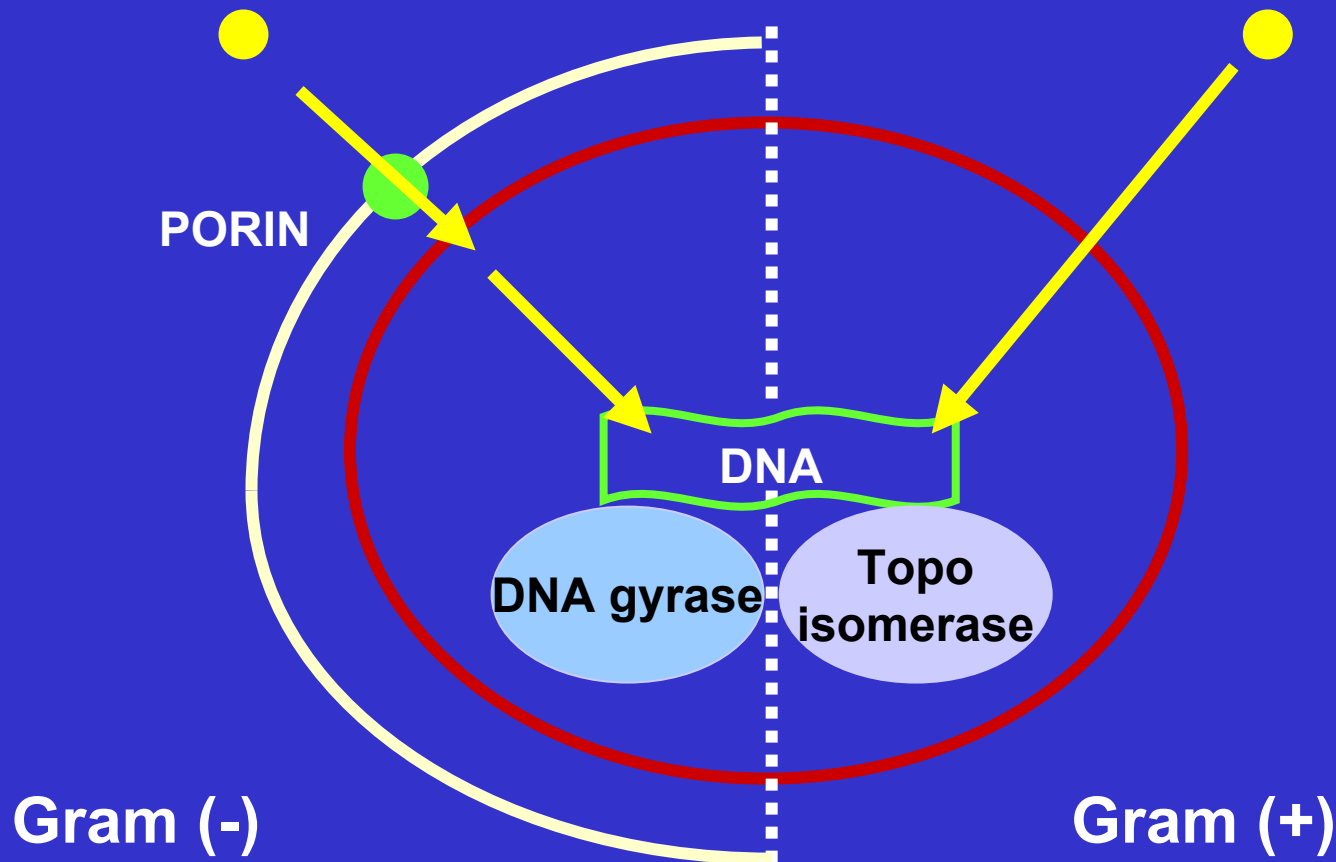
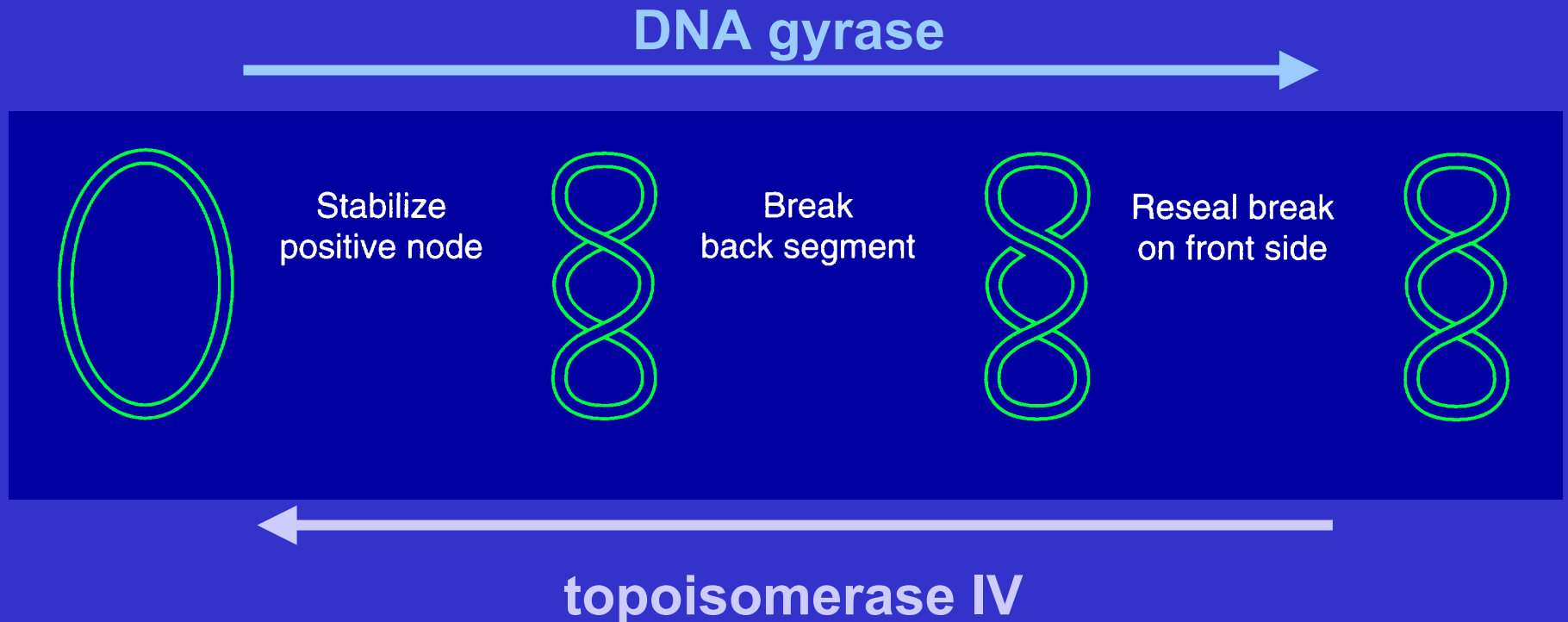


