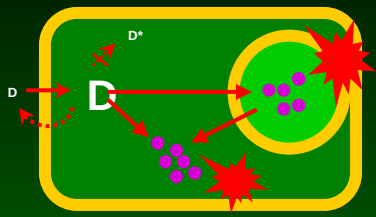
# Models of Intracellular Antibiotic Transport

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www.facm.ucl.ac.be

# Why intracellular antibiotics ?

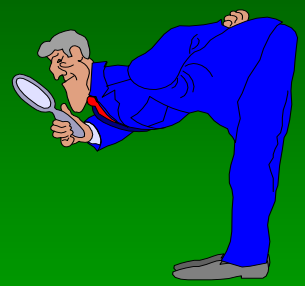
# Intracellular antibiotics: the issues

1. which bacteria and where ?

2. which antibiotics accumulate ?

3. influx vs efflux ?

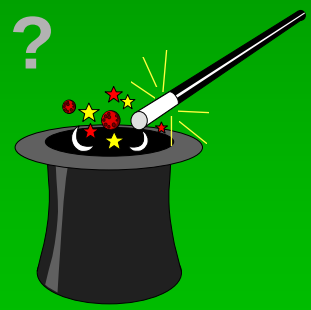
3. where are antibiotics in cells ?



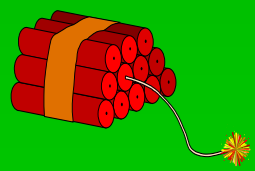
4. intracellular expression of activity ?

5. bacterial responsiveness ?

6. cooperation with host defenses ?



7. any toxicity ?



# Which antibiotics accumulate in cells ?

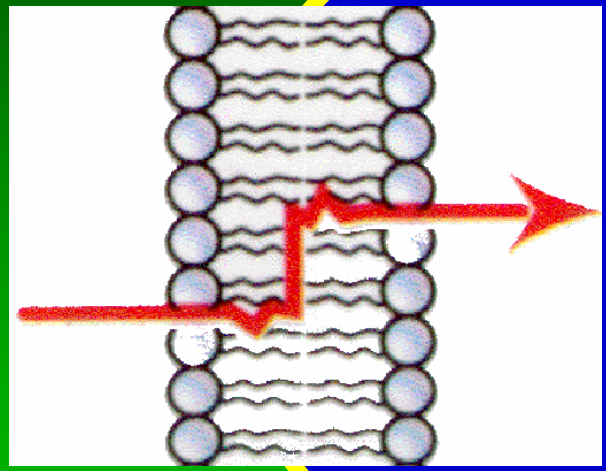
- **beta-lactams: ≤ 1x**
- **aminoglycosides: <1 to 2 x**
- **ansamycins: 2-3 x**
- **tetracyclines: 2-4 x**
- **fluoroquinolones: 5 - 20 x**
- **macrolides: 4 to > 100 x ***
- **glycopeptides: 1 to 400 x !! ****

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

# How do antibiotics penetrate in cells ?

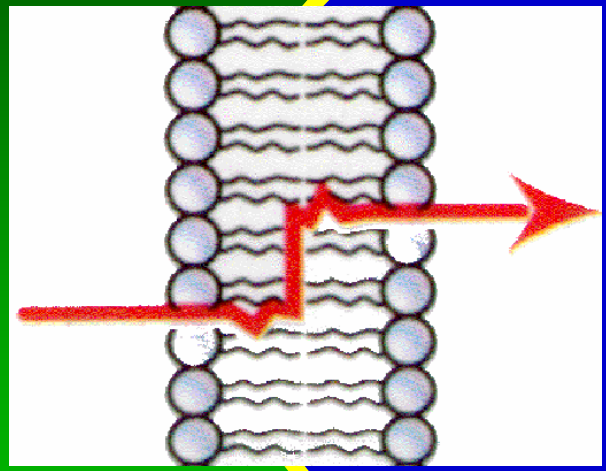
- trans-membrane influx
  - diffusion
  - carrier mediated
- endocytosis

# Entry by diffusion ...



- amphiphilic compounds
- fast
- non-saturable
- no competition by analogues

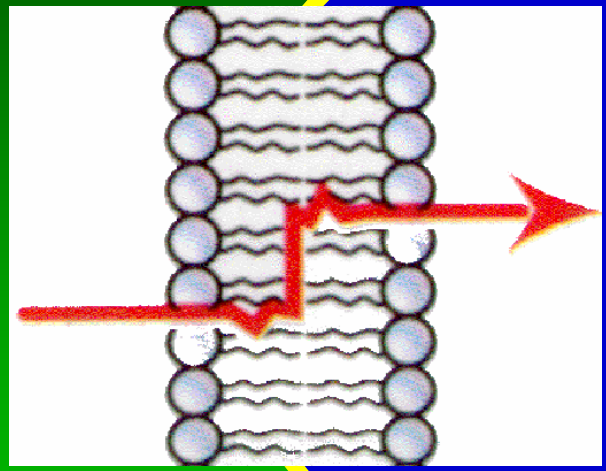
# Entry by diffusion: some examples…

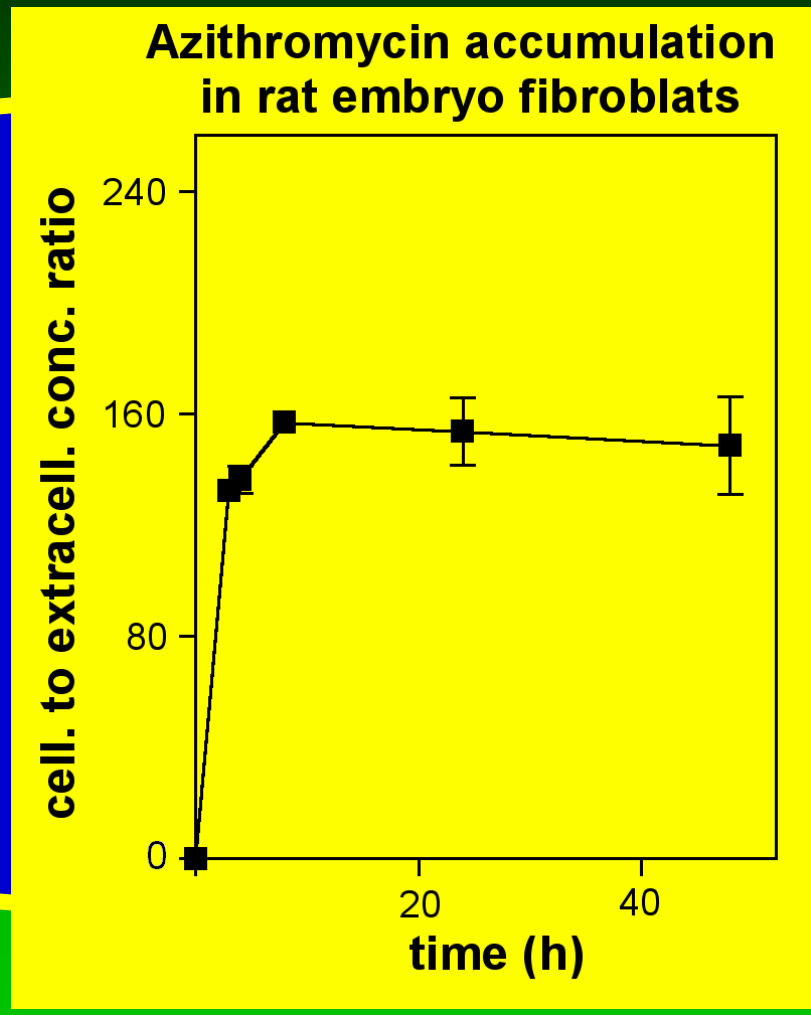


- macrolides
- fluoroquinolones
- tetracyclines
- ansamycines
- β-lactams,
- ...

