How do antibiotics play with multidrug transporters in eucaryotic cells ?

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ATP-Binding Cassette transporters

ABCs transporters

ABCA **ABCB** **ABCC** ABCD ABCE ABCF **ABCG**

P-gp **MRPs** **BCRP**

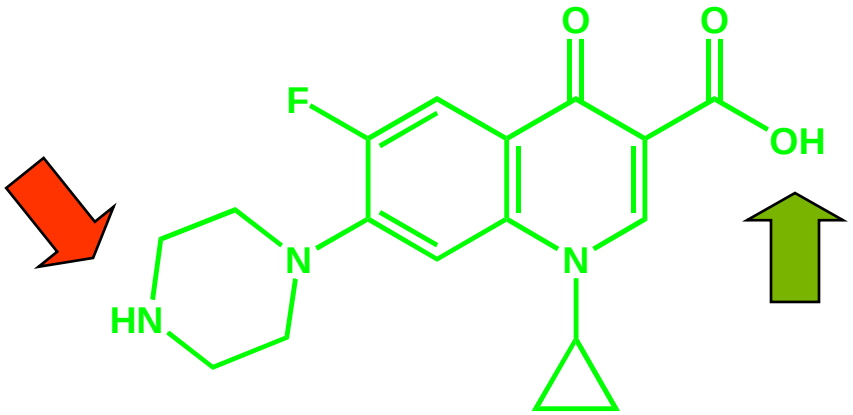
multidrug transporters

Die Bremer Stadtmusikanten

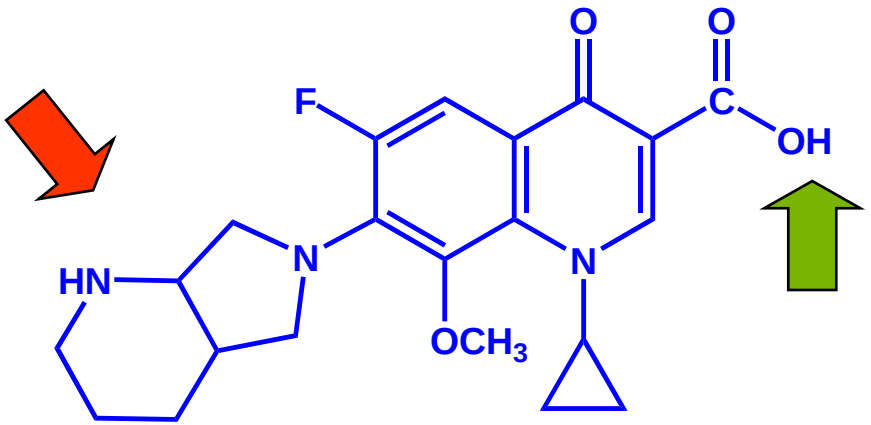
Are antibiotics substrates for MDR transporters in eucaryotic cells ?

Fluoroquinolone antibiotics

ciprofloxacin

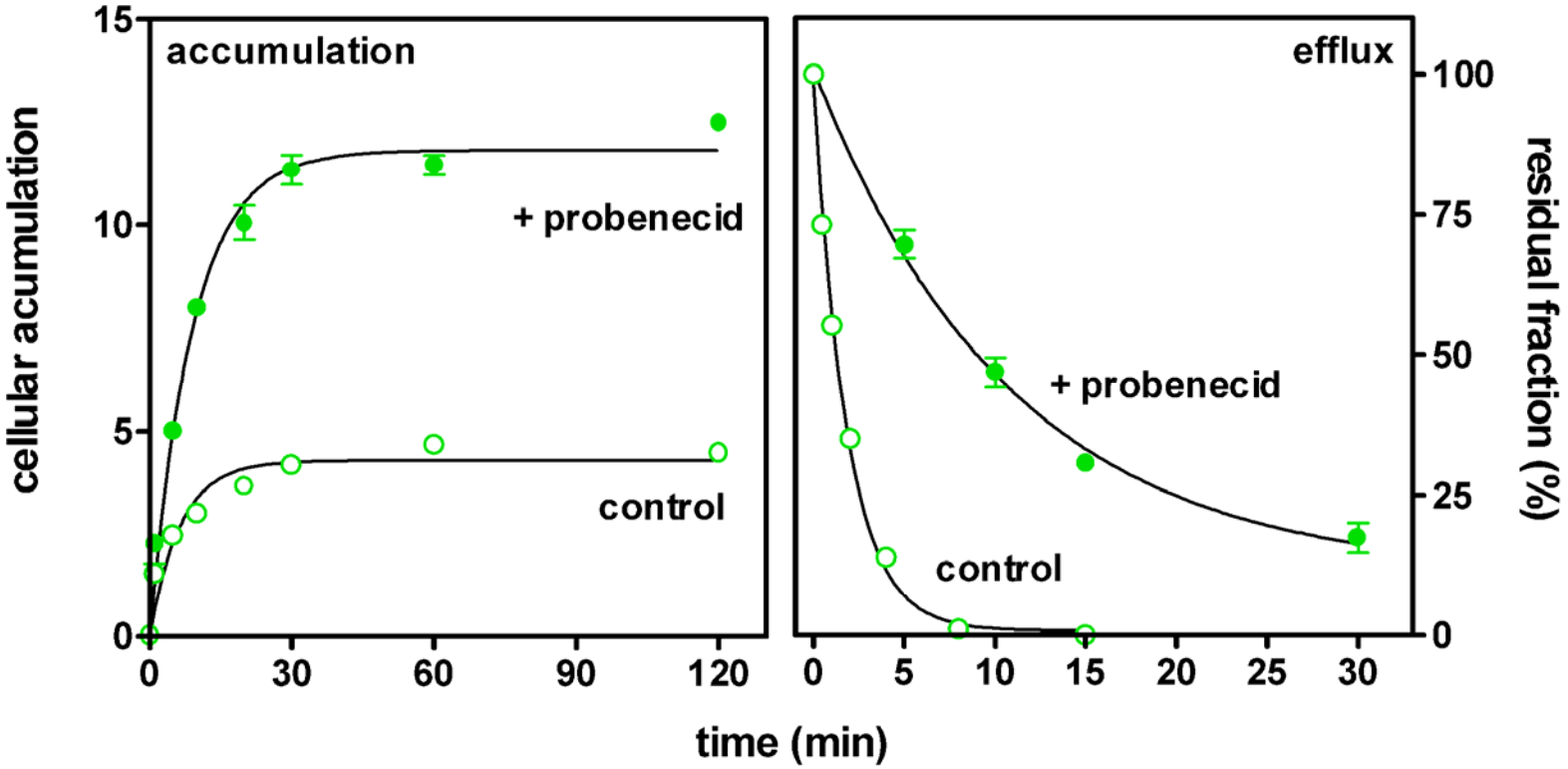


moxifloxacin



Kinetics of accumulation and efflux for ciprofloxacin

both accumulation and efflux markedly affected by probenecid (inhibitors of Mrps)

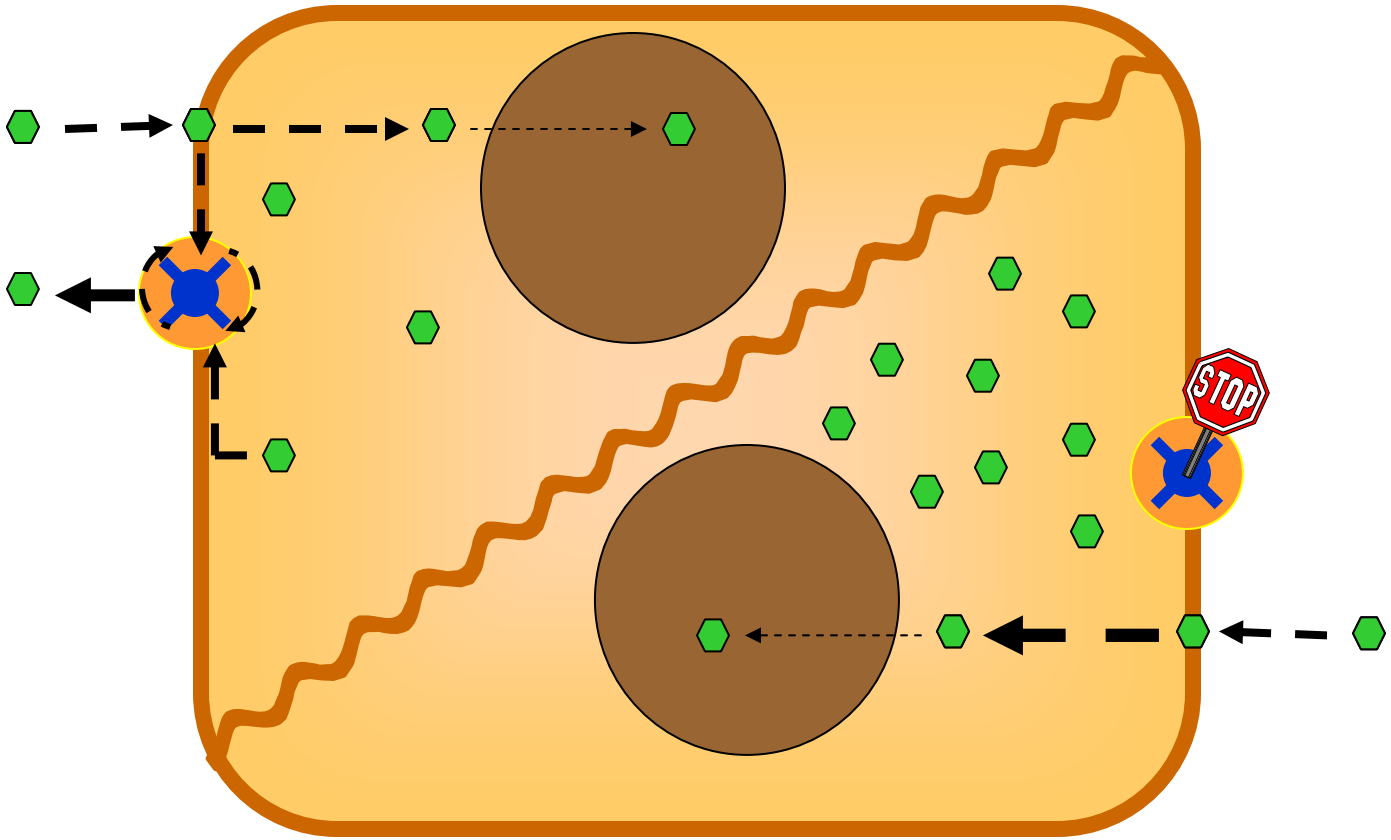


extracell. conc. 17 mg/L; probenecid 5 mM

Michot et al. (2004) AAC 48:2673-82

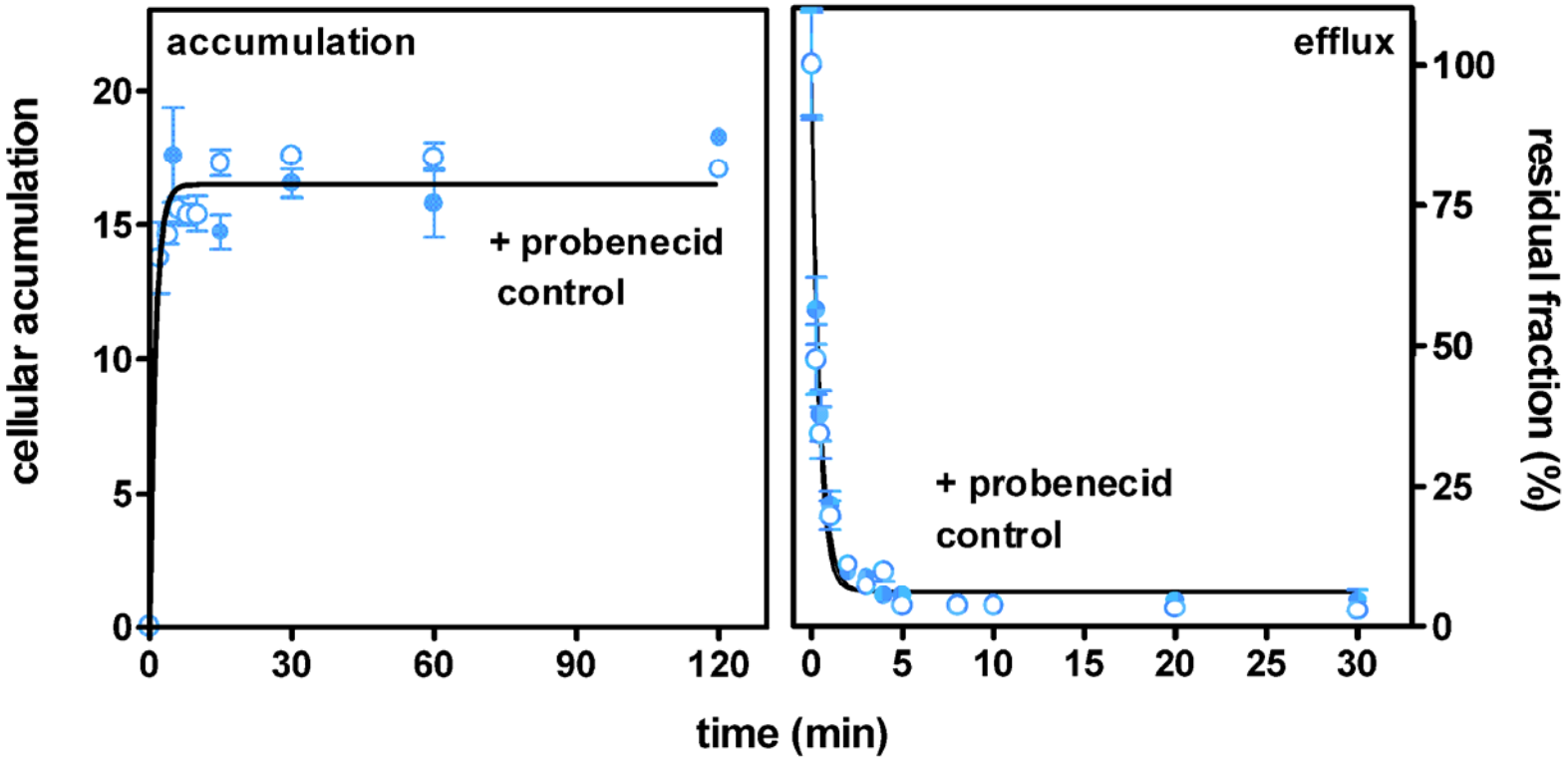
Ciprofloxacin, classical model

Kolaczkowski & Goffeau (1997) Pharmacol. Ther. 76:219-42



Kinetics of accumulation and efflux for moxifloxacin

neither accumulation nor efflux affected by probenecid

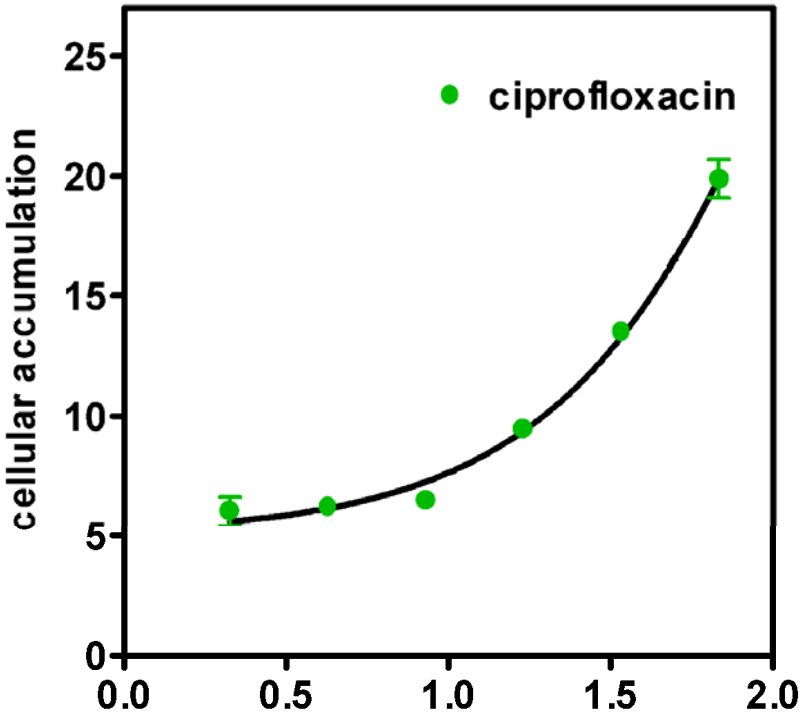


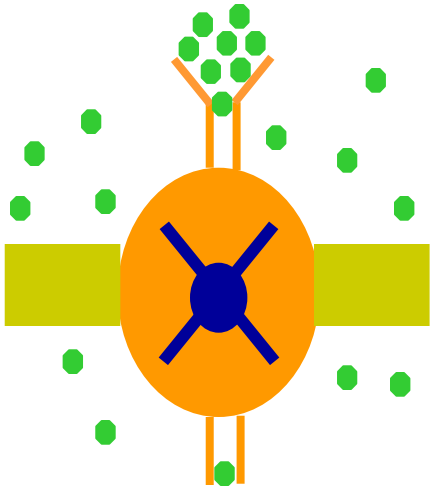
extracell. conc. 17 mg/L; probenecid 5 mM