# Mechanism of action of fluoroquinolones: the basics...



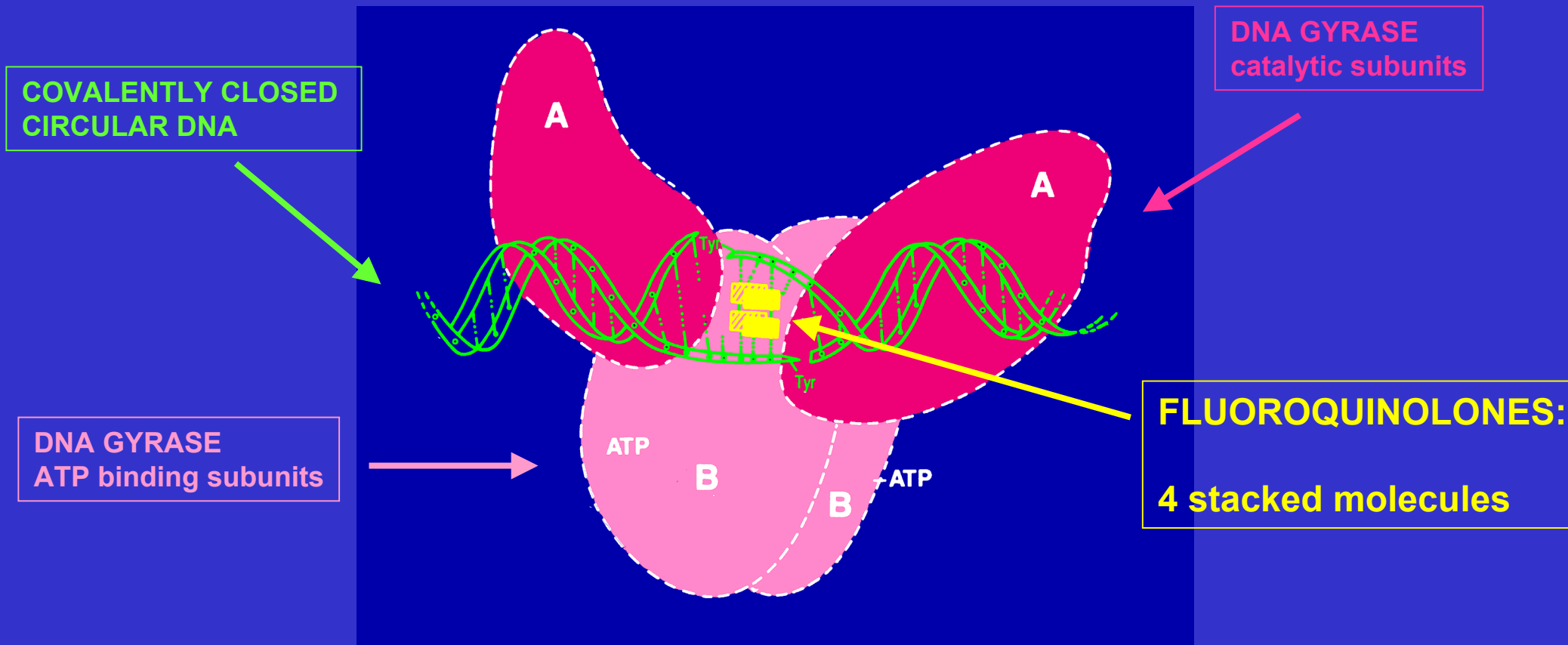
## 2 key enzymes in DNA replication:



