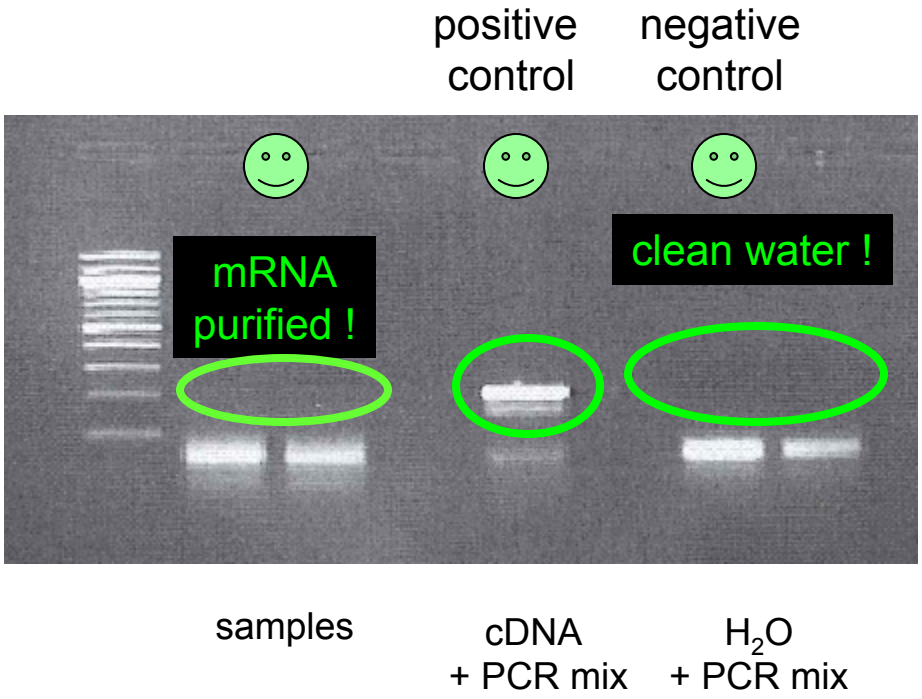
shows that your reagents are not contaminated

A practical example in our lab …

Absence of DNA in mRNA samples for real time PCR

Nucleic acids —purification→ **mRNA** —reverse transcription→ **cDNA** —Amplication→ **Evaluation of gene expression level**



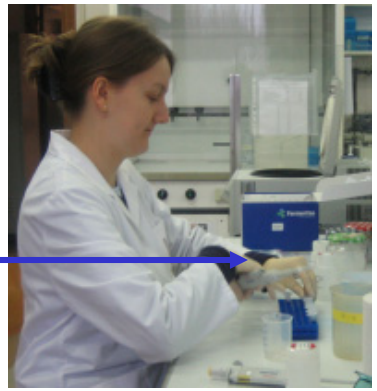


How to prevent contamination

wear gloves … and change when needed !

Contamination source could be the skin !

→ DNA/RNAase could be present

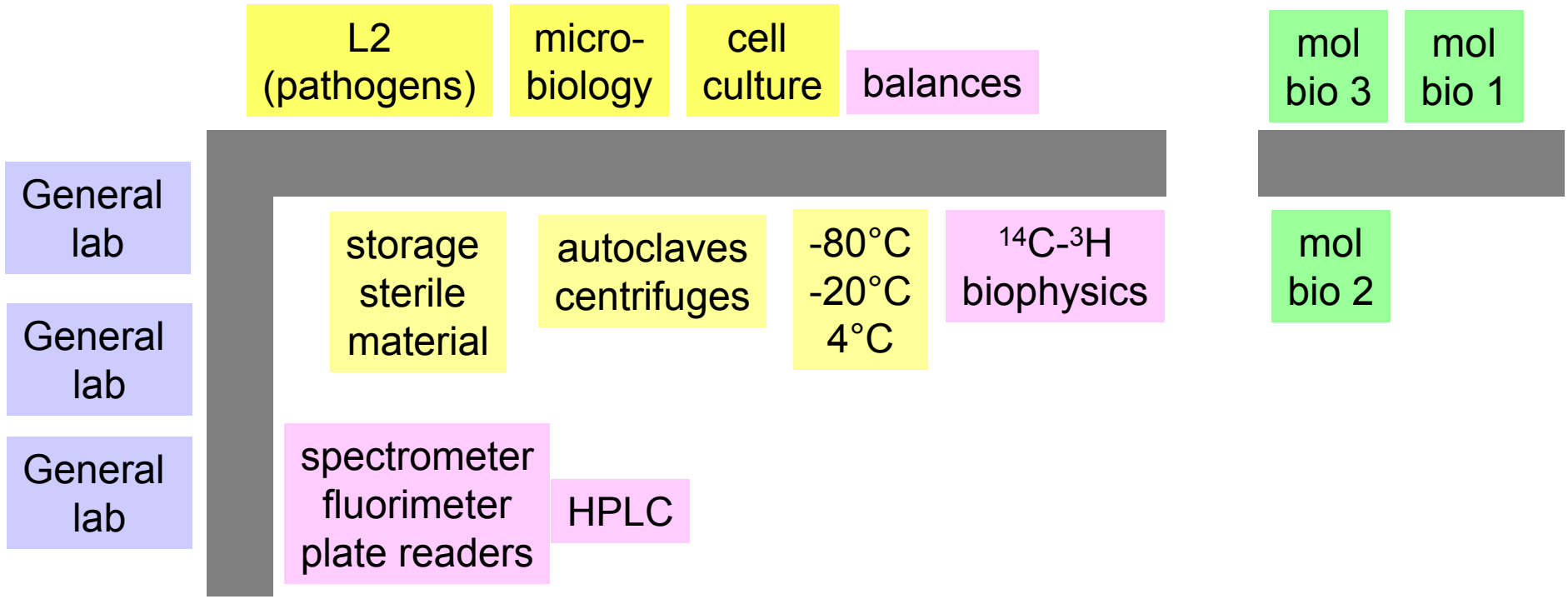


→ proteins could be present

→ western blot detect specific proteins only

→ may be a problem in global analyses of proteome (detection of proteins absent from the sample itself)

A practical example in our lab ….

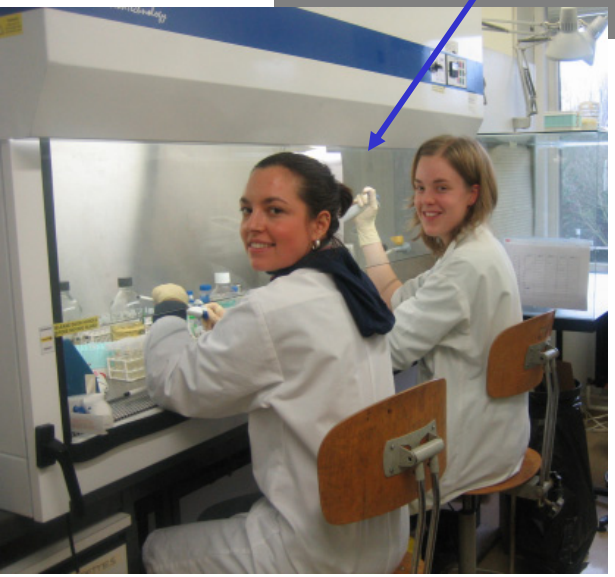


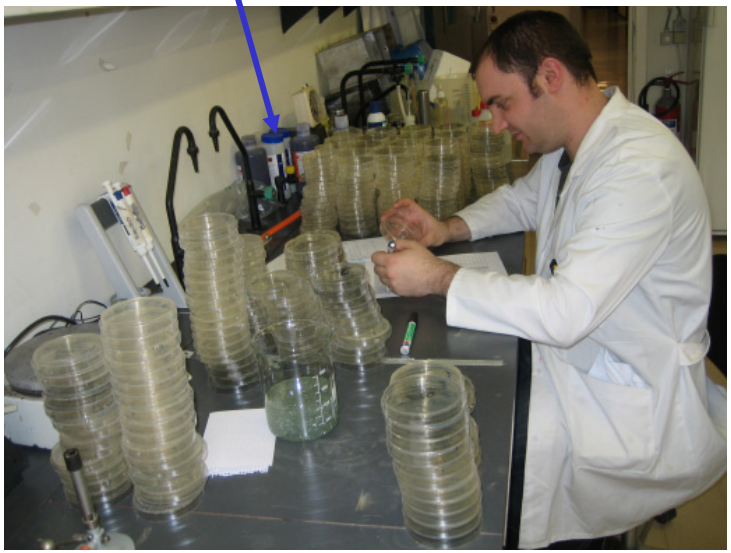
A practical example in our lab ….

L2 (pathogens)

micro-biology

cell culture





A practical example in our lab ….

Pre-PCR steps in a lab 1

mol bio 3

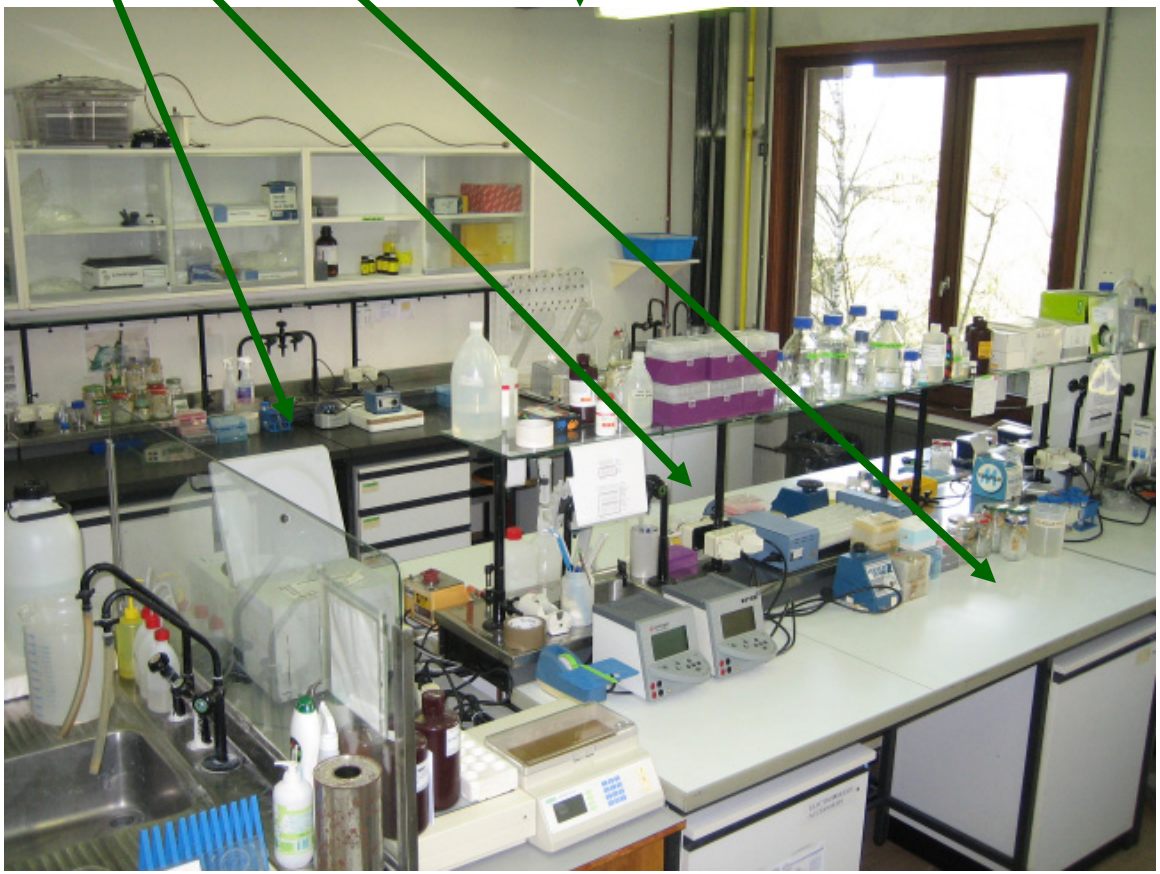
mol bio 1

mol bio 2

A practical example in our lab ….

Lab 2 organized with specific benches

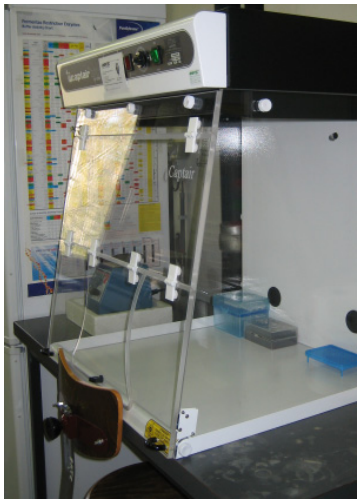
- Proteins
- DNA
- RNA



mol bio 3

mol bio 1

mol bio 2



A practical example in our lab ….

PCR and post-PCR in lab 3

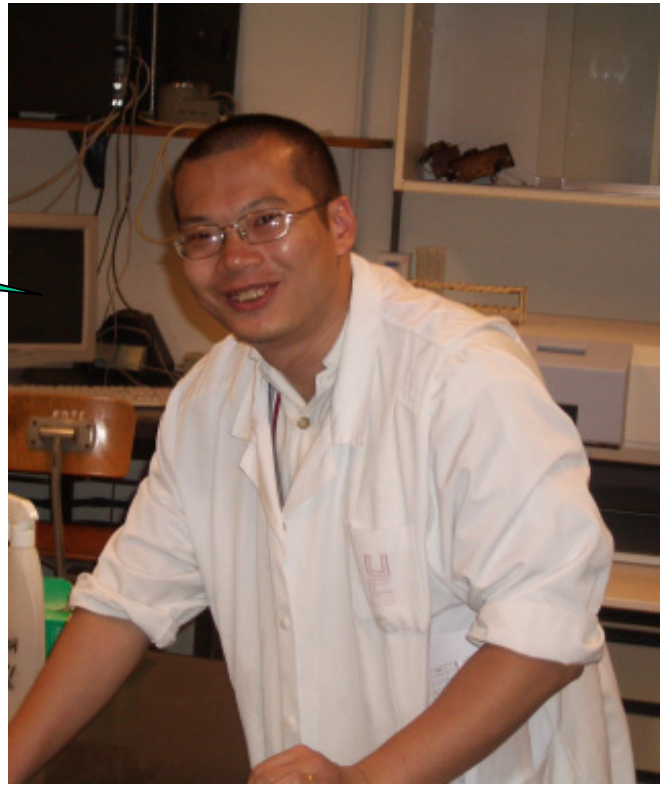
mol bio 3

mol bio 1

mol bio 2

A practical example in our lab ….

Nice place to work !



Don’t forget computers …

Many machines are now piloted with computers …

A series of databases on the web …

Database	URL	Description
Nucleotide Sequence		
GenBank	www.ncbi.nlm.nih.gov	All publicly available nucleotide and protein sequences
EMBL Nucleotide Sequence Database	www.ebi.ac.uk/embl.html	All publicly available nucleotide and protein sequences
DNA Data Bank of Japan (DDBJ)	www.ddbj.nig.ac.jp	All publicly available nucleotide and protein sequences
DNA Sequences: Genes, Motifs and Regulatory Sites		
TIGR Gene Indices	www.tigr.org/tdb/tgi.shtml	Organism-specific databases of EST and gene sequences
ExInt	http://sege.ntu.edu.sg/wester/iekb/	Exon-intron structure of eukaryotic genes
TRANSFAC	http://www.gene-regulation.com	Transcription factors and binding sites
RDP	rdp.cme.msu.edu	Ribosomal database project: rRNA sequences data
Gene Expression		
PIR	pir.georgetown.edu	A collection of protein sequence databases
SWISS-PROT	www.expasy.ch/sprot	Curated protein sequence databases
PROSITE	www.expasy.ch/prosite	Biologically-significant protein patterns and profiles
Pfam	www.sanger.ac.uk/Software/Pfam/	Sequence alignments and profile hidden Markov models
Carbohydrate		
CCSD	bssv01.lancs.ac.uk/gig/pages /gag/carbbank.htm	Complex carbohydrate structure databases (CarbBank)
Protein Structure		
PDB	www.rcsb.org/pdb/	All available 3D structures of proteins and nucleic acids
Genomics		
GO	www.geneontology.org	Gene onthology consortium database
KEGG	www.genome.ad.jp/kegg	Databases of genes, proteins, and metabolic pathways
EcoCyc	ecocyc.org	*E. coli* K-12 genes, metabolic pathways, transporters, regulation
Ensembl	www.ensembl.org	Annotated information on eukaryotic genomes

C. Have a responsible person with skills in molecular biology to conduct projects

C. Have a responsible person with skills in molecular biology

Why ?

- design of a molecular biology experiment is not easy if you are not an expert
  - loss of time
  - loss of money

- data interpretation requires appropriate background

- quality of the data critically depends on
  - the way the experiment was performed
  - the quality of the controls

- many fields are highly competitive; be efficient !
- science is progressing exponentially in that field [databases !!!]

- molecular biology is highly expensive !
  - competitive grant applications need to be submitted ….

C. Have a responsible person with skills in molecular biology

How ?

- Think twice to the best approach to answer your specific question
- Study in depth literature and take examples from high quality papers to design your experiments
- Make use of existing tools → kits
  → strains, cell lines, plasmids, …
- Combine molecular biology with biochemistry/pharmacology to link genetic or proteomic changes with phenotype

2. Make use …

« Magic bullets » meeting; Nuremberg, Germany

Some applications in our team

Pharmacokinetics :

antibiotics and multidrug transporters eucaryotic cells:
Identification of transporters and modulation of their expression
[cellular biology, drug analysis, real-time PCR, western blot, proteomics, genomics]

Antibiotic resistance :

Identification and characterization of resistance by efflux
[microbiology, real time PCR, gene disruption]

Pharmacodynamics :

Modulation of antibiotic activity against intracellular bacteria
[cellular biology, microbiology, cell fractionation, proteomics]

Other examples of integrated pharmacology

Pharmacological, proteomic and genomic characterization of fluoroquinolone transporters in eucaryotic cells

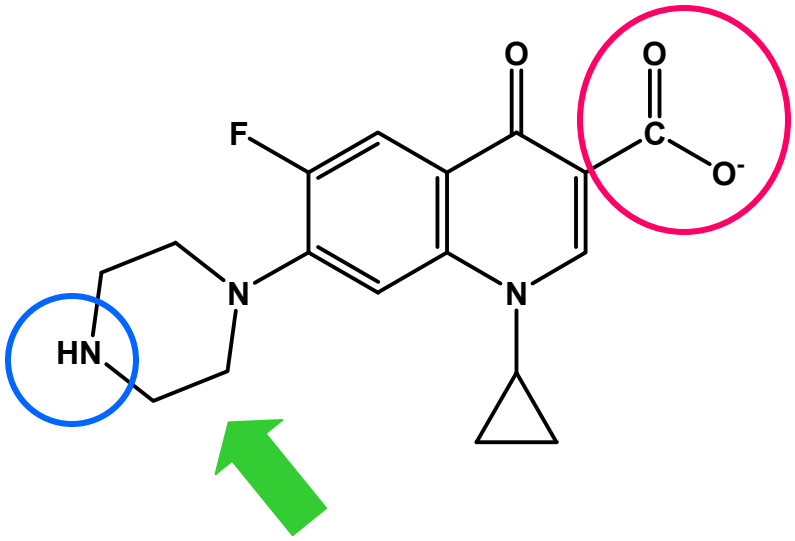
- **Pharmacologie cellulaire et moléculaire, UCL, Bruxelles**
  C. Vallet, N.E. Caceres and B. Marquez
  supervision: M.P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke
- **Laboratorium voor Eiwitbiochemie en Eiwitengineering, Ugent, Ghent**
  M. Aerts; supervision: B. Devreese
- **Centre de Génétique, cliniques St Luc, UCL, Bruxelles**
  Geneviève Ameye, Hélène Antoine-Poirel