Michot et al. AAC (2005) 49:2429-37

Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin

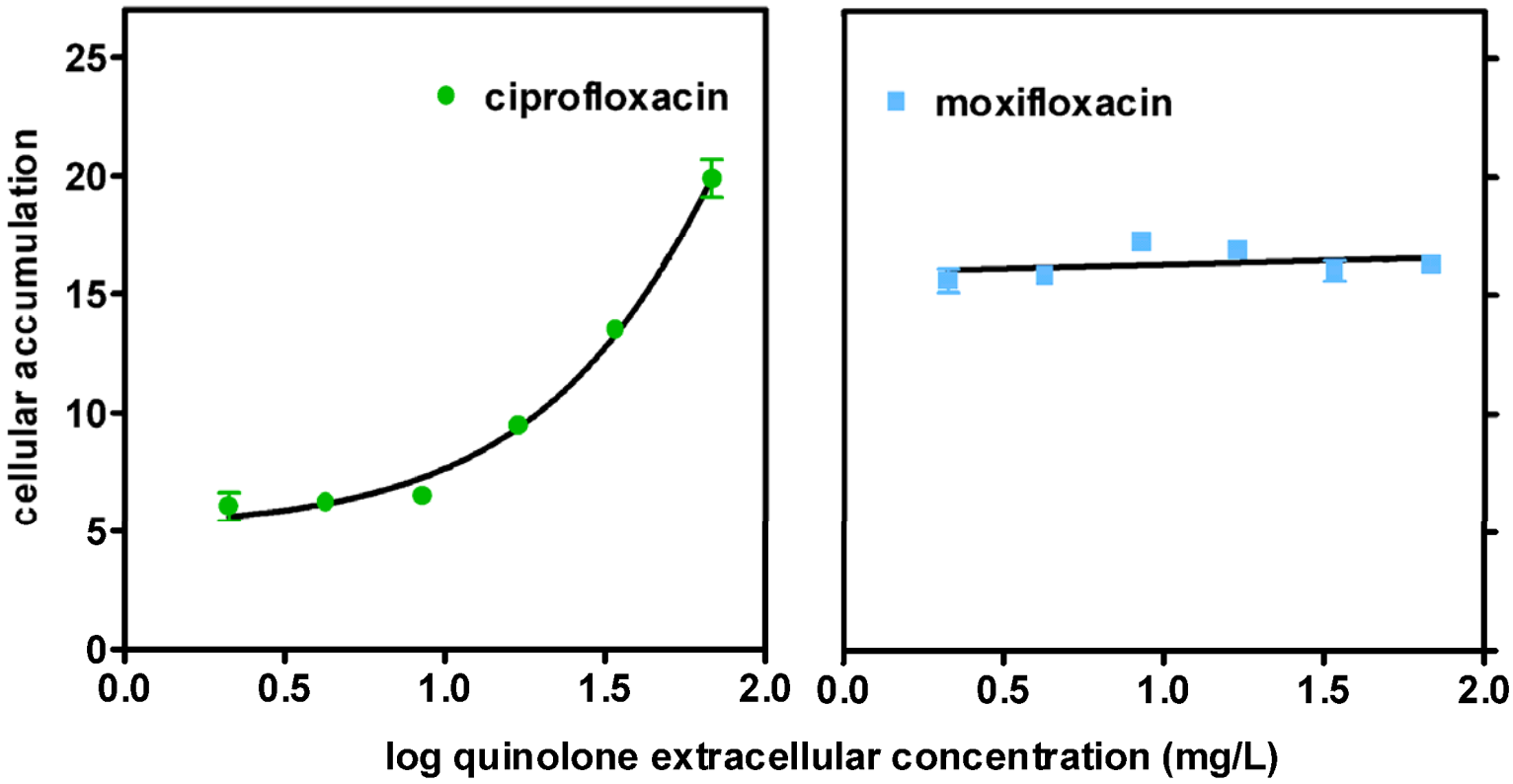




Michot et al. AAC (2005) 49:2429-37

Quinolones as inhibitors of ciprofloxacin efflux

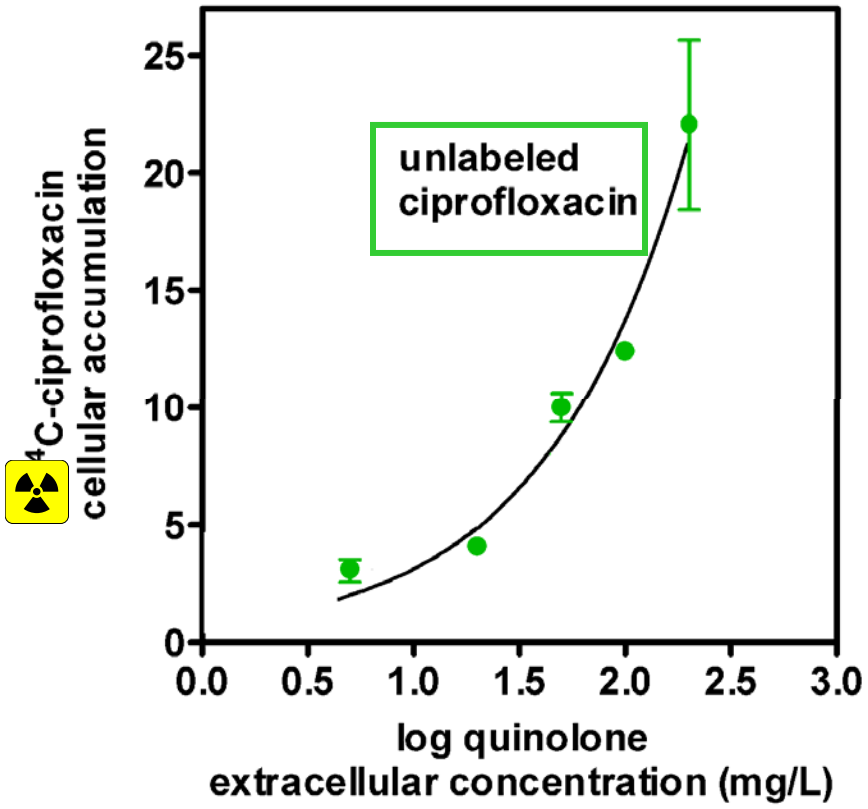
- ciprofloxacin efflux inhibited by ciprofloxacin
- moxifloxacin not affected

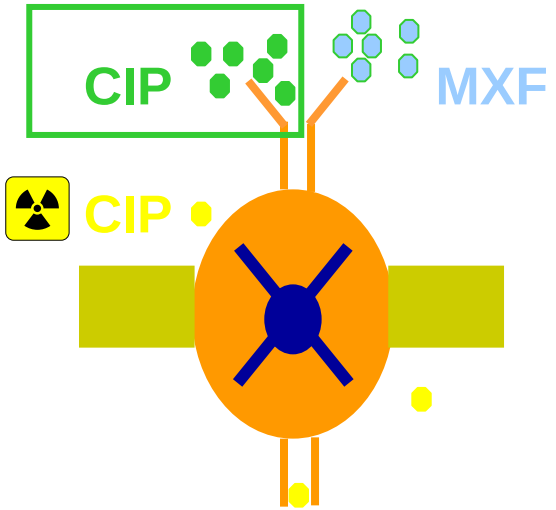


Michot et al. AAC (2005) 49:2429-37

Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin
  moxifloxacin

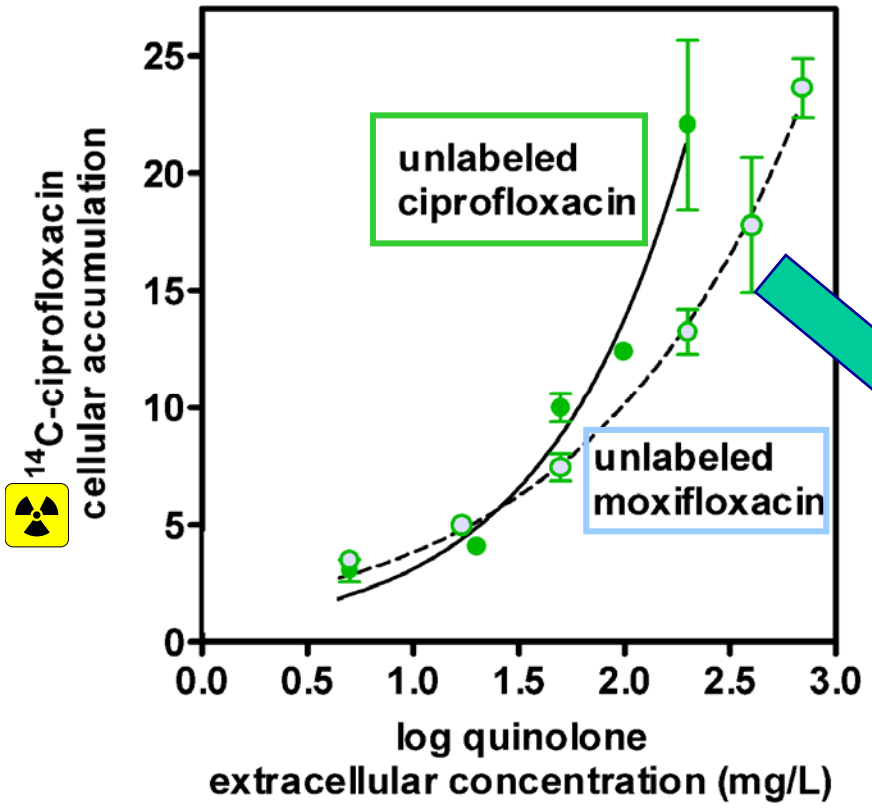


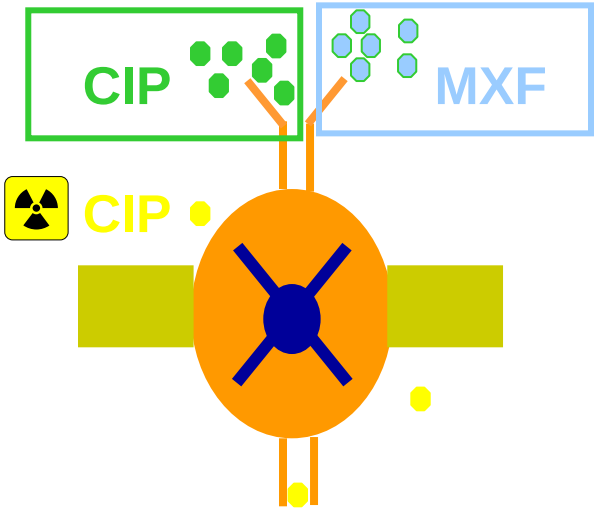


Michot et al. AAC (2005) 49:2429-37

Quinolones as inhibitors of ciprofloxacin efflux

- **ciprofloxacin efflux inhibited by ciprofloxacin**
  moxifloxacin



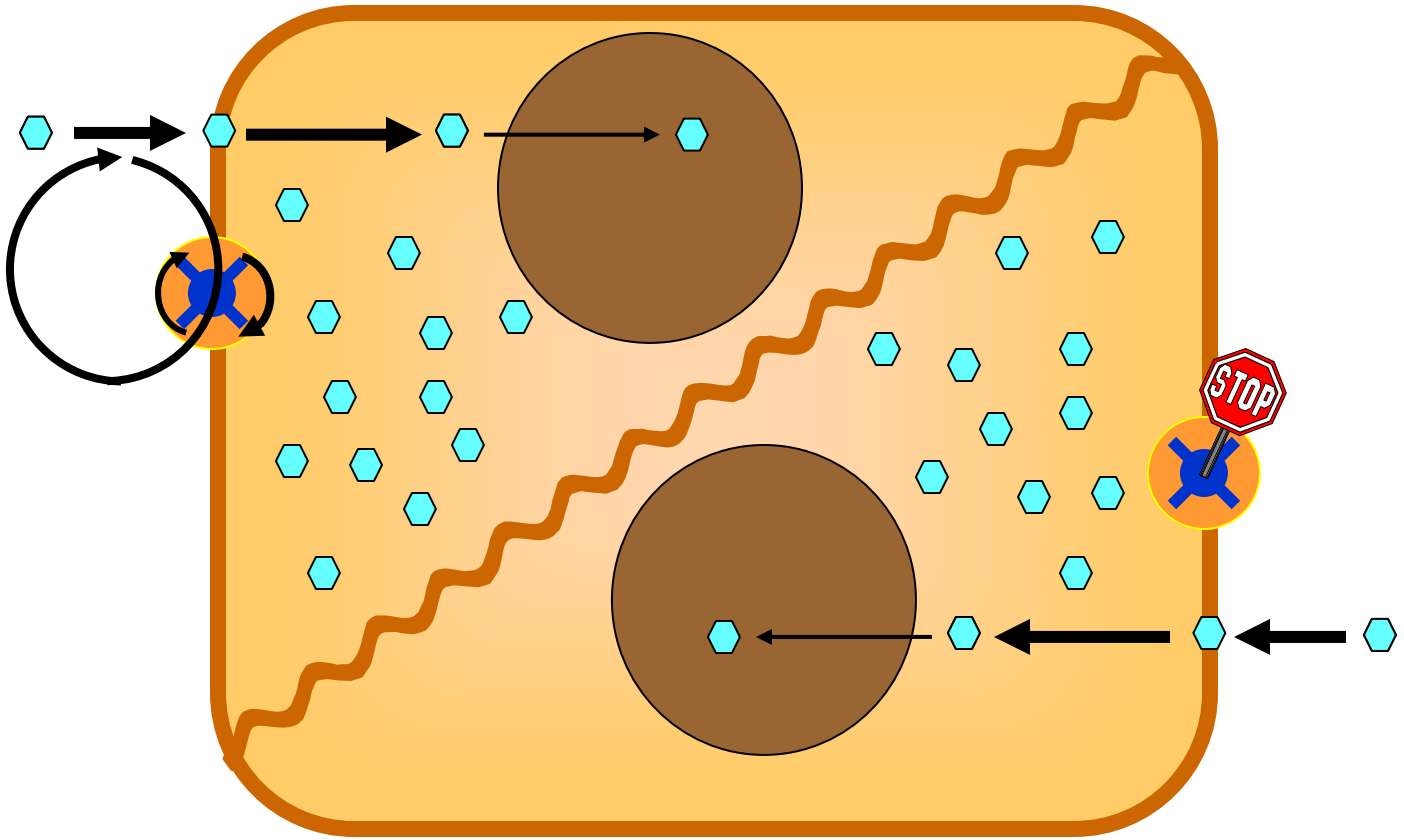


moxifloxacin also able to interact with the transporter !

Michot et al. AAC (2005) 49:2429-37

Moxifloxacin, ‘futile-cycle’ model

Eytan et al. (1996) JBC 271:12897-902



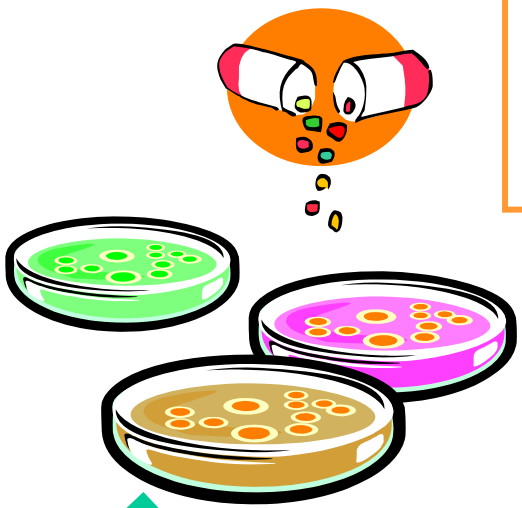
Die Bremer Stadtmusikanten

Can we make eucaryotic cells « resistant » to antibiotics ?