# Entry of azithromycin…

**diffusion**

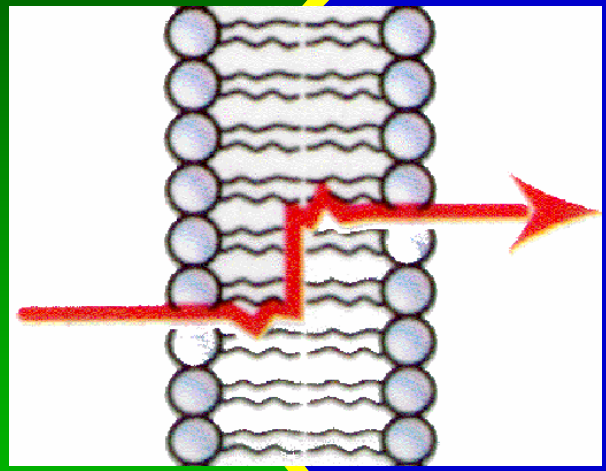


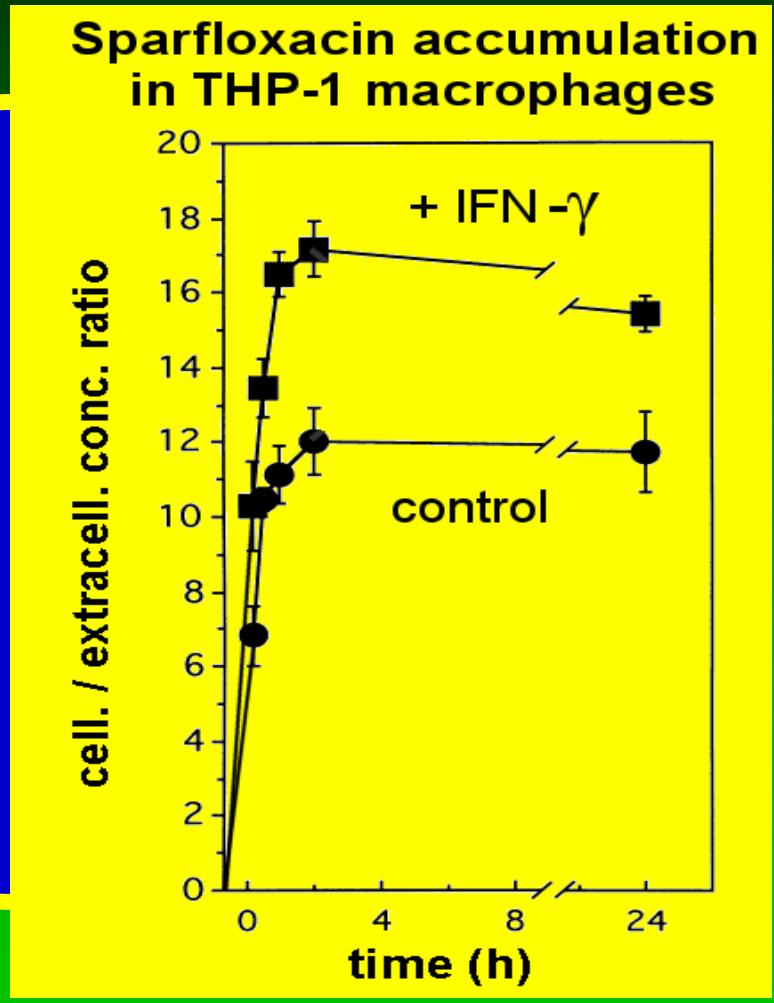



Tyteca et al., EJCB, 2001, in press

# Entry of sparfloxacin …

**diffusion**

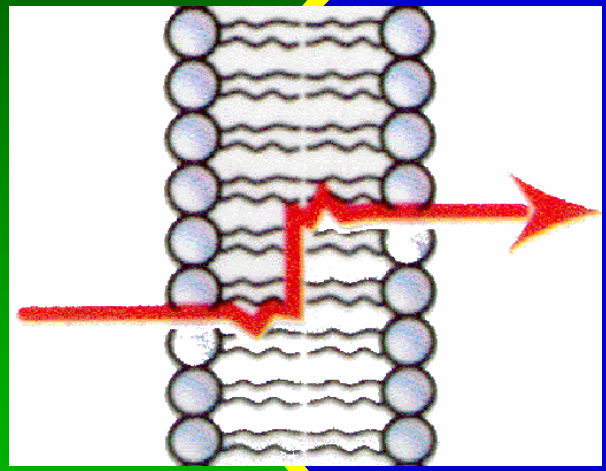


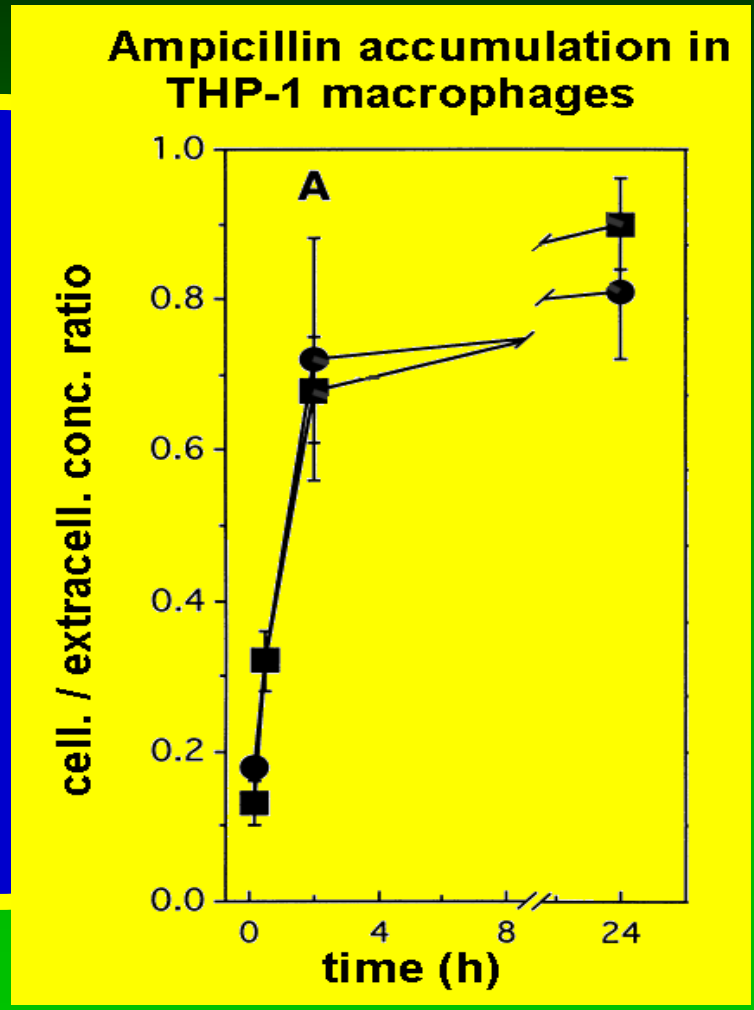



Ouadrhiri et al., AAC, 1999

# Entry of ampicillin …

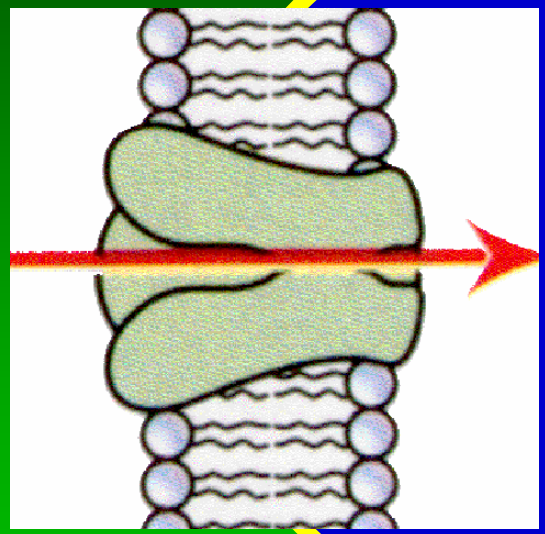
**diffusion**






Ouadrhiri et al., AAC, 1999

# Carrier-meddiated influx ?



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

**only limited evidence for specific transporters is available so far**

# Carrier-mediated influx ?

## the case of HSR-903

0022-3565/99/2891-0079$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 289, No. 1
Printed in U.S.A.

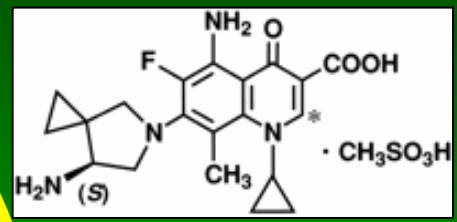
JPET 289:79–84, 1999

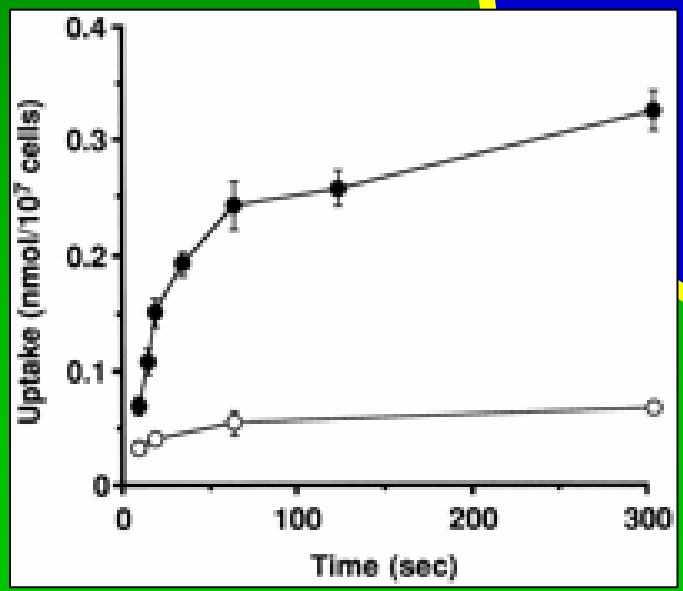
### Carrier-Mediated Lung Distribution of HSR-903, a New Quinolone Antibacterial Agent[1]

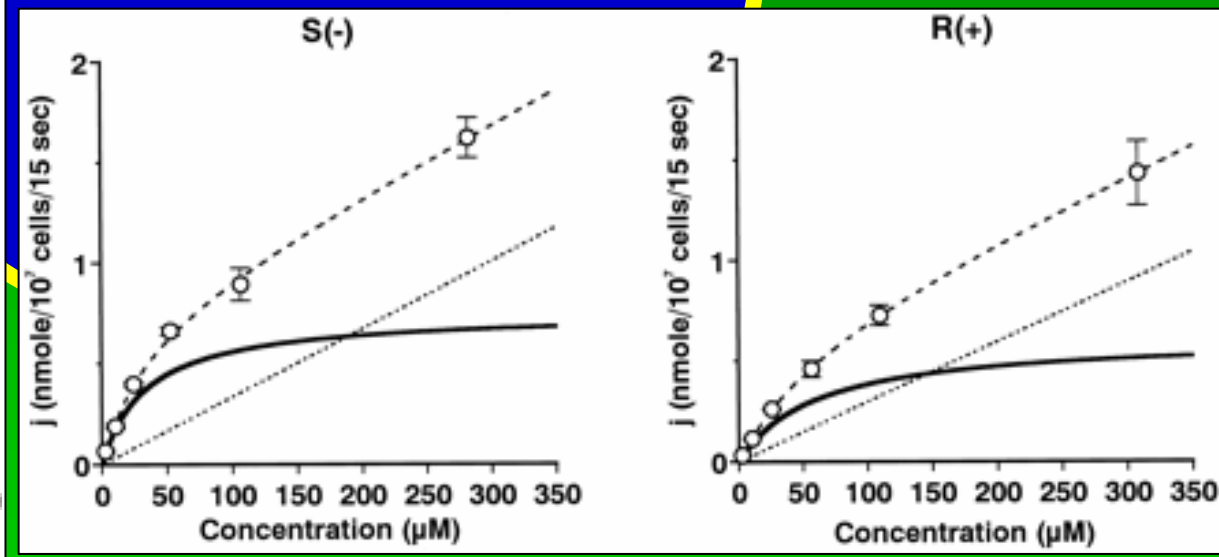
MITSUO MURATA, IKUMI TAMAI, YOSHIMICHI SAI, OSAMU NAGATA, HIDEO KATO, and AKIRA TSUJI

*Department of Pharmacobio-Dynamics, Faculty of Pharmaceutical Sciences, Kanazawa University (M.M., I.T., Y.S., A.T.), Kanazawa, Japan; Research and Development Division, Hokuriku Seiyaku Co. (M.M., O.N., H.K.), Inokuchi, Fukui, Japan; and Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Kawaguchi, Saitama, Japan (I.T., Y.S., A.T.)*

Accepted for publication October 27, 1998 This paper is available online at http://www.jpet.org



# Carrier-mediated influx ?

## the case of HSR-903

TABLE 1

**Inhibitory effect on [$^{14}$C]HSR-903 (10 μM) uptake by unlabeled HSR-903 (*S*-isomer) and its *R*-isomer**

| | Inhibitor concentration | Uptake[a] |
|---|---|---|
| | *μM* | *pmol/10$^7$ cells* |
| Control | 0 | 20.26 ± 1.44 |
| HSR-903 (*S*-isomer) | 50 | 15.23 ± 0.72[b] |
| | 500 | 10.49 ± 0.79[c] |
| *R*-isomer | 50 | 17.68 ± 0.85 |
| | 500 | 11.45 ± 1.31[c] |

Each value indicates mean ± S.E. from four experiments.

[a] Uptake was determined at 15 s.

[b] $P < .05$.

[c] $P < .01$, by ANOVA.

TABLE 3

**Inhibition of [$^{14}$C]HSR-903 (10 μM) uptake by various quinolones (500 μM)**

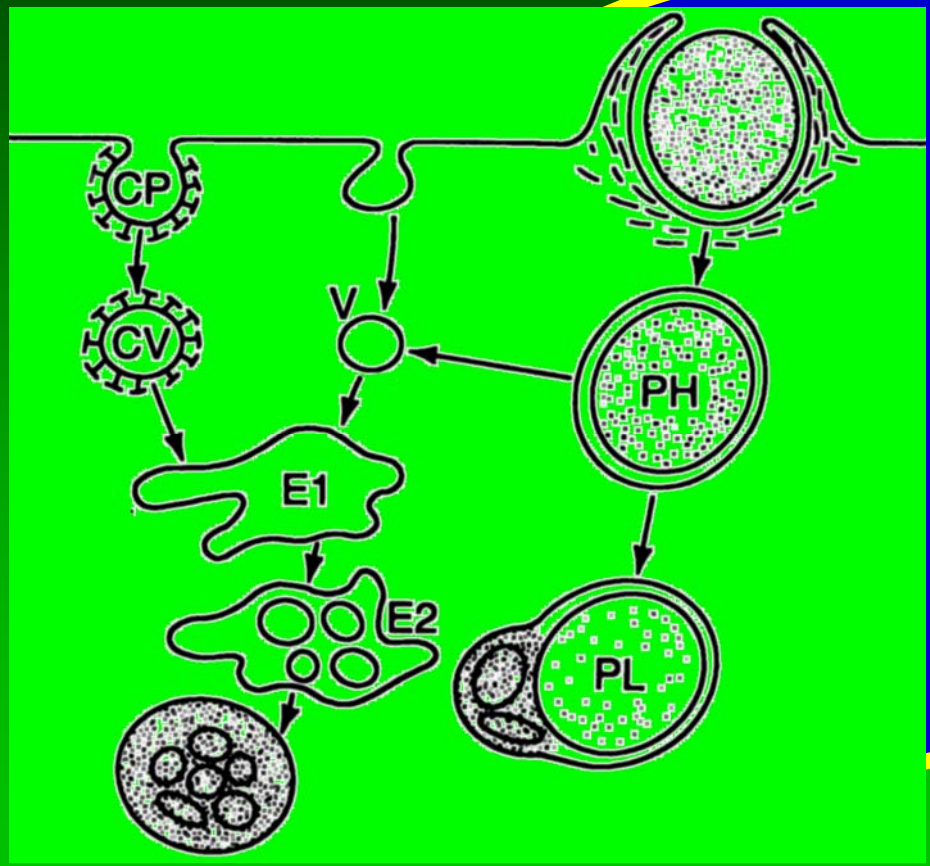
| Inhibitor | Uptake |
|---|---|
| | *% of control* |
| HSR-903 | 51.71 ± 3.78[a] |
| Grepafloxacin | 58.91 ± 4.21[a] |
| Sparfloxacin | 77.01 ± 3.73[b] |
| Levofloxacin | 79.53 ± 5.31 |
| Fleroxacin | 89.85 ± 9.42 |
| Tosufloxacin | 117.97 ± 4.34 |
| Lomefloxacin | 99.23 ± 6.03 |
| Ofloxacin | 88.35 ± 9.81 |
| Enoxacin | 84.91 ± 6.17 |
| Norfloxacin | 90.81 ± 5.14 |

Each value indicates mean ± S.E. from four experiments

[a] $P < .01$.