UCL Université catholique de Louvain

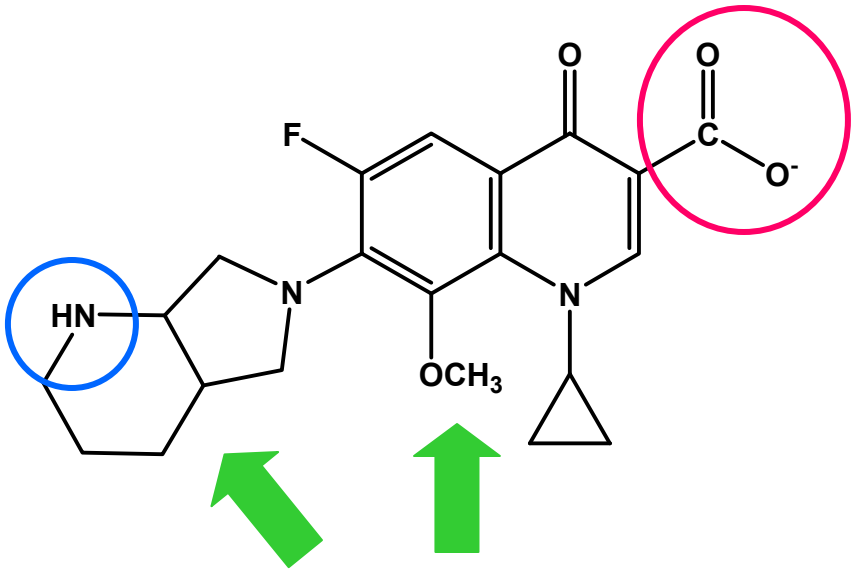
www.facm.ucl.ac.be

Chemical structure of fluoroquinolones

ciprofloxacin



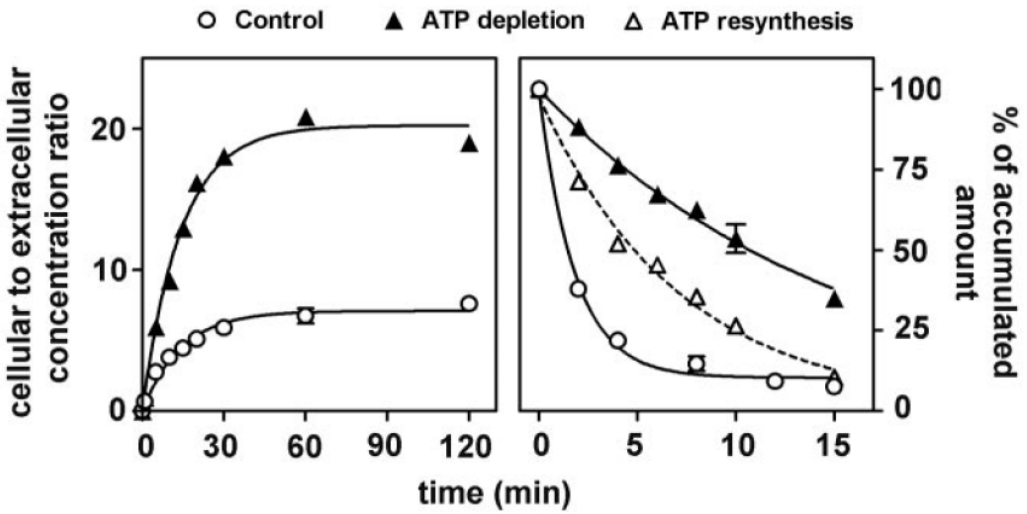
moxifloxacin



Ciprofloxacin is substrate for an MRP-like transporter in J774 macrophages

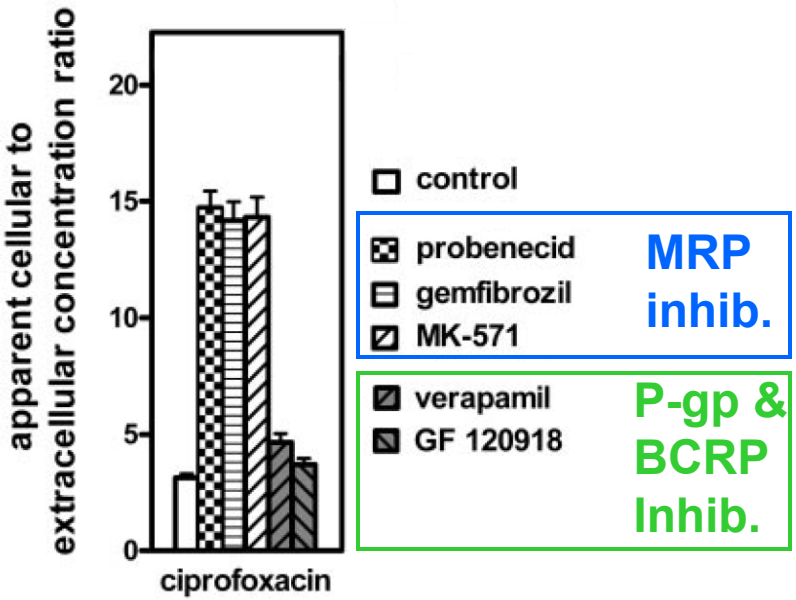
Transport

… ATP-dependent



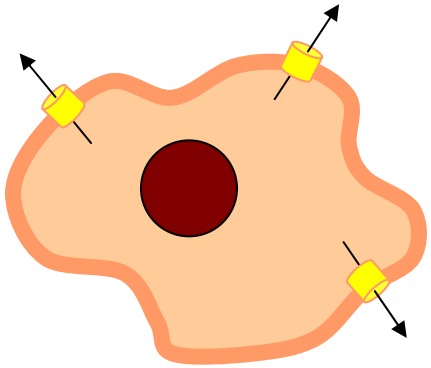
… reduced by inhibitors

- of anion transporters
- of MRP



Michot et al., AAC (2004) 48:2673-2682 ; (2005) 49:2429-2437

Identification of ciprofloxacin transporter: « resistant cells » as a tool

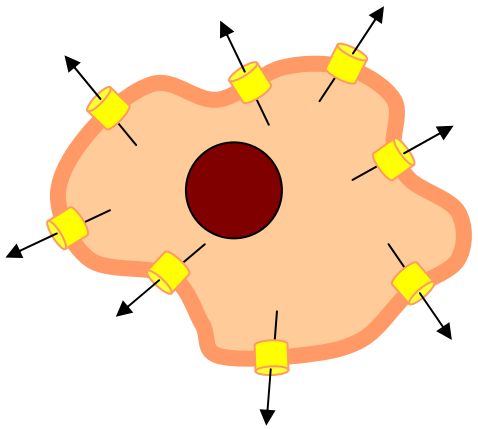


0.1 mM

0.15 mM

Chronic exposure to increasing concentrations of ciprofloxacin

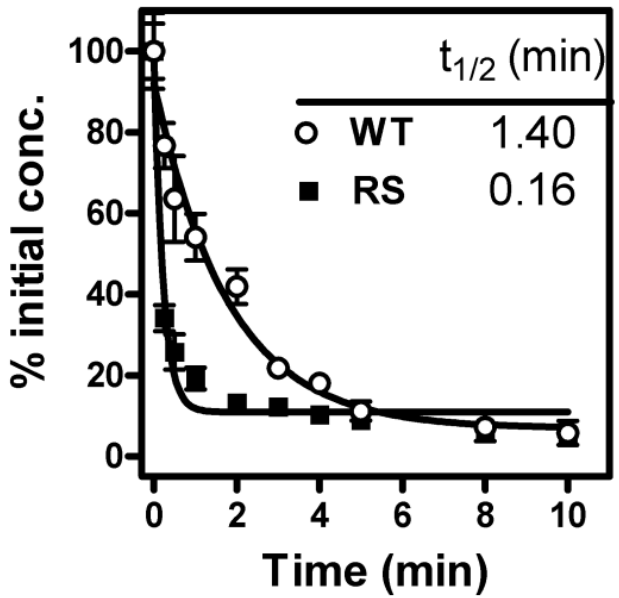
0.2 mM



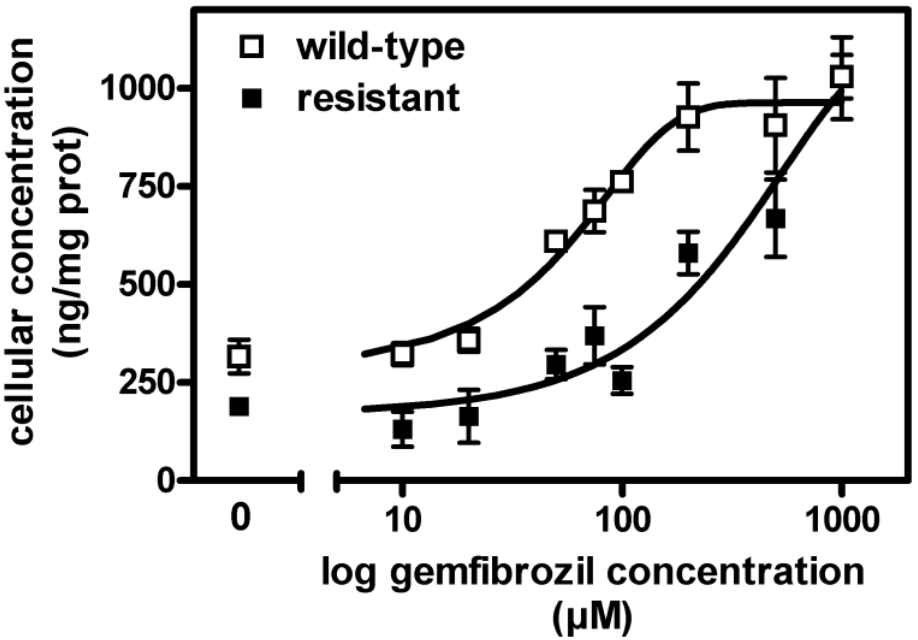
Michot et al., AAC (2006) 50:1689-1695

Ciprofloxacin-resistant cells : phenotypic analysis

↗ efflux



↗ gemfibrozil IC_{50}

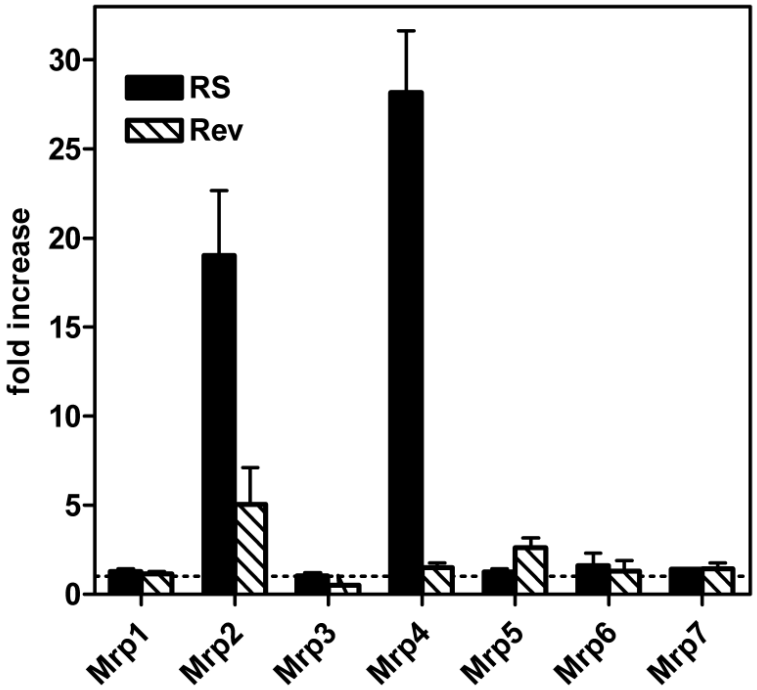


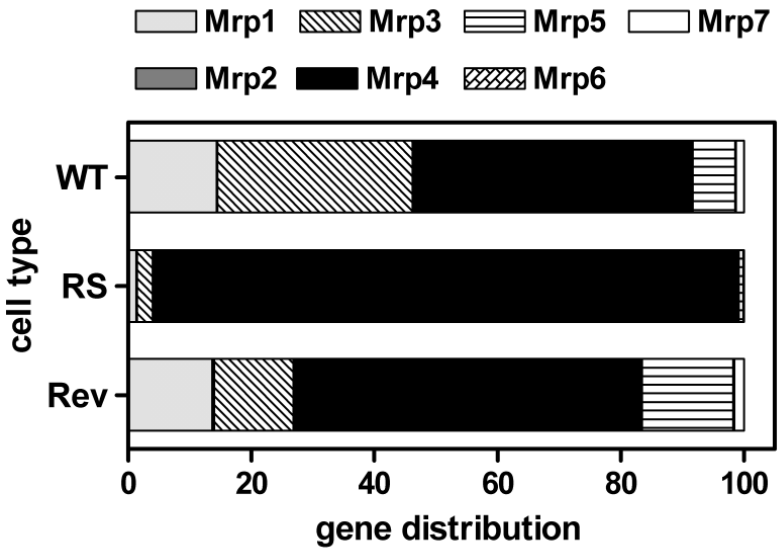
Marquez et al., AAC (2009) 53: 2410-2416

Ciprofloxacin-resistant cells : genomic analysis

ARNm expression levels by Real-Time PCR

↗ expression of Mrp2 and Mrp4, BUT Mrp4 from far most abundant





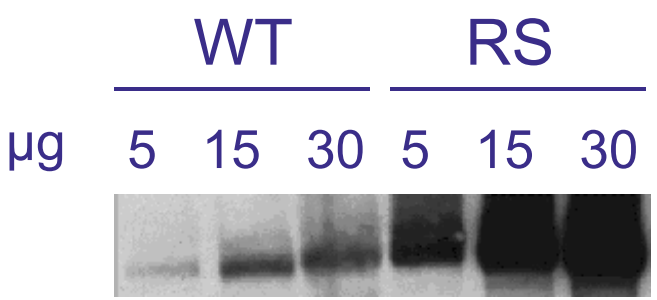
Marquez et al., AAC (2009) 53: 2410-2416

Ciprofloxacin-resistant cells : proteomic analysis

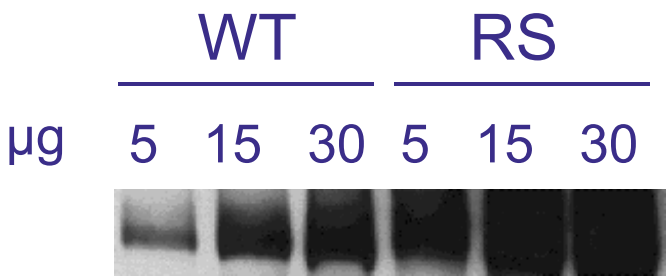
Detection of the corresponding proteins by

Western-Blot of membrane fractions

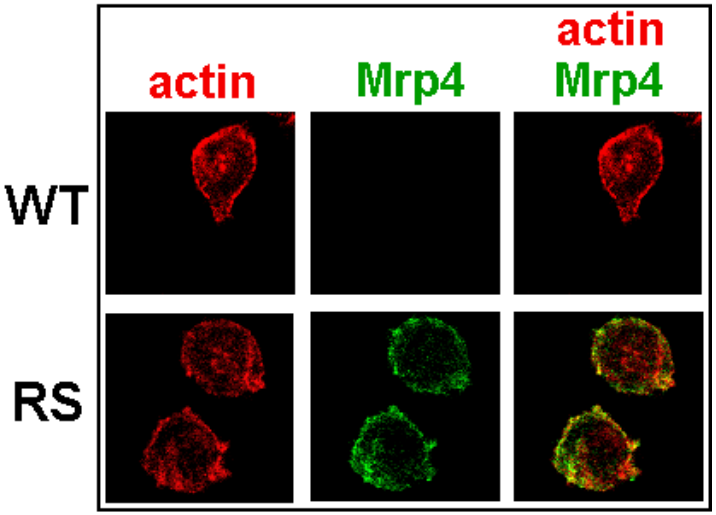
Mrp2



Mrp4



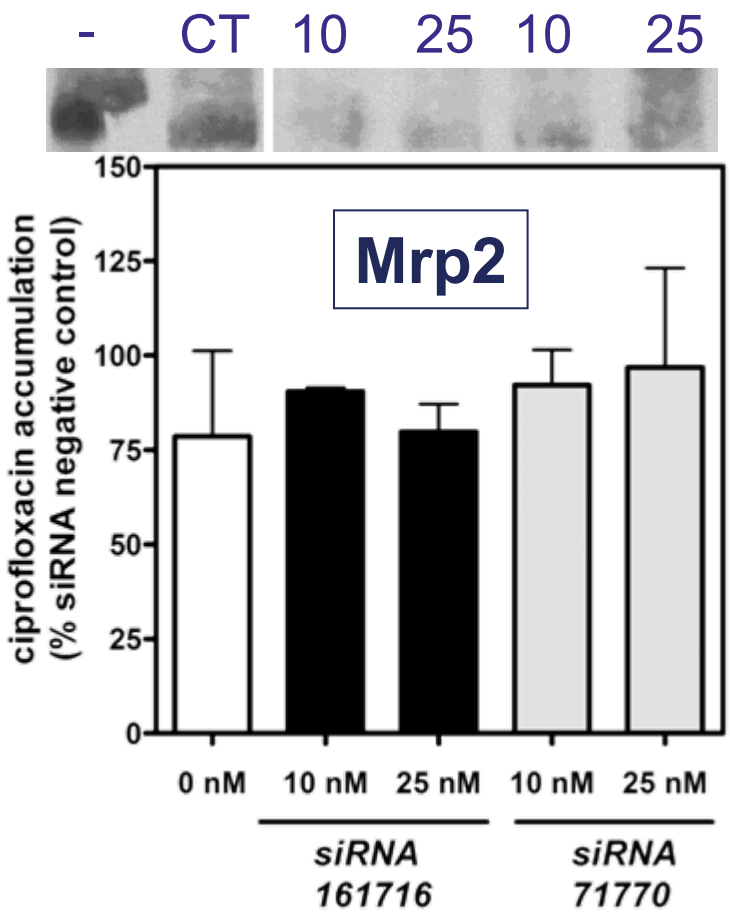
Confocal microscopy

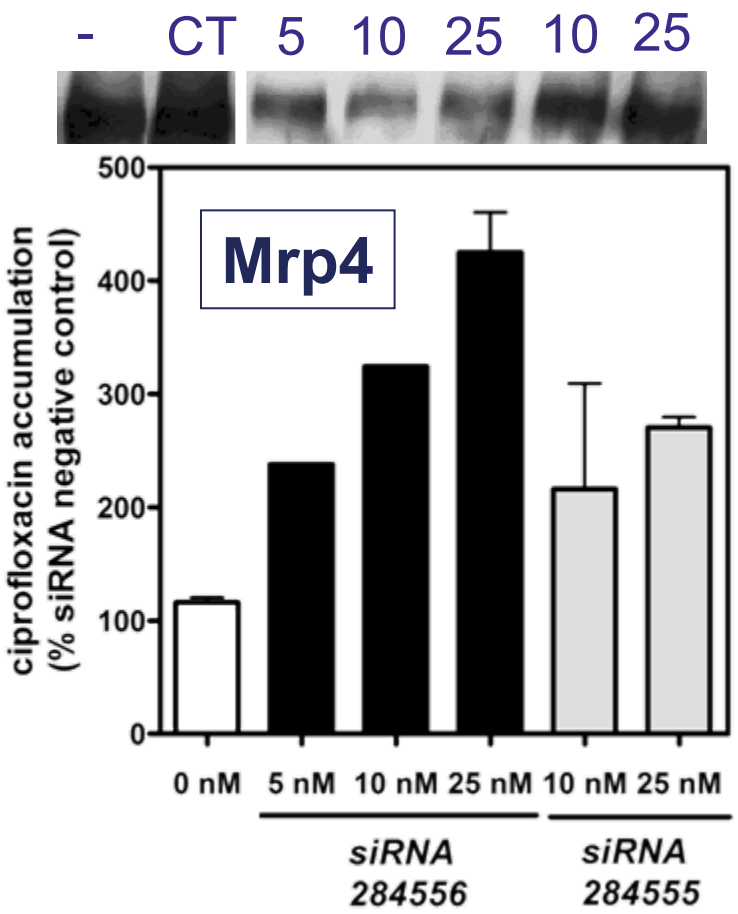


Marquez et al., AAC (2009) 53: 2410-2416

Ciprofloxacin-resistant cells : which is the ciprofloxacin transporter ?

Silencing of MRP expression in resistant cells by siRNA





Marquez et al., AAC (2009) 53: 2410-2416

Stable Isotope Labeling Aminoacid in Culture

WT CIP-macrophages

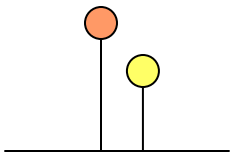
$^{13}C_6$-Lys
$^{13}C_6$-Arg

$^{12}C_6$-Lys
$^{12}C_6$-Arg

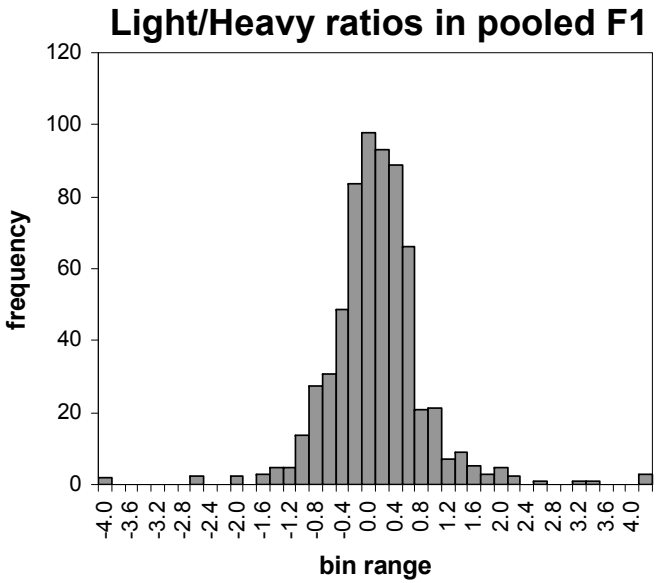
sample mixing 1:1

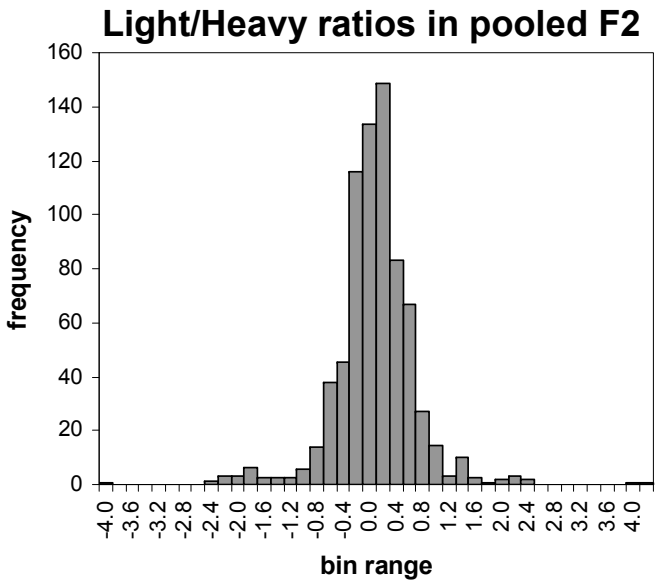
protein digestion

identification in mass spectrometry
and determination of the relative abundance

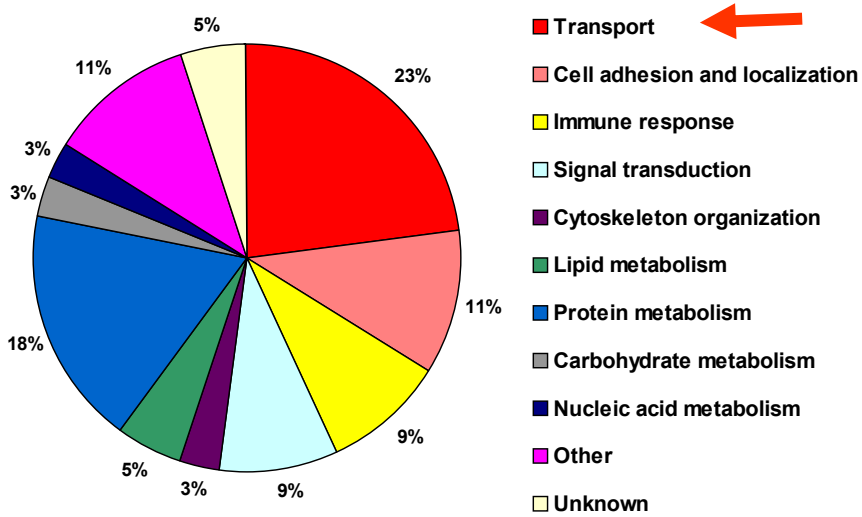


SILAC: proteins with modified expression

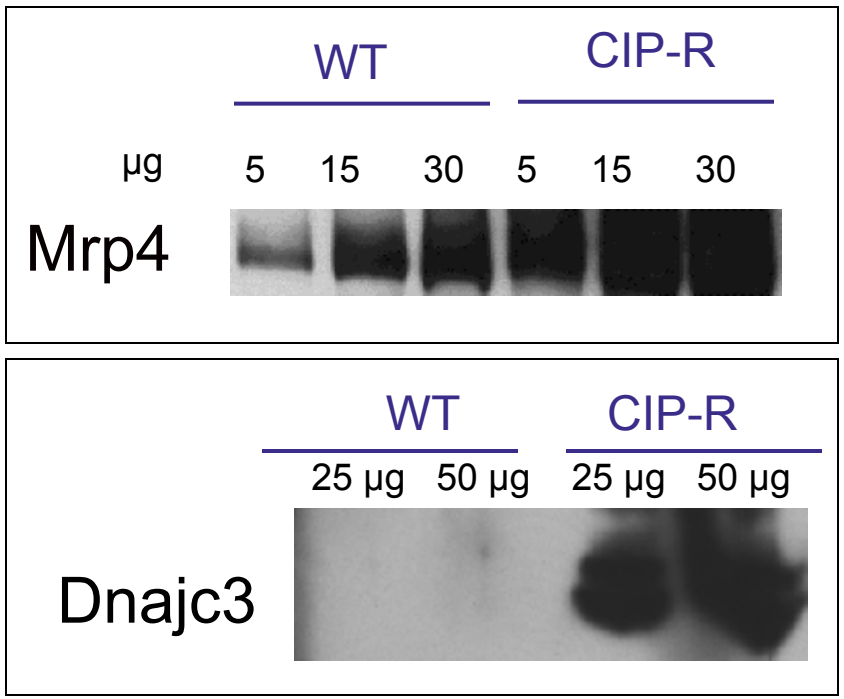


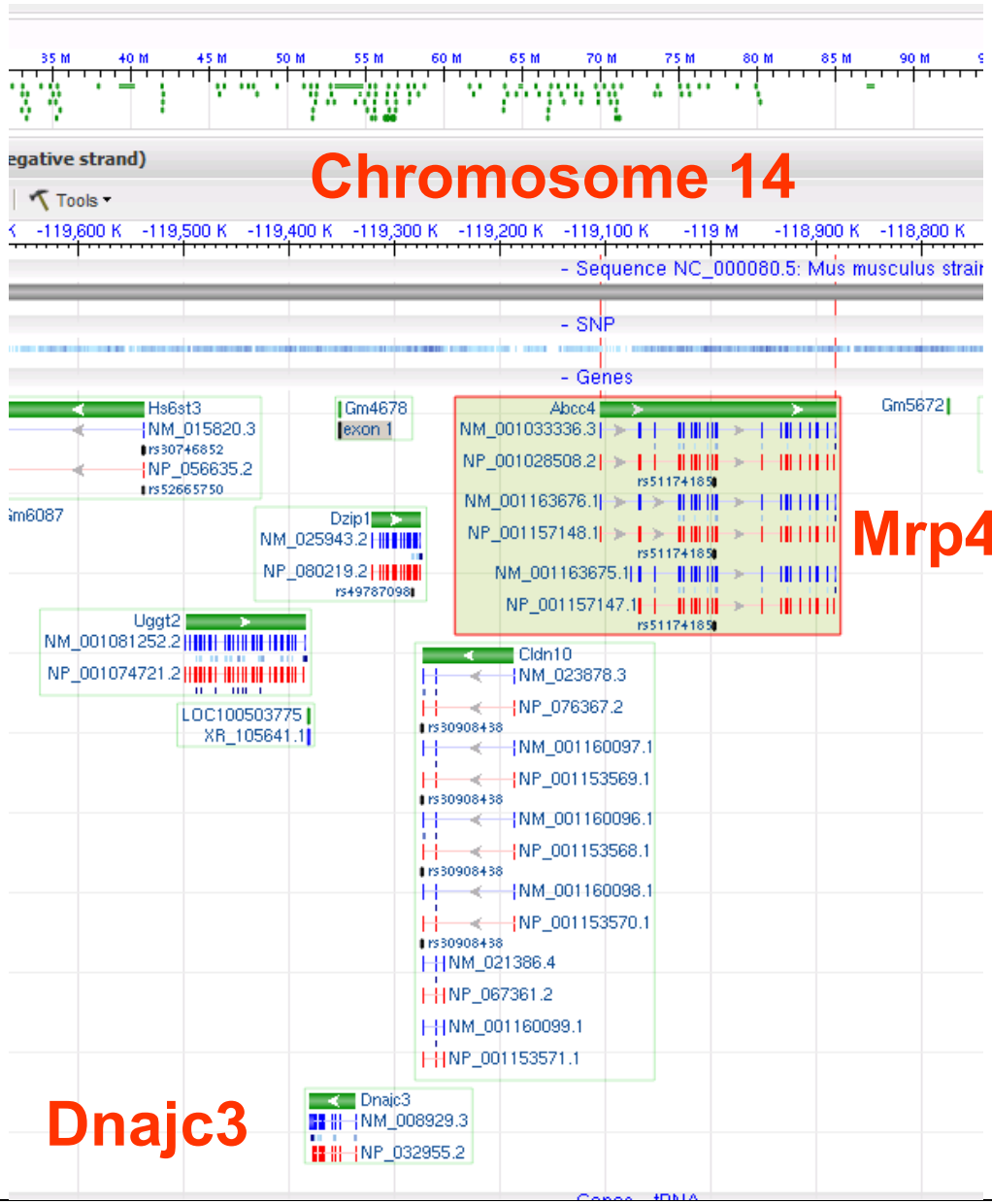


Biological function of proteins with differential abundance



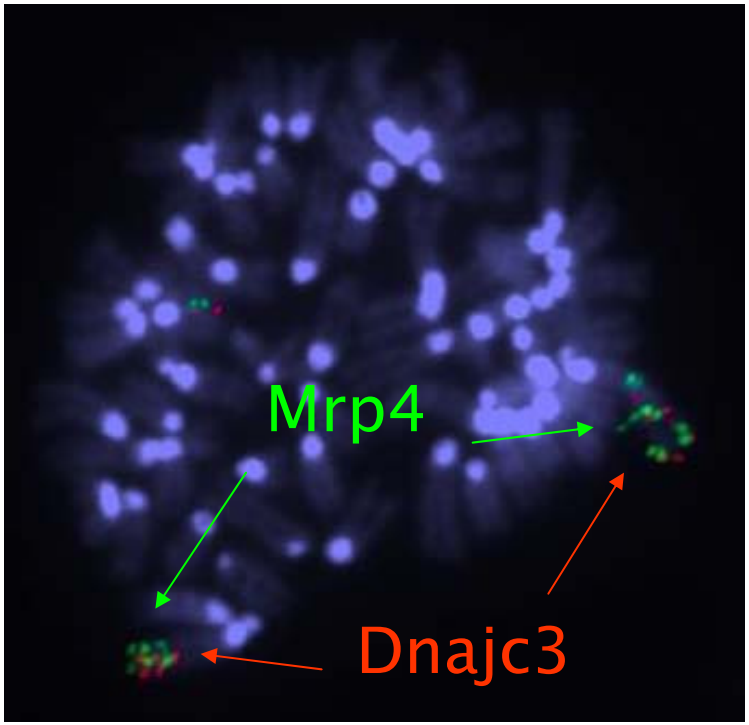
Mrp4 and Dnajc3 are the most upregulated proteins !