A way to further characterize efflux transporters

Over-expression of efflux pumps as mechanism of resistance

How to get resistant cells ?



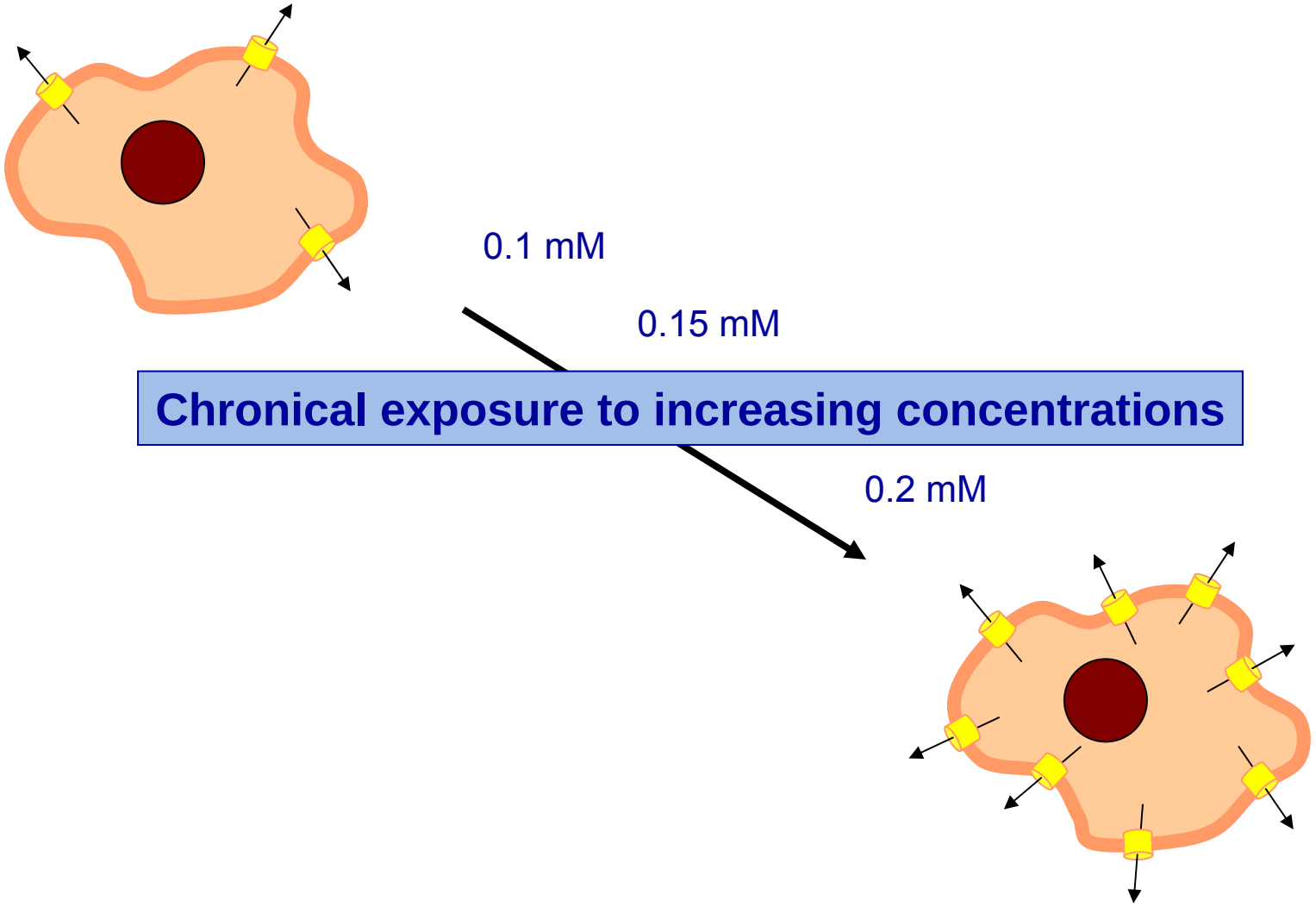
- step-wise increase in AB concentration
- several passages at each step

time and drug consumming!

multifactorial multidrug resistance

Gottesman et al, Methods Enzymol. (1998) 292: 248-58

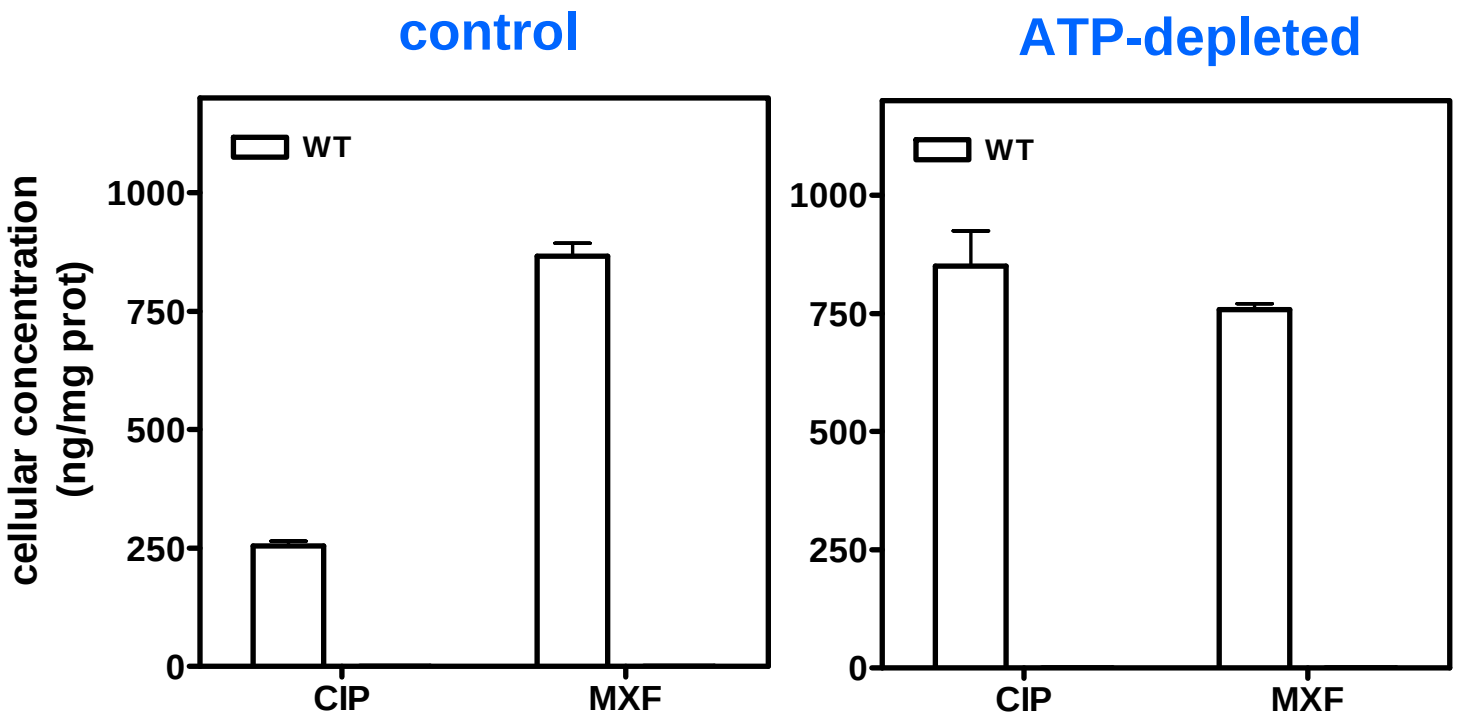
Identification of the ciprofloxacin transporter: « resistant » cells as a tool



Michot et al., Antimicrob. Ag. Chemother. (2006) 50:1689-1695

Ciprofloxacin « resistant » cells: phenotypic analysis

ATP-dependent reduction in cell accumulation of CIP; MXF non affected



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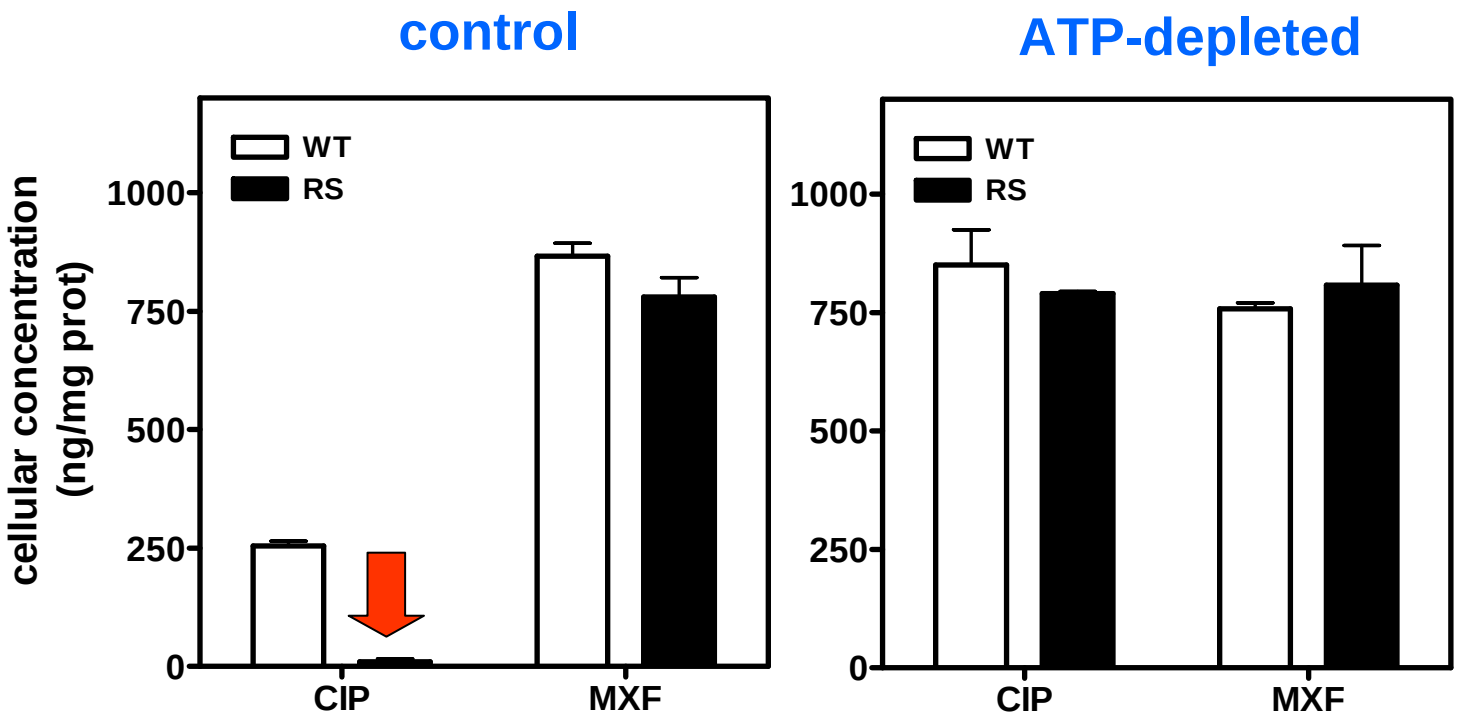
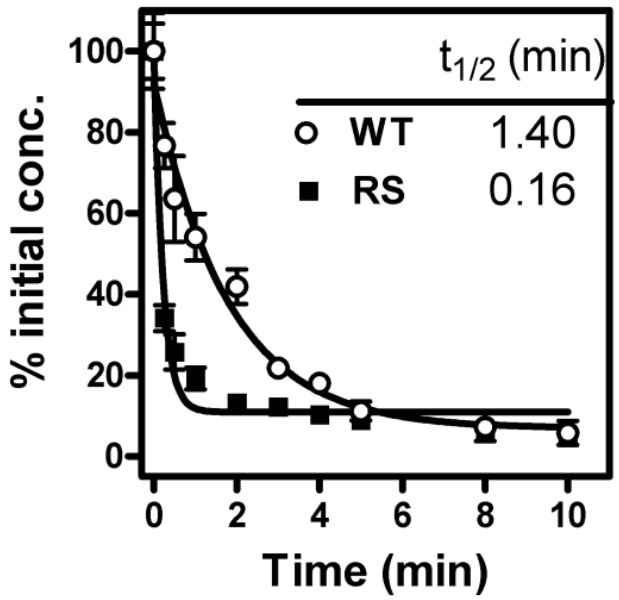


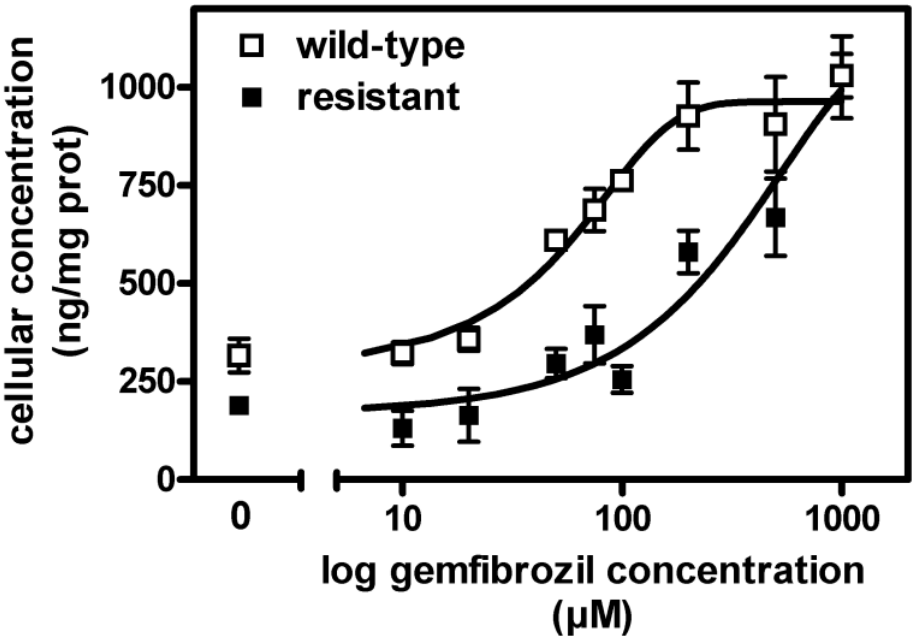
Michot et al., Antimicrob. Ag. Chemother. (2006) 50:1689-1695