[b] $P < .05$, by ANOVA.

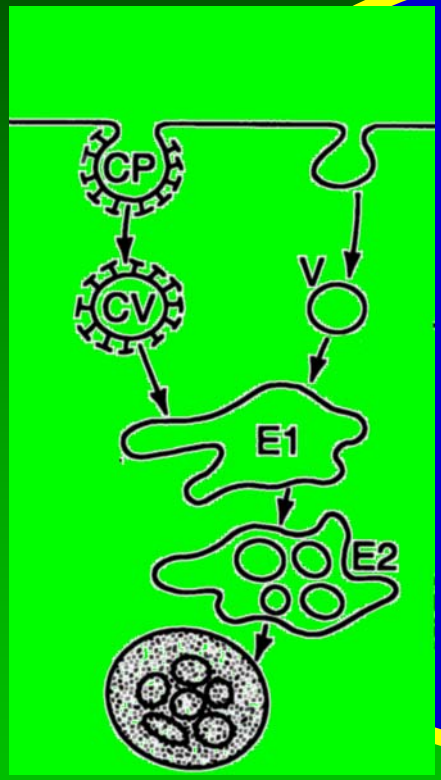
# Endocytosis…




- non-permeant drugs
- slow unless membrane-bound, or receptor-mediated
- confined to vacuolar system