Gene amplification of part of chromosome 14 in CIP-R cells

Mrp4 and Dnaj3 co-amplified in CIP-resistant cells



Active efflux as a mechanism of resistance to fluoroquinolones in *S. pneumoniae*

- **Pharmacologie cellulaire et moléculaire, UCL, Bruxelles**
  L. Avrain, F. El Garch, A. Lismond, S. Delvigne
  supervision: P.M. Tulkens, F. Van Bambeke
- **Unité des agents antibactériens, Institut Pasteur, Paris, France**
  P. Courvalin
- **School of Immunity & Infection, University of Birmingham, UK**
  M. Garvey
  supervision: L. Piddock

UCL Université catholique de Louvain

www.facm.ucl.ac.be

MIC of fluoroquinones

- or + Reserpine as efflux pump inhibitor

FQ / strains	NOR		CIP		LVX		MXF		GMF	
	-R	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**
49619	4		1		1		0.25		0.125	
SP334	**32**		**4**		2		0.5		0.25	
SP335	**64**		**32**		**4**		0.5		**0.5**	
SP295	**16**		2		1		0.125		0.063	
SP13	**64**		**16**		2		0.25		0.25	

- NOR and CIP show elevated MICs in the 4 resistant strains
- LVX MIC is close to the EUCAST Bkpt (± 1 dil) in all strains
- MXF and GMF consistantly show low MICs

El Garch et al., JAC (2010) 65: 2076–2082

MIC of fluoroquinones

- or + Reserpine as efflux pump inhibitor

FQ / strains	NOR		CIP		LVX		MXF		GMF	
	-R	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**
49619	4	2	1	0.5	1	0.5	0.25	0.25	0.125	0.125
SP334	**32**	4	**4**	1	2	1	0.5	0.5	0.25	0.125
SP335	**64**	**8**	**32**	**2**	**4**	**2**	0.5	0.25	**0.5**	0.125
SP295	**16**	2	2	0.5	1	1	0.125	0.125	0.063	0.032
SP13	**64**	**16**	**16**	**2**	2	1	0.25	0.25	0.25	0.125

- reserpine reverses resistance but only partially in 2 strains
- MFX not affected; LVX and GMF poorly affected

→
- efflux contributes to resistance in the 4 strains
- other mechanisms also present in 2 strains

El Garch et al., JAC (2010) 65: 2076–2082

Target mutations ?

FQ / strains	NOR		CIP		LVX		MXF		GMF	
	-R	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**
49619	4	2	1	0.5	1	0.5	0.25	0.25	0.125	0.125
SP334	**32**	4	**4**	1	2	1	0.5	0.5	0.25	0.125
SP335	**64**	**8**	**32**	**2**	**4**		**ParE (Ile460Val)**		**0.5**	0.125
SP295	**16**	2	2	0.5	1	1	0.125	0.125	0.063	0.032
SP13	**64**	**16**	**16**		**ParE (Ile460Val); ParC (Ser79Phe;Lys137Asn)**					

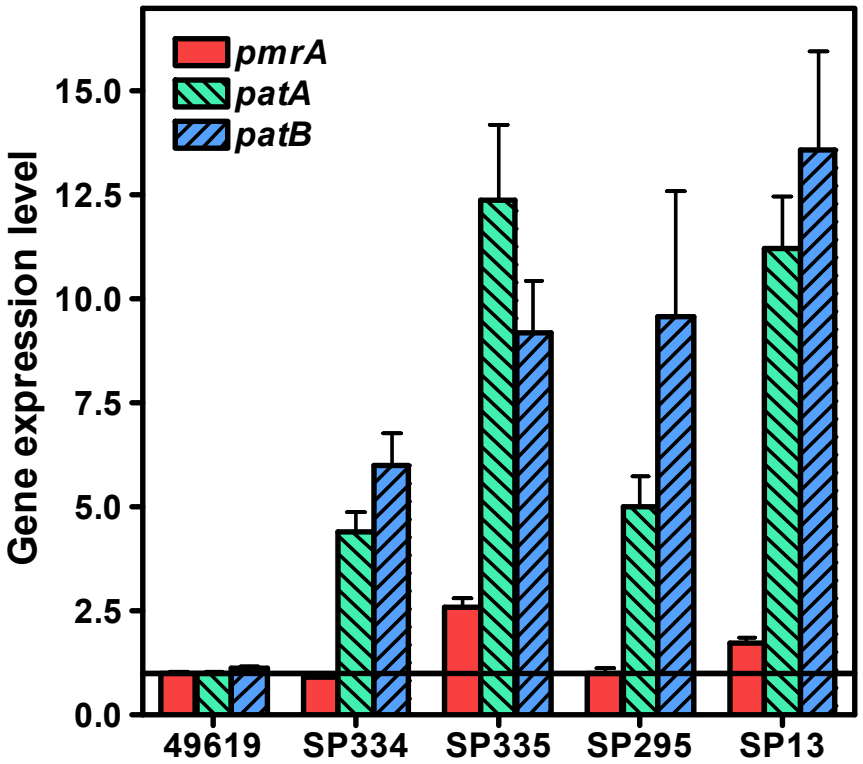
- reserpine reverses resistance but only partially in 2 strains

→

- efflux contributes to resistance in the 4 strains
- target mutations evidenced in 2 strains

El Garch et al., JAC (2010) 65: 2076–2082

Basal expression level of efflux systems

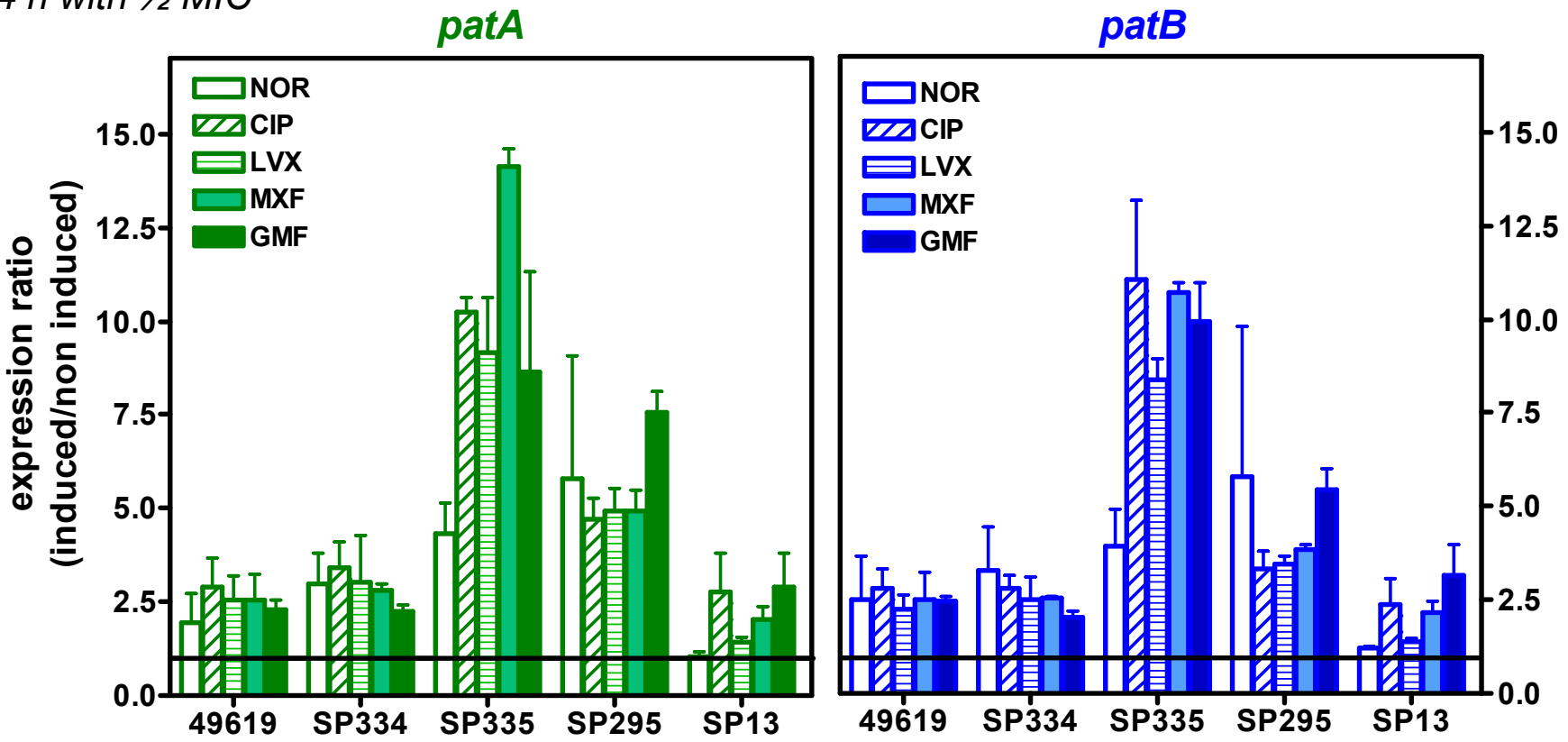


- all strains overexpress *patA*/*patB* to variable level
- SP335 and SP13 show a low level of *pmrA* overexpression

El Garch et al., JAC (2010) 65: 2076–2082

Induced expression level

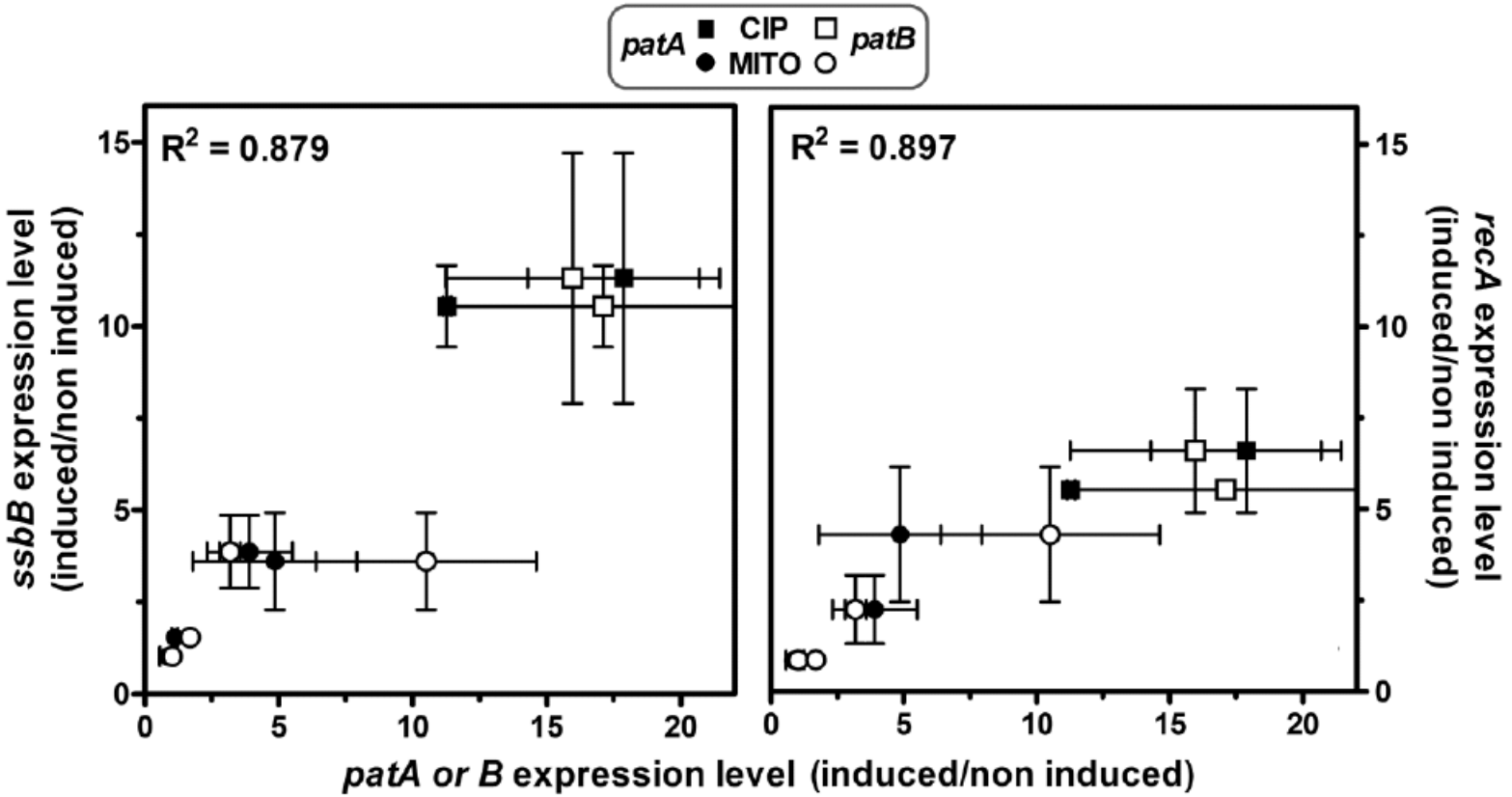
4 h with ½ MIC



- induction of *patA*/*patB*
  - in all strains but to highly variable levels
  - by all FQ, whether substrates or not

El Garch et al., JAC (2010) 65: 2076–2082

Induced expression level … response to stress



- concommitant overexpression og genes involved in response to stress

El Garch et al., JAC (2010) 65: 2076–2082

Efflux by PatA/PatB causes resistance !

Table 1. Susceptibility of *S. pneumoniae* to fluoroquinolones and substrates of efflux pumps in the absence (−R) or presence (+R) of reserpine (20 mg/L)

Strains	Relevant characteristics[a]	Mutations in QRDR	MIC (mg/L) of: norfloxacin −R	norfloxacin +R	ciprofloxacin −R	ciprofloxacin +R	levofloxacin −R	levofloxacin +R	moxifloxacin −R	moxifloxacin +R	gemifloxacin −R	gemifloxacin +R
ATCC49619	Wild-type	None	4	2	0.5	0.5	0.5	0.5	0.125	0.125	0.031	0.031
ATCC49619*patA*	ATCC49619 *patA*::*magellan2*, SPT^R	None	4	2	0.5	0.5	0.5	0.5	0.125	0.125	0.031	0.016
ATCC49619*patB*	ATCC49619 *patB*::*magellan2*, SPT^R	None	2	2	0.5	0.5	0.5	0.5	0.125	0.125	0.031	0.031
ATCC49619*pmrA*	ATCC49619 *pmrA*::*magellan2*, SPT^R	None	4	2	1	0.5	0.5	0.5	0.125	0.125	0.031	0.031
SP334	ATCC49619 after 13-days exposure to ciprofloxacin, CIP^R	None	**32**	4	**4**	0.5	**2**	1	0.25	0.25	**0.125**	0.031
SP334*patA*	SP334 *patA*::*magellan2*, SPT^R	None	4	4	1	0.5	1	1	0.125	0.125	0.063	0.031
SP334*patB*	SP334 *patB*::*magellan2*, SPT^R	None	8	4	1	1	1	1	0.25	0.25	0.063	0.063
SP334*pmrA*	SP334 *pmrA*::*aad9*, SPT^R	None	**32**	4	**4**	0.5	1	0.5	0.25	0.25	**0.125**	0.063

Disruption of patA or patB restores susceptibility to fluoroquinolones in a resistant strain while disruption of pmrA does not

→ efflux of fluoroquinolones mediated by patA/patB *in S. pneumoniae*

El Garch et al., JAC (2010) 65: 2076–2082

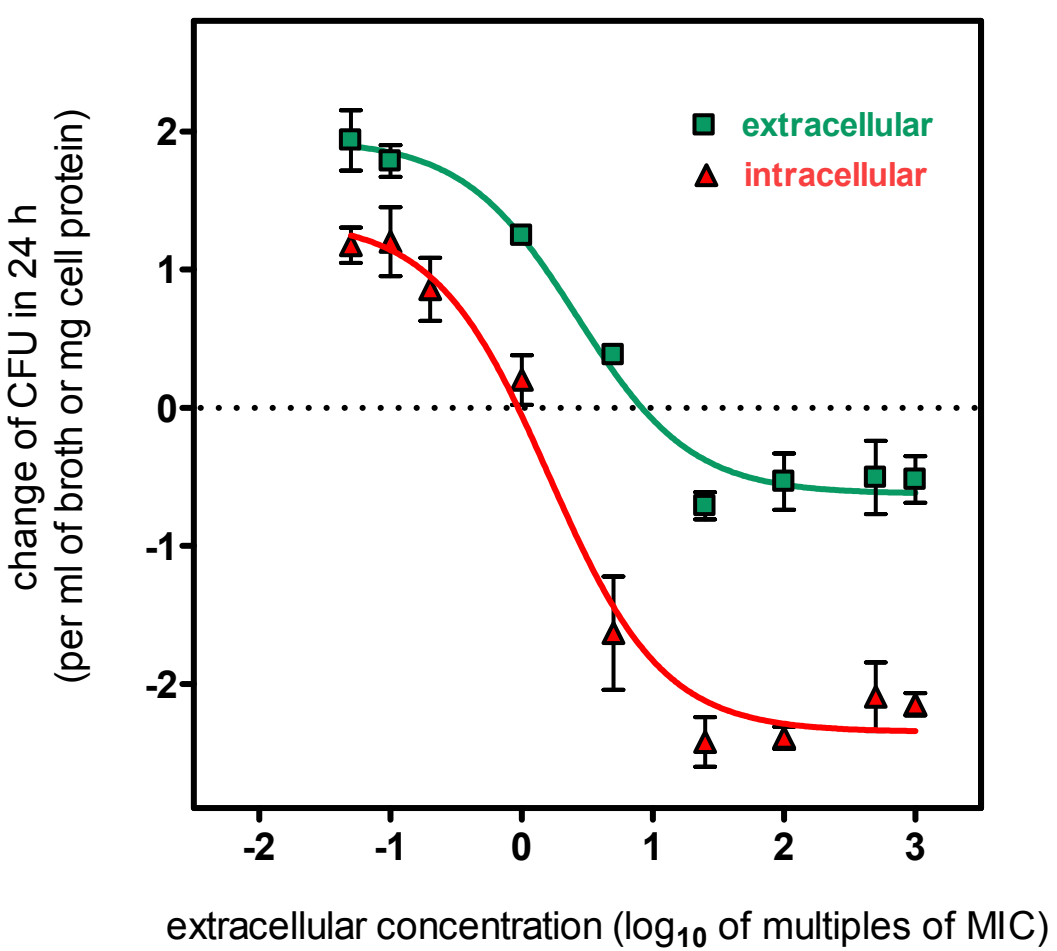
Why do beta-lactams regain activity against intracellular *Listeria monocytogenes* ?