**bacterial DNA is supercoiled**

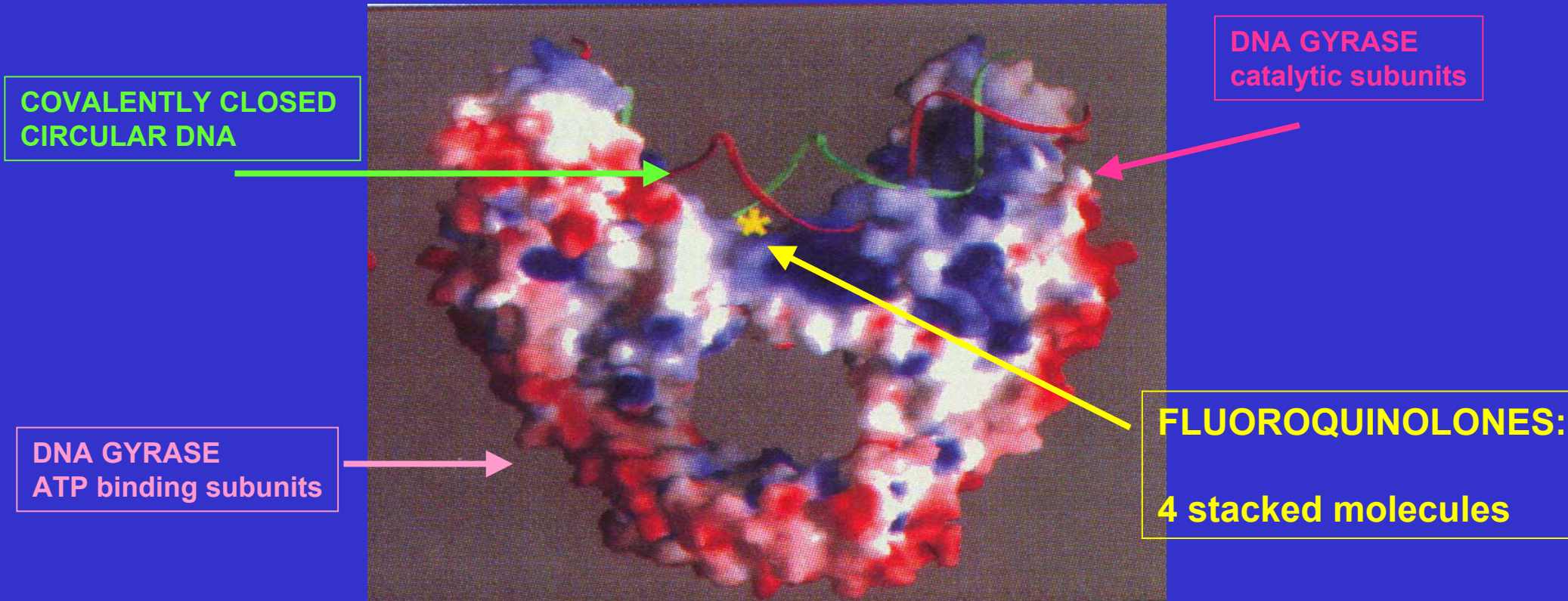
# Ternary complex

## DNA - enzyme - fluoroquinolone



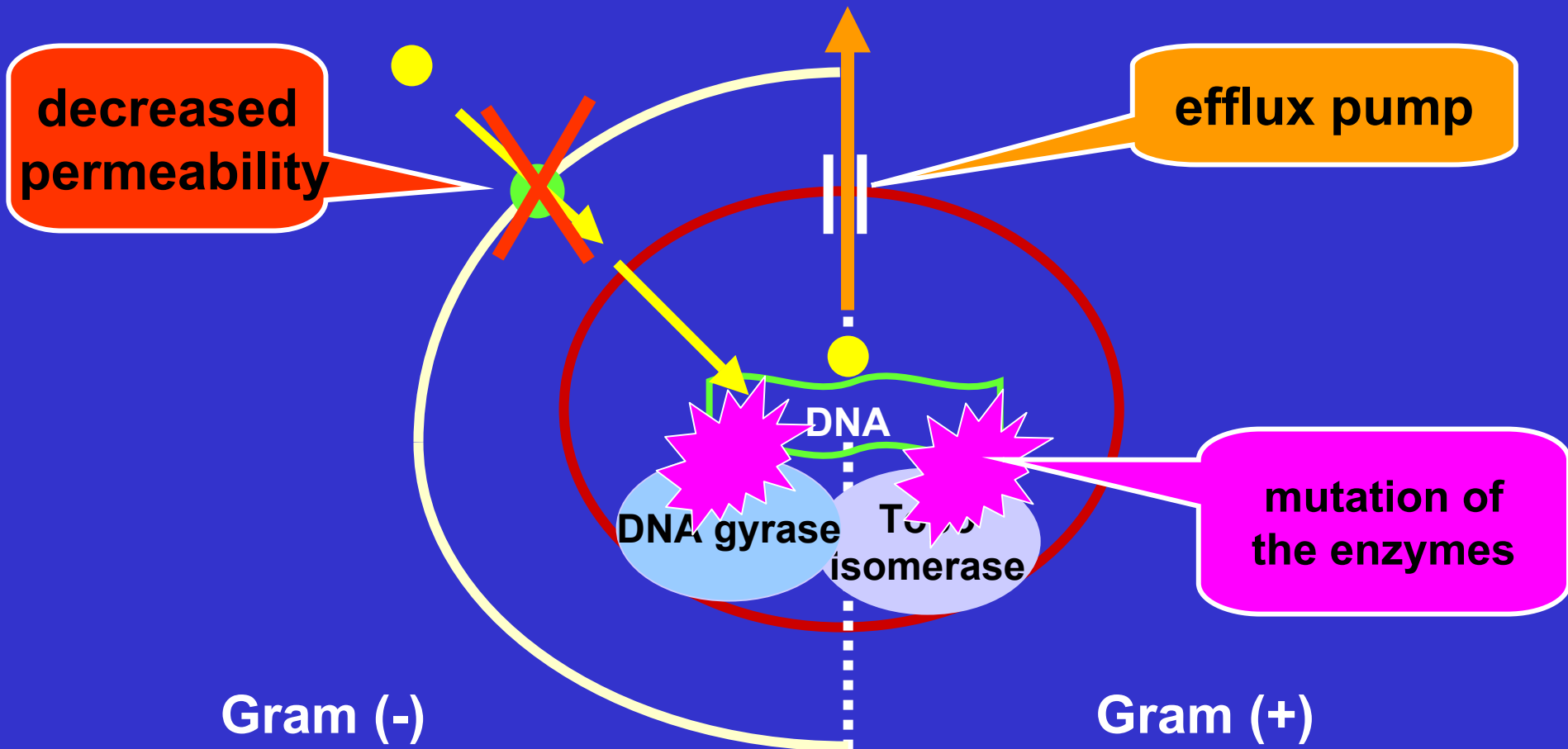
(Shen, *in* Quinolone Antimicrobial Agents, 1993)