# Endocytosis: examples…

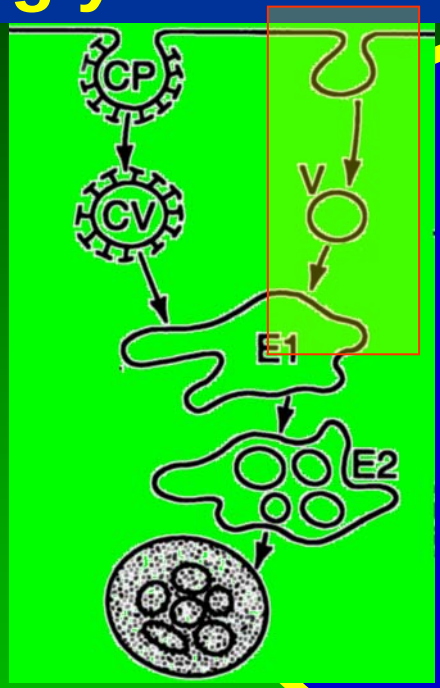
**pinocytosis**

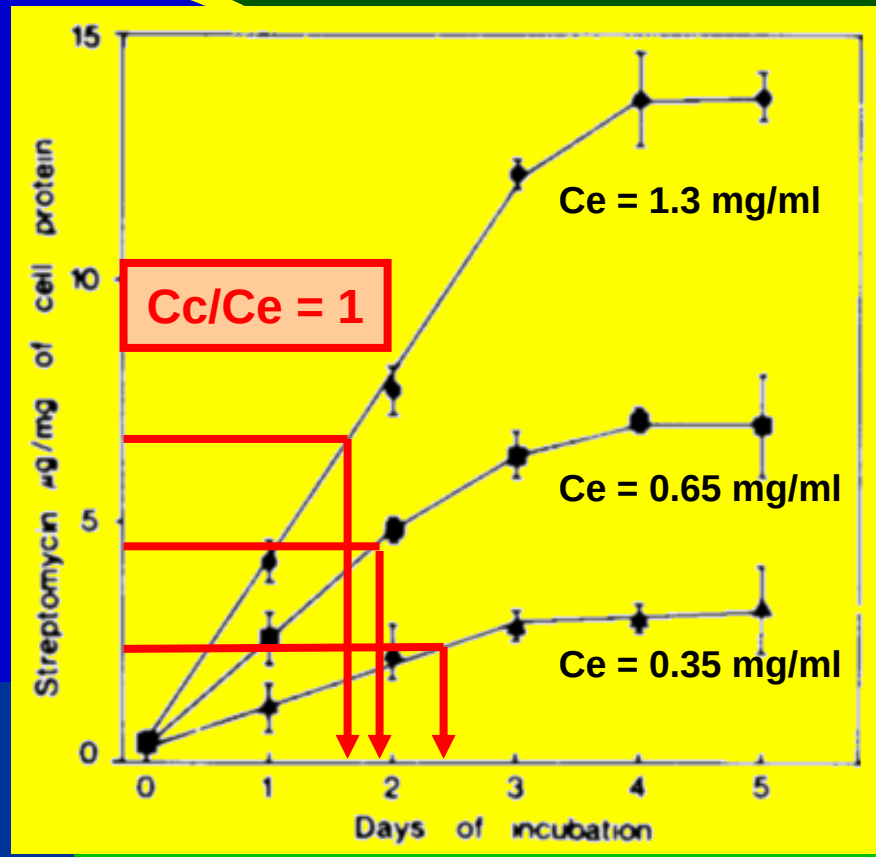



- **aminoglycosides**
- **glycopeptides (?)**

# Fluid-phase endocytosis…

## aminoglycosides in fibroblasts or macrophages

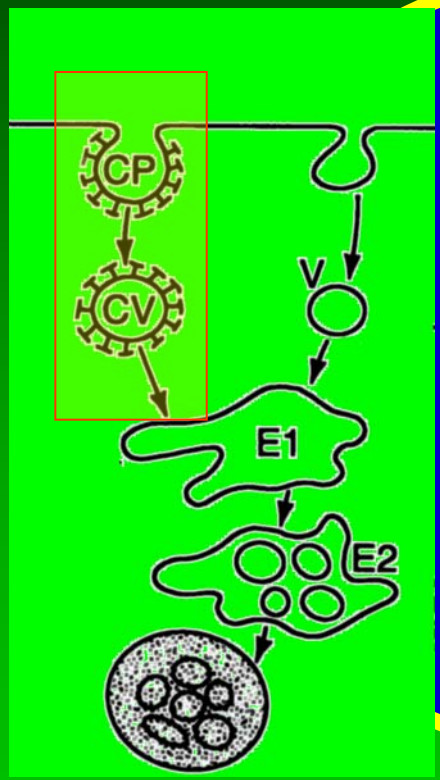



- Slow (days…)
- ill-effective (2-4 fold)
- non-saturable

Tulkens & Trouet, 1978

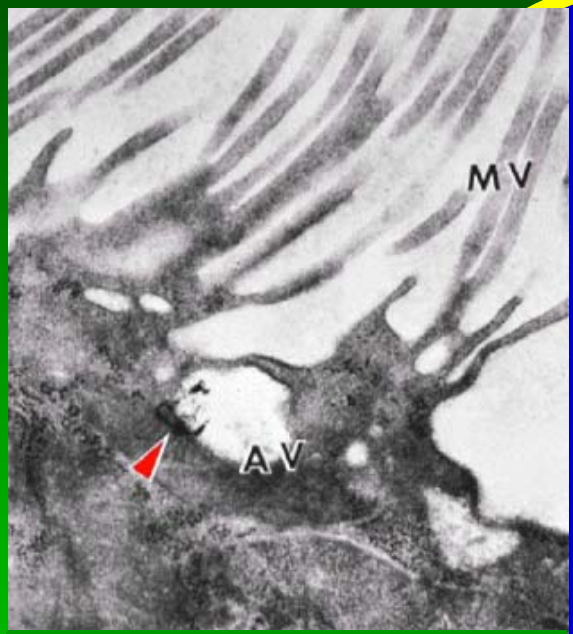
# Receptor-mediated endocytosis…




- fast (days…)
- very effective (100 –fold or more)
- saturable …

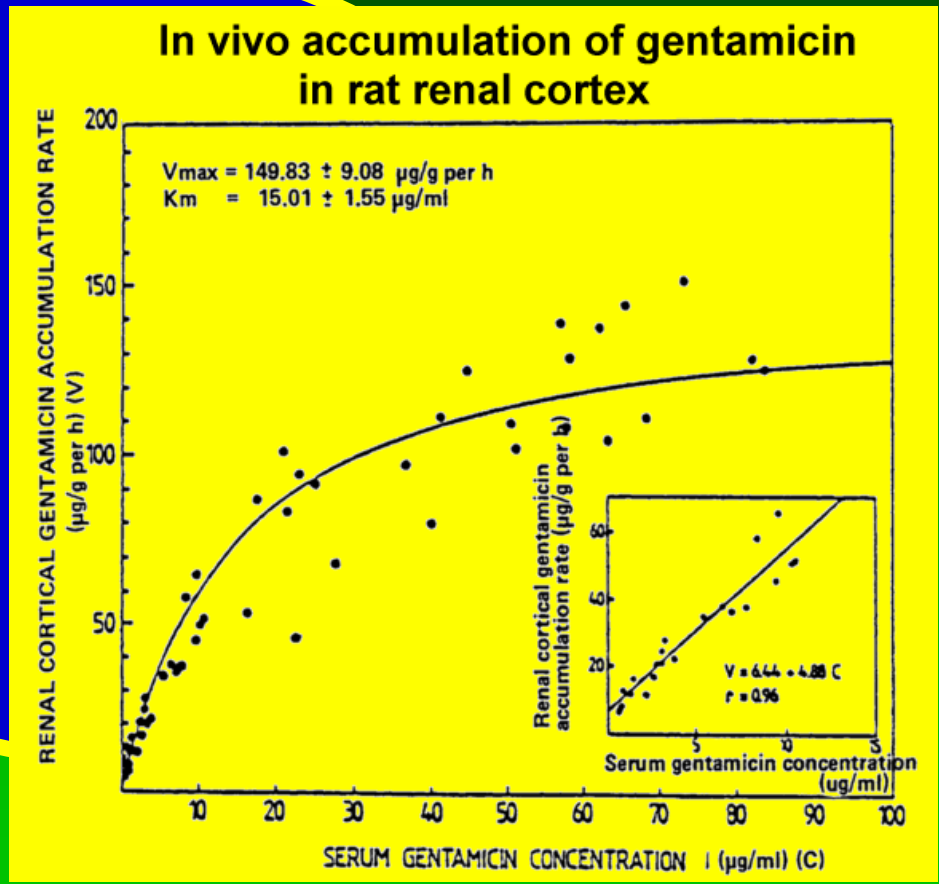
# Receptor-mediated endocytosis…

## entry of aminoglycosides in kidney tubular cells




**binding to**

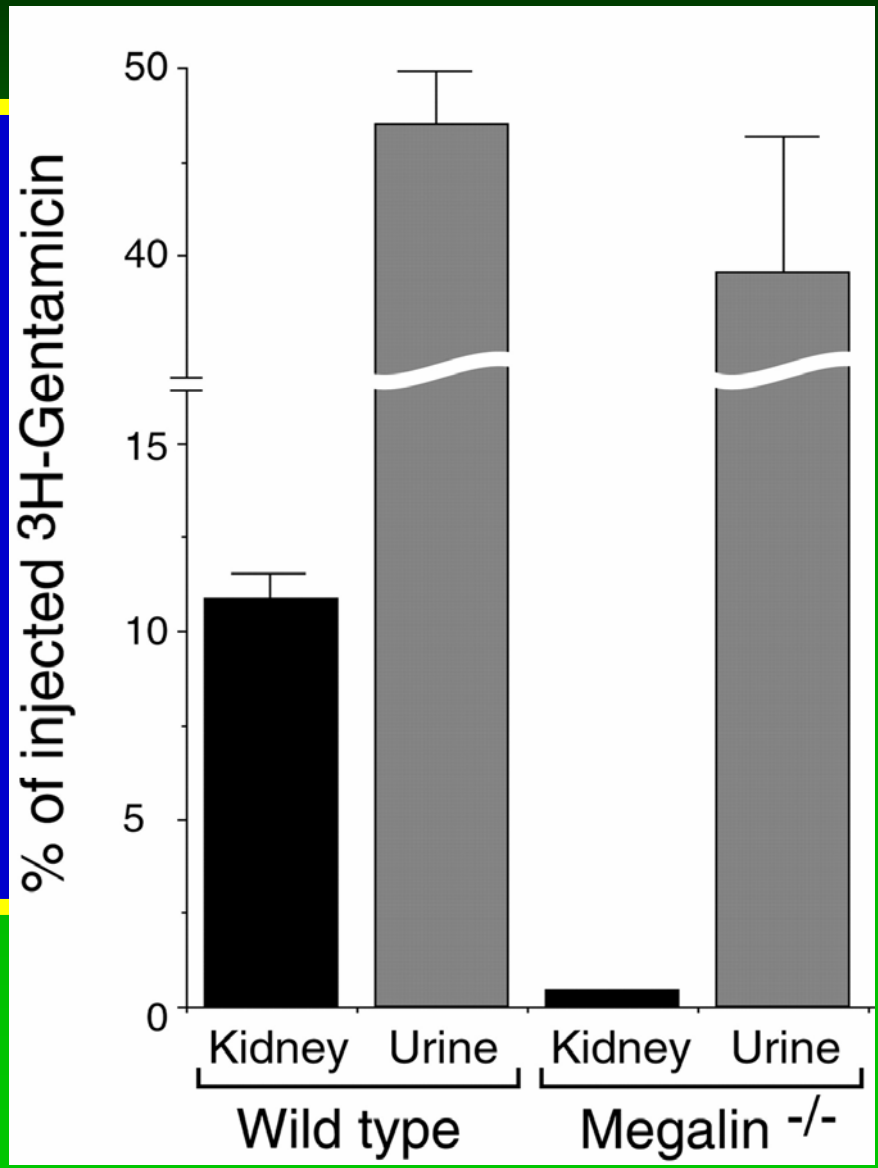
- **megalin**
  (Moeströp et al., 1995)
- **acidic phospholipids**
  (Humes et al, 1983)




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

# Receptor-mediated endocytosis…

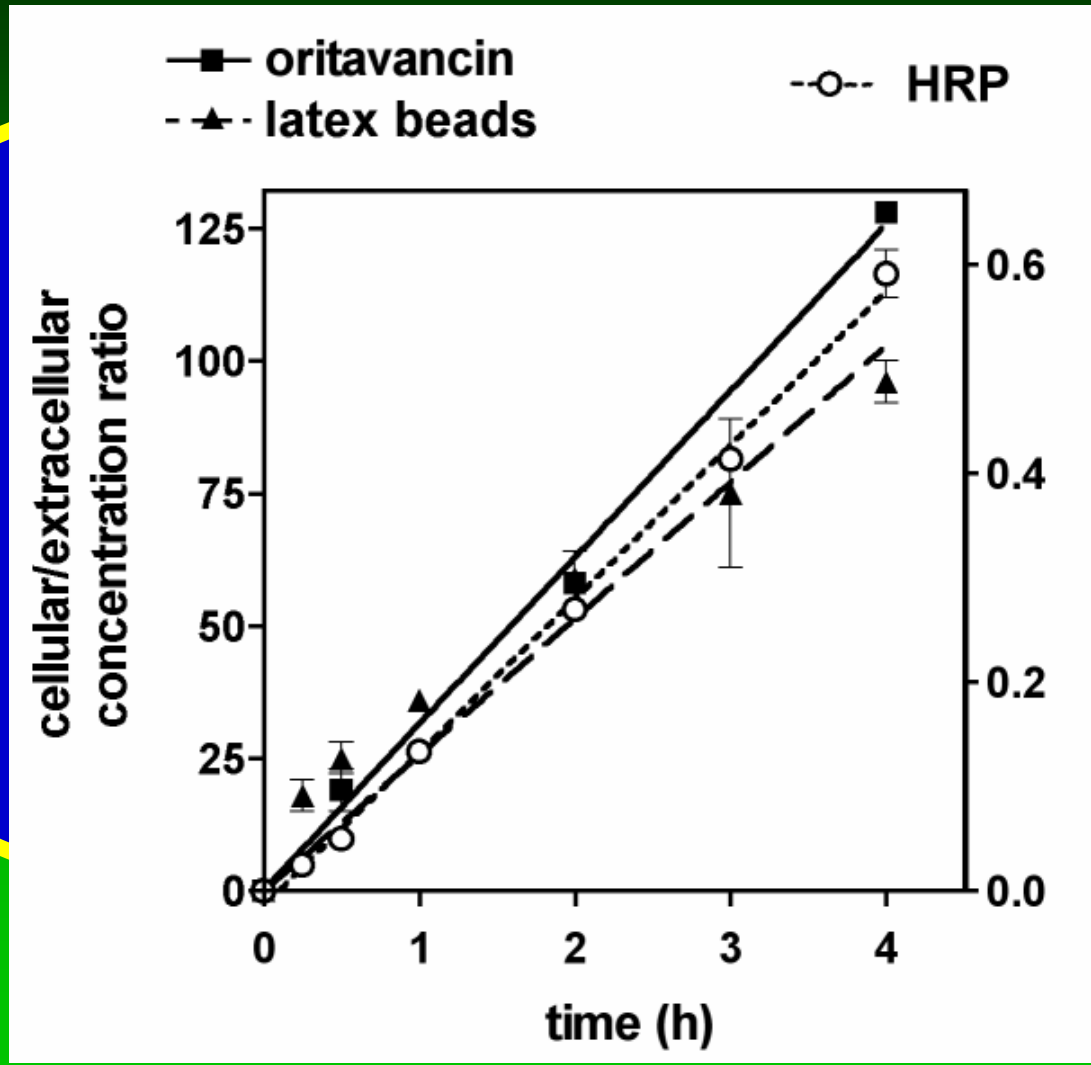
Mice deficient in megalin do not accumulate gentamicin in kidney




Schmitz et al., J. Biol. Chem. 277:618-622, 2002

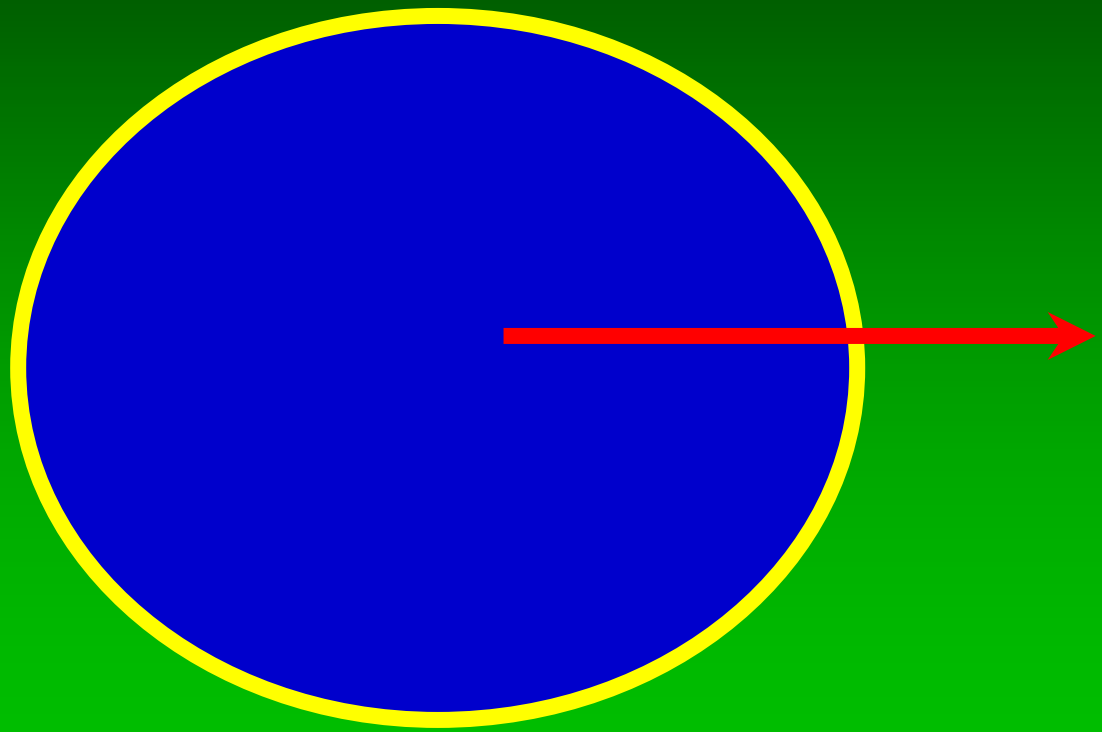
# Membrane-binding-mediated endocytosis ? …

- very effective if tight binding (100 –fold or more)
- continuous over time …

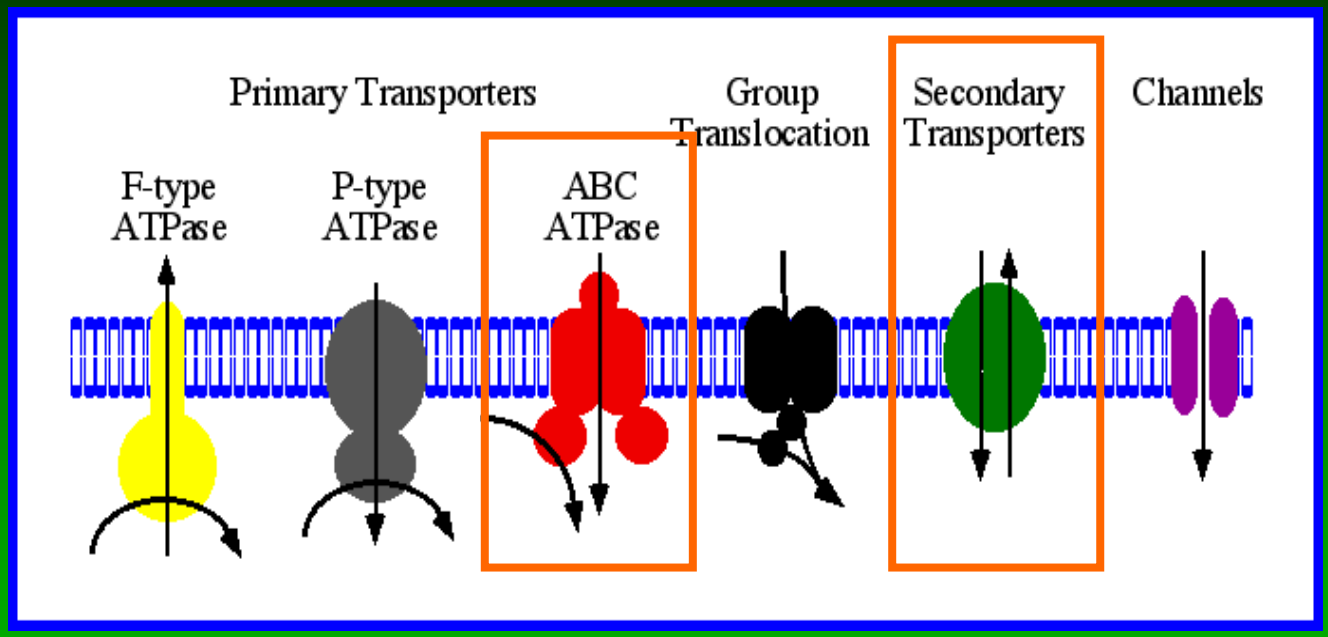



Van Bambeke et al., AAC (2004) 48:2853-2860

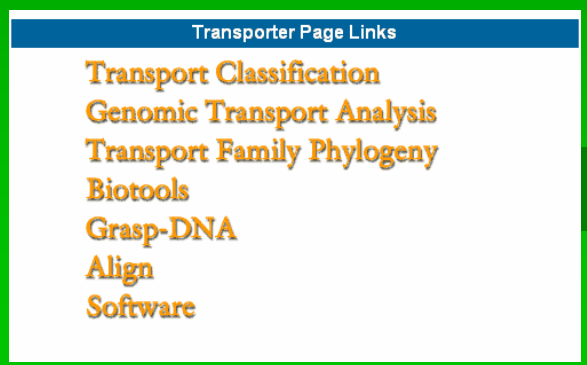
# Antibiotics efflux ?