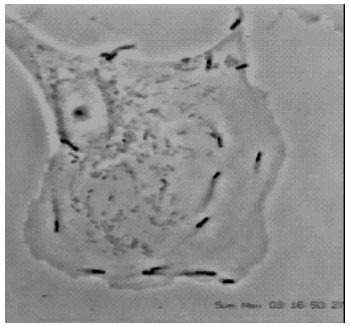
- **Pharmacologie cellulaire et moléculaire, UCL, Bruxelles**
  S. Van de Velde, S. Carryn
  supervision: P.M. Tulkens, F. Van Bambeke
- **Laboratorium voor Eiwitbiochemie en Eiwitengineering, Ugent, Ghent**
  M. Aerts; supervision: B. Devreese
- **Unité de recherche en biologie cellulaire,**
  Facultés Universitaires Notre-Dame de la Paix, Namur, Belgium
  Edouard Delaive, Marc Dieu
  supervision: M. Raes

UCL Université catholique de Louvain

www.facm.ucl.ac.be

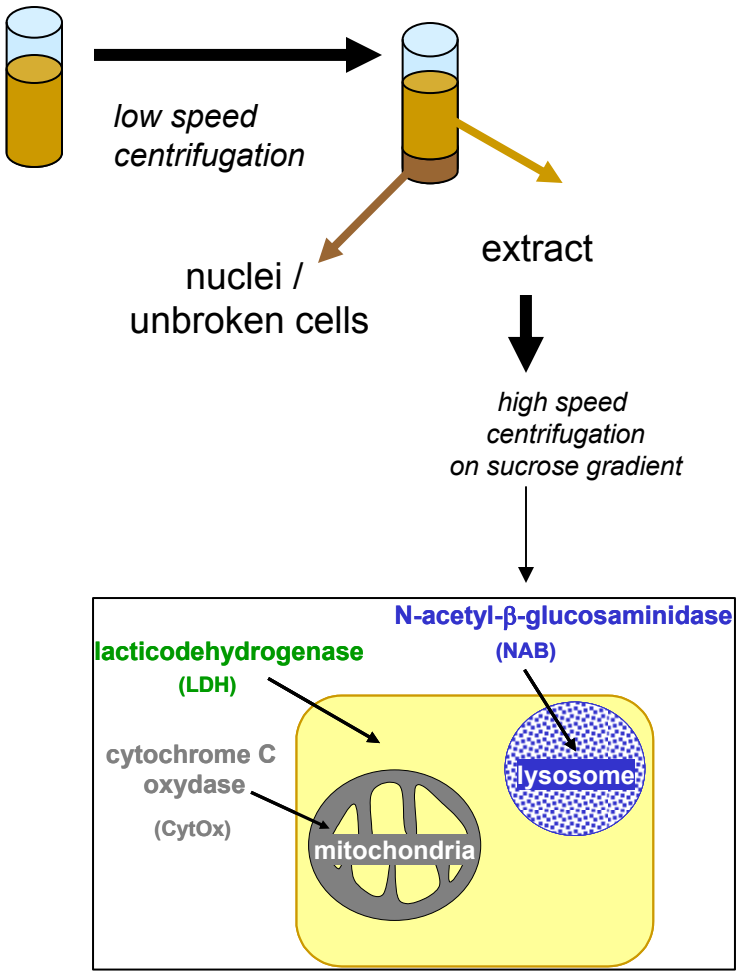
Ampicillin is more active against intracellular than extracellular Listeria

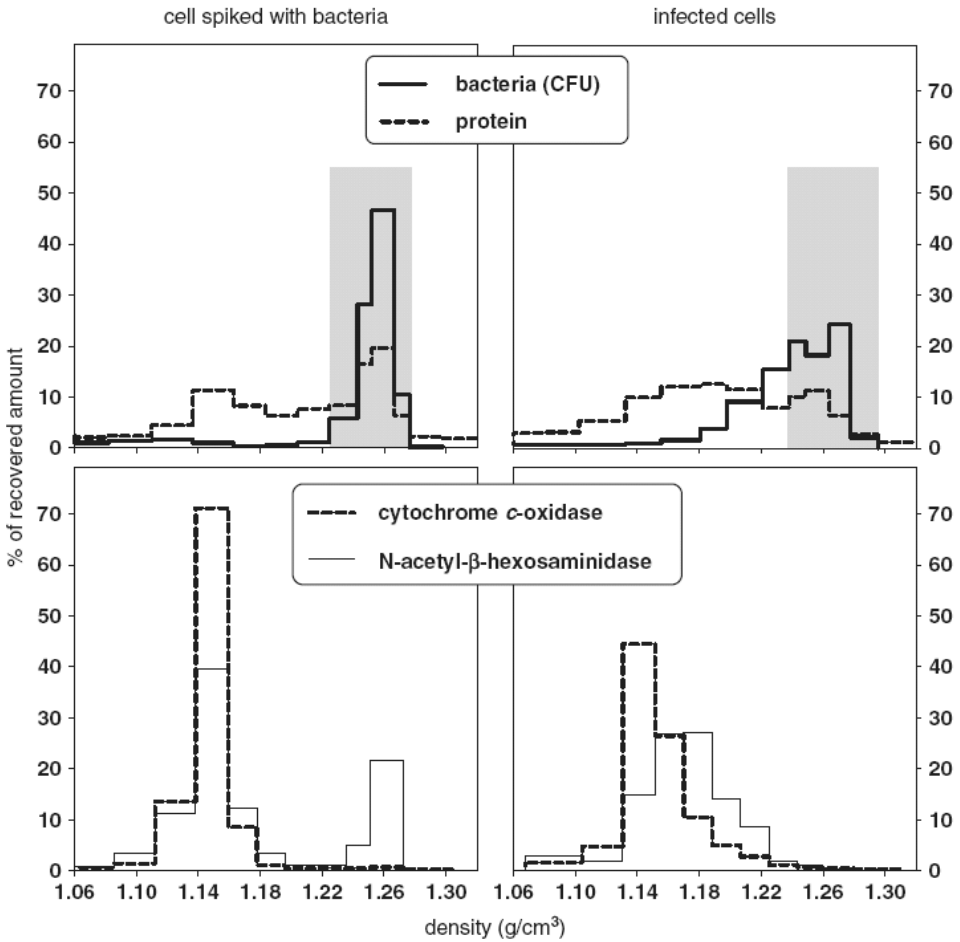




Changes in intracellular metabolism ?

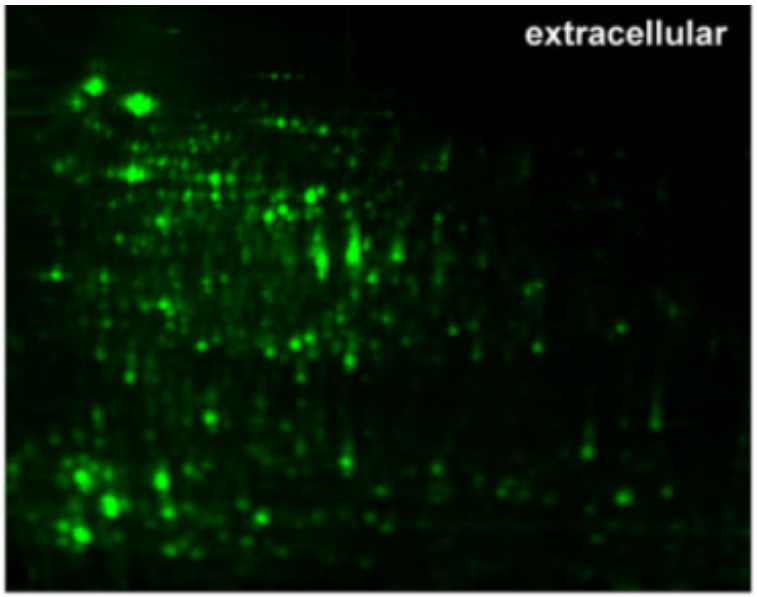
Purifying intracellular Listeria by cell fractionation

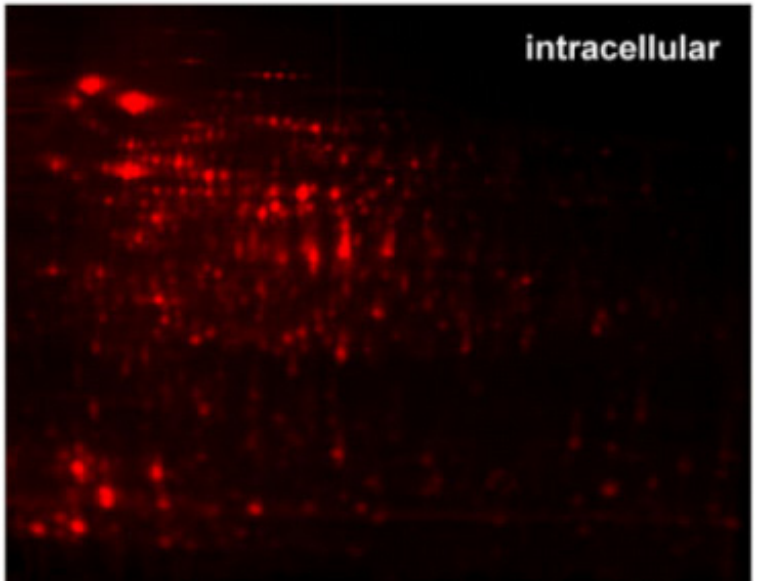


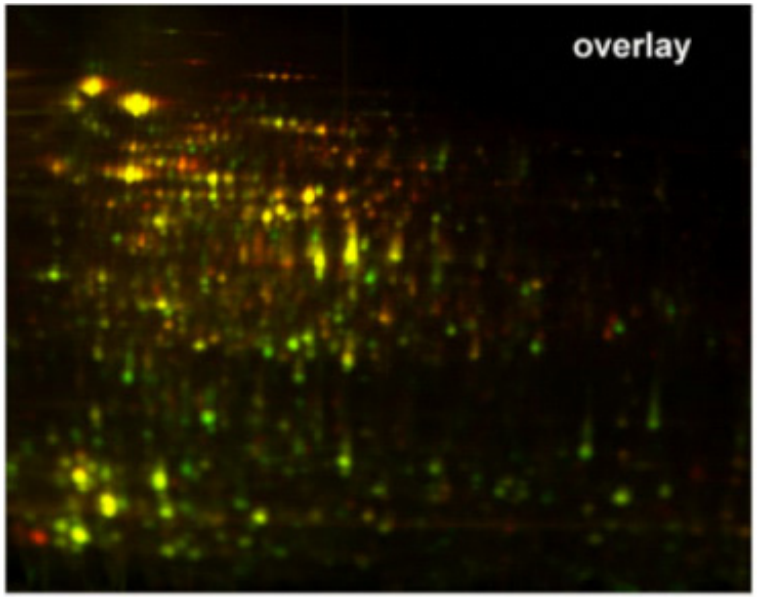


Van de Velde et al., Proteomics (2009) 9, 5484–5496

Separating and quantifying proteins

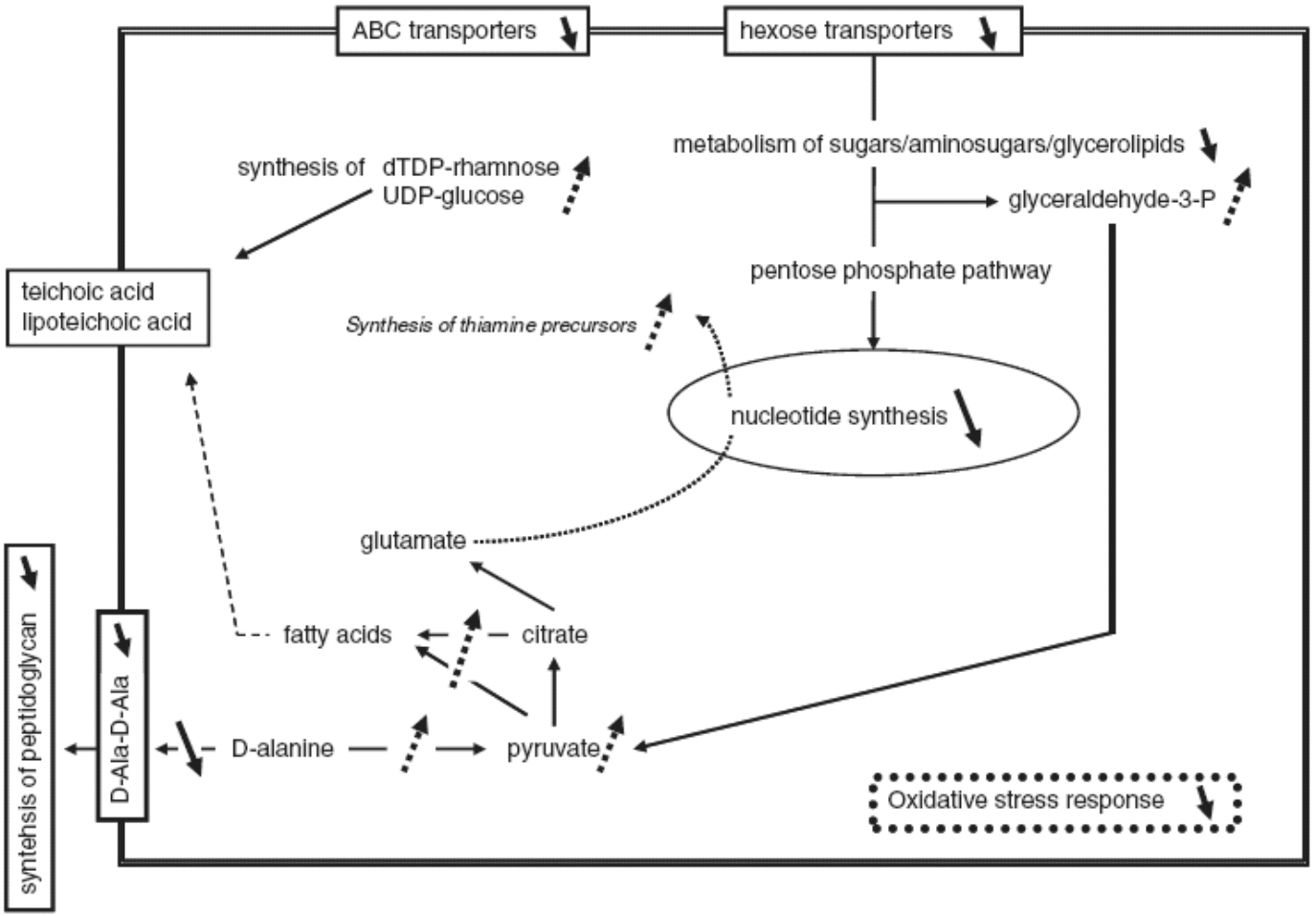






Van de Velde et al., Proteomics (2009) 9, 5484–5496

Interpreting changes in protein expression ….



Target of beta-lactams!

Van de Velde et al., Proteomics (2009) 9, 5484–5496

Examples of integrated programs of molecular pharmacology

Oritavancin :

a new antibiotic with novel mode of action and unusal cellular pharmacokinetic profile

Beta-lactams :

How do beta-lactams regain activity against MRSA in the intracellular milieu

Aminoglycosides :

from molecular mechanisms of toxicity to clinical implications

Pharmacology of oritavancin

- **Pharmacologie cellulaire et moléculaire, UCL, Bruxelles**
  C. Seral, H.A. Nguyen, S. Lemaire, P. Baudoux, O. Domenech
  supervision: M.P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke
- **Faculté d'ingénierie biologique, agronomique et environnementale, Unité de chimie des interfaces, Louvain-La-Neuve**
  Y. Dufrêne

UCL Université catholique de Louvain

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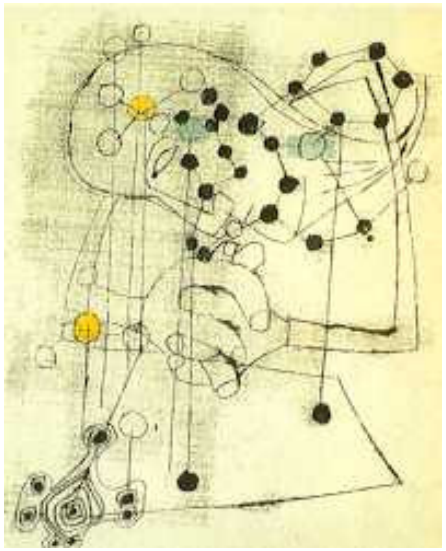
Our main research interests...

cellular pharmacokinetics

cellular pharmacodynamics

antibiotic toxicity

resistance



novel bacterial targets

clinical applications

Antibiotics: from molecules to man

Oritavancin story

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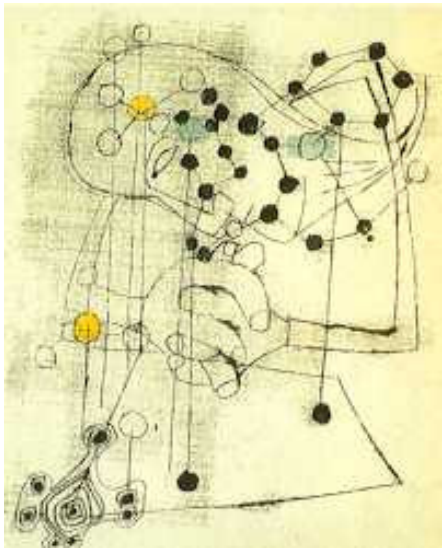

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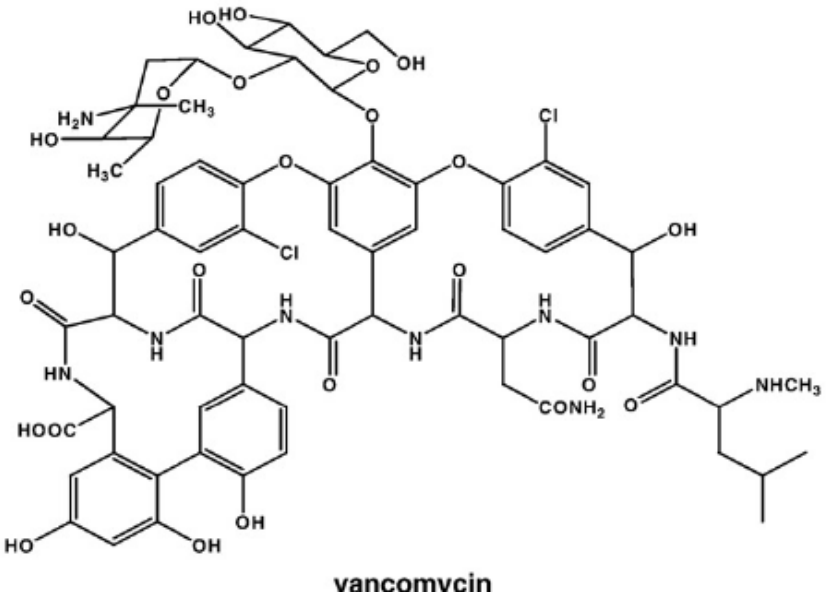
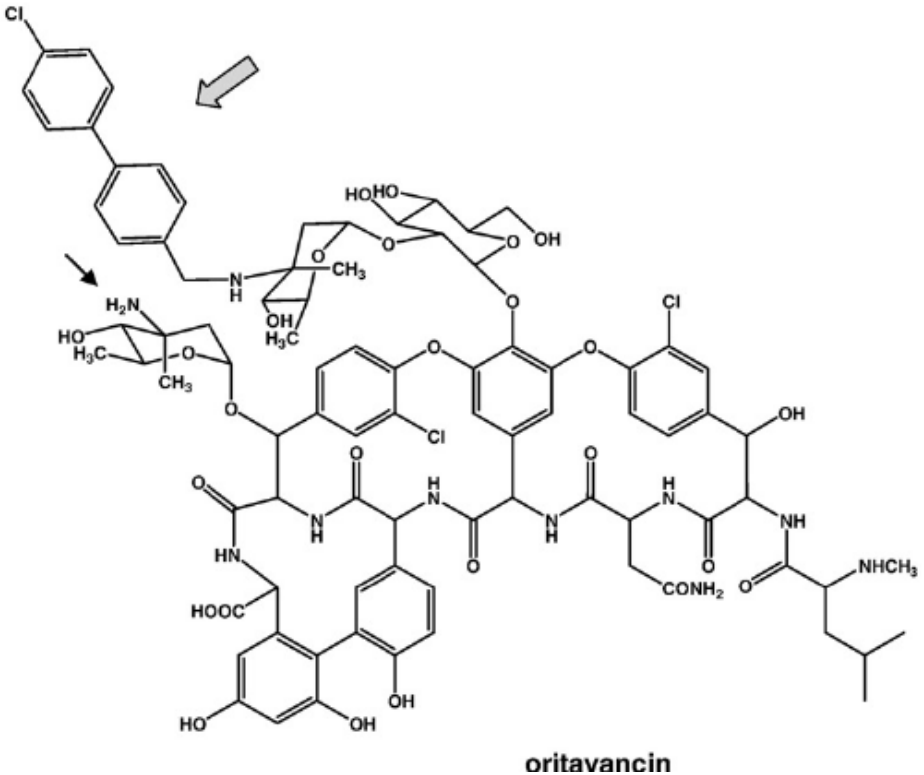
Oritavancin, a novel lipoglycopeptide …

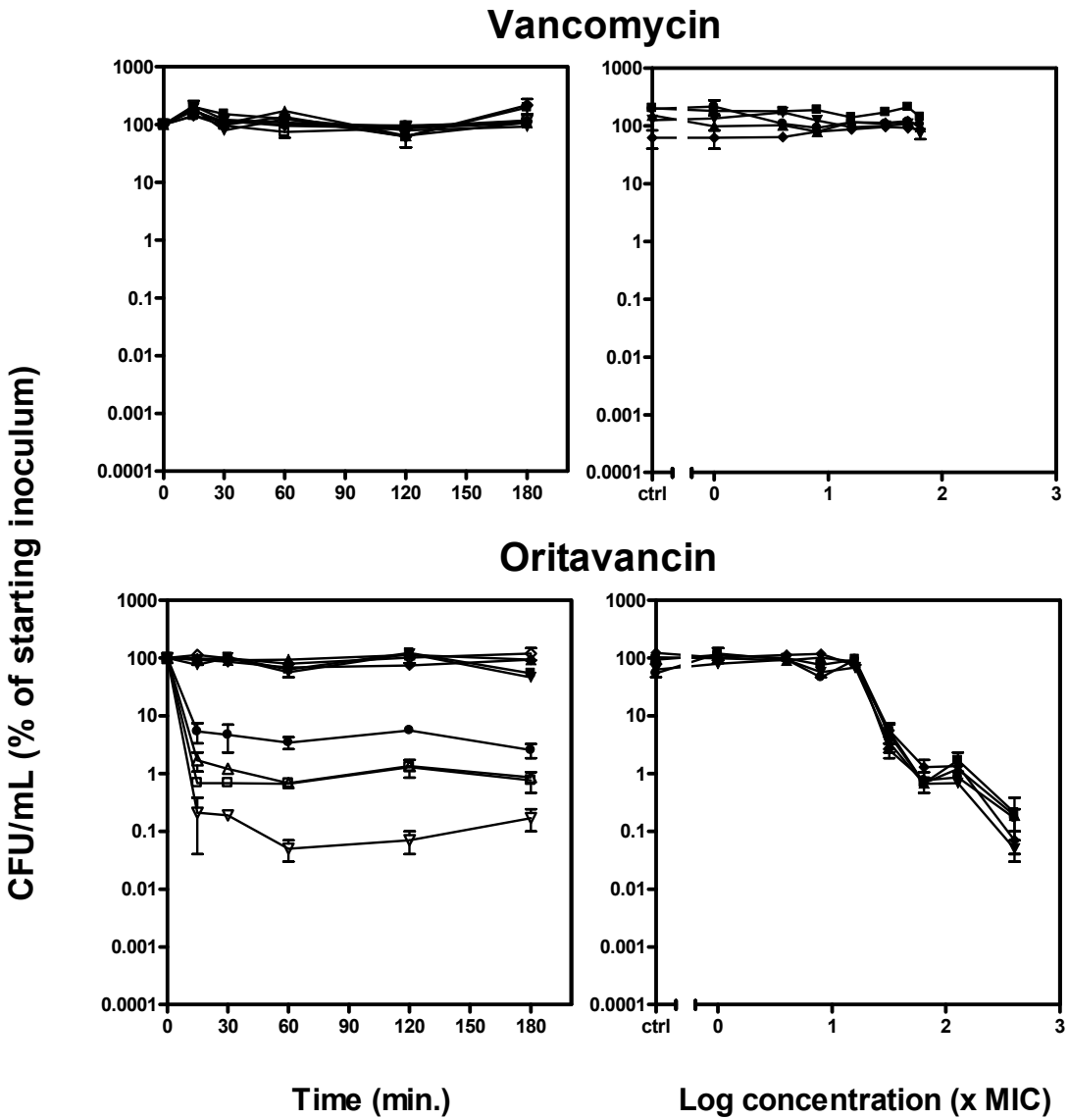


vancomycin



oritavancin

A new mode of action ….