# Ternary complex DNA - enzyme - fluoroquinolone

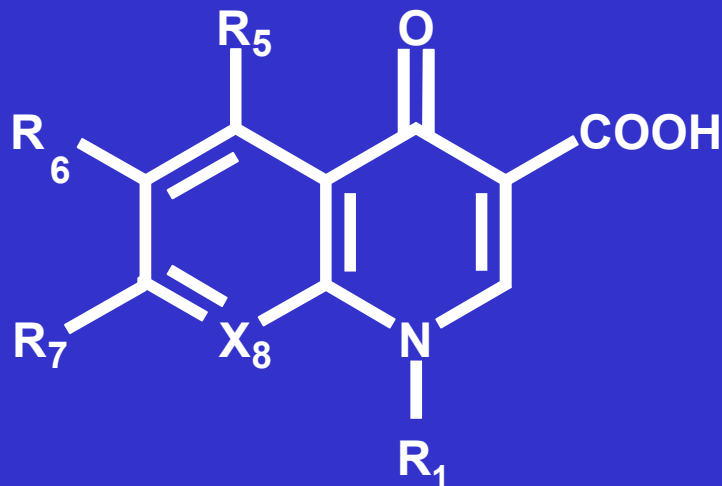


Cabral *et al.*, Nature, 1997

# Resistance to fluoroquinolones: the basics



**Fluoroquinolones are the first entirely  
man-made antibiotics:  
do we understand our molecule ?**



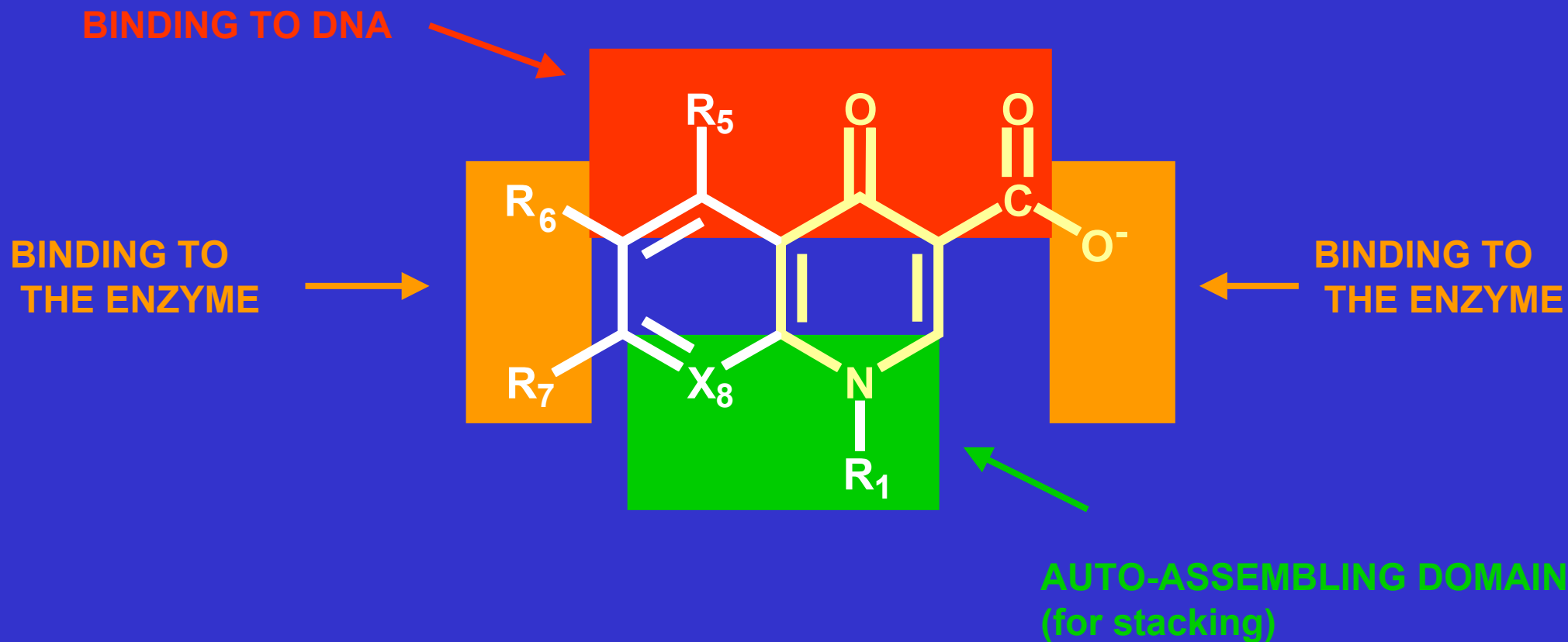
**Don't panic, we will travel together....**

# Chemistry and Activity



This is where all begins...