Ciprofloxacin « resistant » cells: phenotypic analysis

↗ efflux rate



↗ IC_{50} gemfibrozil

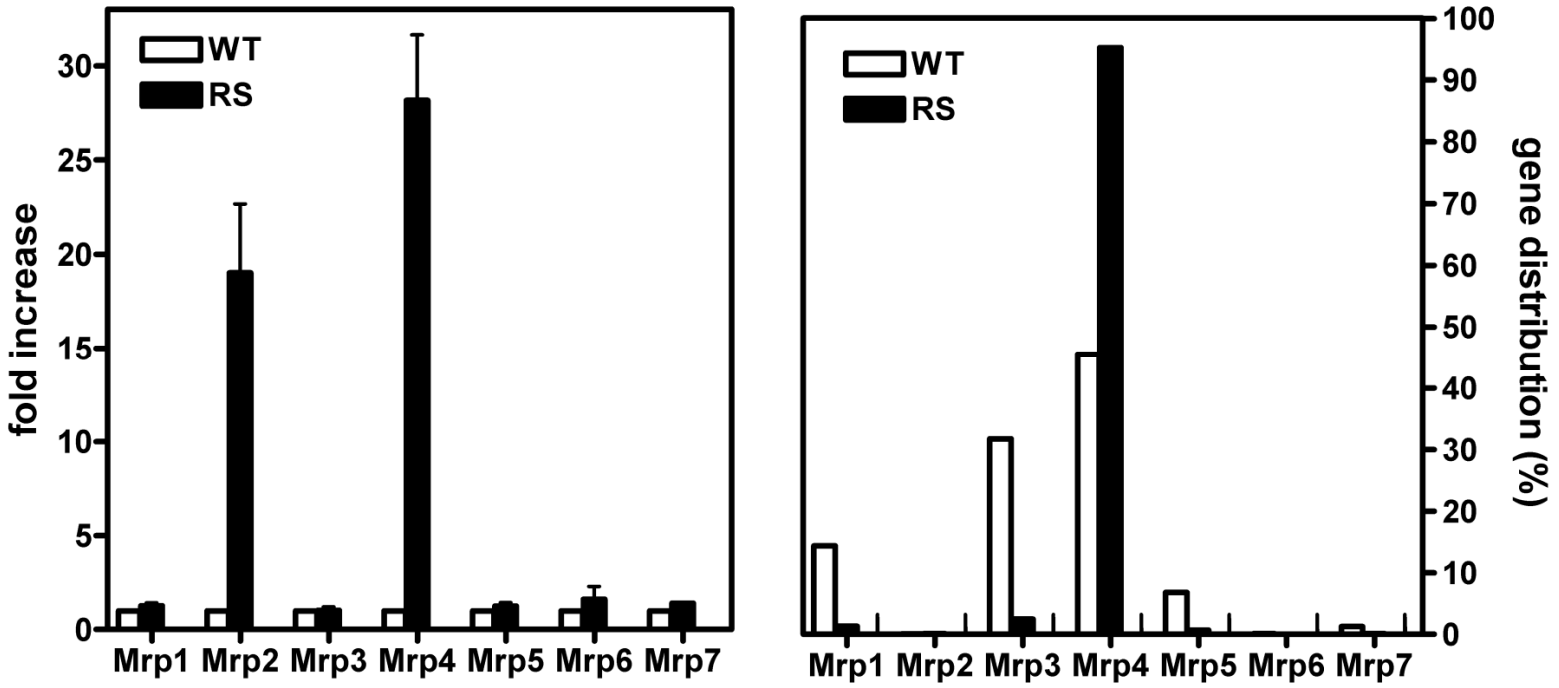


Marquez et al., Antimicrob. Ag. Chemother. (2009) 53:2410-6

Ciprofloxacin « resistant » cells: genotypic analysis

ARNm levels (Real-Time PCR)

↗ expression Mrp2 and Mrp4, but Mrp4 predominates



Marquez et al., Antimicrob. Ag. Chemother. (2009) 53:2410-6

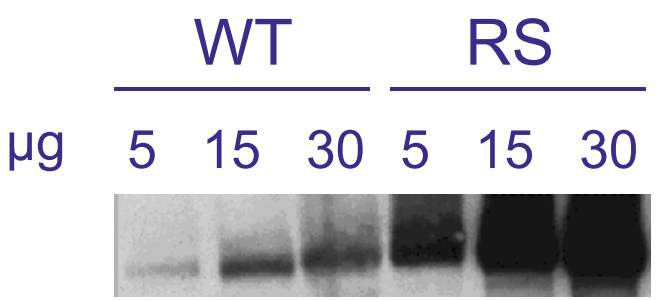
Ciprofloxacin « resistant » cells: proteomic analysis

detection of the proteins by

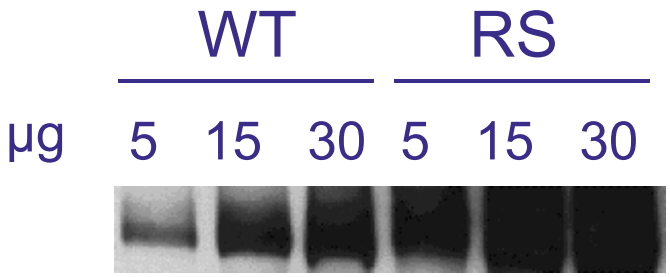
Western-Blot of membrane fraction

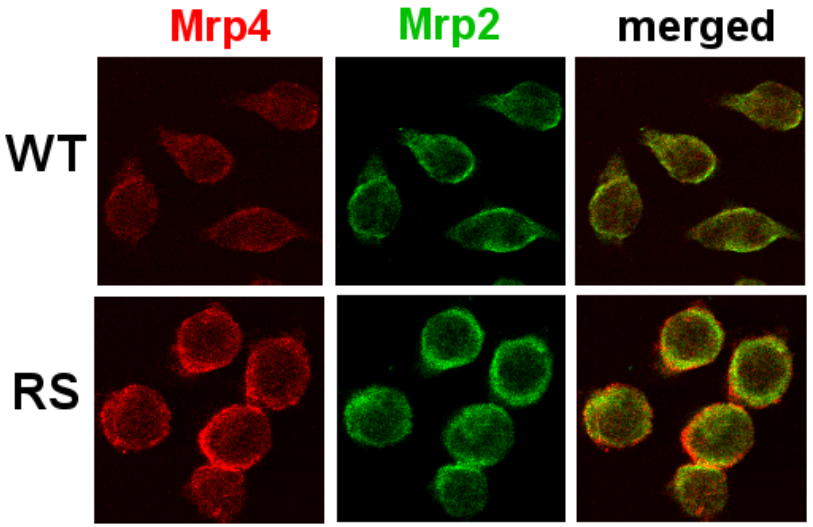
Confocal microscopy

Mrp2



Mrp4





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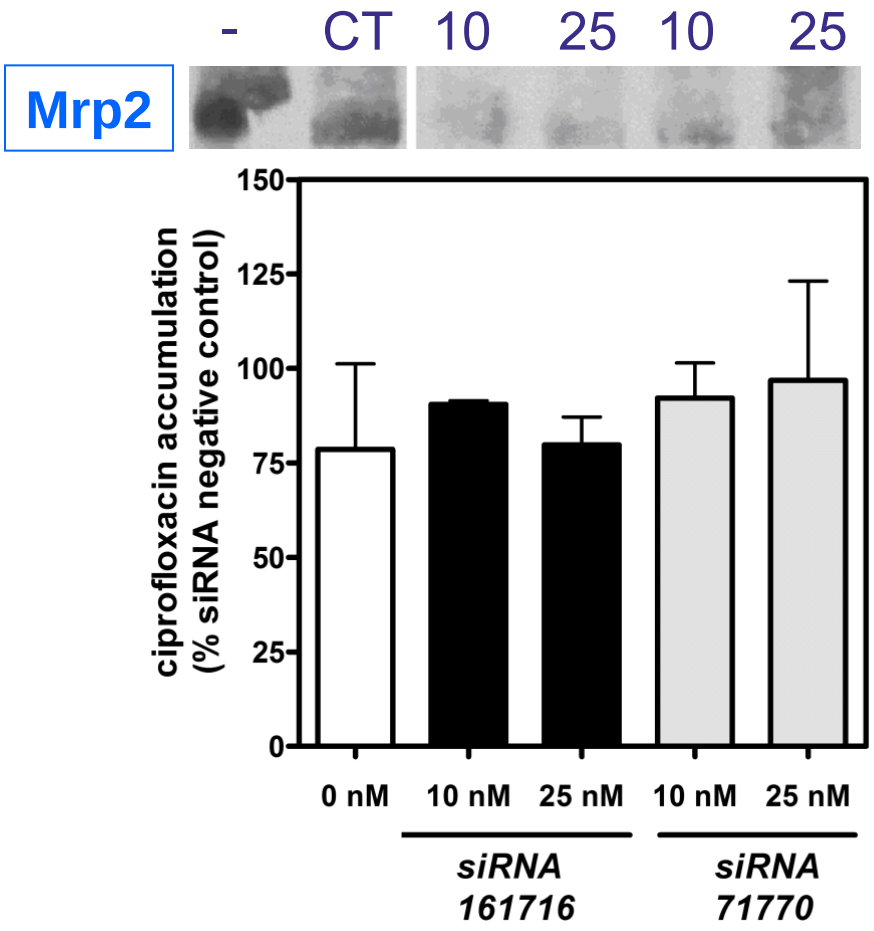
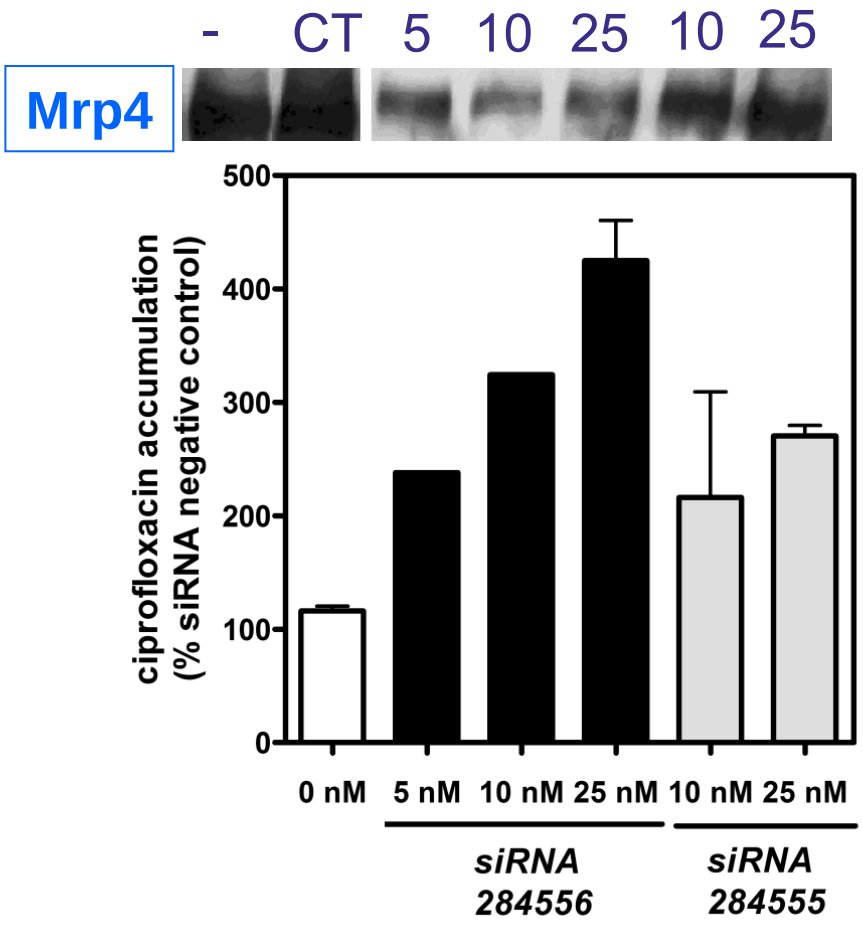
Ciprofloxacin « resistant » cells : which transporter ?

Specific extinction of gene expression by siRNA





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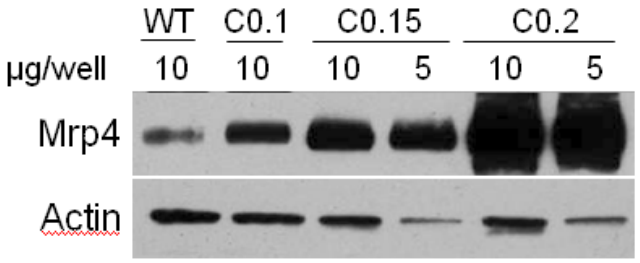
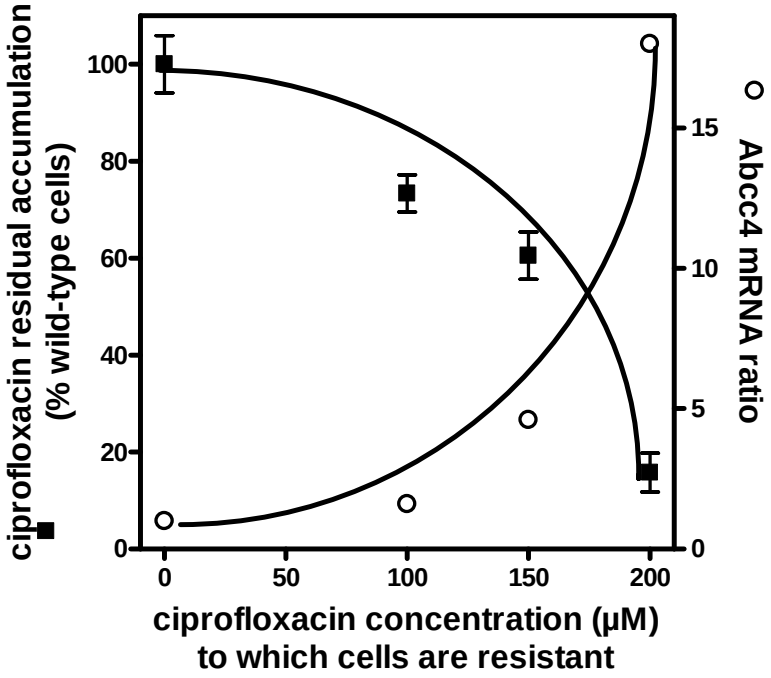
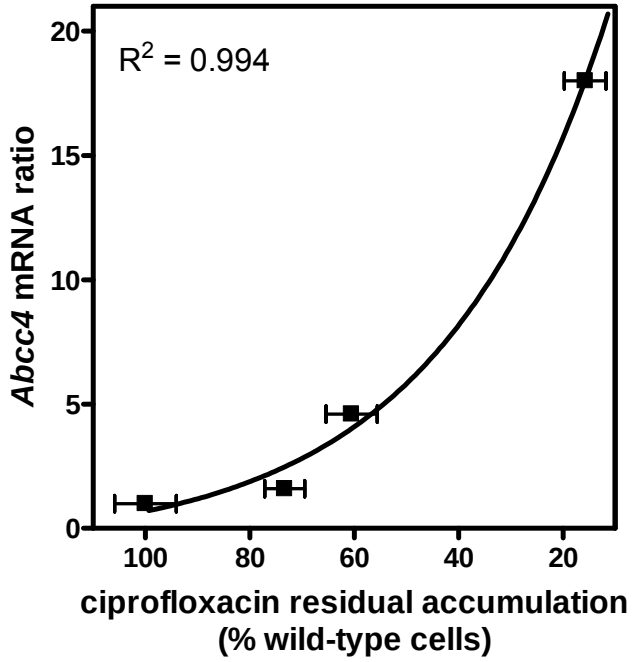
Acquisition of resistance is a stepwise process

Accumulation and Mrp4 expression during selection of resistance

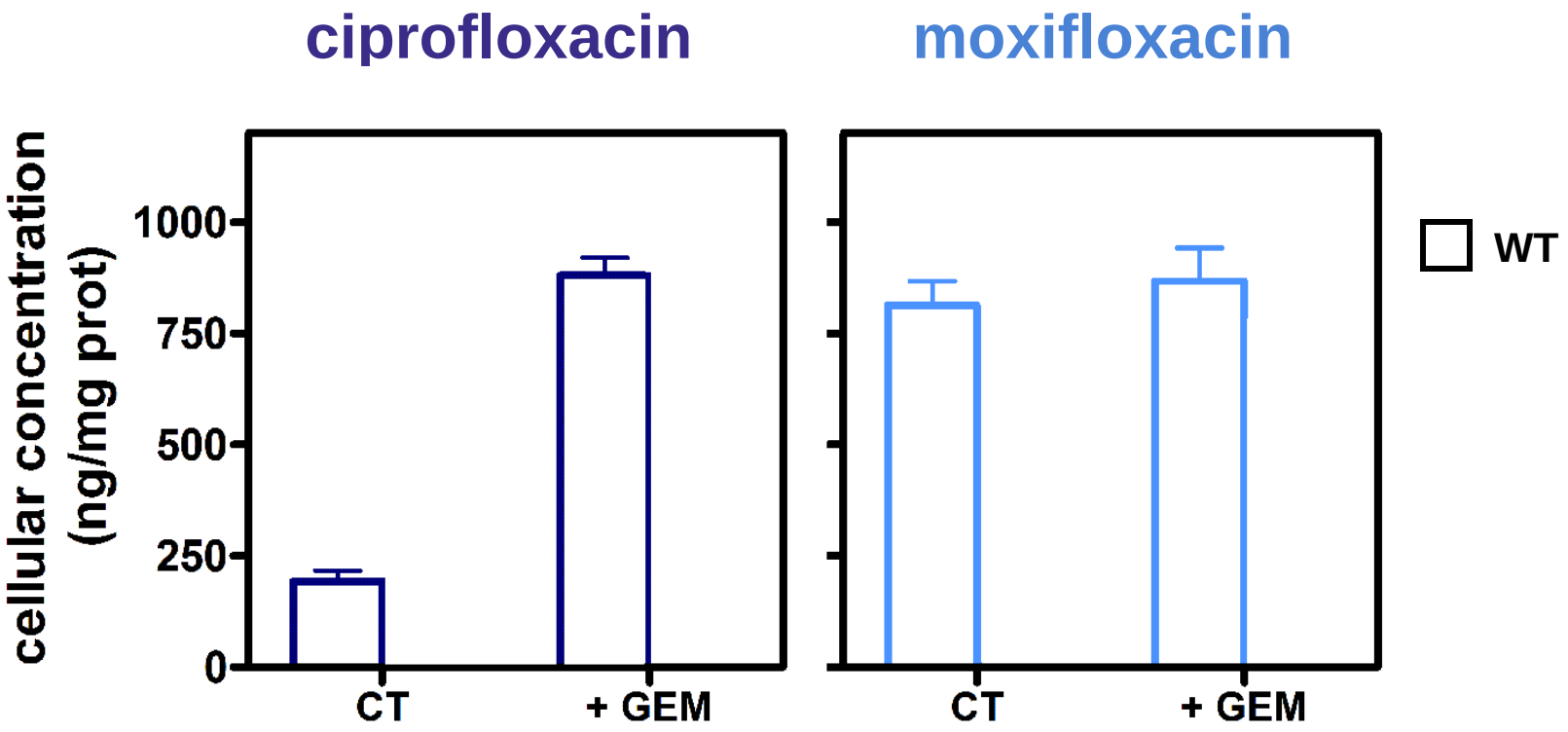






Can we select for moxifloxacin « resistant » cells ?