# Transporters - data bases




main drug transporters




http://www-biology.ucsd.edu/~msaier/transport/

Saier, 2000

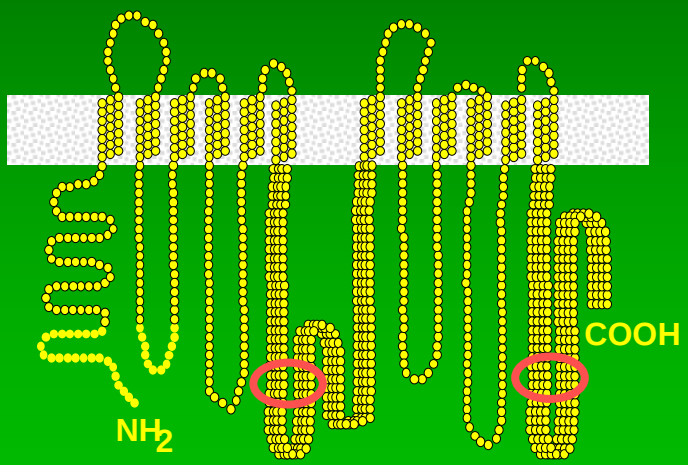
# Transporters involved in the efflux of antibiotics from eucaryotic cells

| superfamily | transporter | physiol. substrates | antibiotics |
|---|---|---|---|
| ABC | MDR1 | phospholipids | fluoroquinolones<br>macrolides<br>β-lactams<br>tetracyclines<br>streptogramins |
| | MRP1 | phospholipids<br>leukotrienes<br>conjugates | fluroquinolones<br>macrolides<br>rifamycins |
| | MRP2 | conjugates | fluoroquinolones<br>β-lactams |
| MFS | NPT1 | phosphates | β-lactams |
| OAT | OATP1 | bile salts<br>steroids | β-lactams |

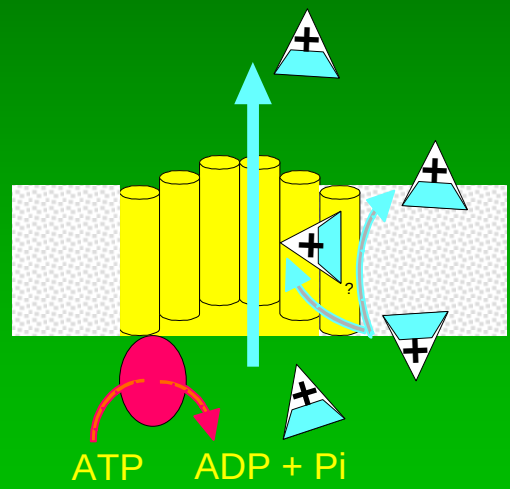
# Most frequent antibiotic-pumps in eucaryotes (1/2)

## Multiple Drug Resistance (MDR also known as PgP)

### TOPOLOGY




### MECHANISM




### ANTIBIOTICS

tetracyclines
fluoroquinolones
erythromycin
lincosamides
rifampicin

chloramphenicol

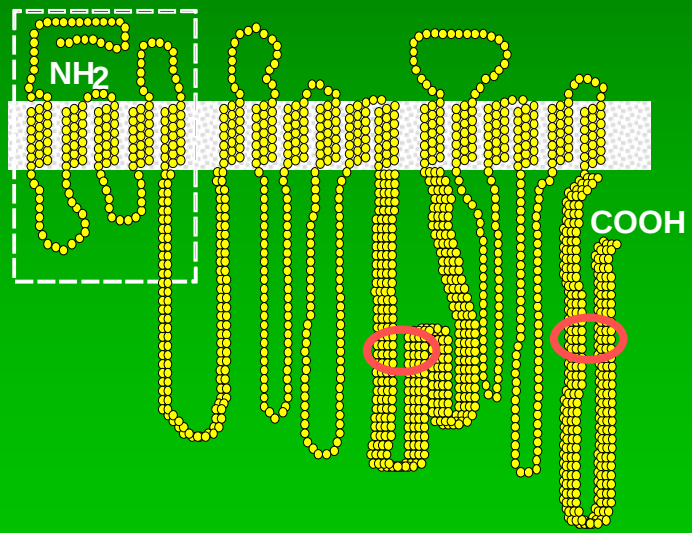
aminoglycosides

Van Bambeke et al.,Biochem. Pharmacol. 2000

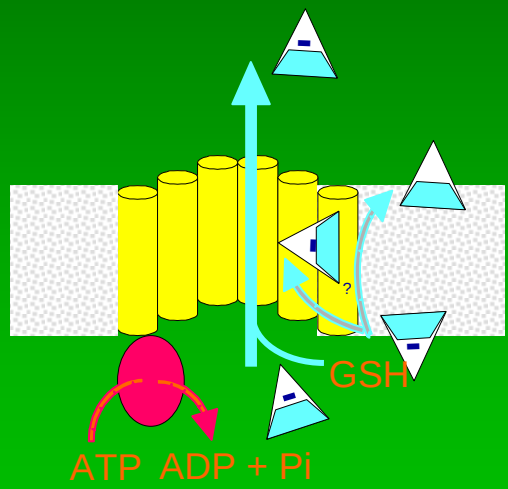
# Most frequent antibiotic-pumps in eucaryotes (2/2)

## Multidrug Resistance Proteins (MRP)

### TOPOLOGY




### MECHANISM




### ANTIBIOTICS

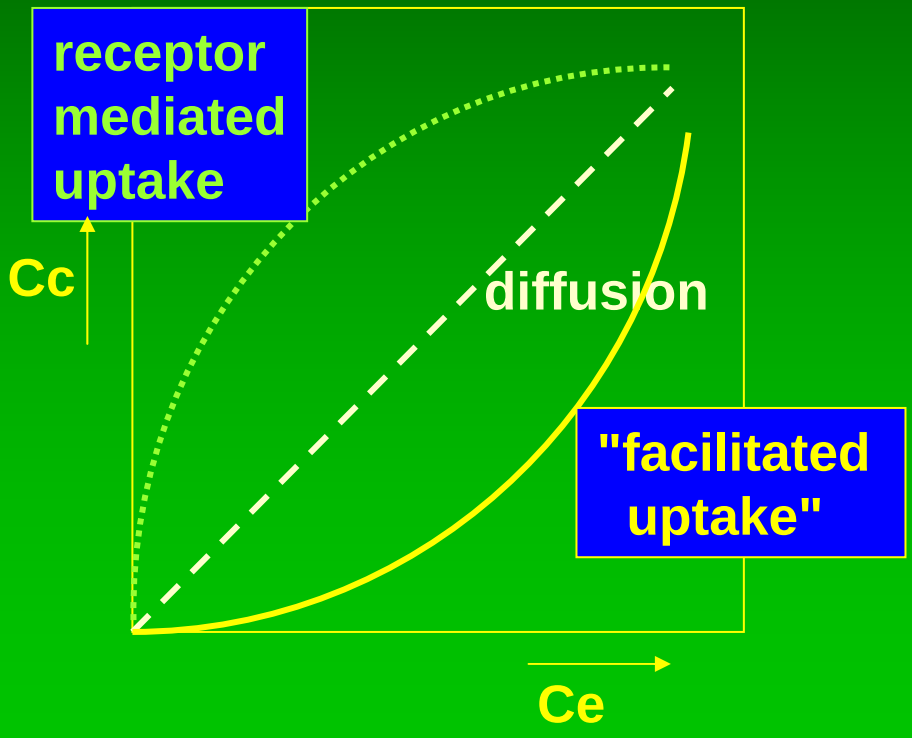
fluoroquinolones

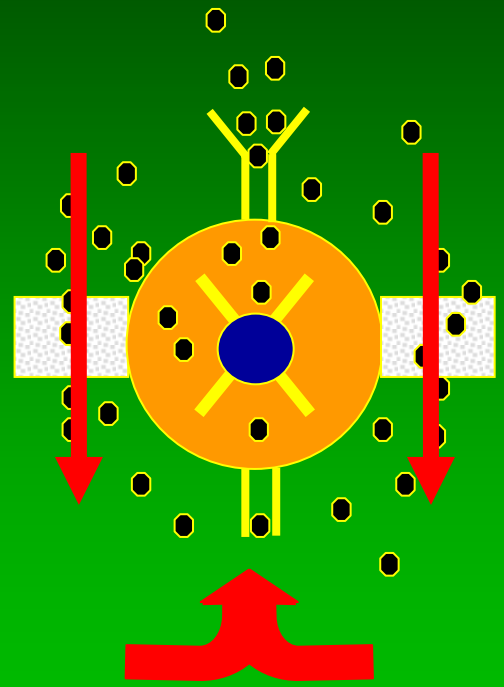
tetracyclines
macrolides

Van Bambeke et al.,Biochem. Pharmacol. 2000

# Evidencing active efflux ...

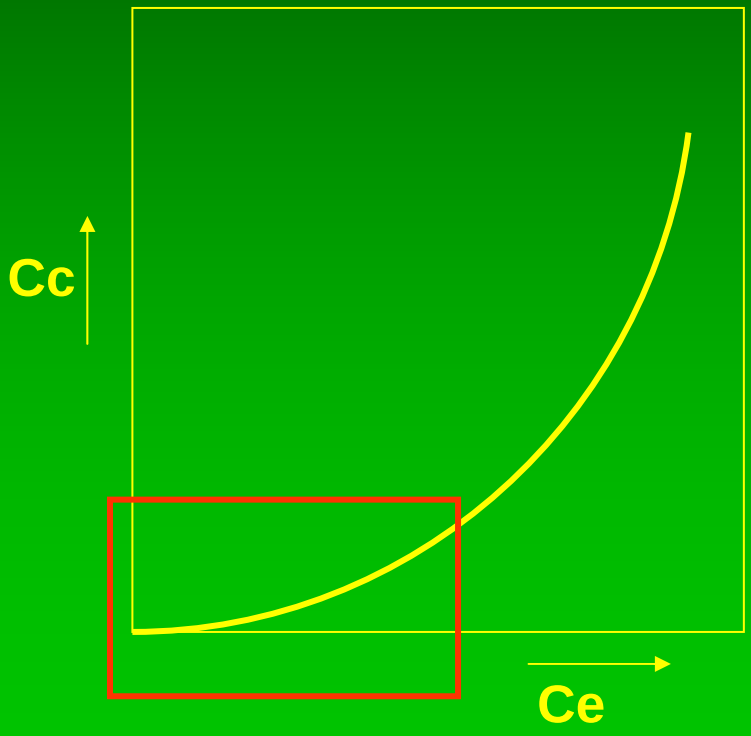
**non linear accumulation kinetics ...**