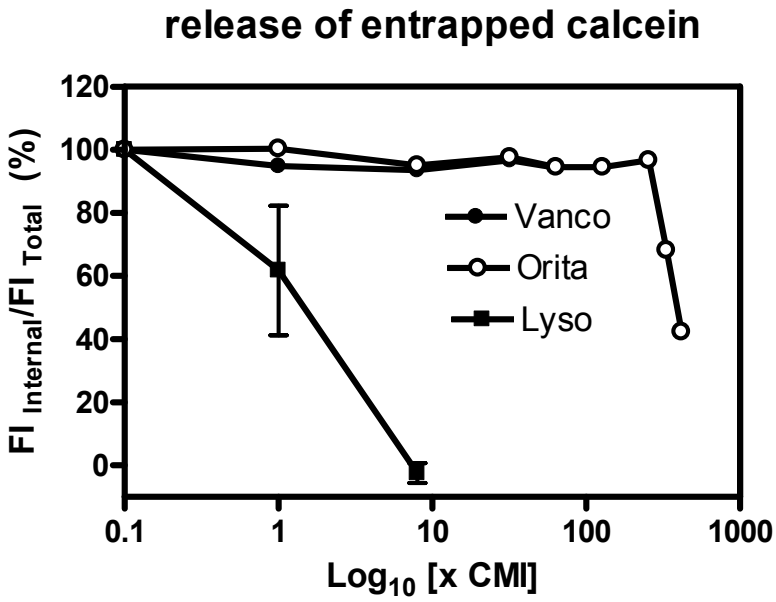


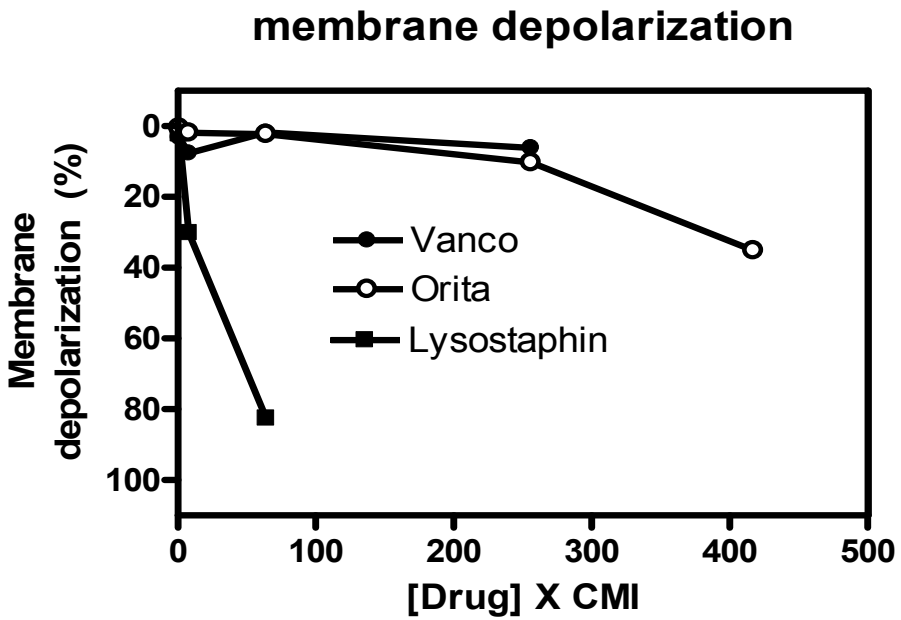
Bacteriostatic effet

Time- and concentration-dependent killing

Baudoux, Nguyen et al. ICAAC 2009, Poster C1 1354

A new mode of action ….

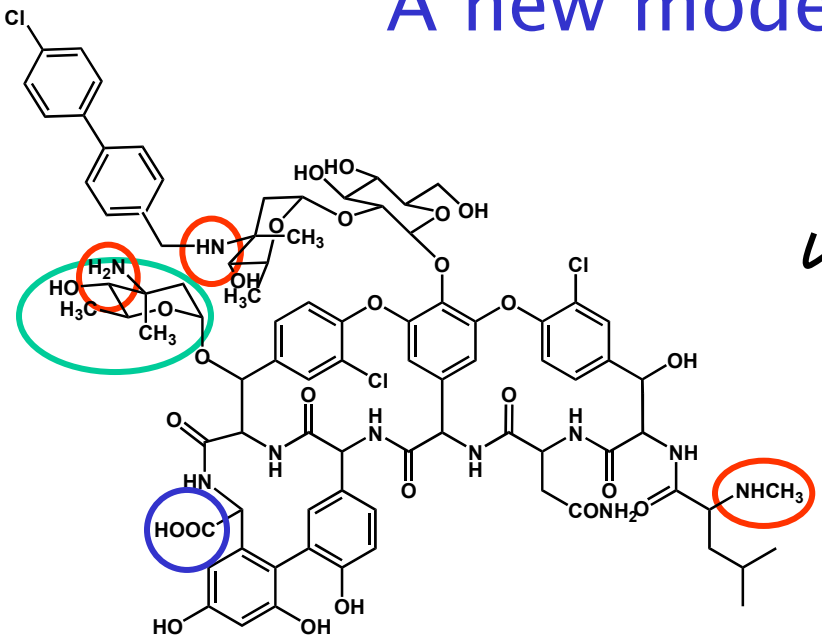


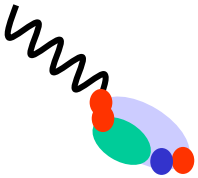


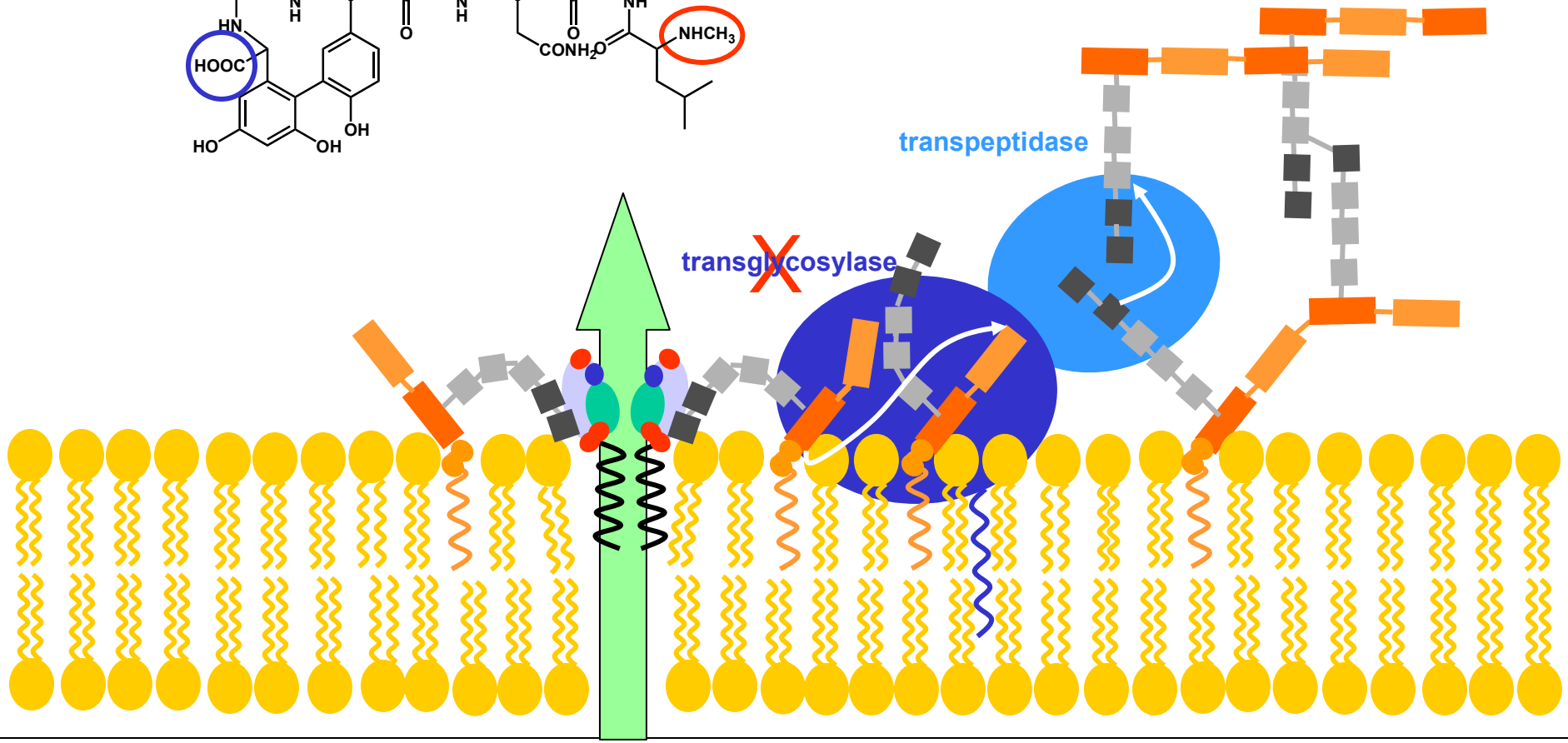
Membrane permeabilization and depolarization

Baudoux, Nguyen et al.
ICAAC 2009, Poster C1 1354

A new mode of action ….







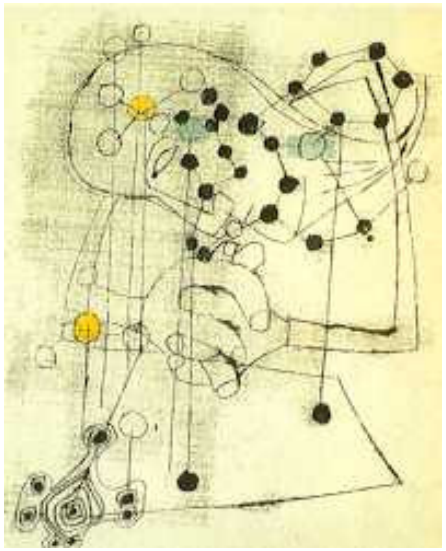
Our main research interests...

cellular pharmacokinetics

cellular pharmacodynamics

antibiotic toxicity

resistance



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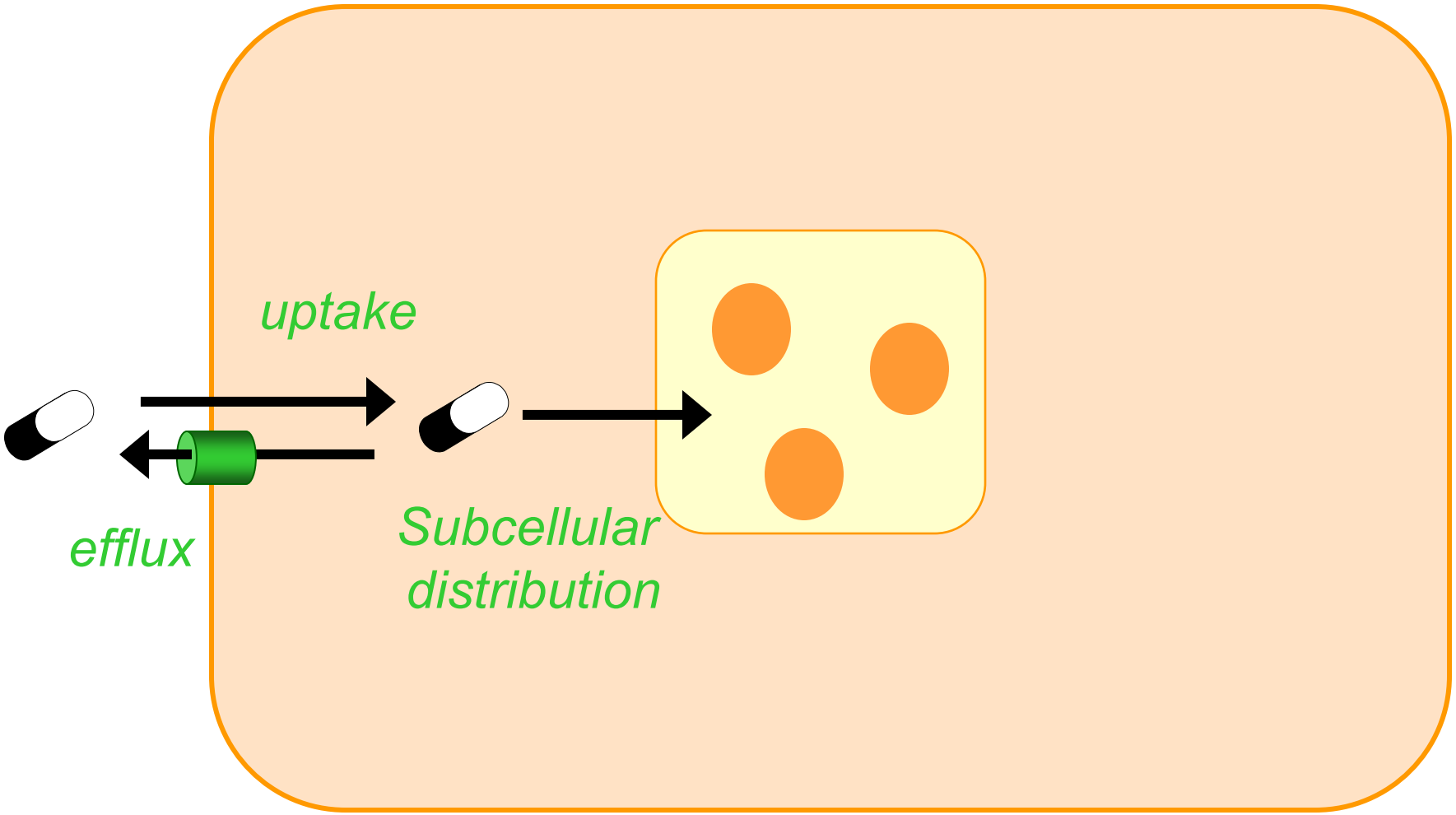
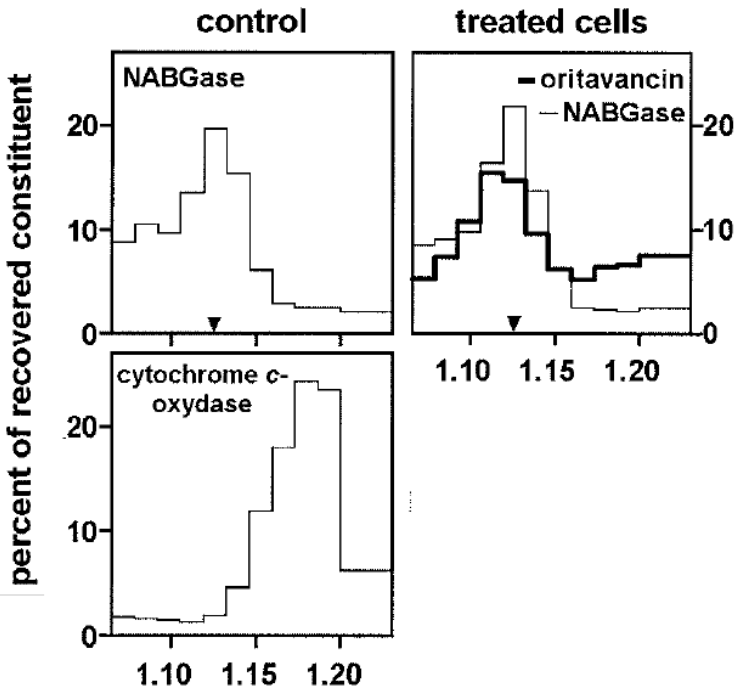


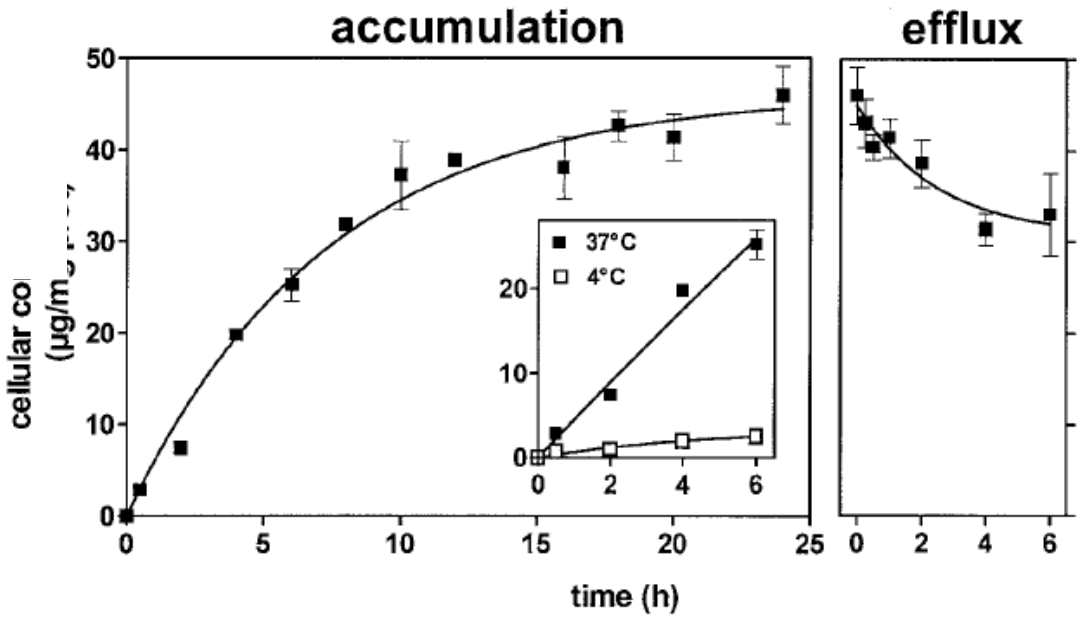
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

Cellular pharmacokinetics of oritavancin



Lysosomal tropism

Huge cellular accumulation



Van Bambeke et al. AAC (2004) 48:2853-2860

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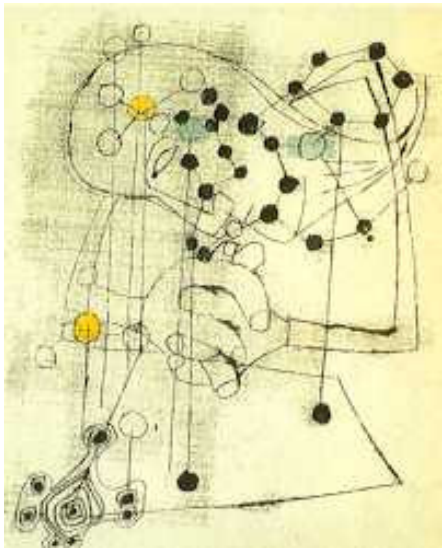

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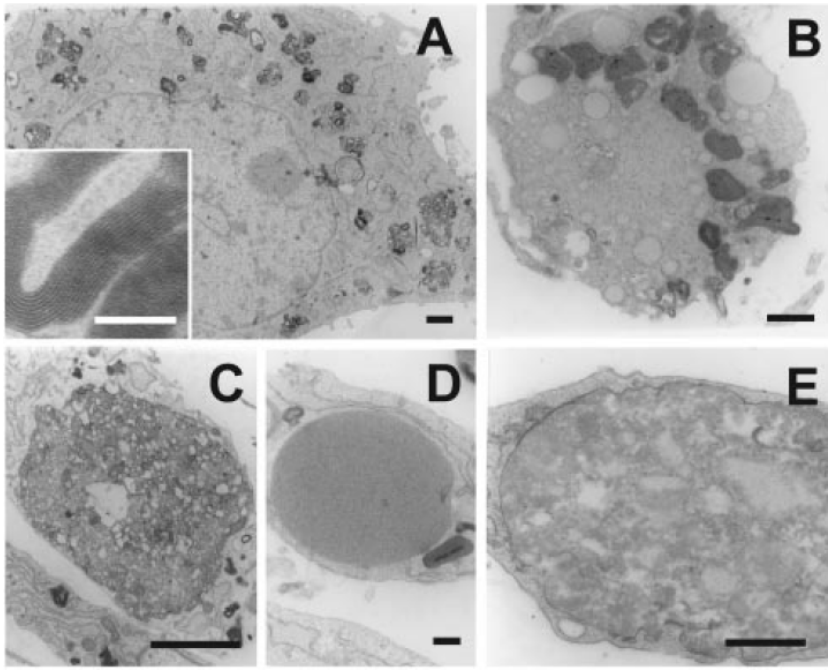
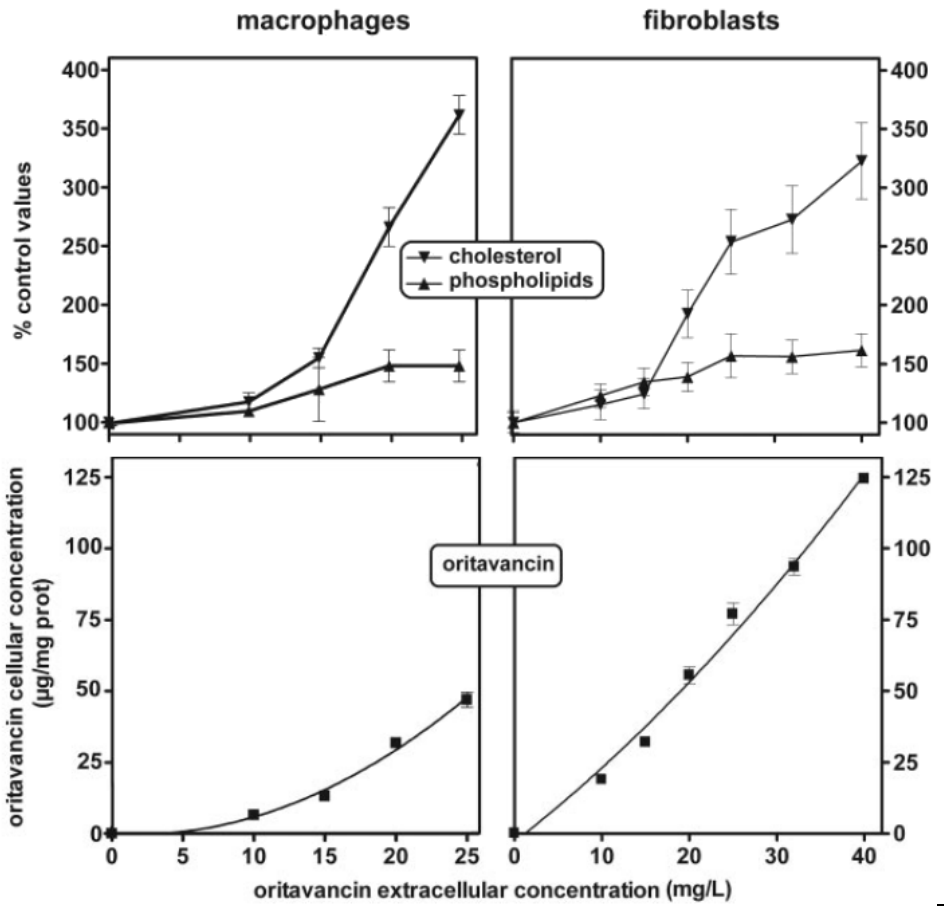


Mixed
storage disorder

Accumulation of lipids



Van Bambeke et al. AAC (2005) 49:1695-1700

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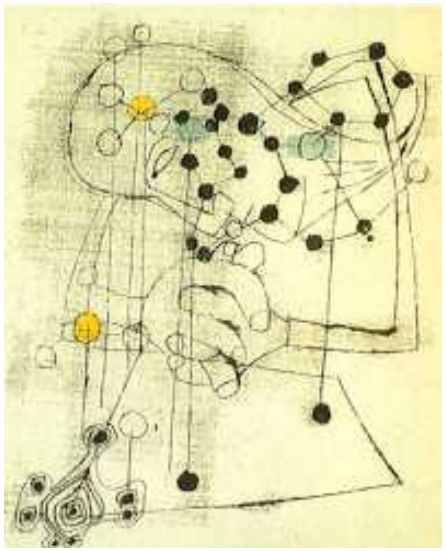