# The pharmacophore common to all fluoroquinolones



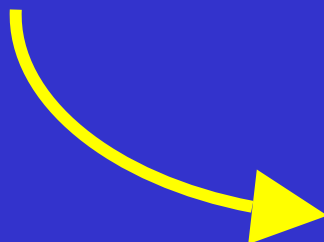


# From chloroquine to nalidixic acid...

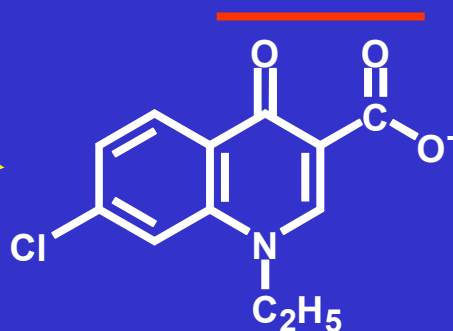


chloroquine

1939



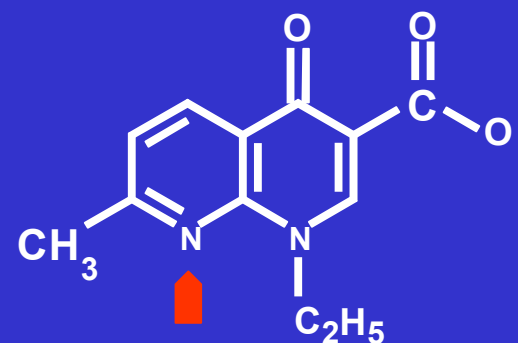
1958



7-chloroquinoline  
(synthesis intermediate  
found to display  
antibacterial activity)

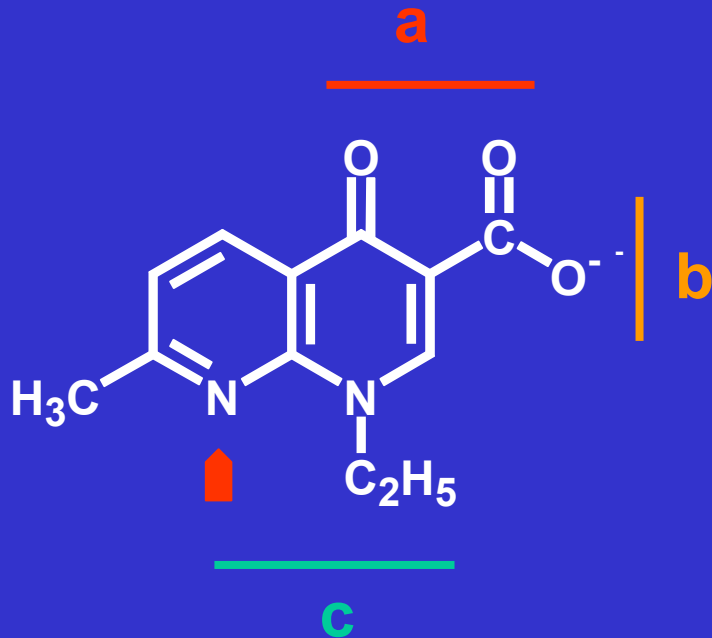



nalidixic acid



1962

# Nalidixic acid \*

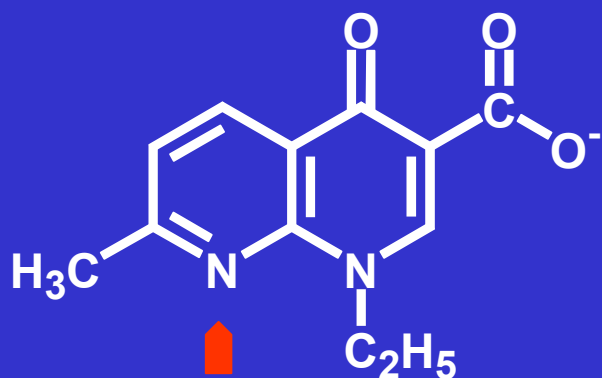


- typical chemical features of fluoroquinolones (a, b, c)  
BUT a naphthridone  
(N at position 8: )
- limited usefulness as drug
  - narrow antibacterial spectrum (*Enterobacteriaceae* only)
  - short half-life (1.5h)
  - high protein binding (90%)