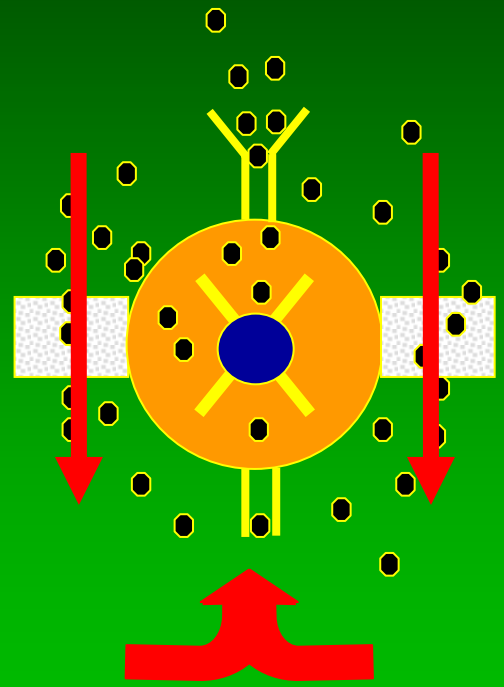


# Evidencing active efflux ...

**non linear accumulation kinetics ...**

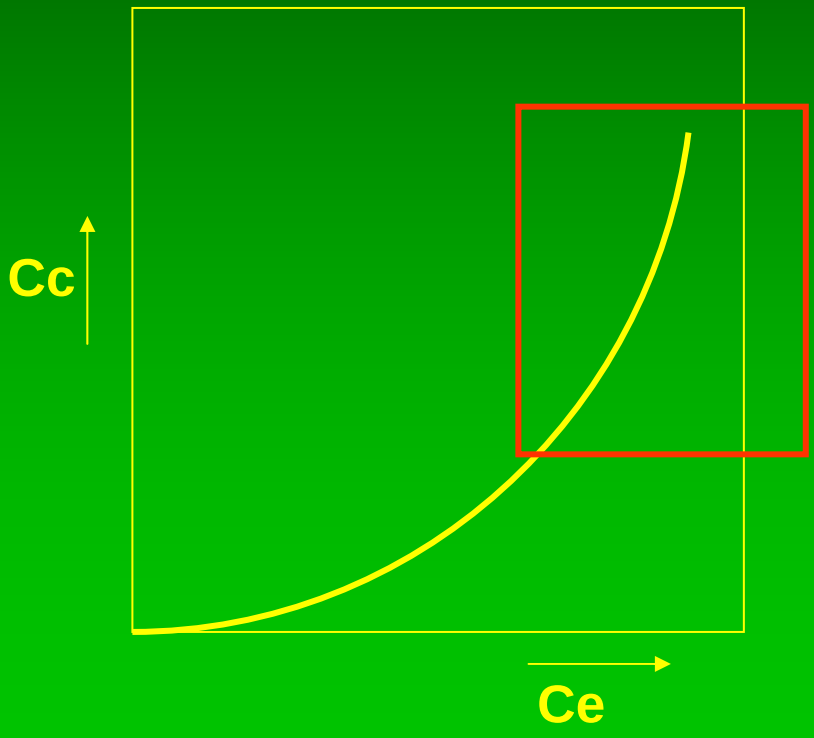


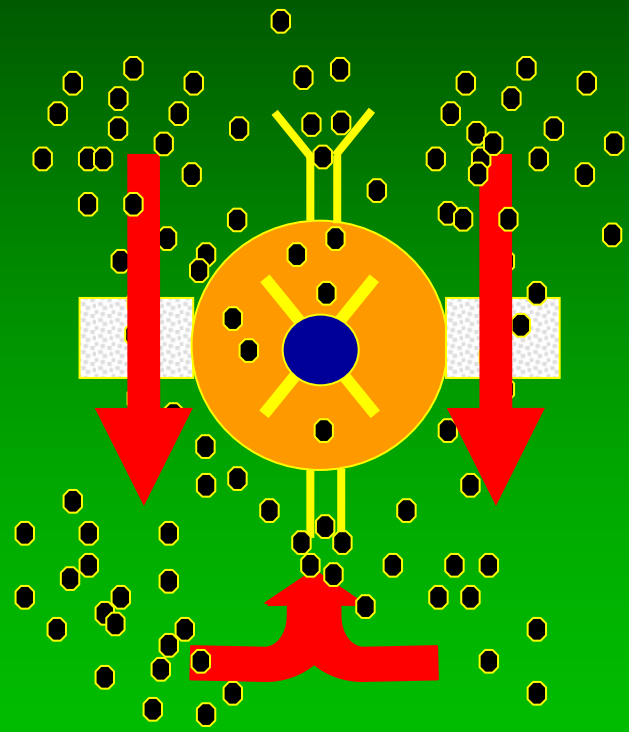



**at low concentrations, most of the drug is rexported …**

# Evidencing active efflux ...

**non linear accumulation kinetics ...**



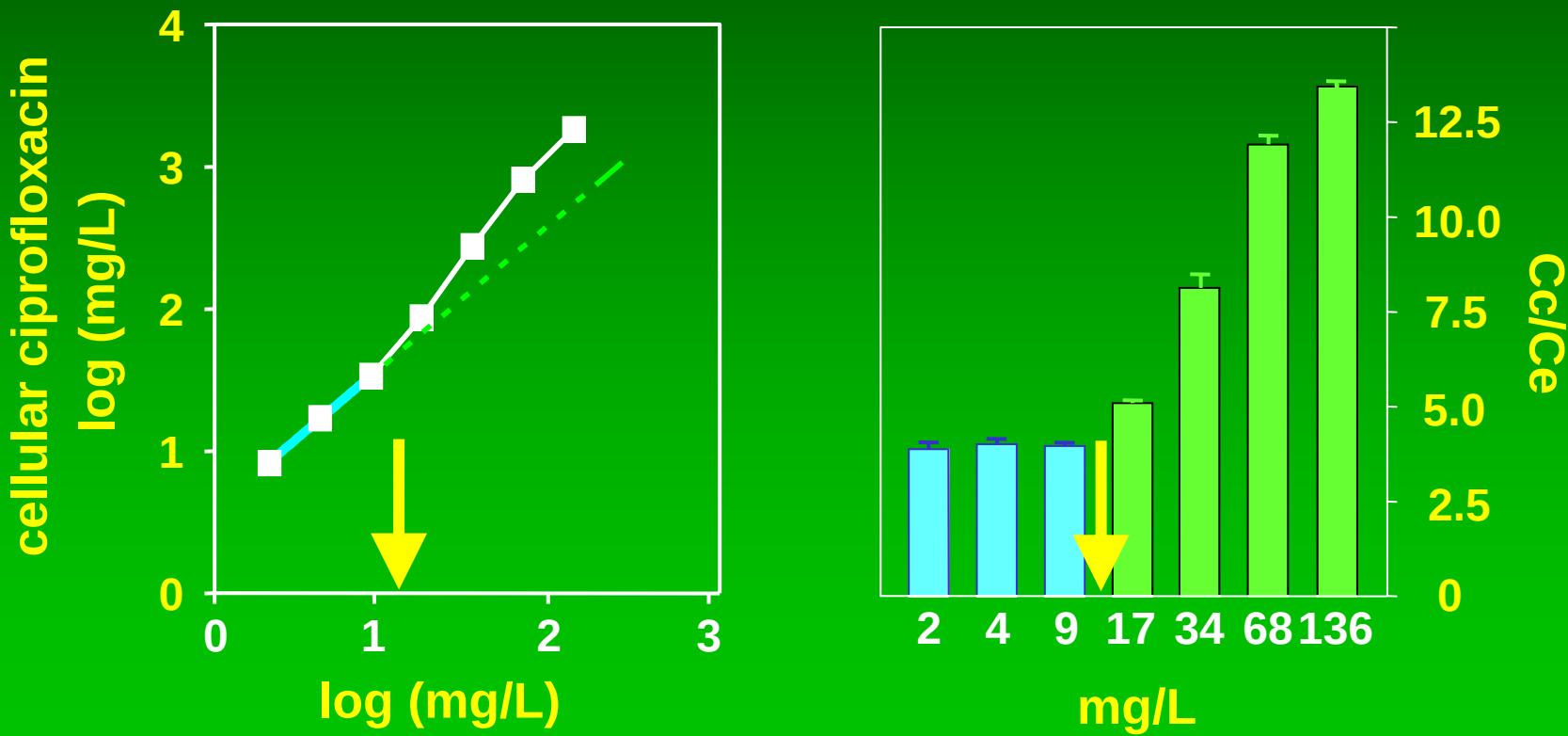



**at large concentrations, efflux becomes saturated**

# Evidencing efflux of ciprofloxacin

**Ciprofloxacin accumulation in J774 macrophages is facilitated upon increase of its extracellular concentration**

Michot et al., AAC (2004) 48:2673-2682



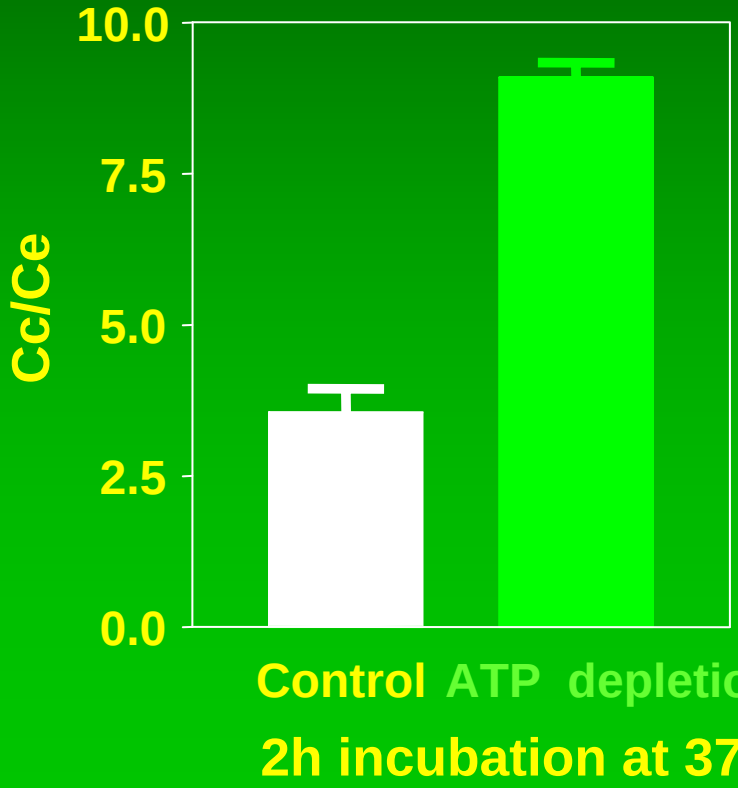

**extracellular [ciprofloxacin] - 2h incubation at 37°C**

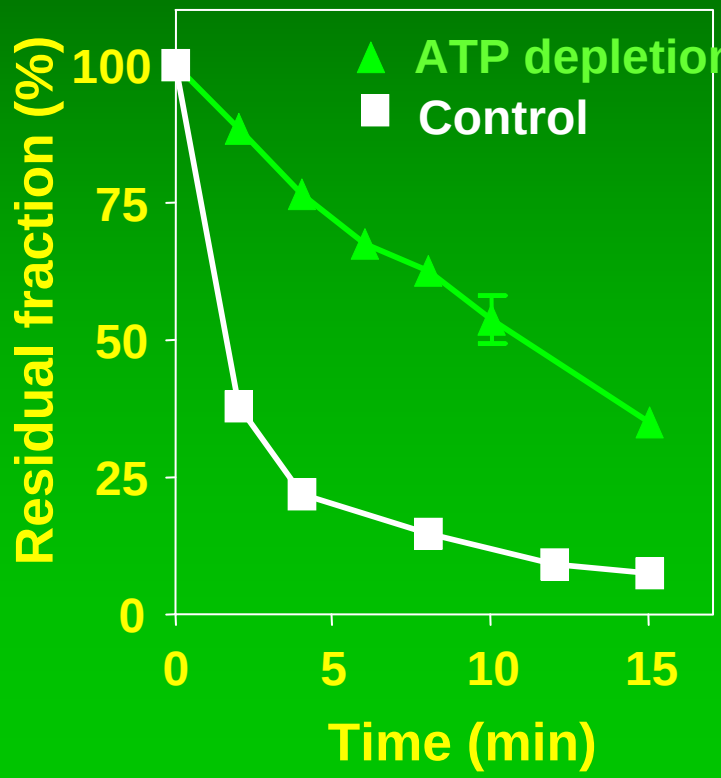
# Characterization of the transporter(s)