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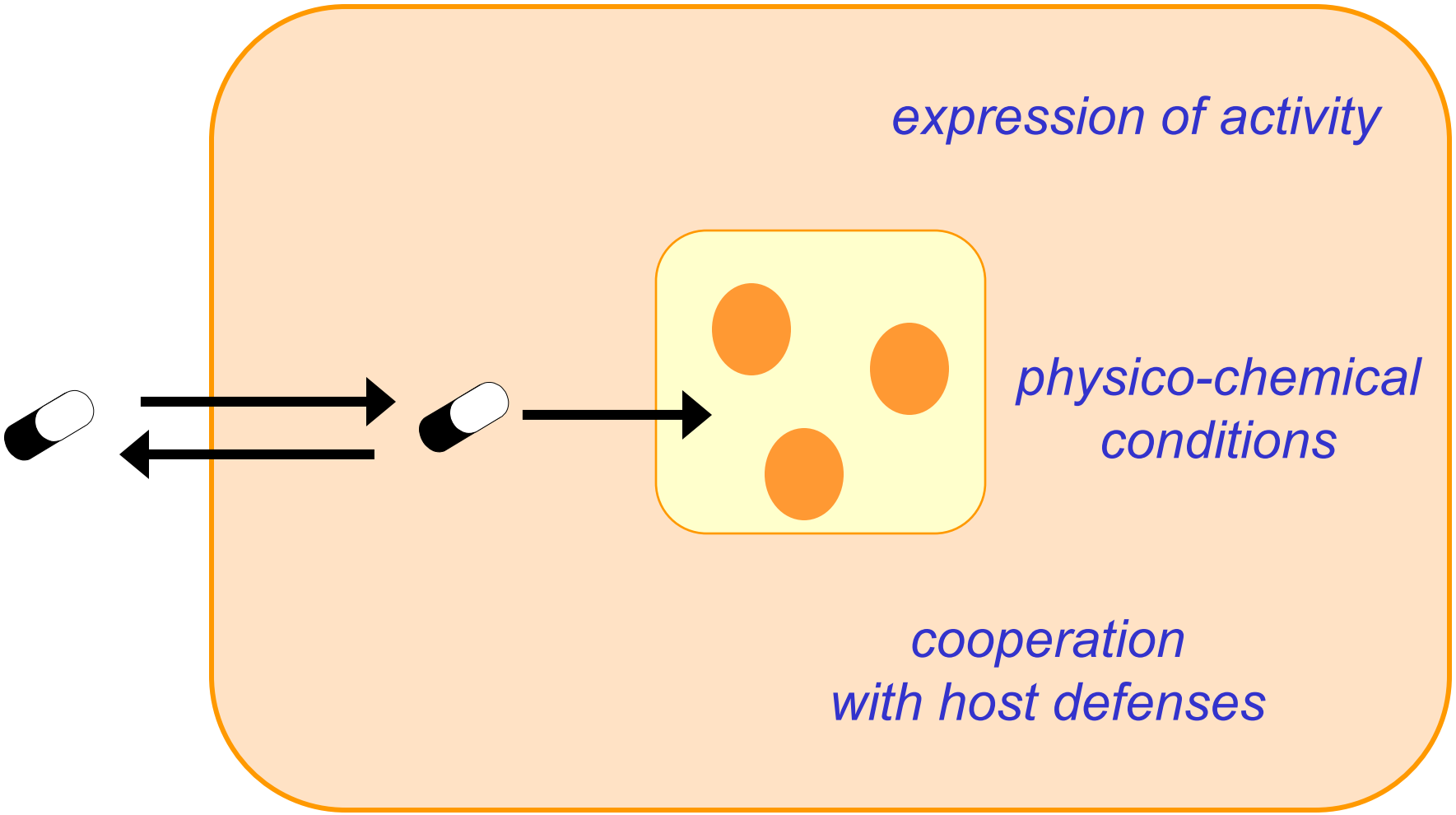
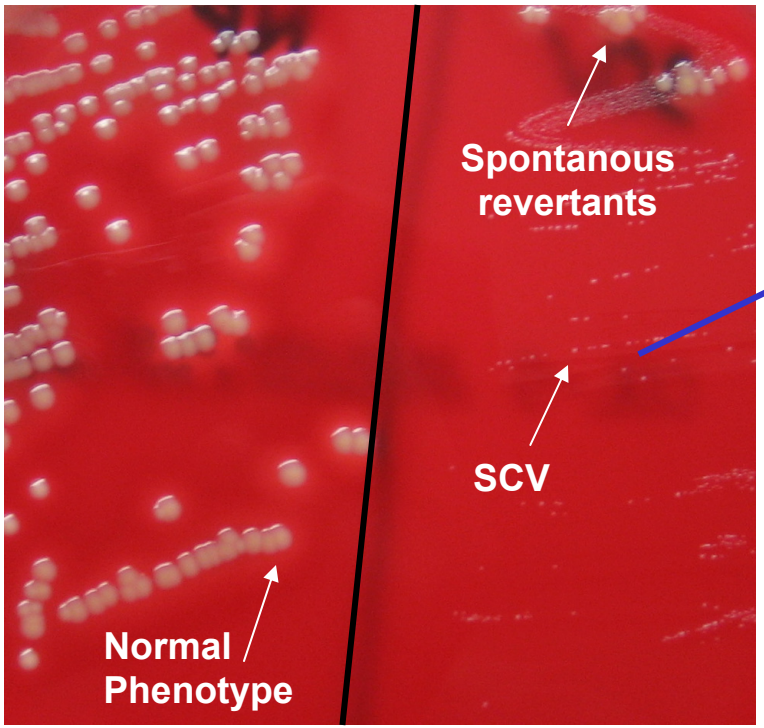


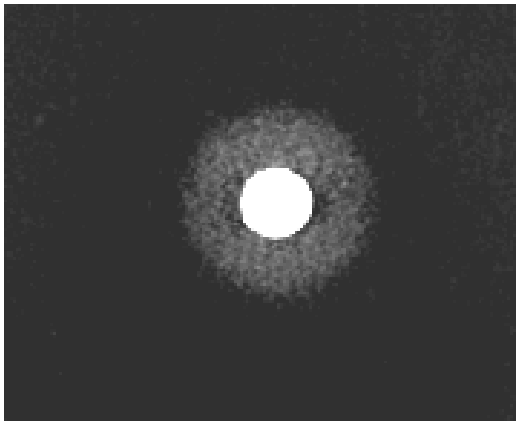
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

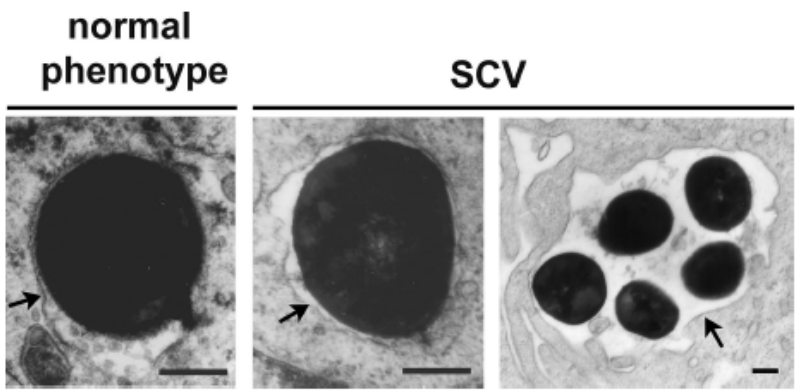
SCV isolated from a cystic fibrosis patient

Vergison et al. JAC (2007) 59:893-9

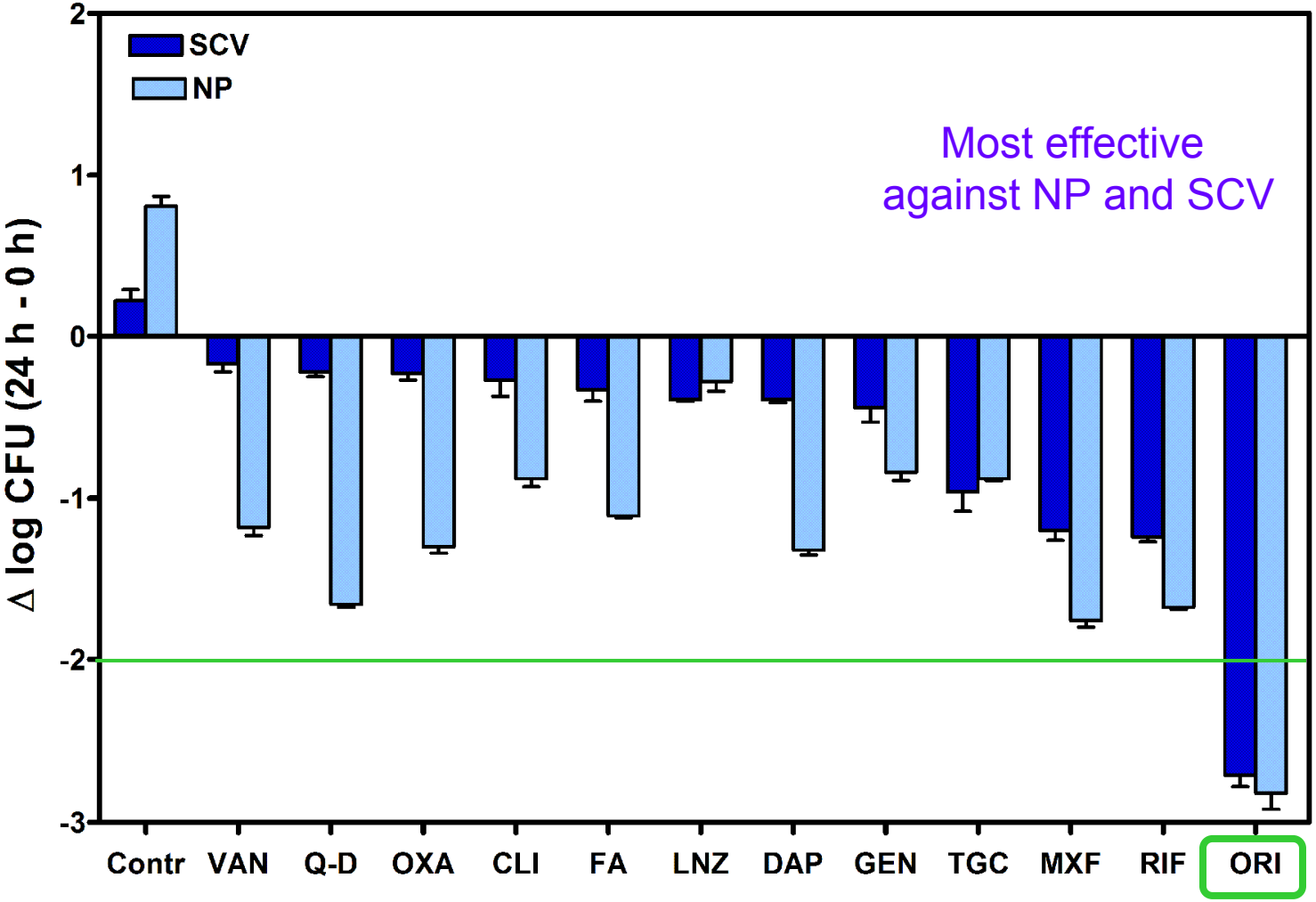


Thymidine dependent



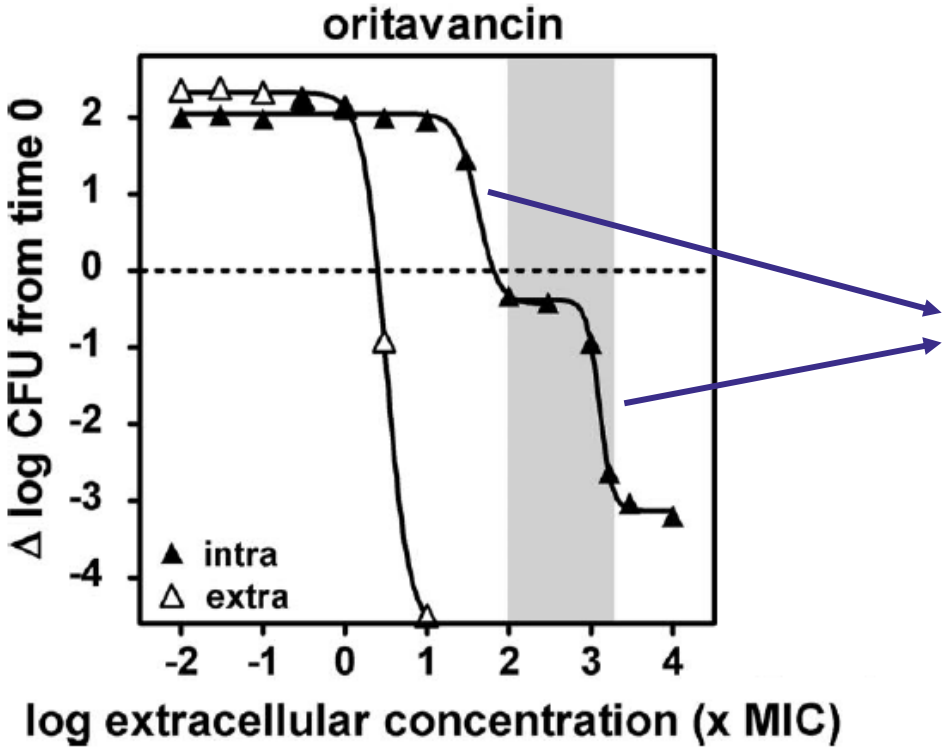


Comparison of antibiotics at their human Cmax



Nguyen et al, AAC (2009) 53:1434–1442

Dose effect relationship



Dual mode of action ?

Nguyen et al, AAC (2009) 53:1434–1442

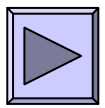
Conclusion of the oritavancin story and links with the clinics ….

• highly bactericidal and active against resistant strains thanks to a novel mode of action
→ Useful for infection by super bugs

• high accumulation in cells
→ Prolonged half life for once a day or once a week administration

• active against intracellular staphylococci
→ Interest for persistent infections

• lysosomal storage disorder
→ Significance in the clinics ?
→ FDA asks for more safety data before registration ….



How do beta-lactams regain activity against MRSA intracellularly ?

- **Pharmacologie cellulaire et moléculaire, UCL, Bruxelles**
  S. Lemaire
  supervision: P.M. Tulkens, F. Van Bambeke
- **Department of Chemistry and Biochemistry, Université de Notre-Dame Notre Dame, IN**
  C. Fuda, S. Mobashery
- **Centre d'ingénierie des protéines, Université de Liège**
  B. Jooris

UCL Université catholique de Louvain

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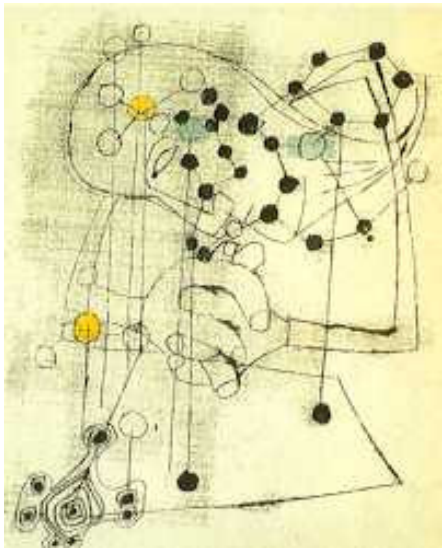

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Beta-lactam activity against intracellular MRSA

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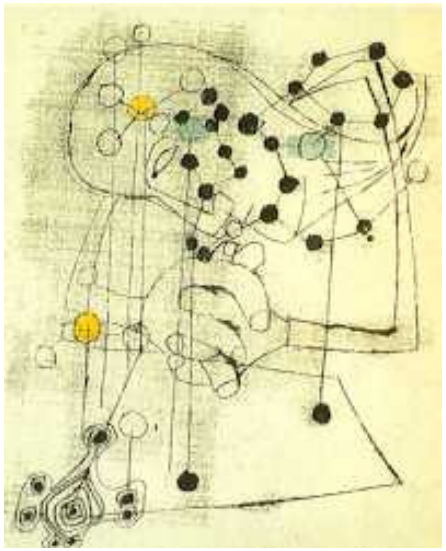
cellular pharmacodynamics

resistance

antibiotic toxicity



novel bacterial targets

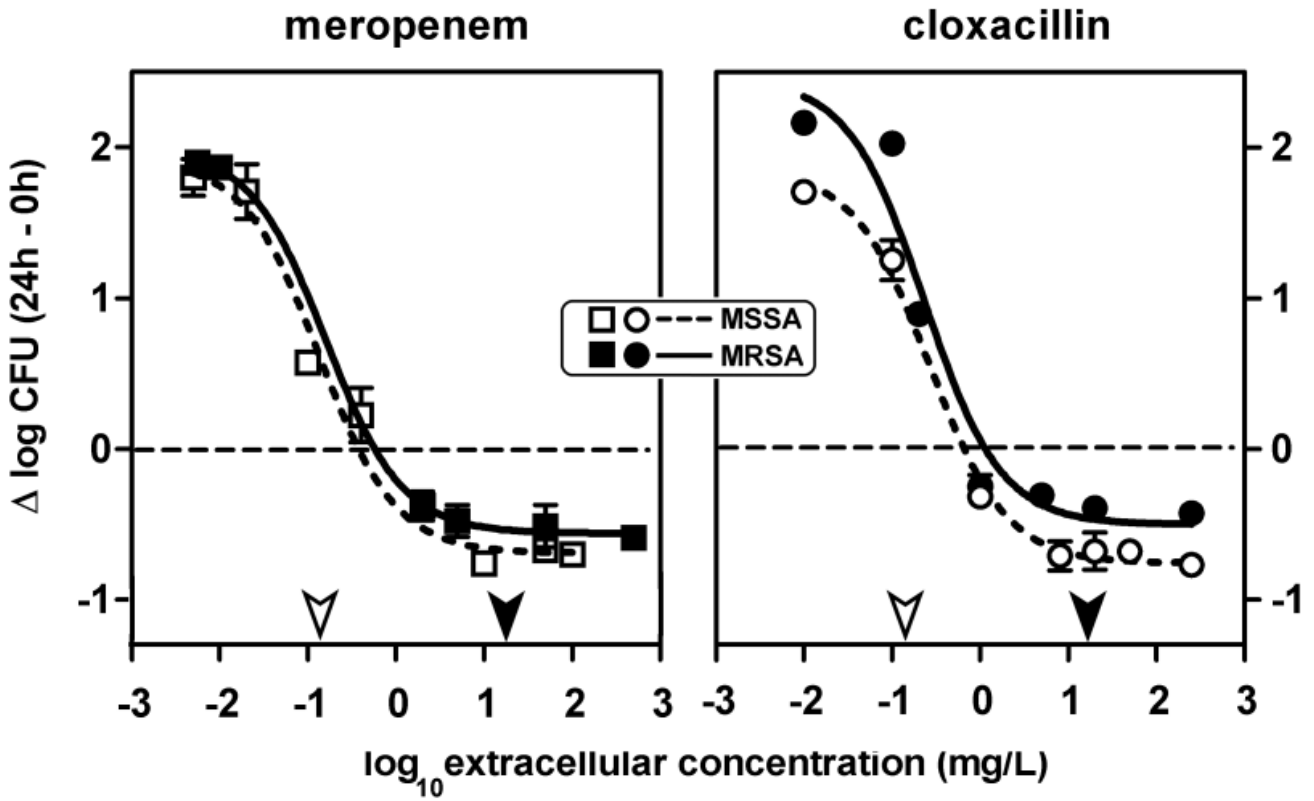
clinical applications

Antibiotics: from molecules to man

Beta-lactam activity against intracellular MRSA

MRSA *vs.* MSSA: intracellular activity of β-lactams

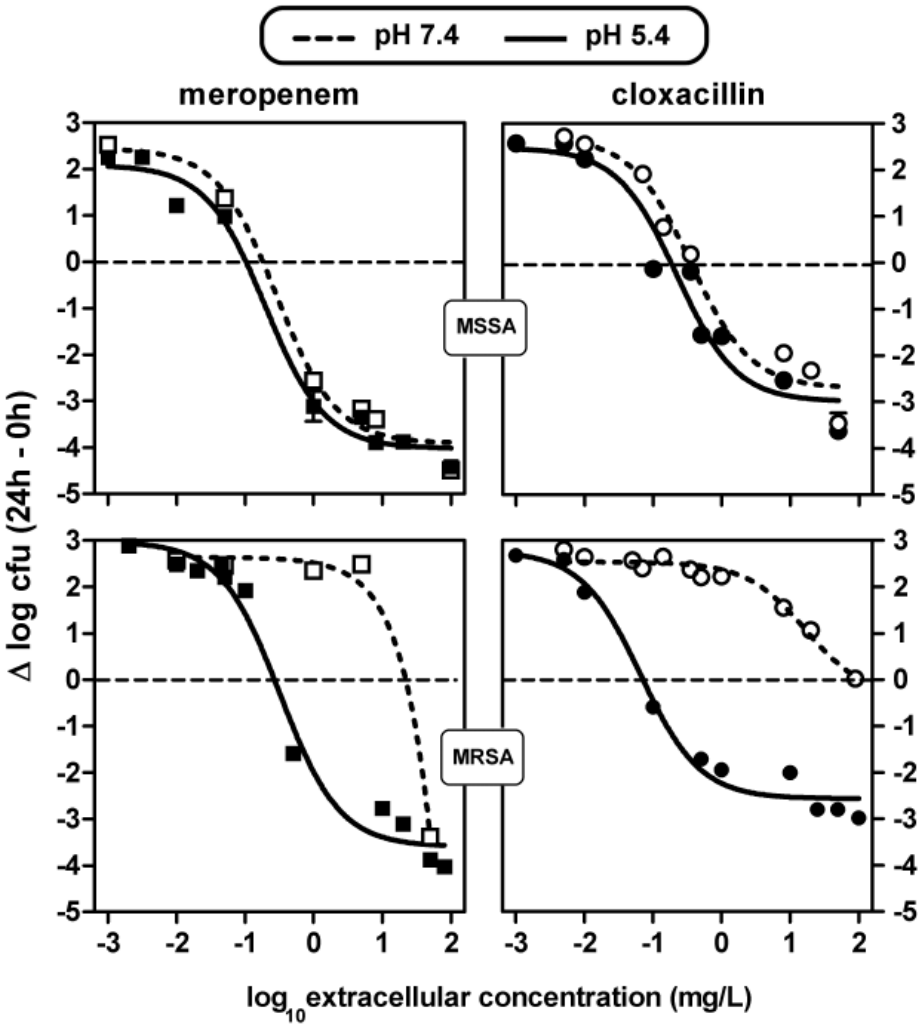
MRSA are as susceptible as MSSA to β-lactams when intracellular !



Lemaire et al., AAC (2007) 51:1627-32

MRSA *vs.* MSSA: extracellular activity of β-lactams

MRSA are as susceptible as MSSA in broth at acidic pH

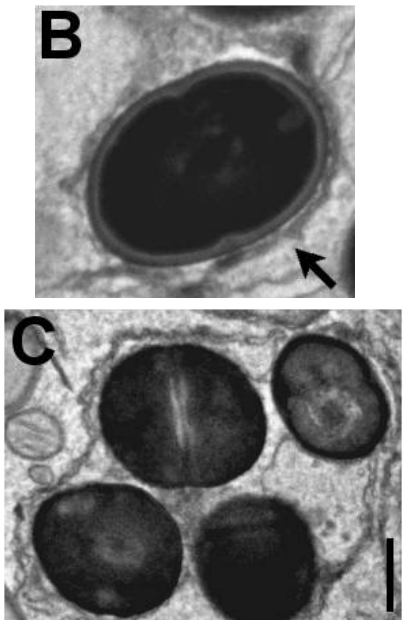


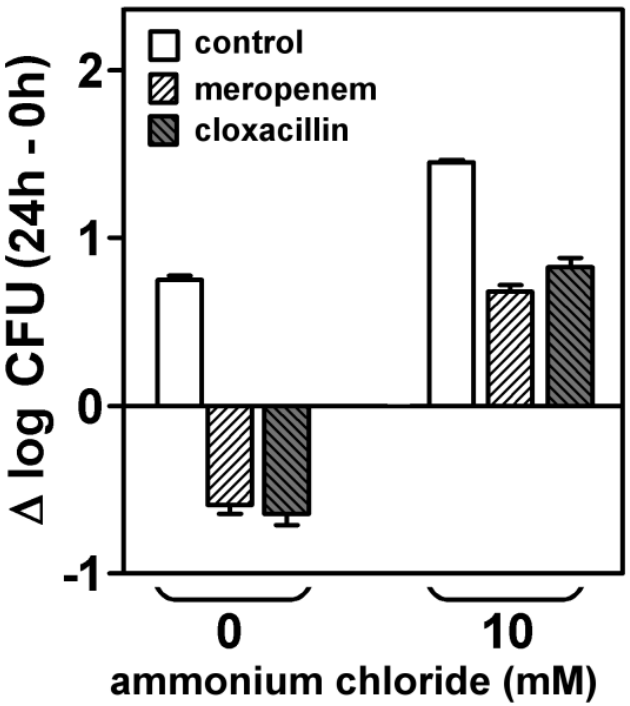
Lemaire et al., AAC (2007) 51:1627-32

MRSA *vs.* MSSA: extracellular activity of β-lactams

Neutralization of lysosomes makes intracellular MRSA resistant to β-lactams !