FQ accumulation and gemfibrozil effect



Vallet et al., FEBS-ABC meeting. (2010)

Can we select for moxifloxacin « resistant » cells ?

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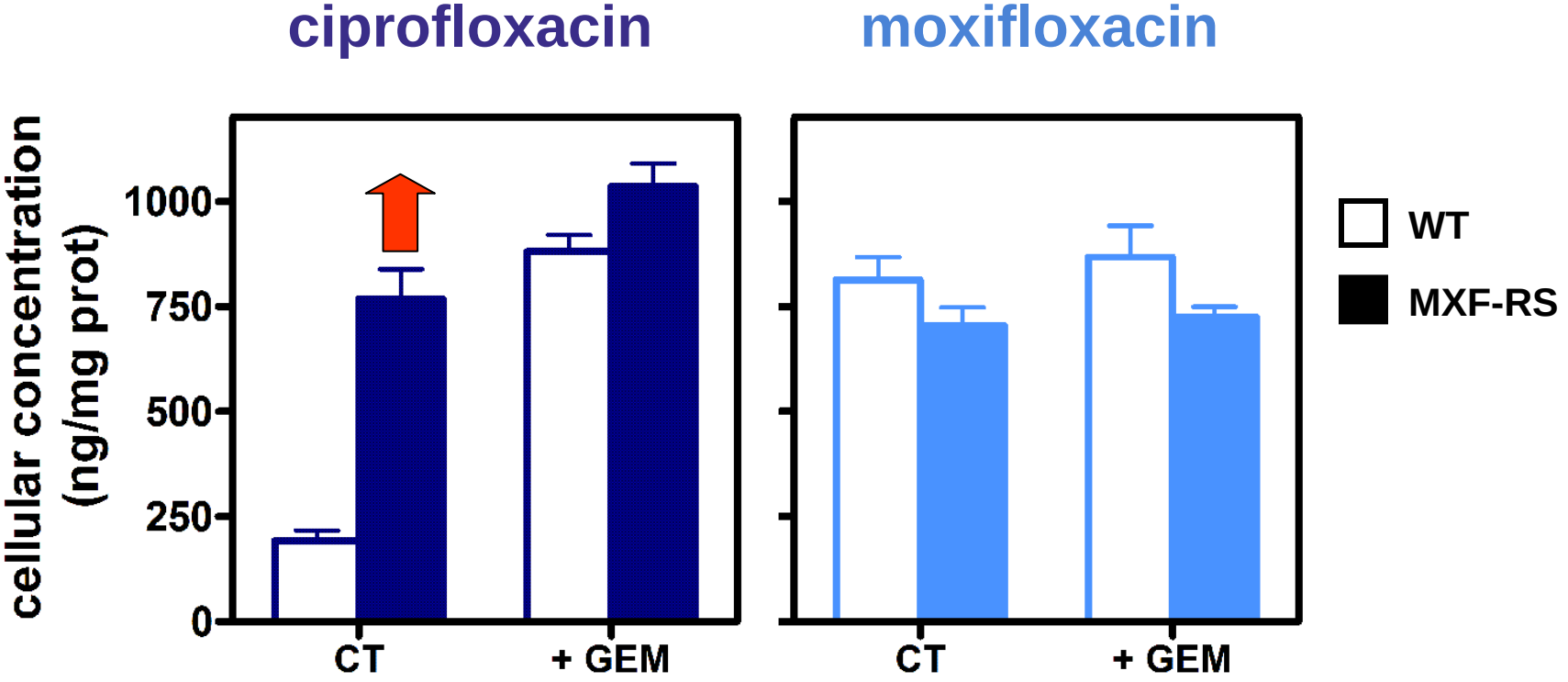

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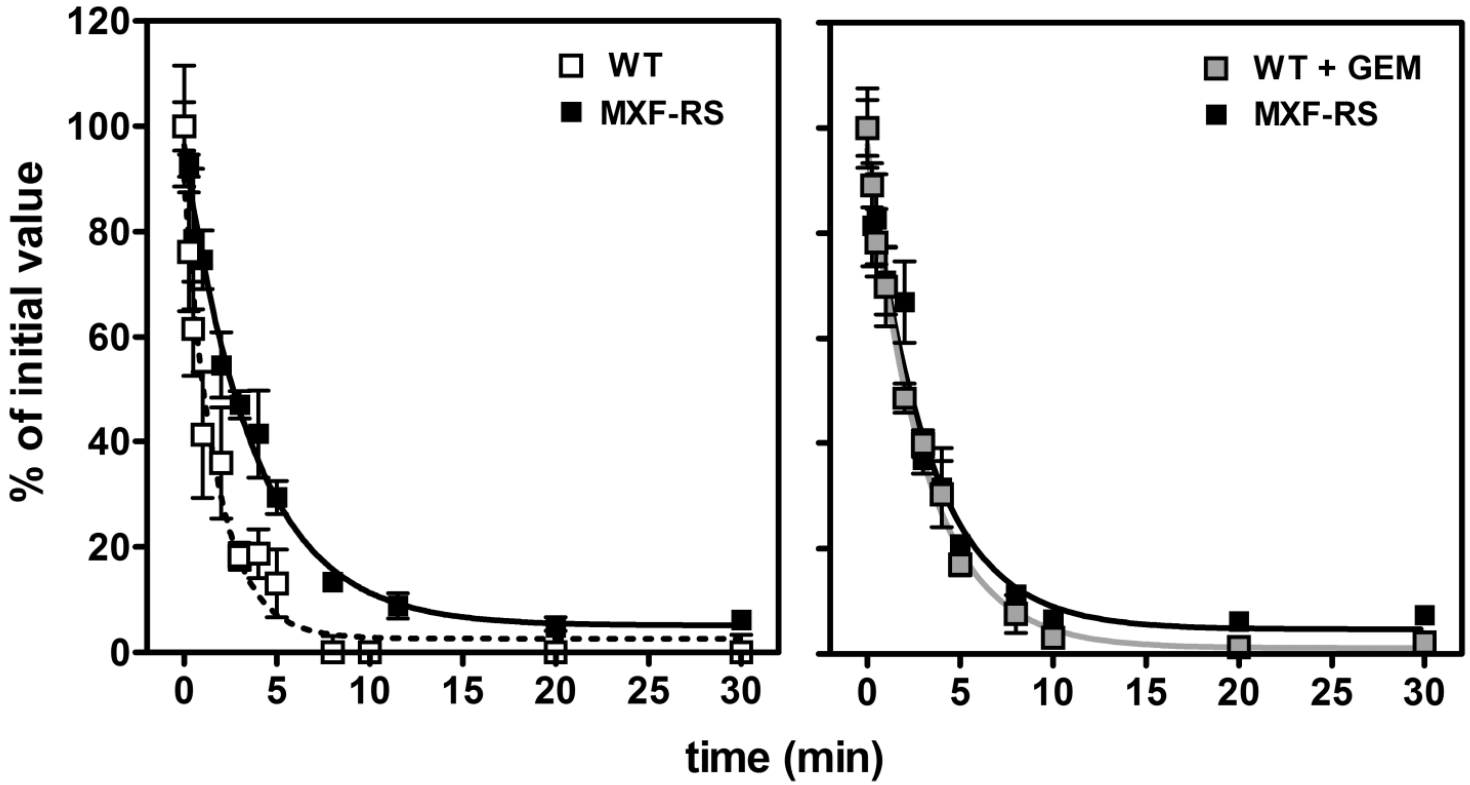
Can we select for moxifloxacin « resistant » cells ?

Ciprofloxacin efflux



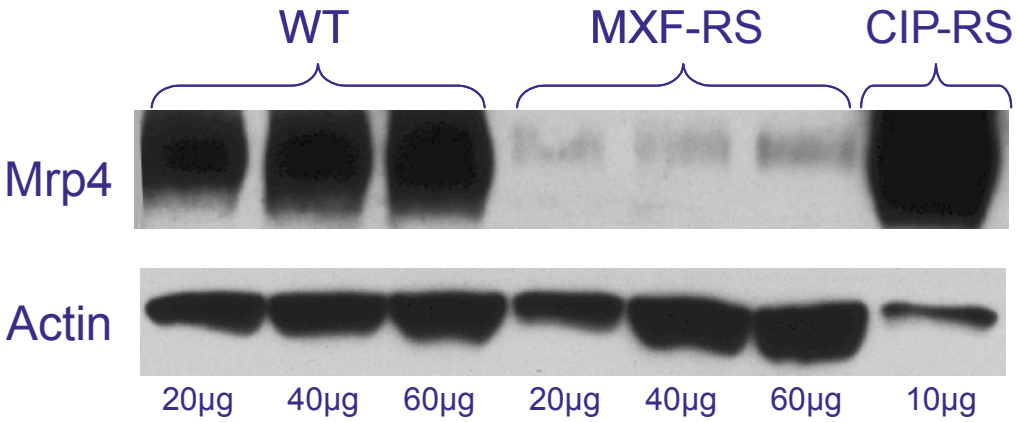
$T_{1/2}$ WT >> $T_{1/2}$ WT+GEM = $T_{1/2}$ MXF-RS

Vallet et al., FEBS-ABC meeting. (2010)

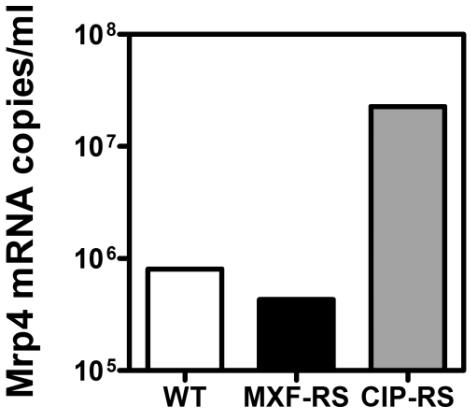
Moxifloxacin-exposed cells are « anti » resistant!

Mrp4 expression

Western-Blot

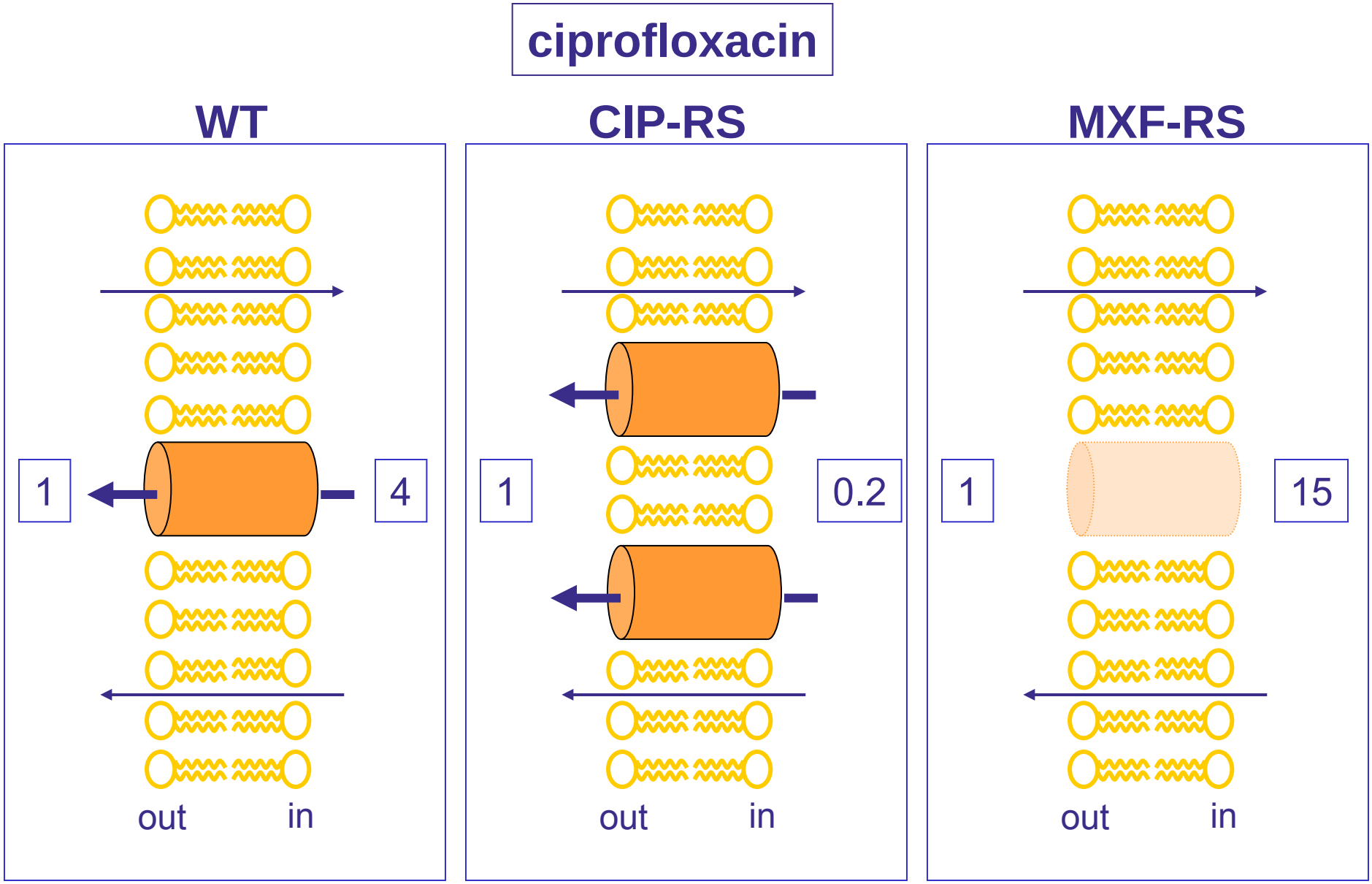


ARNm (Real Time PCR)