\* Belg. pat. 612,258 to Sterling Drugs, 1962

# From nalidixic acid to the 1st fluoroquinolone (1 of 4)

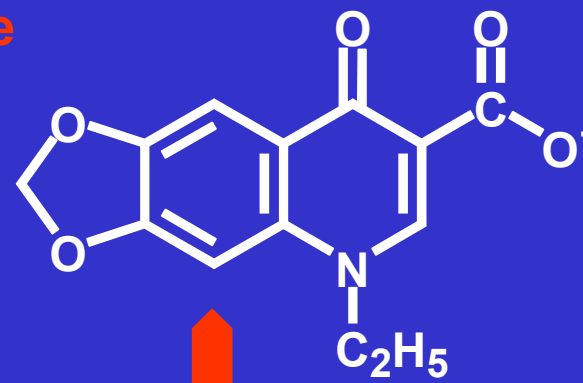
nalidixic acid



1. modify naphthyridone  
into quinolone



oxolinic acid \*



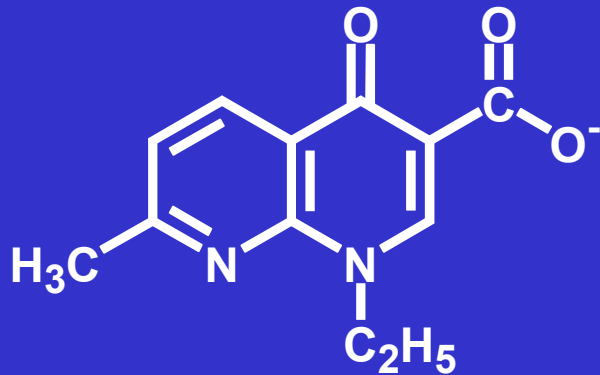
shows reduced protein binding...

\* Ger. pat. to Warner Lambert, 1967

\* quinoleine

# From nalidixic acid to the 1st fluoroquinolone (1 of 4)

nalidixic acid



2. discovery of  
flumequine \*



flumequine \*



shows weak but broad  
Gram(-) activity

\* Ger pat. to Rikker Labs, 1973

\* benzo-quinolizine

# From nalidixic acid to the 1st fluoroquinolone (1 of 4)

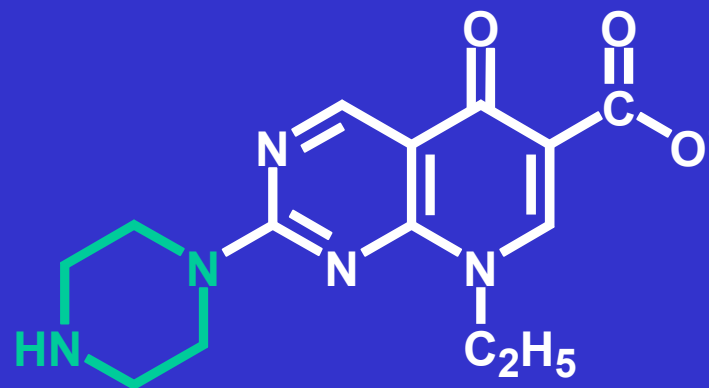
nalidixic acid



3. introduce a  
piperazine \*



pipemidic acid \*



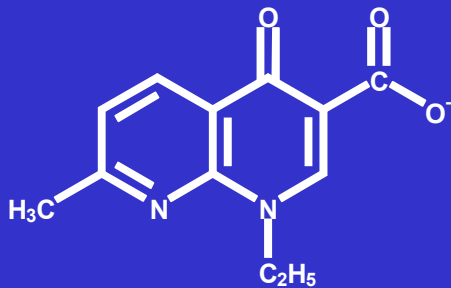
shows longer half-life...