MRSA are inside [acidic] vacuoles





Lemaire et al., AAC (2007) 51:1627-32

PBP2a conformation is modified by acidic pH

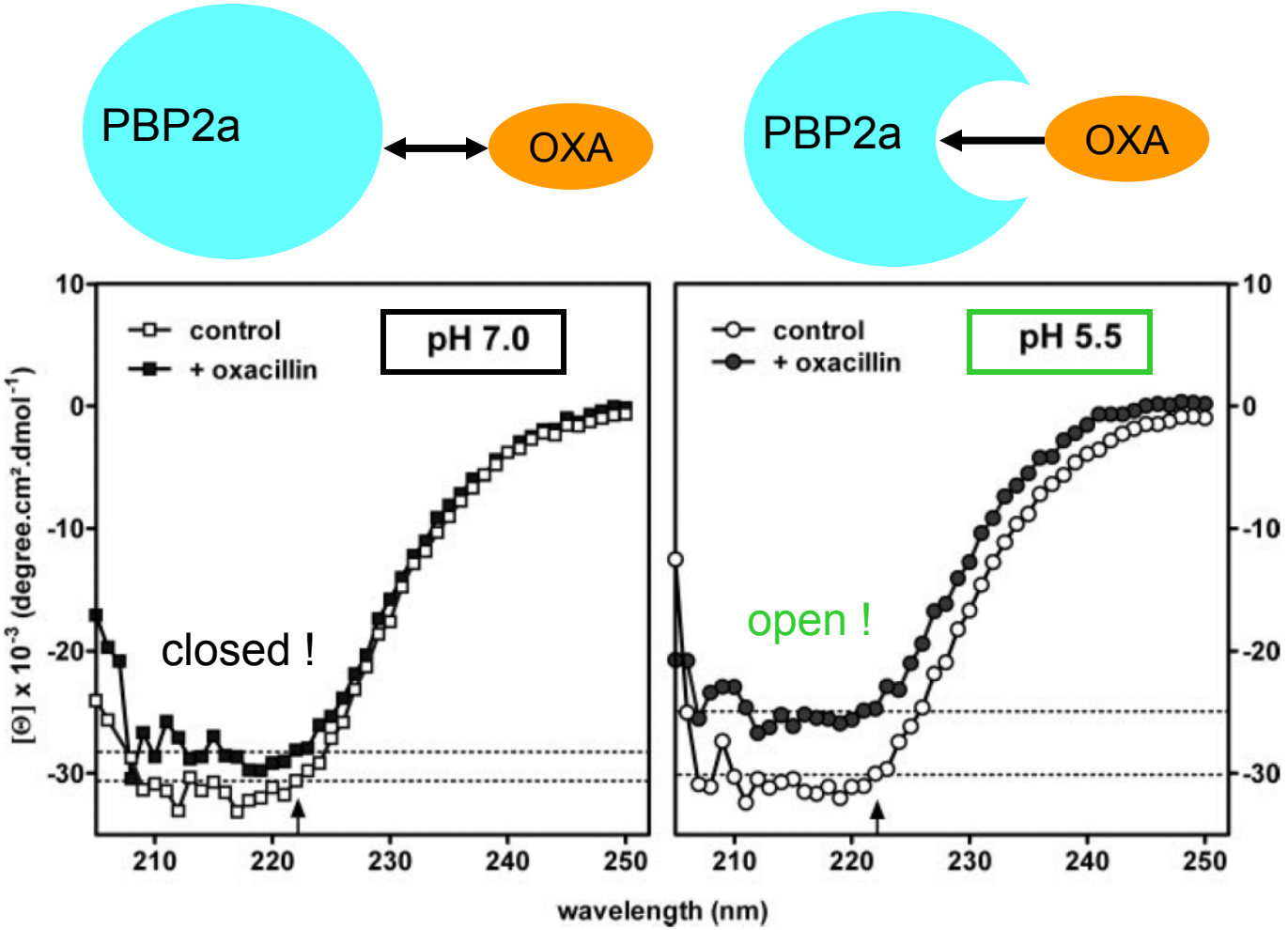
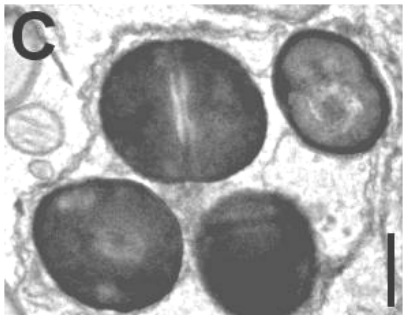
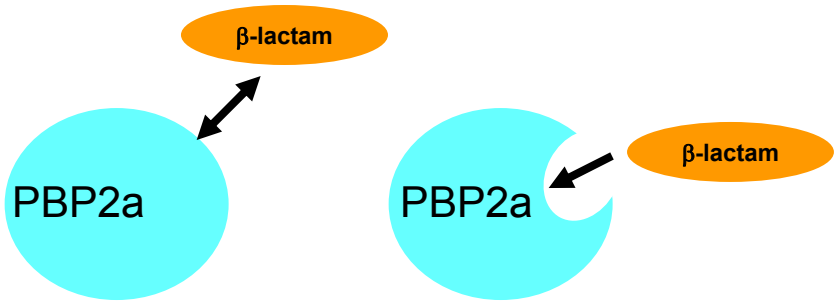


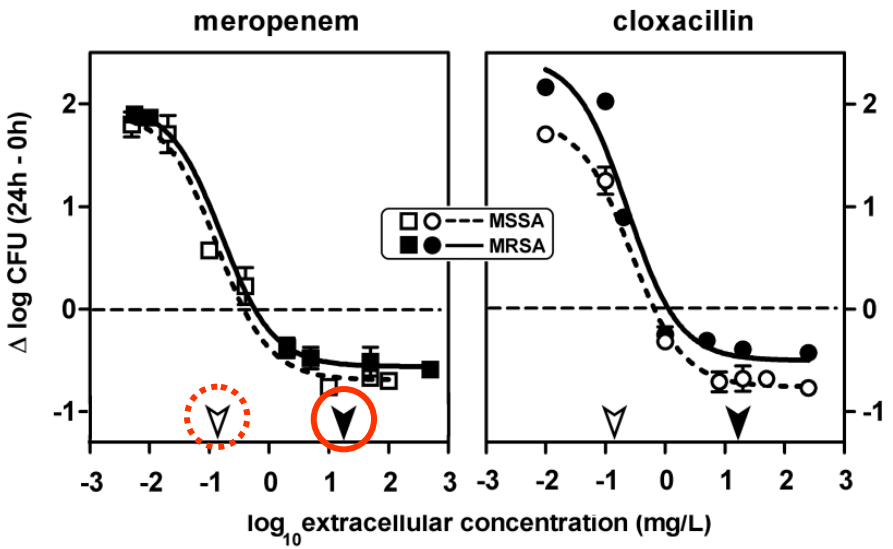
FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at 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) 283:12769-76

Impact of intraphagosomal acid pH on the expression of methicillin-resistance in *S. aureus*







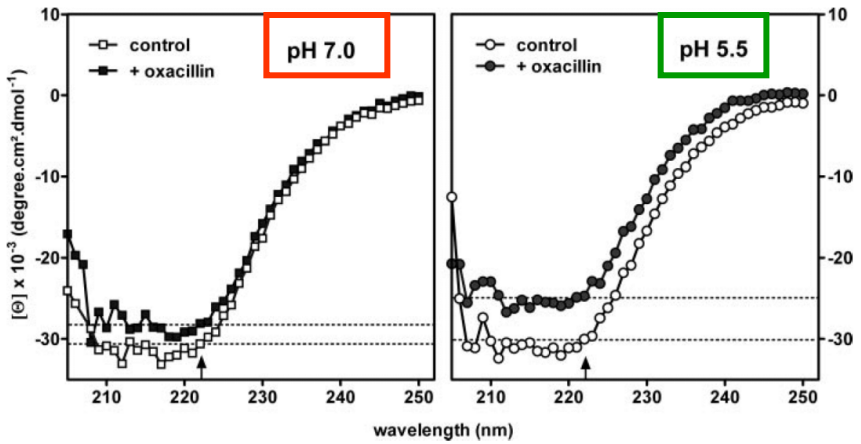


FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at 222 nm (*vertical arrow* on the *abscissa*) for each condition. The spectrum of oxacillin has been subtracted from all data points.

Lemaire et al., Antimicrob. Agents and Chemother, (2007) 51:1627-32; J. Biol. Chem. (2008) 283:12769-76

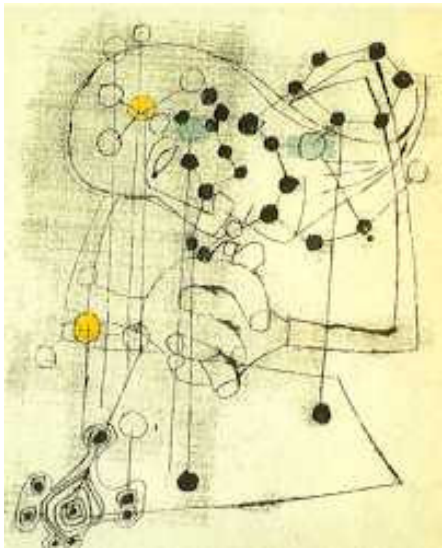
Our main research interests...

cellular pharmacokinetics

cellular pharmacodynamics

antibiotic toxicity

resistance



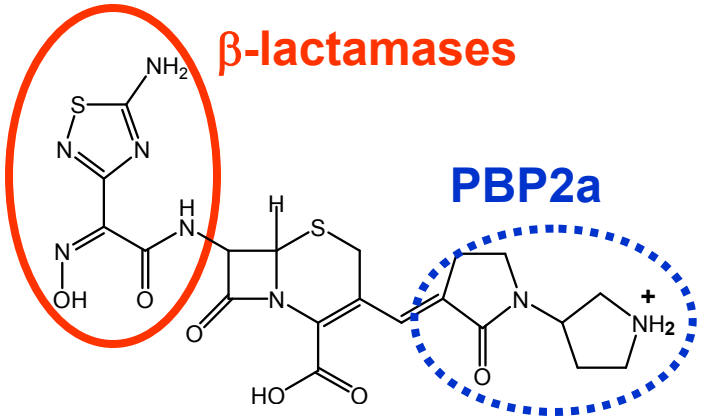
novel bacterial targets

clinical applications

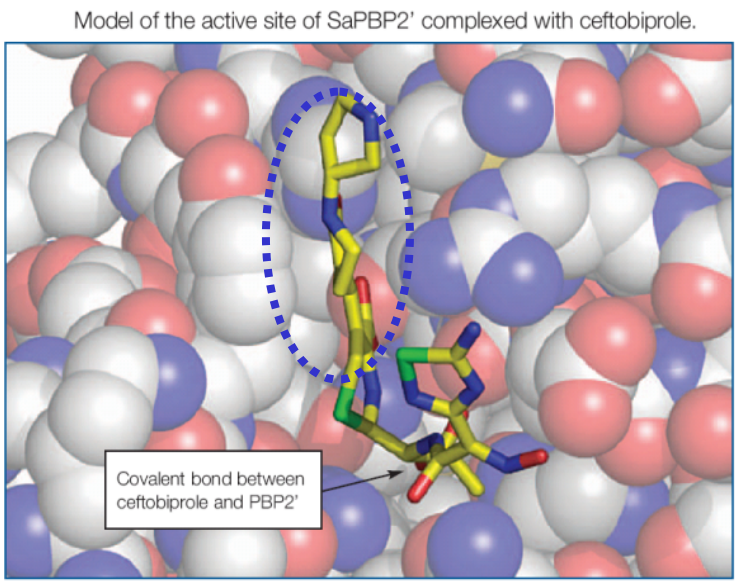
Antibiotics: from molecules to man

ceftobiprole

Compound	Rates of hydrolysis by purified β-lactamases: Class A — *Staphylococcus aureus* PC 1
Ro 63-9141	0.93
Ceftriaxone	19
Cephalothin	200
Penicillin G	10,000



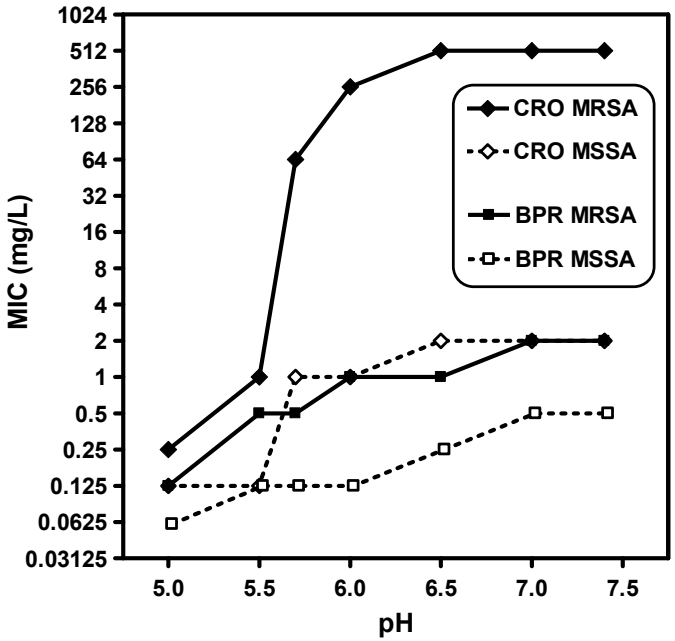
Compound	Affinity for PBPs: IC_{50} for competition with fluorescein-labeled ampicillin (μM) — *Staphylococcus epidermidis* PBP 2′
Ro 63-9141	0.87
Ceftriaxone	115
Imipenem	>500
Methicillin	>500



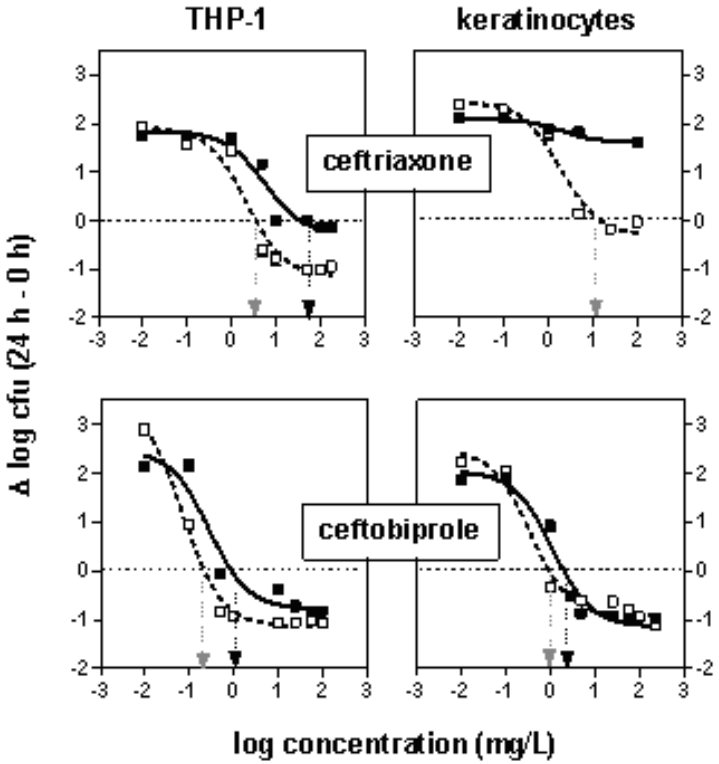
open conformation

Lovering et al., ECCMID (2006) P1586
Hebeisen et al., AAC (2001) 45:825-31

ceftobiprole



Ceftobiprole MIC is not markedly influenced by pH



Ceftobiprole is as active against intracellular MSSA and MRSA

Lemaire et al. AAC (2009) 53:2289-2297

Conclusion of the MRSA story and links with the clinics ….

- Intracellular medium can modulate the expression of resistance mechanisms
- conformation of PBP2a is critical for activity
- design of new beta-lactams able to « open » PBP2a
- ceftobiprole rejected but ceftaroline accepted by the FDA in 2010

Nephrotoxicity of aminoglycosides: from molecular mechanisms to clinical implications

- **Pharmacologie cellulaire et moléculaire, UCL, Bruxelles**
  H. Servais, S. Denamur
  supervision: M.P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke
- **Unité de biologie cellulaire, UCL**
  D. Tyteca, P. Vandersmissen
  supervision: P. Courtoy

UCL Université catholique de Louvain

www.facm.ucl.ac.be

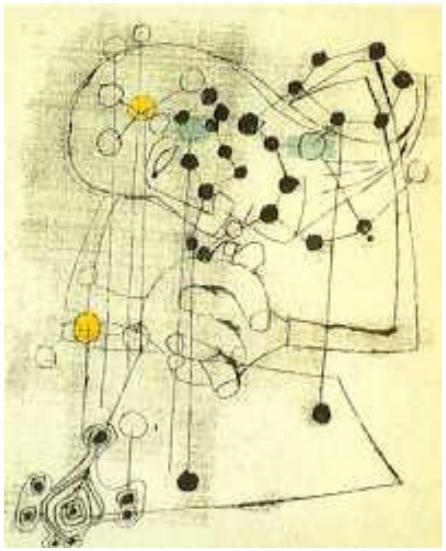
Our main research interests...

cellular pharmacokinetics

cellular pharmacodynamics

antibiotic toxicity

resistance



novel bacterial targets

clinical applications

Antibiotics: from molecules to man

Nephrotoxicity of aminoglycosides

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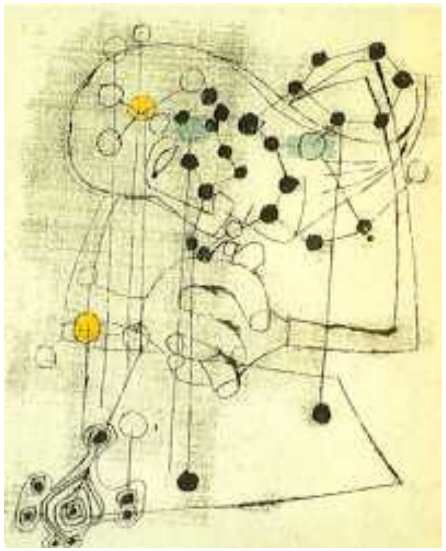
resistance



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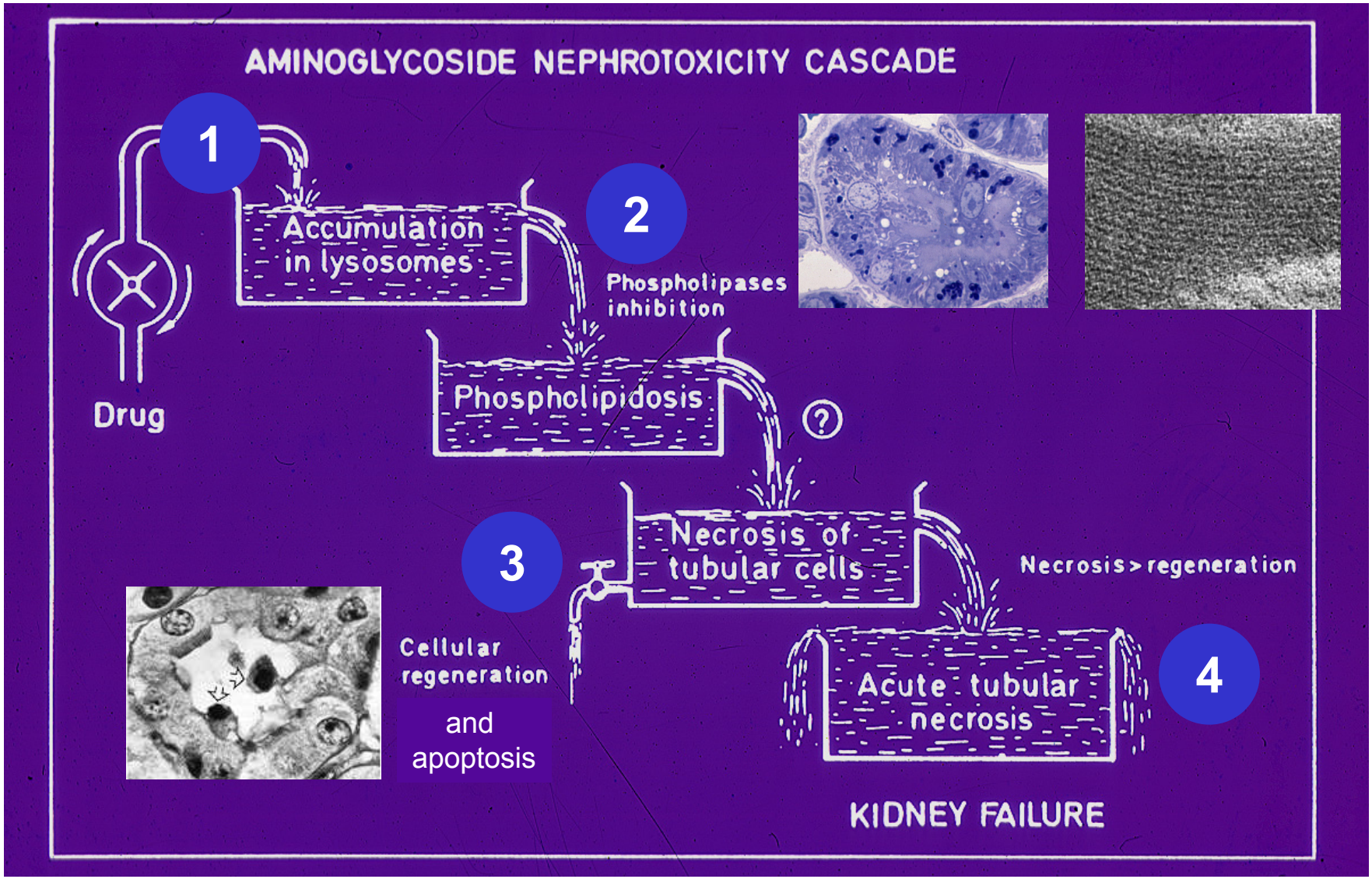
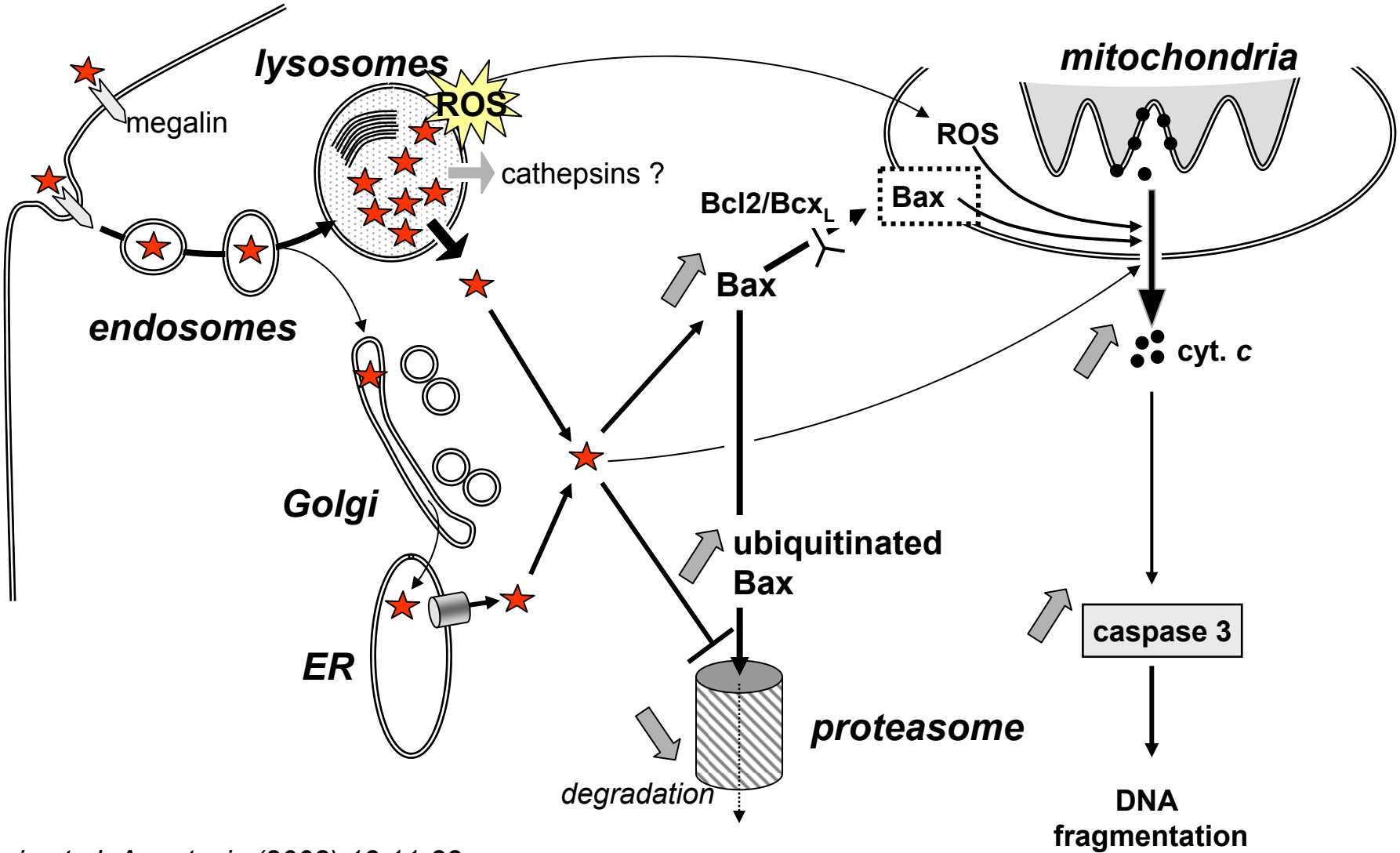
nephrotoxicity cascade



Tulkens, Am. J. Med. (1986) 80(6B): 105-114

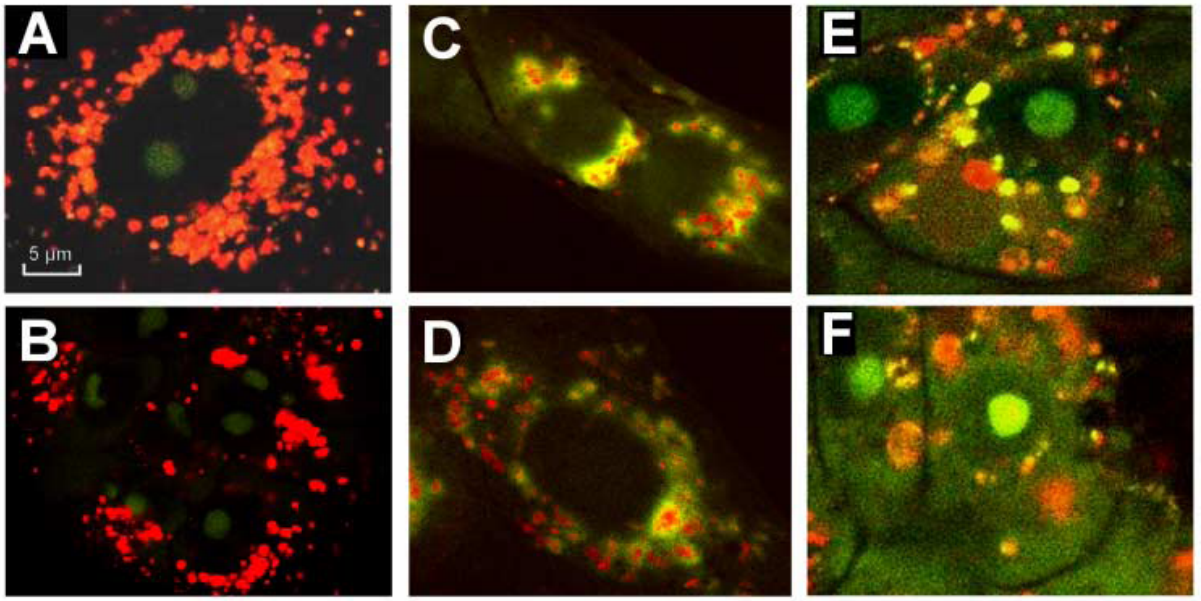
Aminglycoside-induced apoptosis



Servais et al. Apoptosis (2008) 13:11-32.