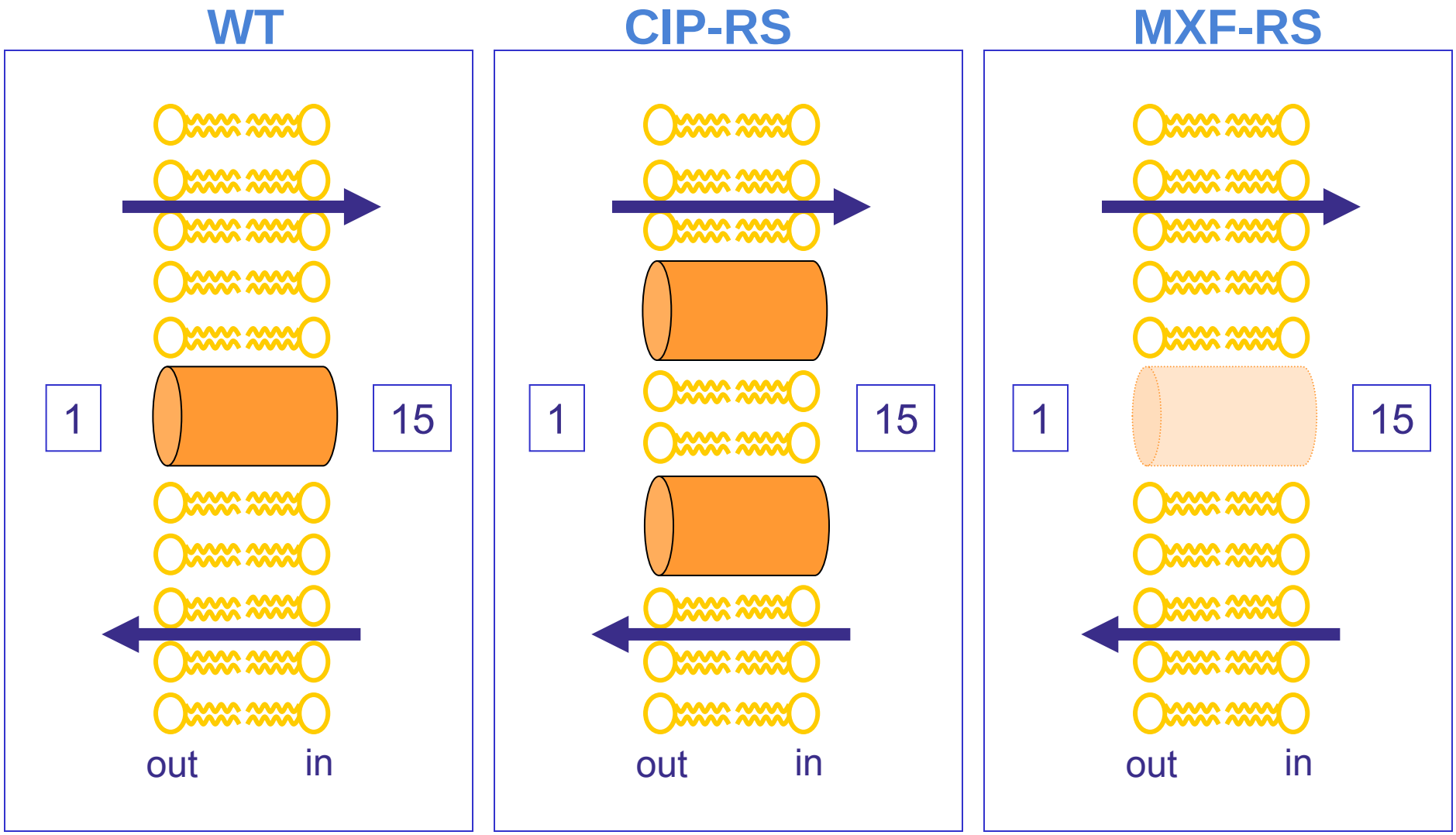
Vallet et al., FEBS-ABC meeting. (2010)

Fluoroquinolone transport: model



Fluoroquinolone transport: model

moxifloxacin

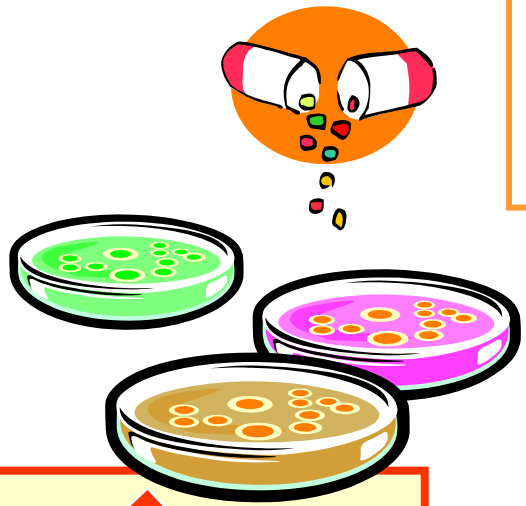


Die Bremer Stadtmusikanten

Efflux …
is it enough for resistance ?

Efflux pumps … and what next ?

How to get resistant cells ?



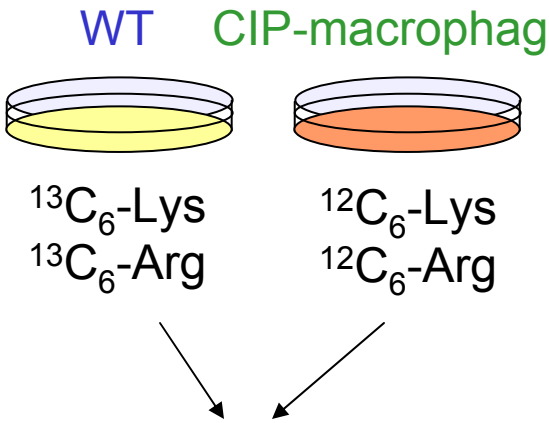
- step-wise increase in AB concentration
- several passages at each step

time and drug consumming!

multifactorial multidrug resistance

Gottesman et al, Methods Enzymol. (1998) 292: 248-58

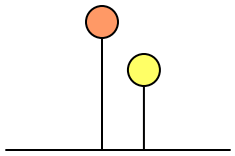
Stable Isotope Labeling Aminoacid in Culture



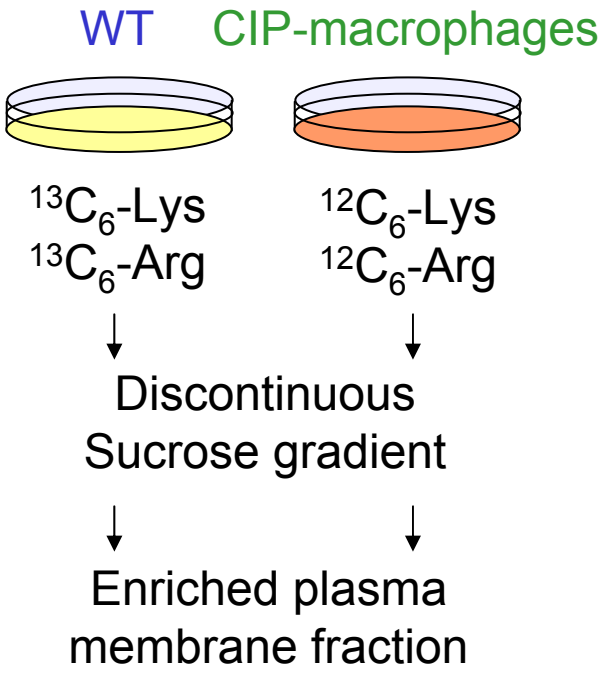
sample mixing 1:1

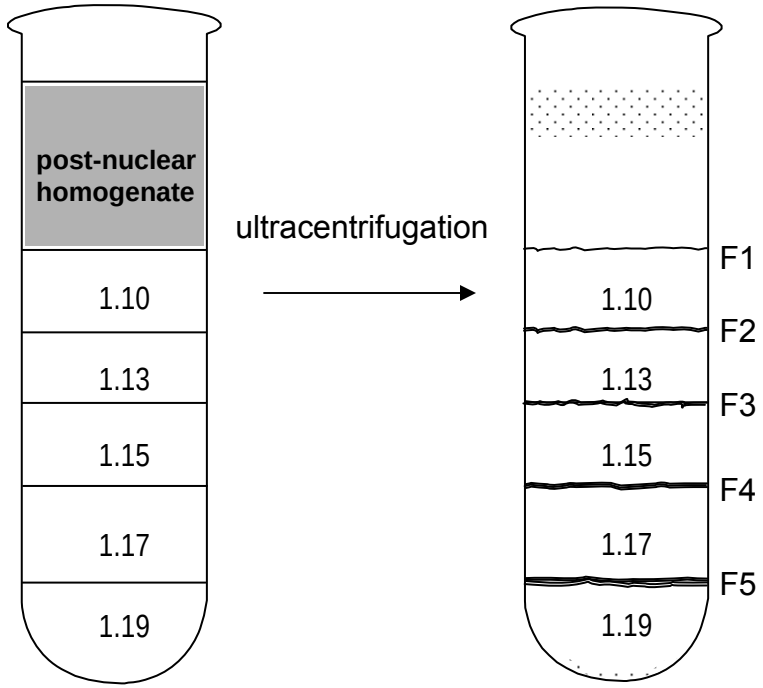
protein digestion

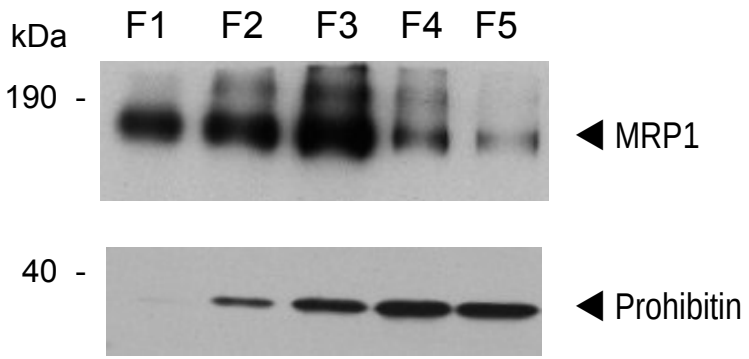
identification in mass spectrometry
and determination of the relative abundance



Stable Isotope Labeling Aminoacid in Culture







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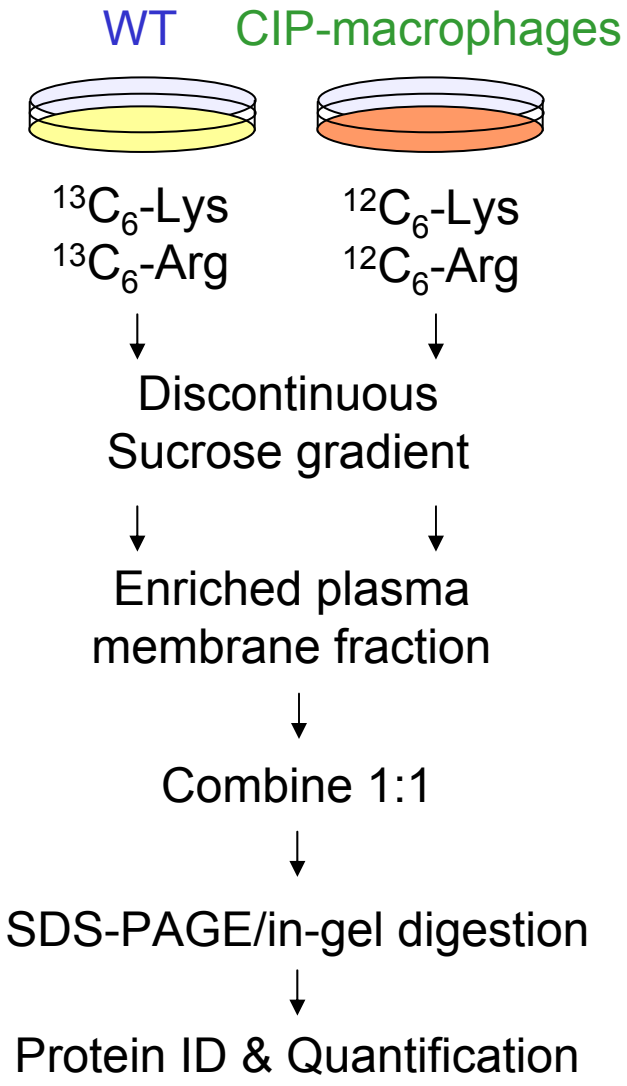
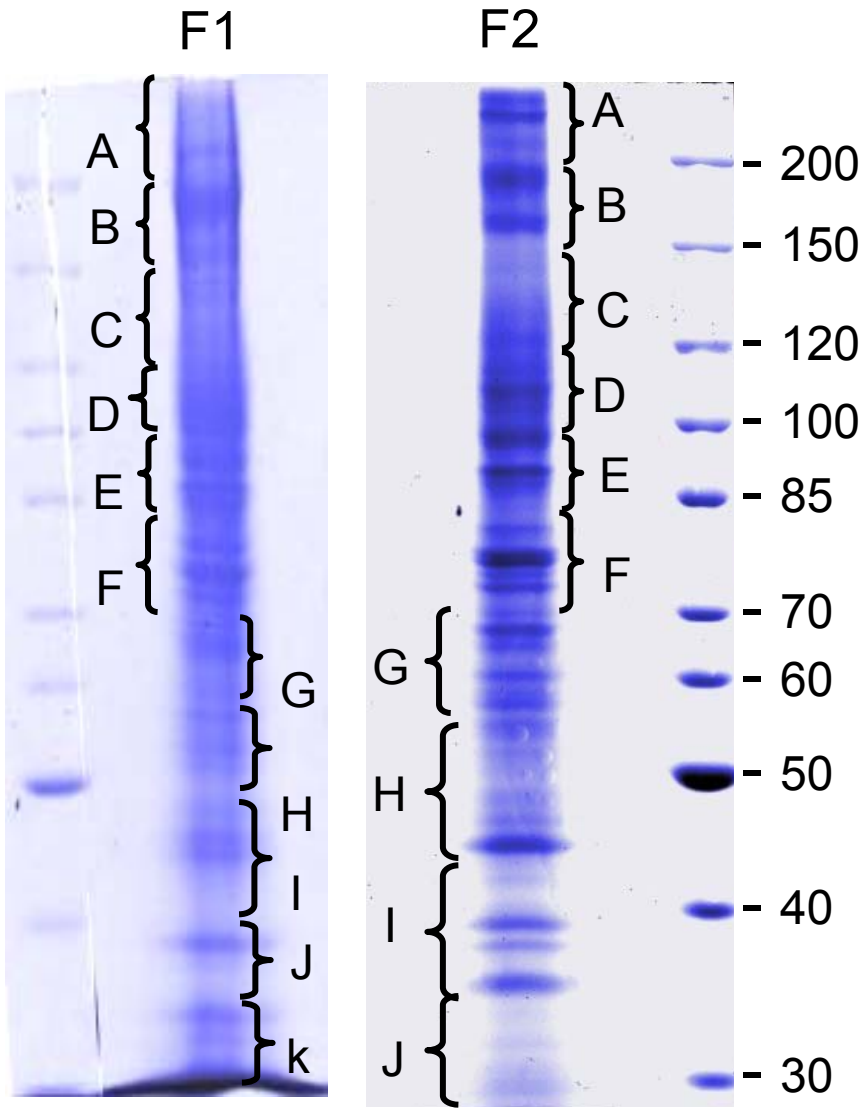




SILAC: global data

⇨ Identification of 900 proteins with 3 uniques peptides

Among proteins detected in both fractions

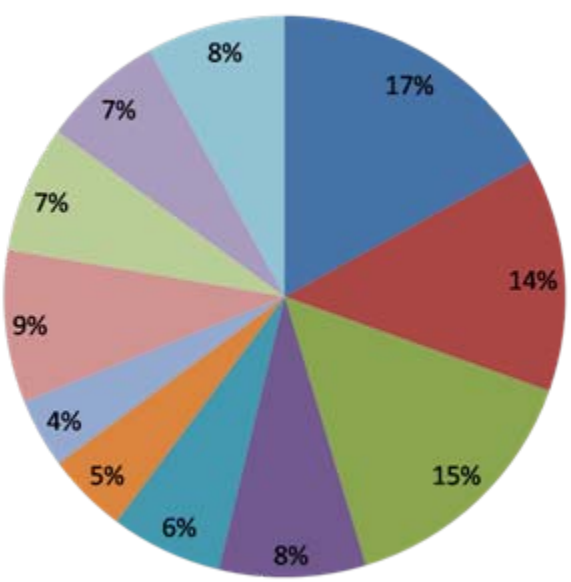
- ↳ 15 ↗ expression in CIP-macrophages as compared to WT
- ↳ 13 ↘ expression in CIP-macrophages as compared to WT