**ATP depletion increases ciprofloxacin accumulation and decreases ciprofloxacin efflux in J774 macrophages**

Michot et al., AAC (2004) 48:2673-2682

**Ce = 50 µM (17µg/ml)**

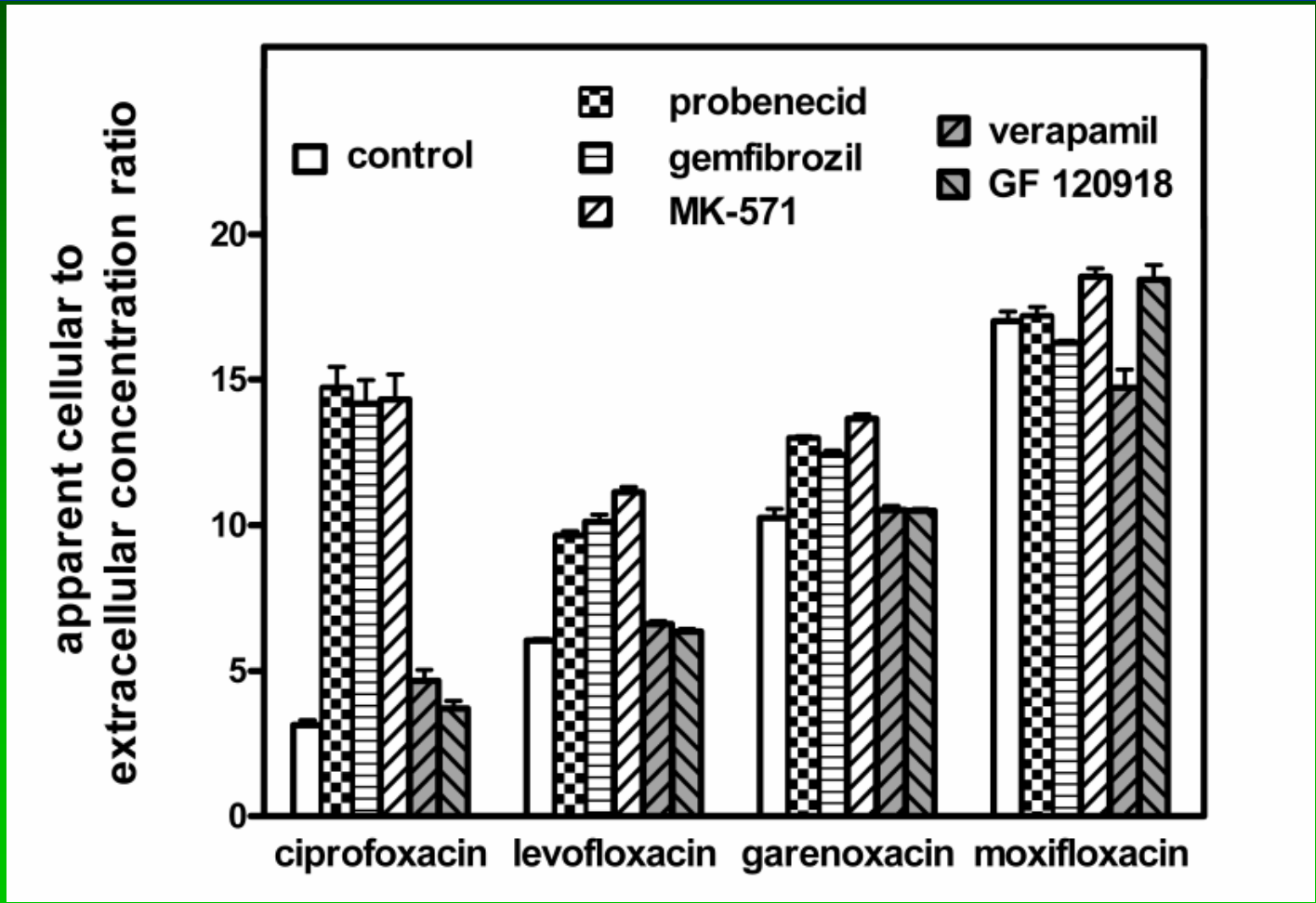


# Efflux vary among closely related derivatives and may be impaired by (apparently) unrelated substances

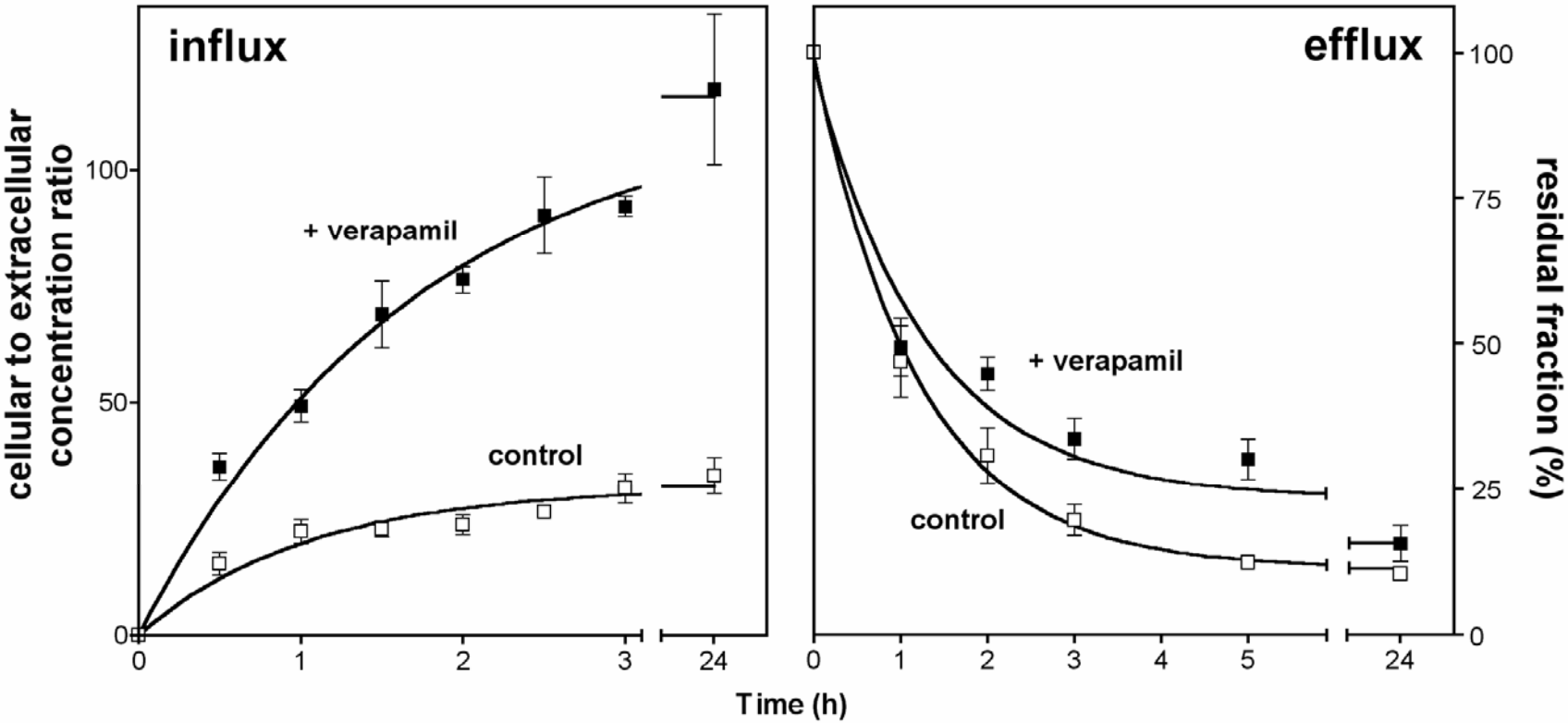
## accumulation of quinlones in J774 macrophages and influence of P-gp and MRP inhibitors

Michot et al. AAC (2005) 49:2429-2437



# Evidencing efflux of azithromycin macrolides (through P-gp) …

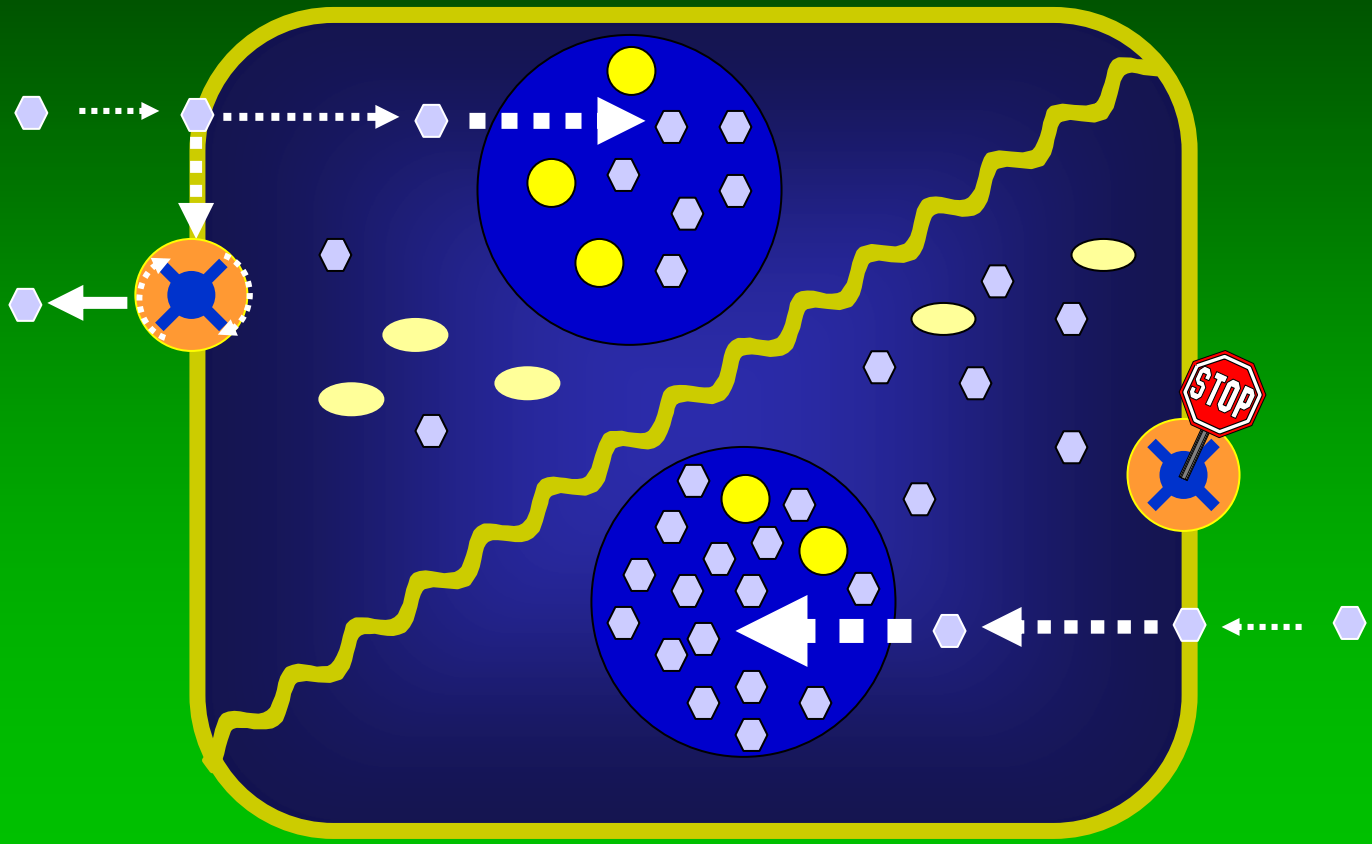
**Kinetics of influx and efflux of azithromycin in J774 murine macrophages with or without 20 µM verapamil.**