Molecular mechanisms of apoptosis

Gentamicin allows for a partial relocalisation
of acridine orange in the cytosol

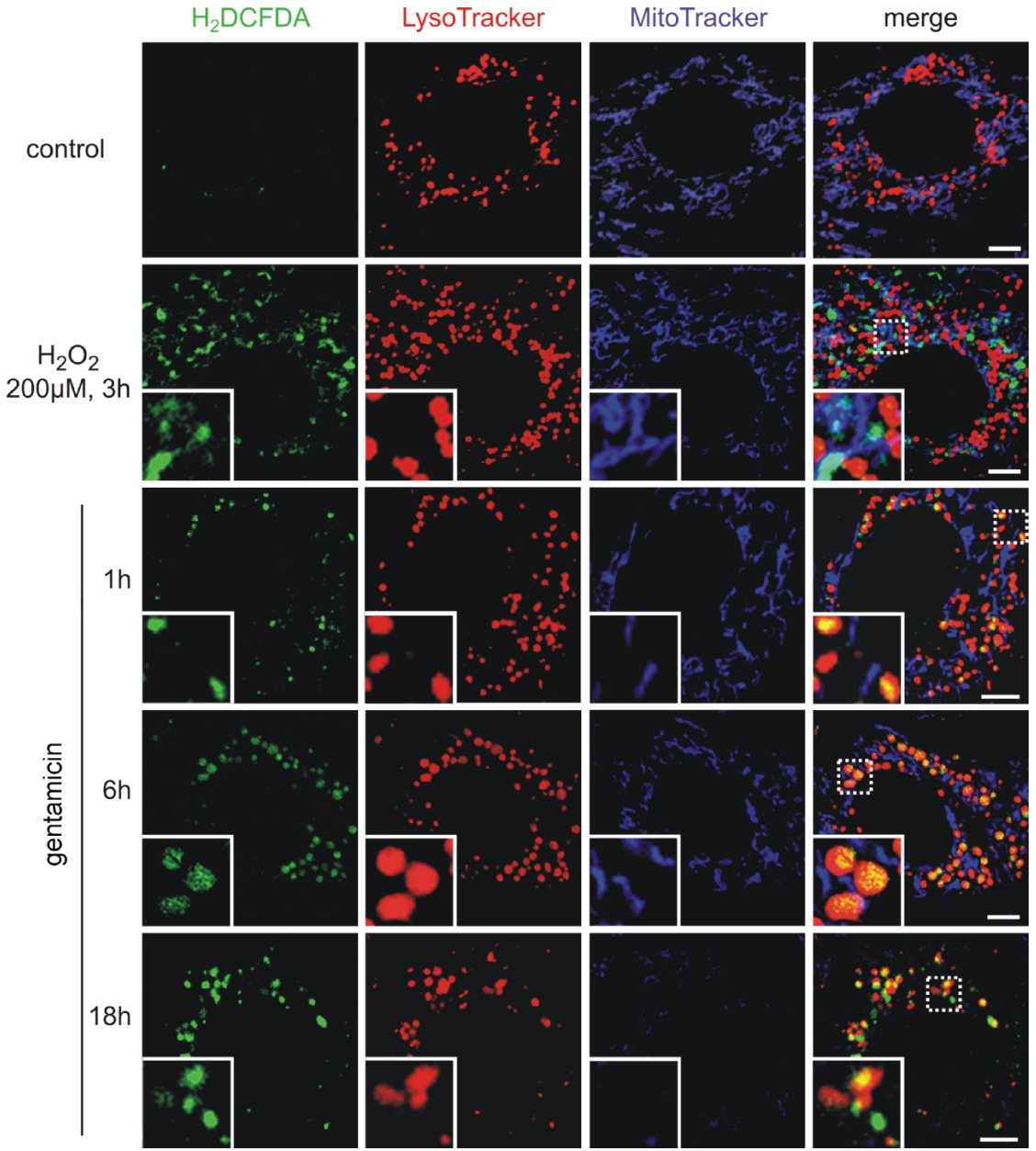


control | gentamicin | MSDH (positive control)

Servais et al. TAP (2005) 15:321-333 .

Molecular mechanisms of apoptosis

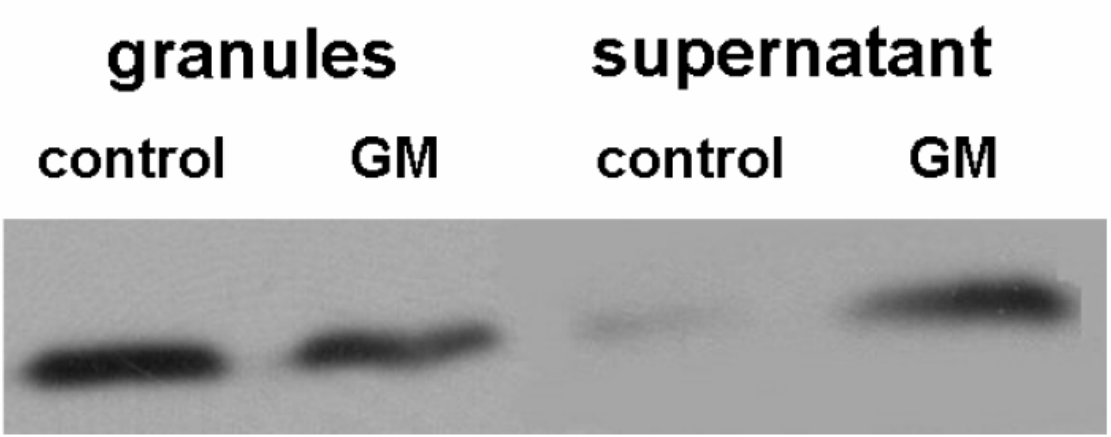
Gentamicin induces
ROS production
In lysosomes



Denamur et al, FRMB, under revision

Molecular mechanisms of apoptosis

Gentamicin allows for a partial relocalisation
of cytochrome C from mitochondria to the cytolsol



Servais et al. TAP (2005) 15:321-333 .

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cellular pharmacokinetics

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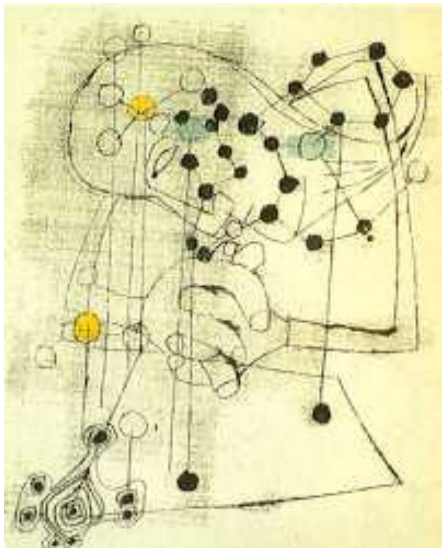
resistance



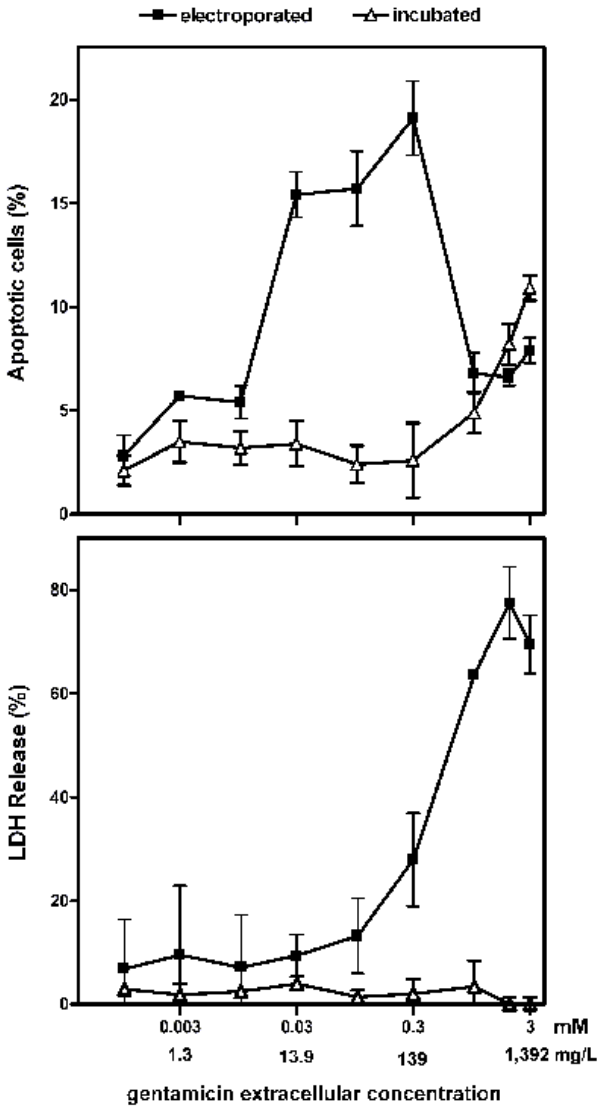
novel bacterial targets

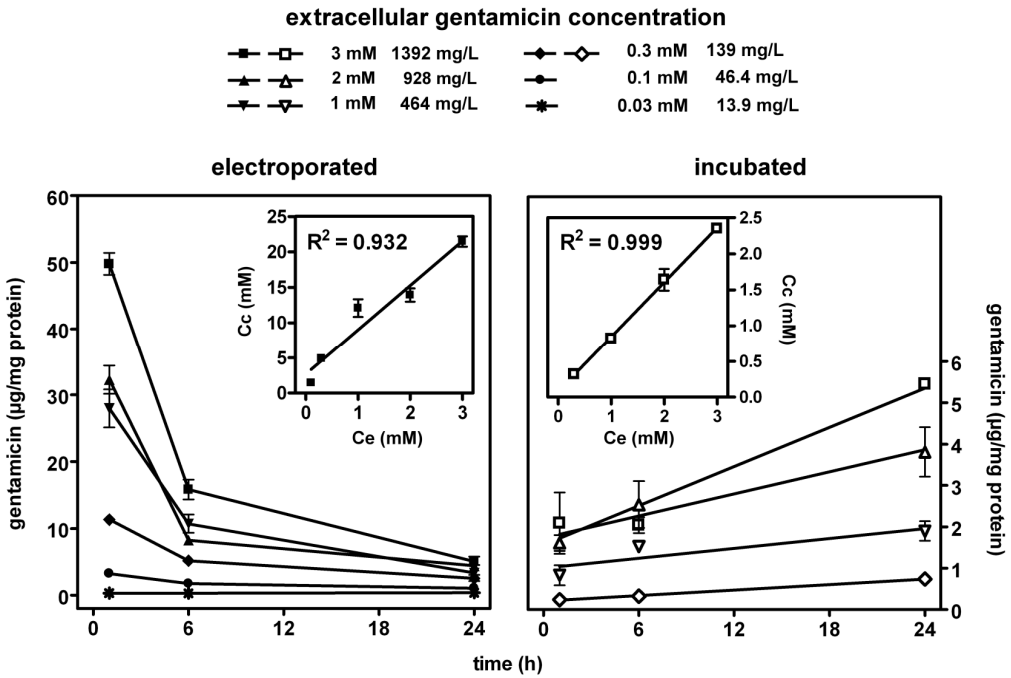
clinical applications

Antibiotics: from molecules to man

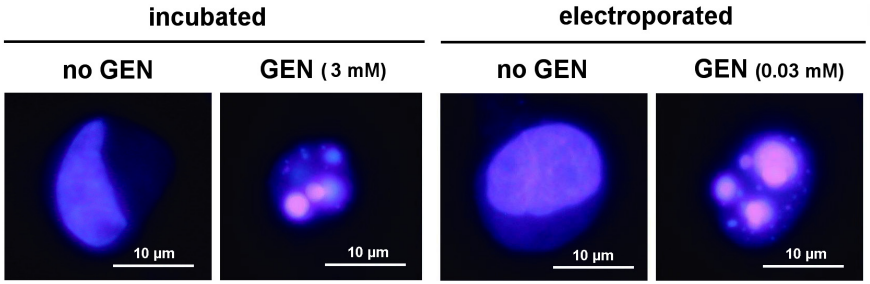
Nephrotoxicity of aminoglycosides

Relationship between accumulation and apoptosis





Introducing gentamicin in the cytosol by electroporation markedly increases toxicity



Servais et al. AAC (2006) 50:1213-1221

Our main research interests...

cellular pharmacokinetics

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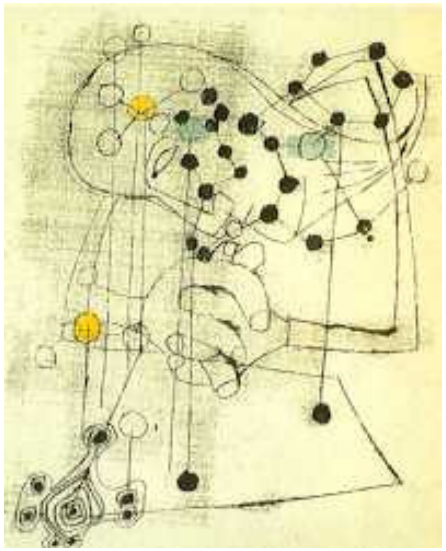
antibiotic toxicity

resistance



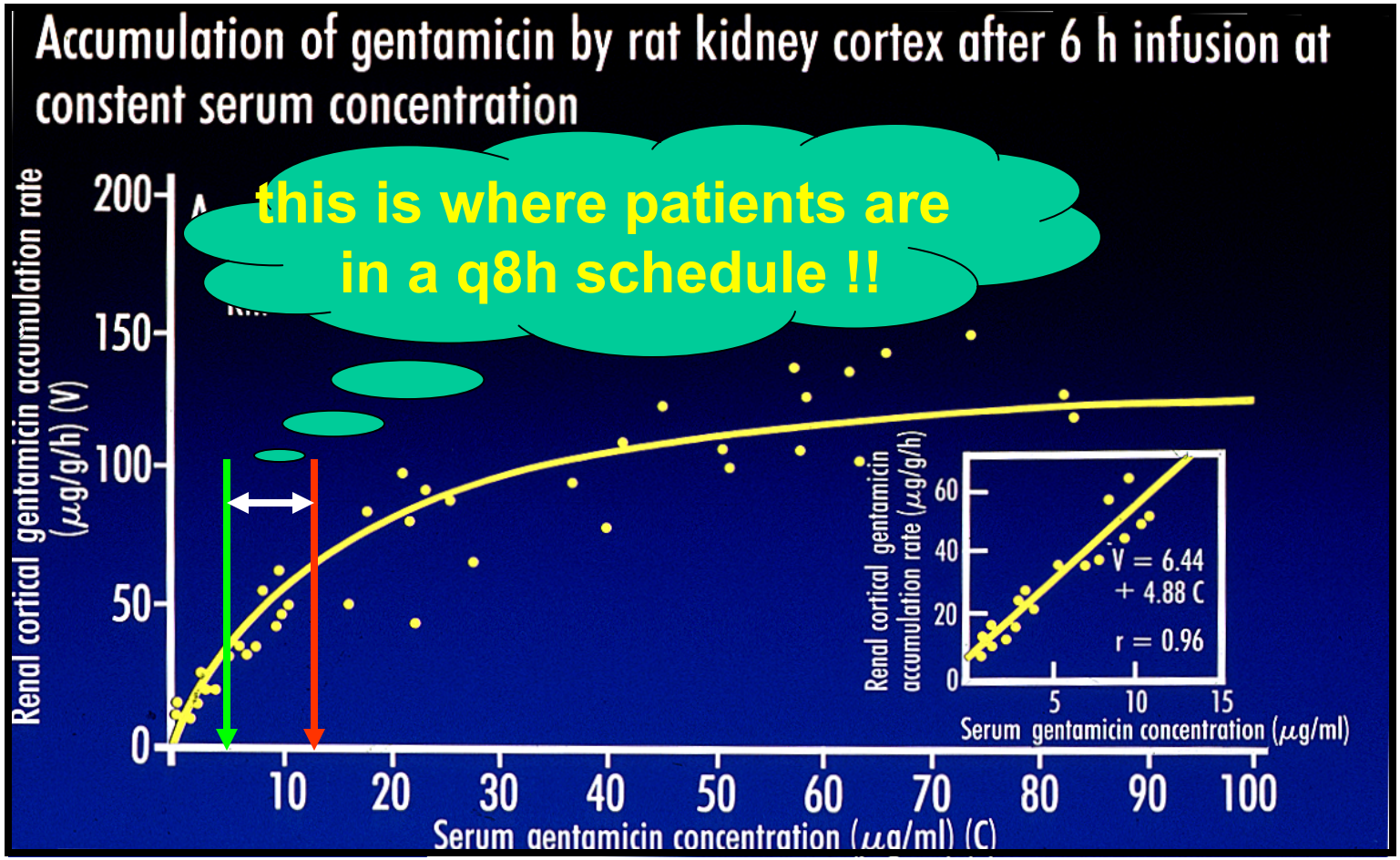
novel bacterial targets

clinical applications

Antibiotics: from molecules to man

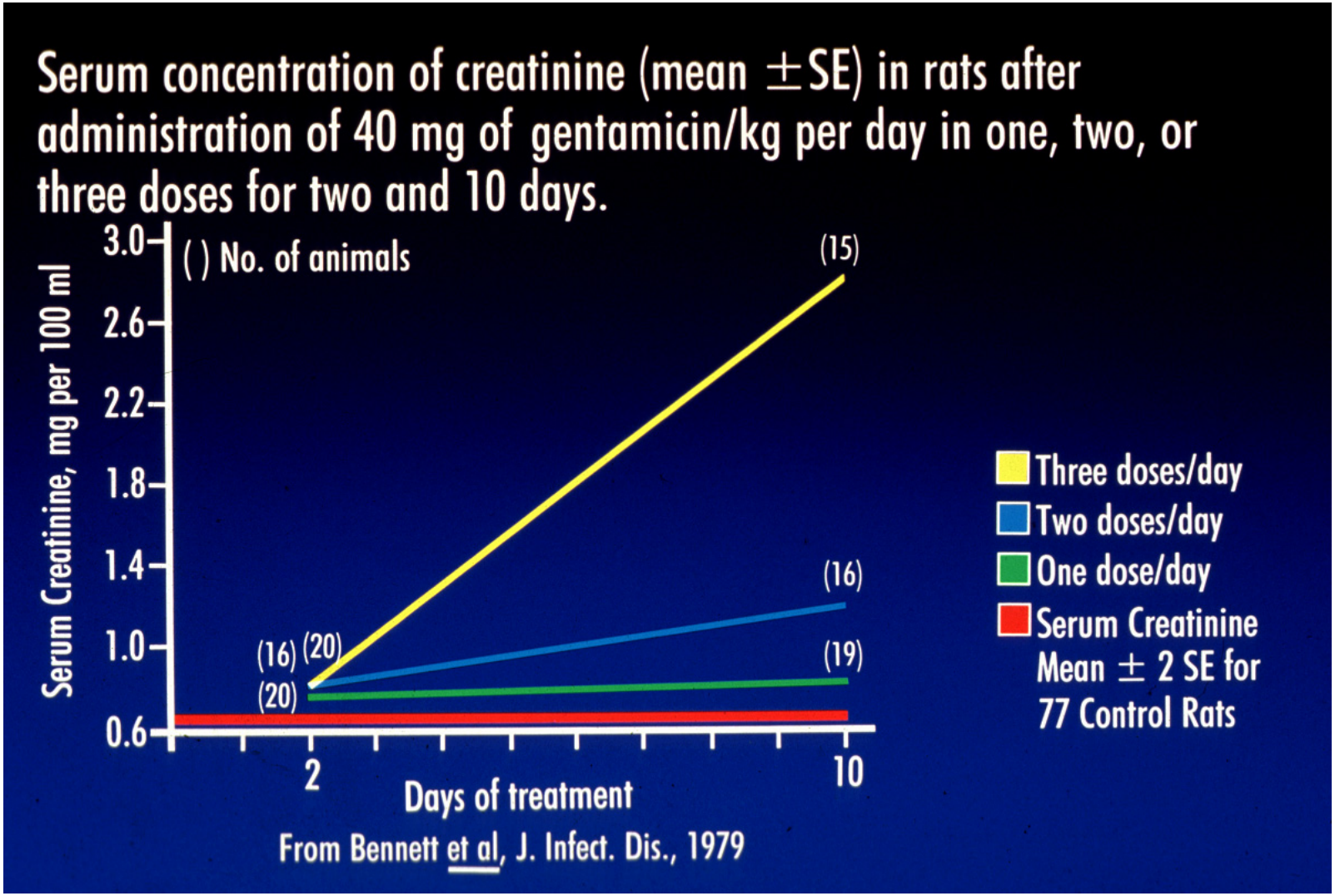
Nephrotoxicity of aminoglycosides

Once a day administration as a way to reduce toxicity



* Giuliano *et al.*, J. Pharm. Exp. Ther., 1986

Once a day administration as a way to reduce toxicity



Conclusion of the aminoglycoside story and links with the clinics ….

- aminoglycoside apoptosis is mediated by lysosomal destabilization
  → Lysosomal phospholipidosis as a protecting factor ?

- electroporation as a way to screen toxic potential of new molecules

- once-a-day administration reduces toxicity and at the same time improves activity ….

Happy researchers in our cellular and molecular pharmacology group …

Happy researchers in our cellular and molecular pharmacology group …

Hope it may help you to be as successful as Hoang Anh was in Europe …