Among proteins detected in one of the two fractions

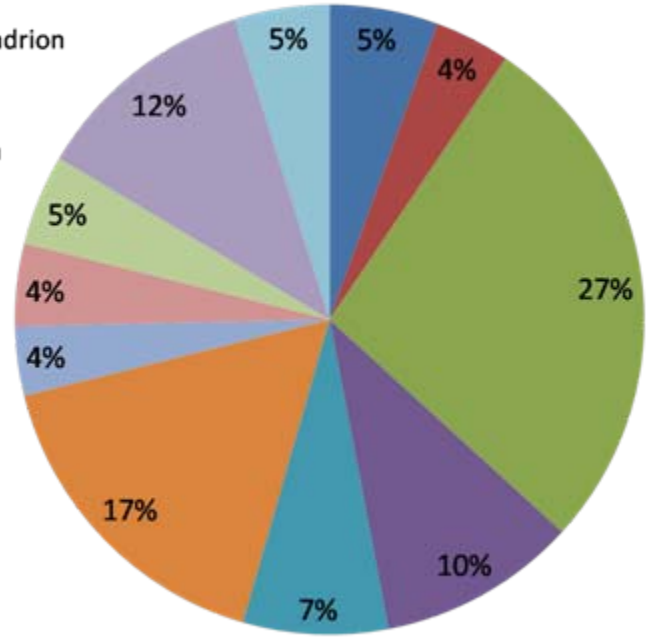
- ↳ 37/36 ↗ expression in CIP-macrophages as compared to WT
- ↳ 29/34 ↘ expression in CIP-macrophages as compared to WT

Marquez et al, FEBS 2009

Proteins with modified expression in CIP-resistant cells

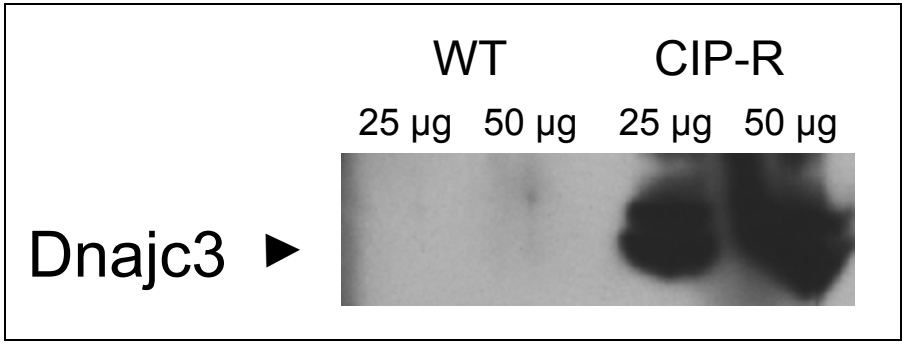


- Plasma membrane
- Unknown membrane protein
- ER/Golgi
- Endosome/Lysosome
- Cytoskeleton
- Ribosome
- Extracellular
- Nucleus
- Mitochondrion
- Cytosol
- Unknown



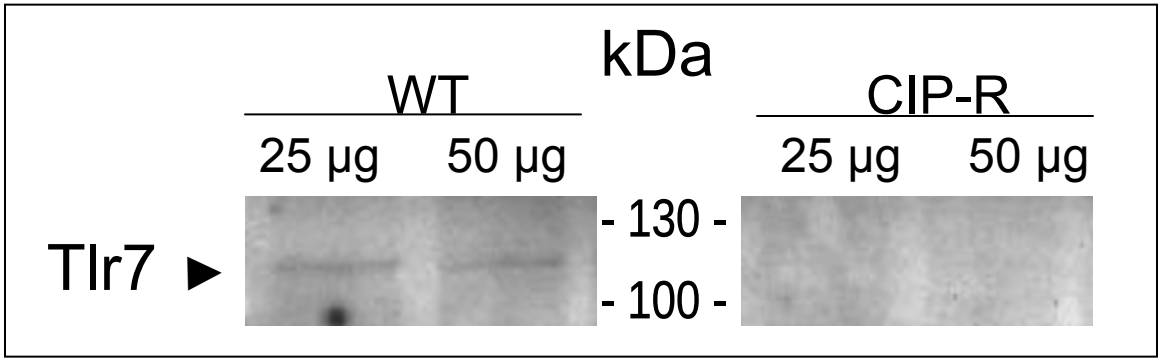
- Cell adhesion and localization
- Immune response
- Transport
- Signal transduction
- Cytoskeleton organization
- Protein metabolism
- Carbohydrate metabolism
- Lipid metabolism
- Nucleid acid metabolism
- Other
- Unknown

Proteins with modified expression in CIP-resistant cells

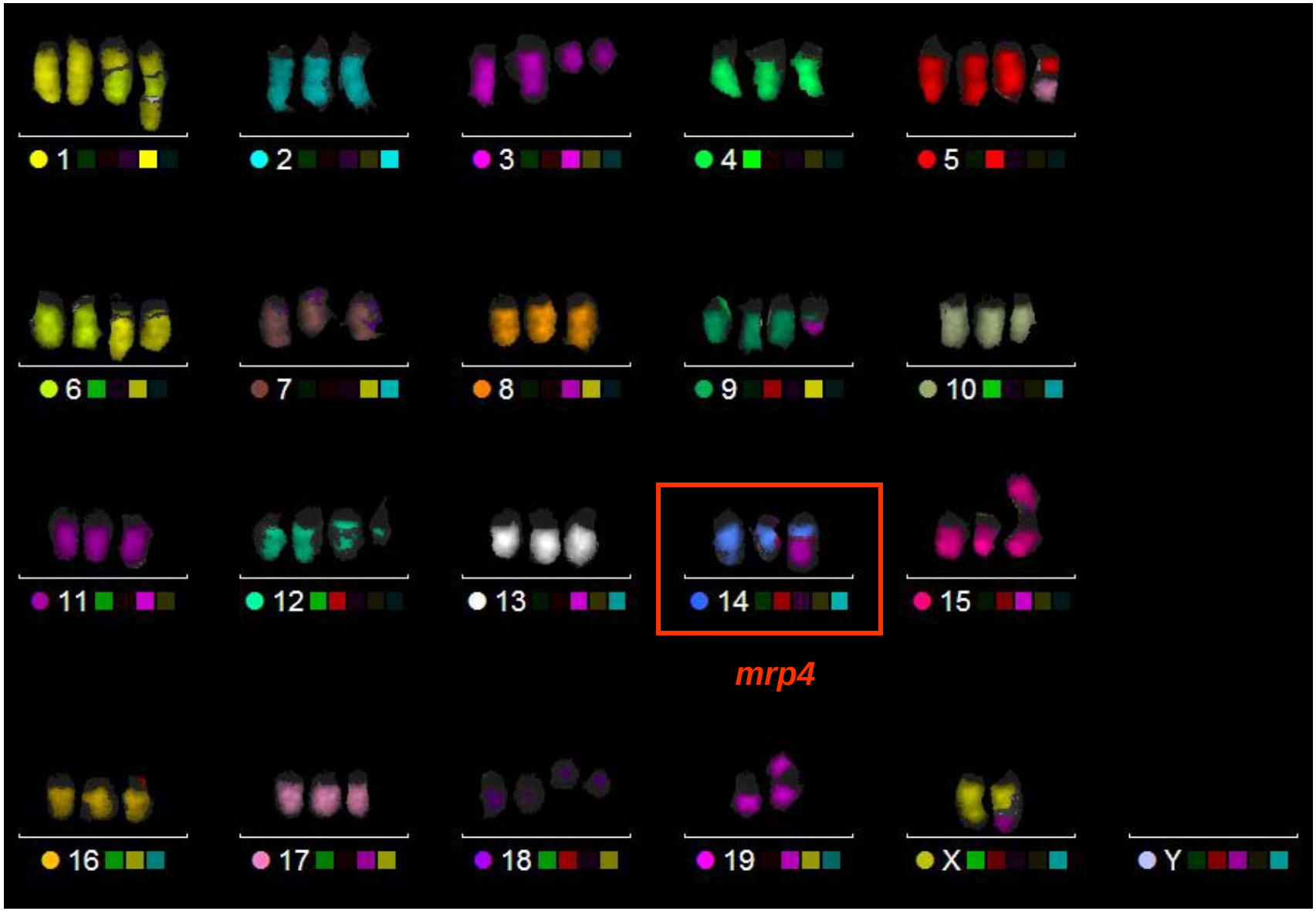


On the same chromosome as *mrp4* !

Transports nucleosides/tides (substrates for Mrp4 !)

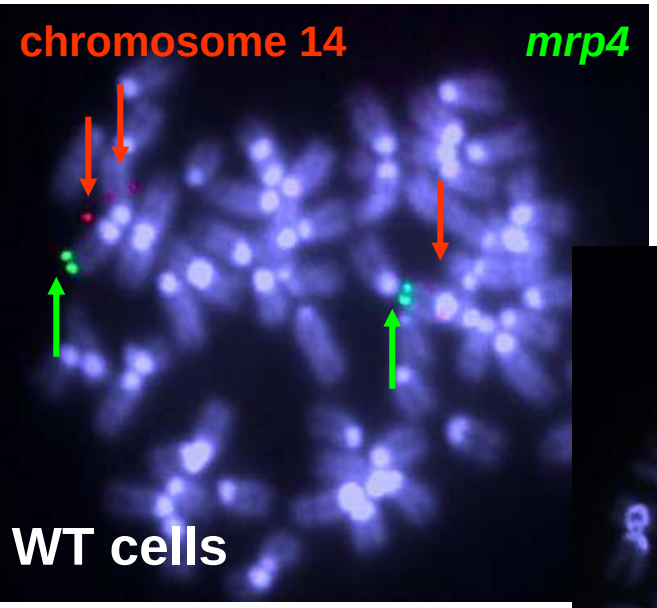


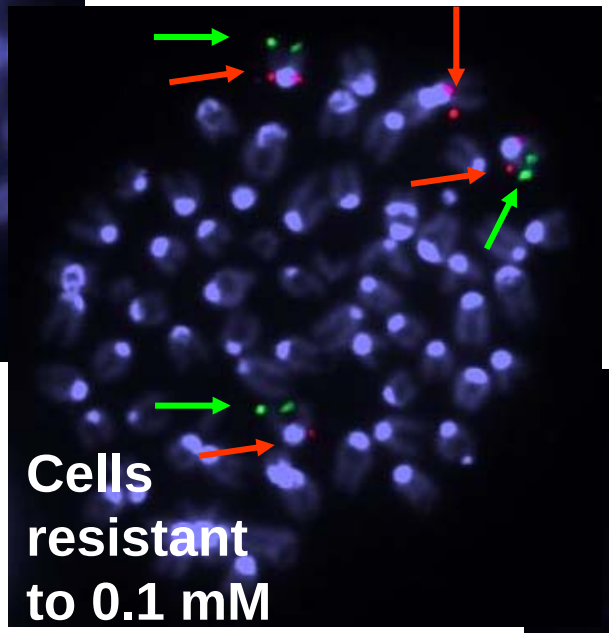
Karyotype of J774 cells

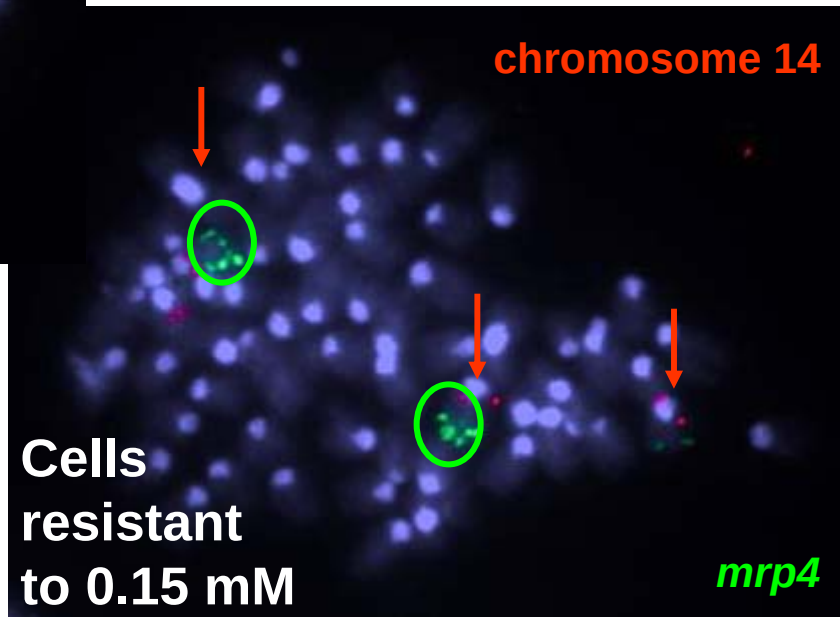


Gene amplification in CIP-resistant cells

FISH analysis



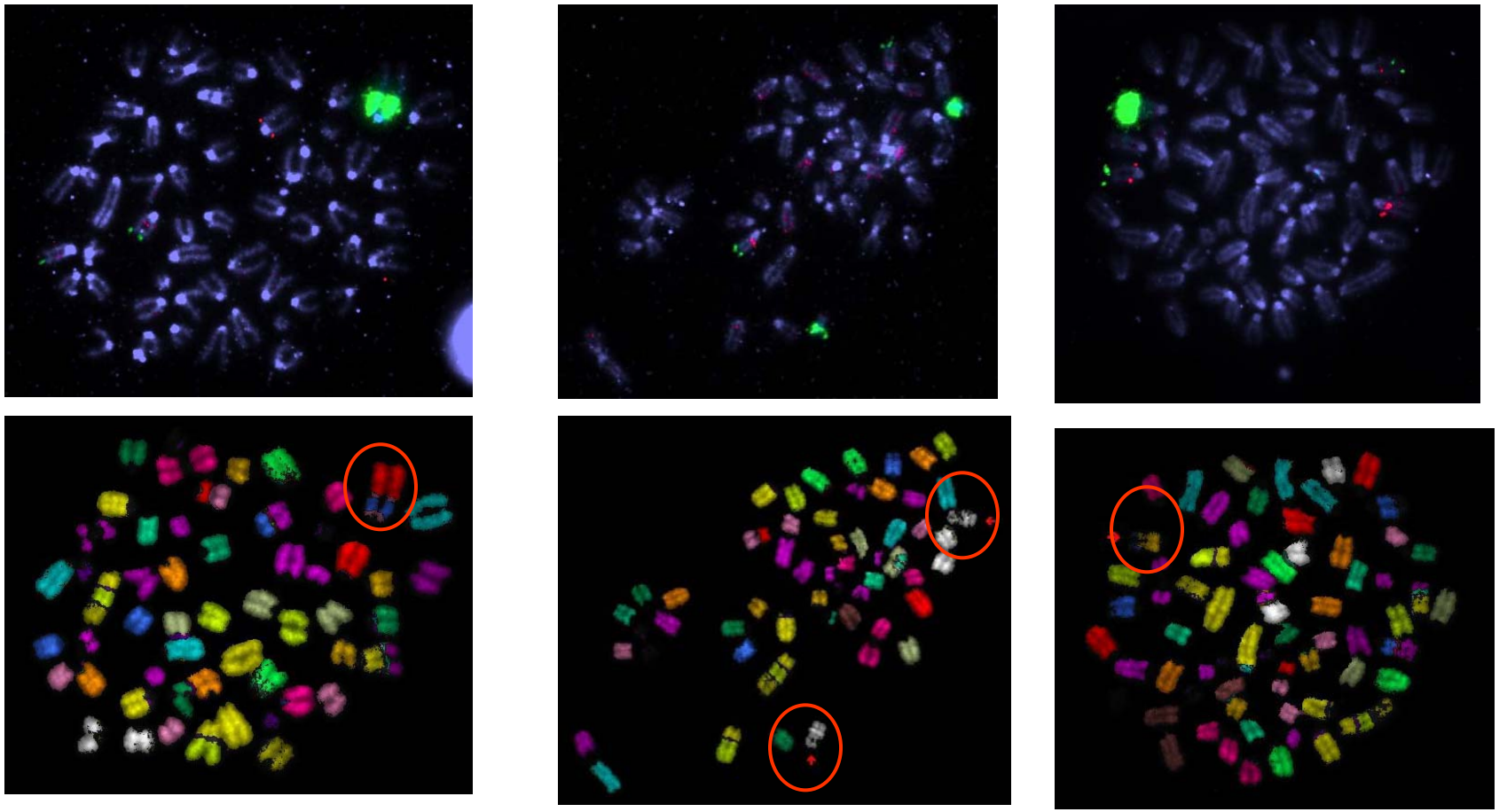




Gene amplification in CIP-resistant cells

mFISH analysis of CIP-R cells

Heterogeneity of cell population !