Seral et al., AAC (2003) 47:1047-1051

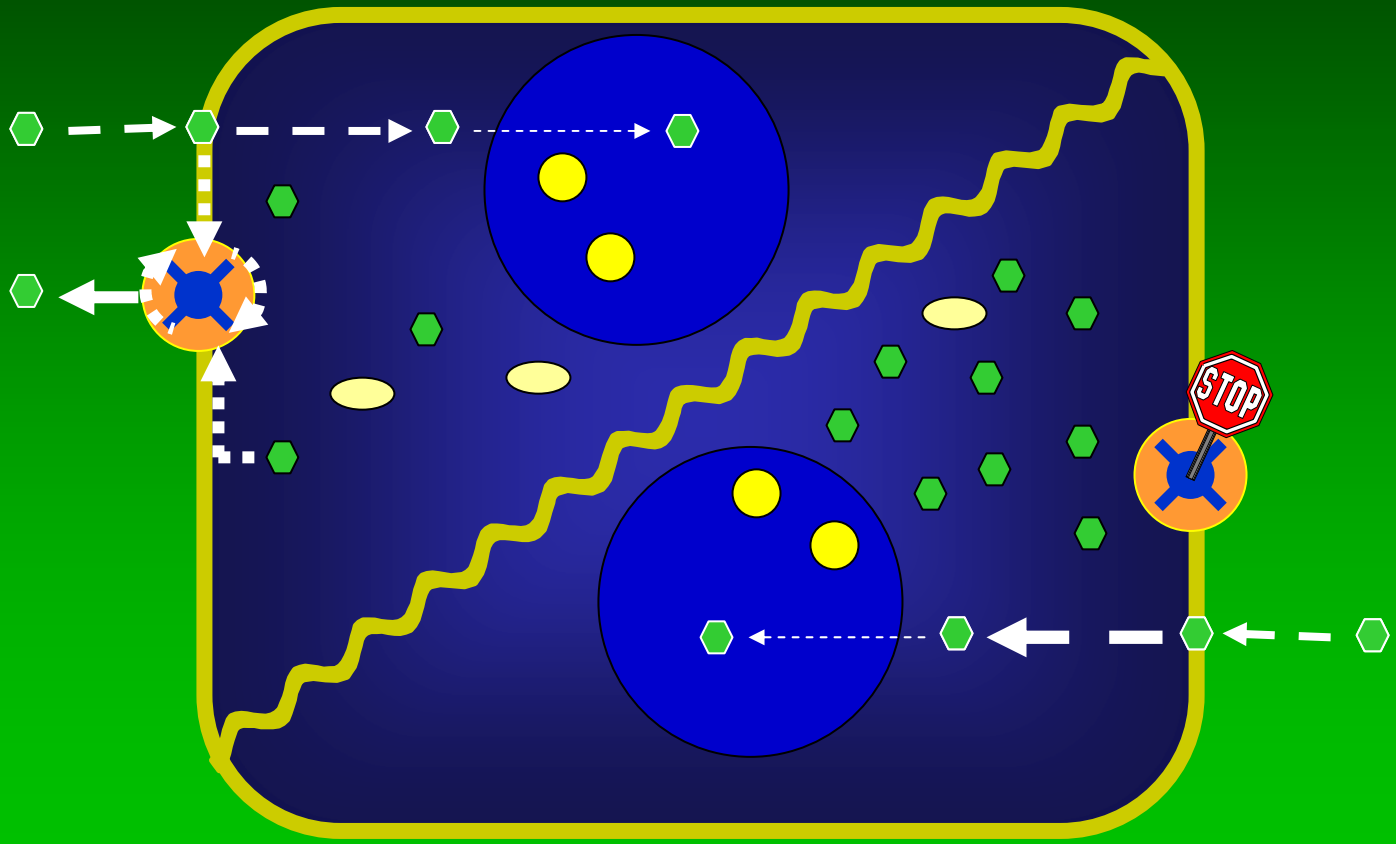
# Azithromycin follows the a ‘kick-back’ model

Gaj *et al*. (1998) Biochem. Pharmacol. 55:1199-211

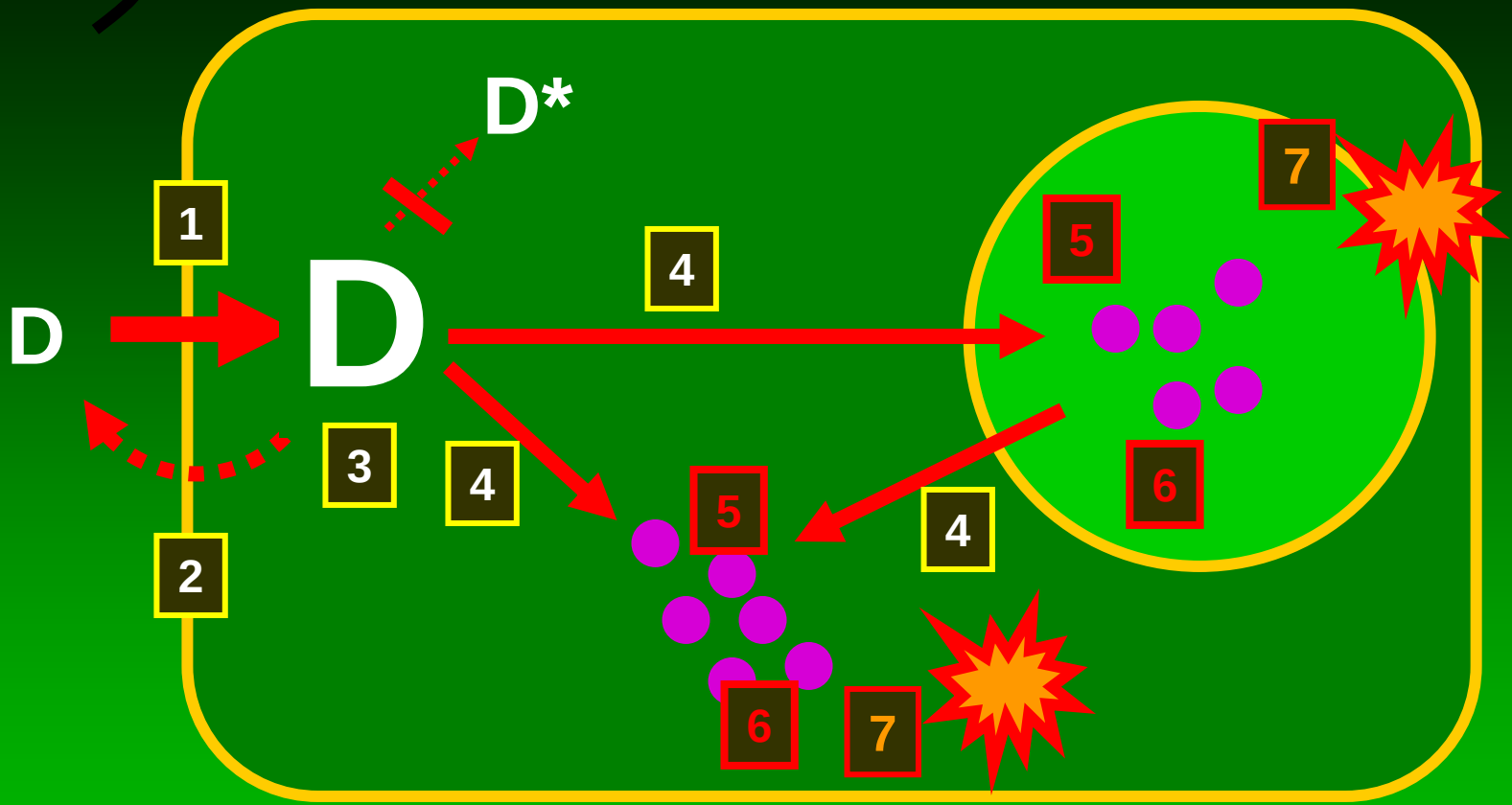


# Ciprofloxacin is follwing the classical model

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



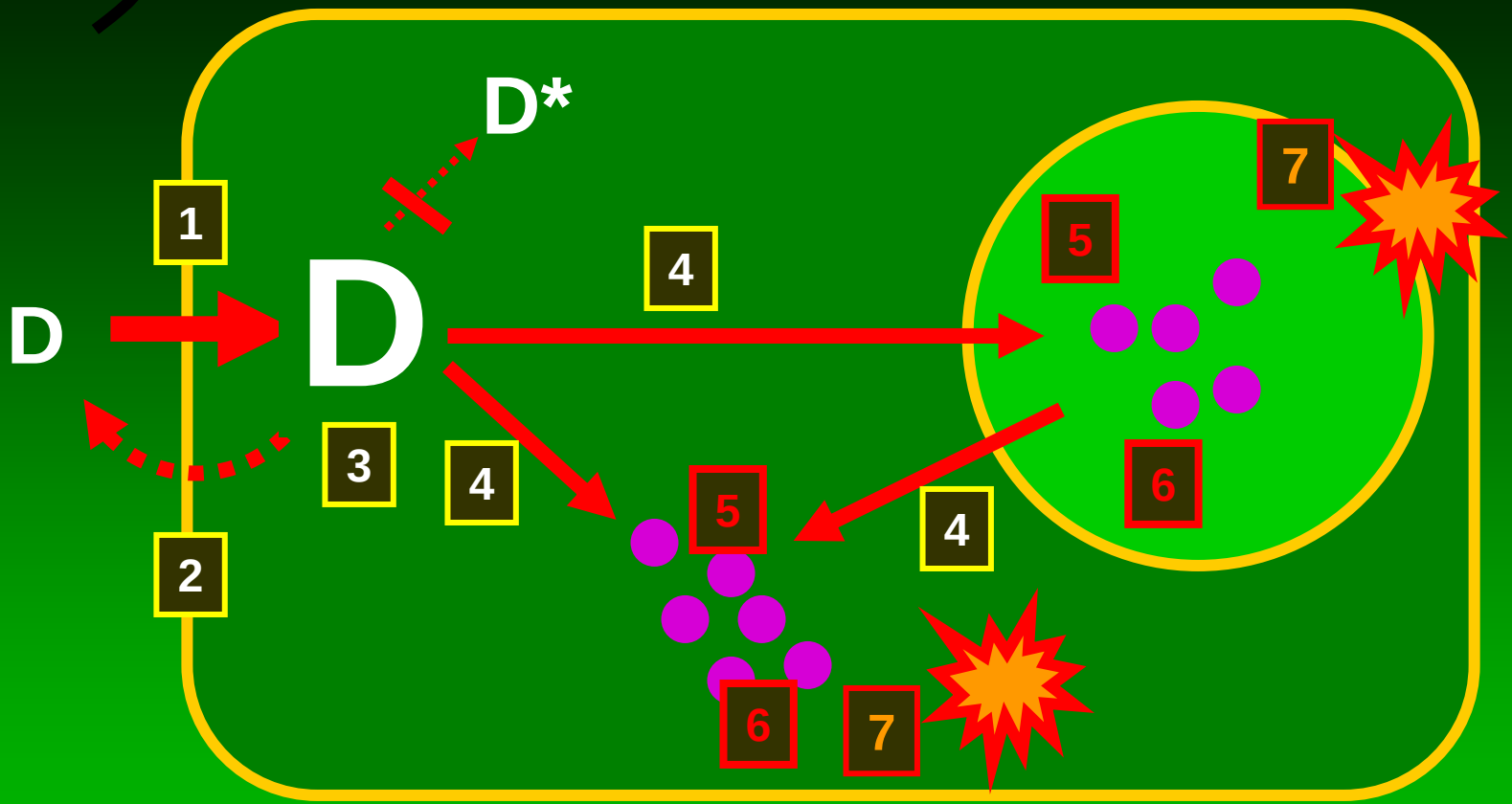
# Any relation to activity ?




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

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

# Co-workers on all this stuff ...

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