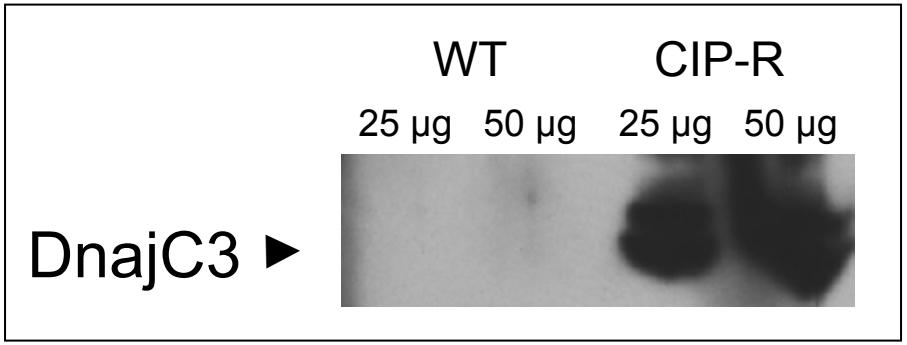


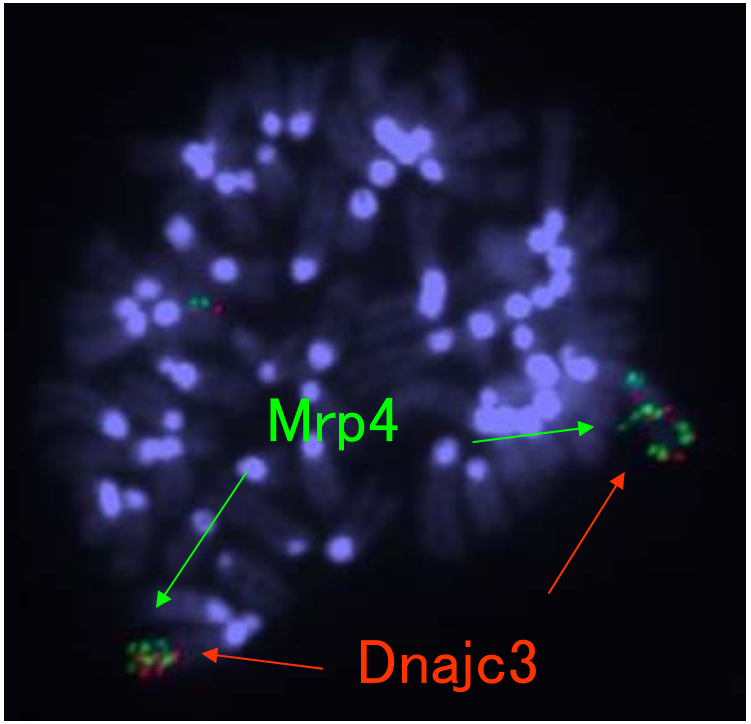
chromosome 5 chromosome 13 chromosome 16

Gene amplification in CIP-resistant cells

FISH analysis of CIP-R cells

Mrp4 and DnajC3 co-amplified in CIP-resistant cells

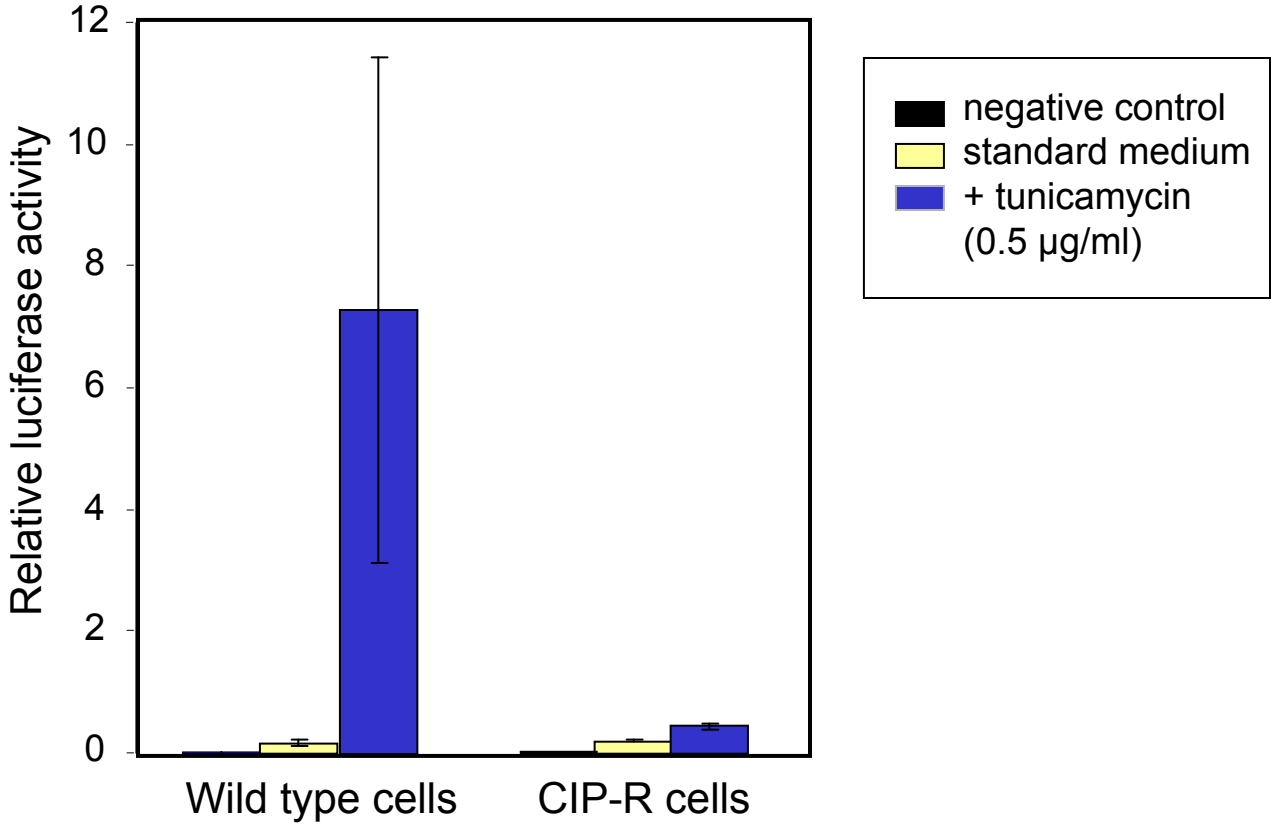




Implication of co-amplified genes in resistance

DnajC3 protects CIP-resistant cells from ER stress

Quantification of ER-stress signaling with a Dual Luciferase Assays of cells transfected with Cignal ERSE reporter



and in moxifloxacin-resistant cells ?

Die Bremer Stadtmusikanten

Expression of ABC transporters

TaqMan Low Density Array of ABC transporters

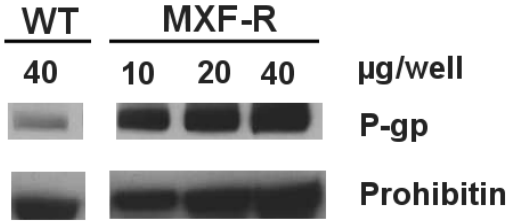
gene	Cell line	
	ciprofloxacin-resistant	moxifloxacin-resistant
Abca1	-1.53[a]	-3.51
Abca2	-1.13	-1.12
Abca3	1.10	-1.56
Abca4	nd	nd
Abca5	-1.85	2.31
Abca6	nd	nd
Abca7	-1.36	-1.20
Abca8a	nd	nd
Abca8b	(1.39)	76.20
Abca9	(-17.45)	(-10.48)
Abca13	1.66	2.02
Abca14	nd	nd
Abca15	nd	nd
Abcb1a*	(-1.38)	76.35
	(-1.89)	84.42
Abcb1b	1.03	1.72
Abcb2	1.10	4.30
Abcb3	-1.35	3.17
Abcb4	1.01	2.48
Abcb6	-1.12	-1.22
Abcb8	1.00	-1.25
Abcb9	6.08	9.83
Abcb10	1.40	1.43
Abcb11	(1.15)	(1.19)

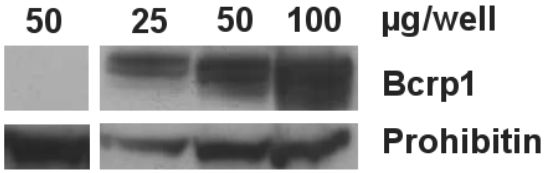
gene	Cell line	
	ciprofloxacin-resistant	moxifloxacin-resistant
Abcc1	1.08	-1.26
Abcc2*	(1.44)	(-1.26)
	(9.25)	(5.09)
Abcc3	-1.15	1.09
Abcc4	14.59	-1.82
Abcc5	-1.26	1.29
Abcc6	nd	nd
Abcc7	nd	nd
Abcc8	(1.22)	(7.74)
Abcc9	nd	nd
Abcc10	1.01	-1.40
Abcc12	nd	nd
Abcd1	1.35	2.22
Abcd2	1.10	1.79
Abcd3	-1.04	-1.51
Abcd4	-1.43	-2.03
Abce1	-1.04	-1.00
Abcf2	-1.01	1.21
Abcf3	-1.08	1.11
Abcg1	-1.93	-4.92
Abcg2*	(1.12)	108.41
	(1.17)	99.47
Abcg3	nd	nd
Abcg4	(1.62)	(-1.07)
Abcg5	nd	nd
Abcg8	nd	nd

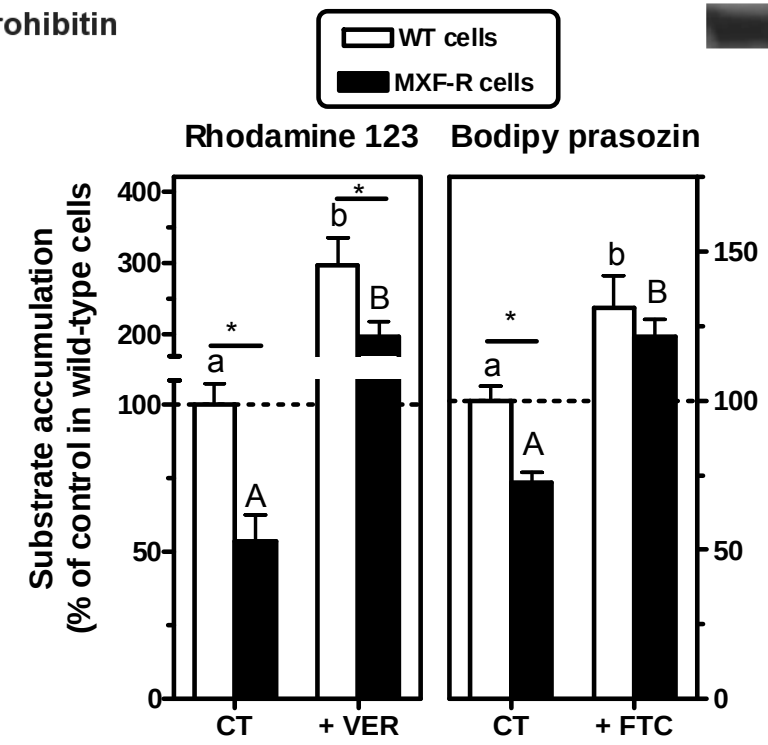
Vallet et al., FEBS-ABC meeting. (2010)

Overexpression of MDR transporters

P-gp and Bcrp are functional



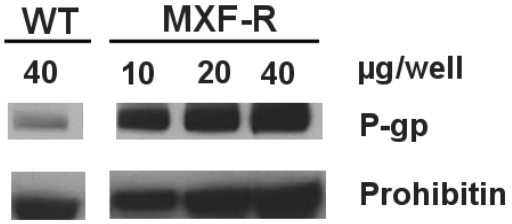


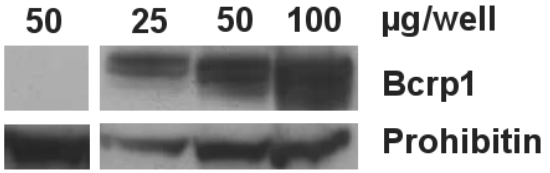


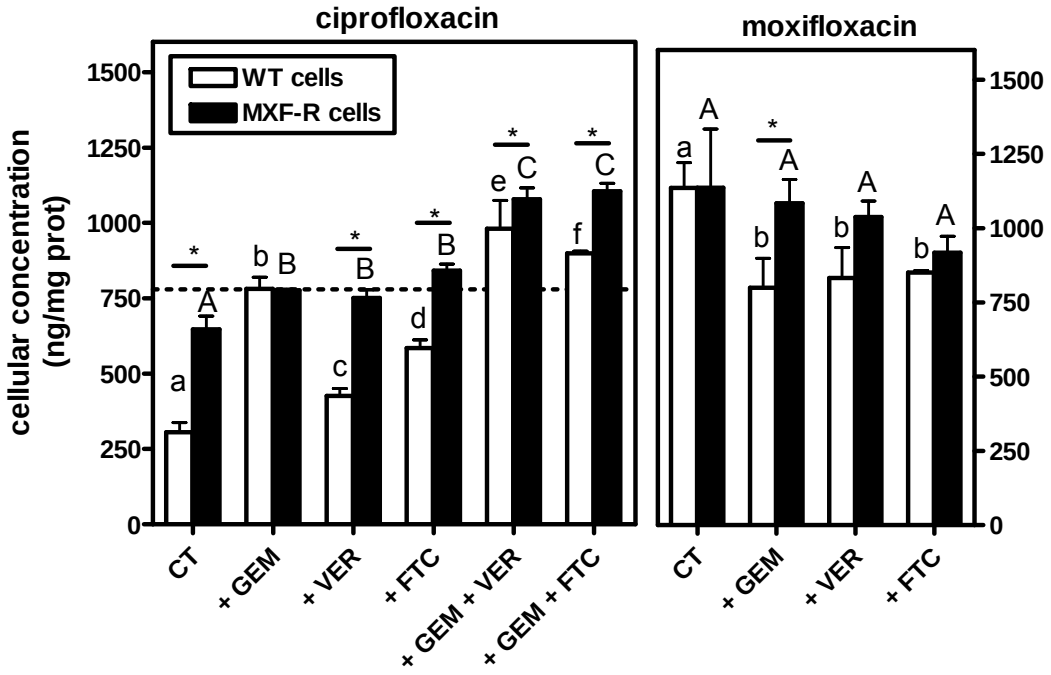
Vallet et al., FEBS-ABC meeting. (2010)

Overexpression of MDR transporters

P-gp and Bcrp are functional
but do not play a major role in FQ accumulation







Vallet et al., FEBS-ABC meeting. (2010)

Take home message

Antibiotics are also substrates for efflux in eucaryotic cells

Die Bremer Stadtmusikanten

Take home message

Die Bremer Stadtmusikanten

Closely related molecules behave very differently with respect to transport

Take home message

Die Bremer Stadtmusikanten

Non substrates may induce a 'anti-resistant' phenotype

Take home message

Die Bremer Stadtmusikanten

Efflux is only part of the response to drug exposure

Coworkers …

- **B. Devreese and M. Aerts**

Proteomics

Laboratory for Protein Biochemistry and Biomolecular Engineering,
Ghent University, Belgium

- **E. Jacquet and N. Nhiri**

TaqMan Low Density Array

Institut de Chimie des Substances Naturelles,
CNRS, Gif sur Yvette, France

- **G. Ameye and H. Antoine-Poirel**

FISH

Centre de Génétique,
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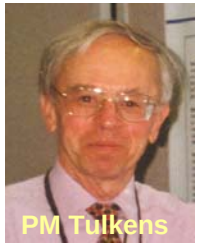
Brussels Stadtmusikanten for this story …



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