\* Ger. Pat. to Roger Bellon, 1974

\* pyrido-2-3-pyrimidine

# From nalidixic acid to the 1st fluoroquinolone (1 of 4)

nalidixic acid

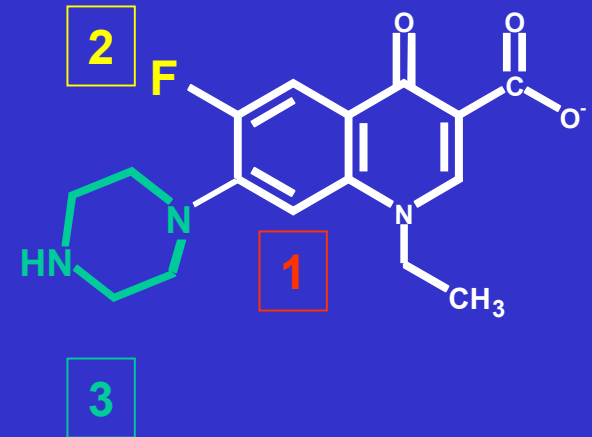


combine all 3  
features \*...



1978

norfloxacin \*



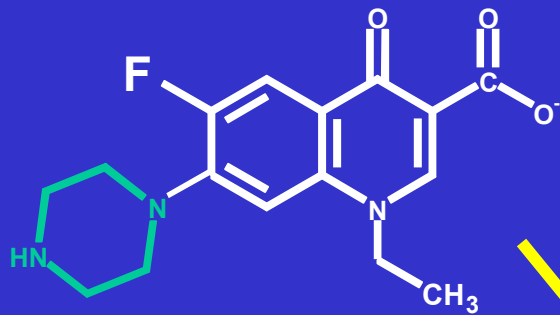
broader Gram(-) activity  
less protein binding (50%)  
longer half-life (3-4h)

\* Belgian patent 863,429, 1978 to Kyorin

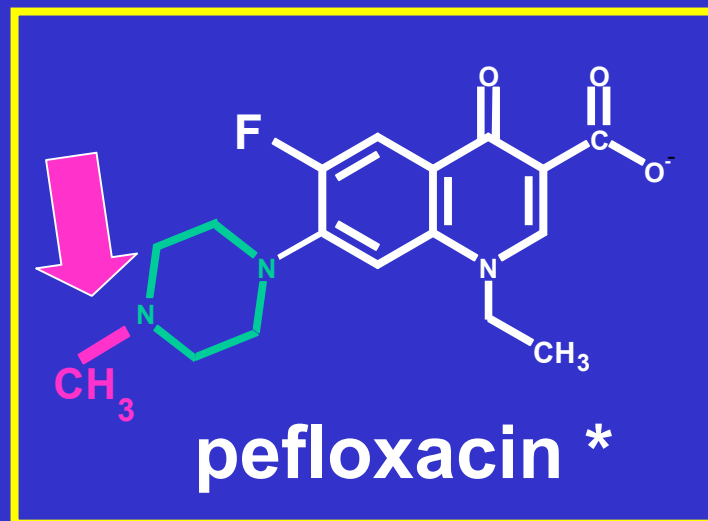
\* 6-fluoro-7-pyrimidino-quinoleine

# From norfloxacin to the other 1st generation fluoroquinolones: pefloxacin

norfloxacin



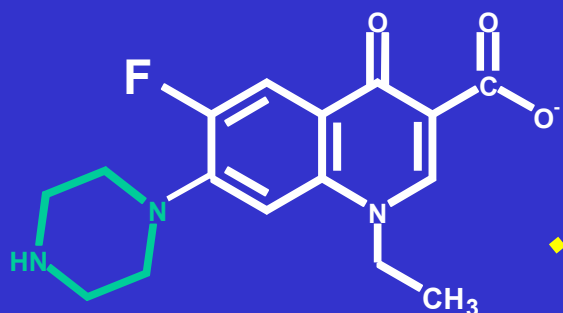
**Add a methyl  
to still increase  
half-life**



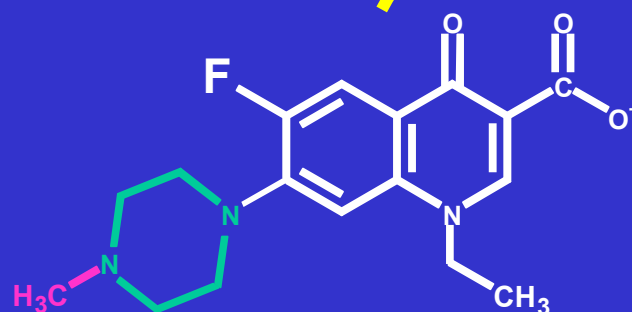
\* Ger. pat. 2,840,910 to  
Roger Bellon/Dainippon, 1979

# From norfloxacin to the other 1st generation fluoroquinolones: ofloxacin

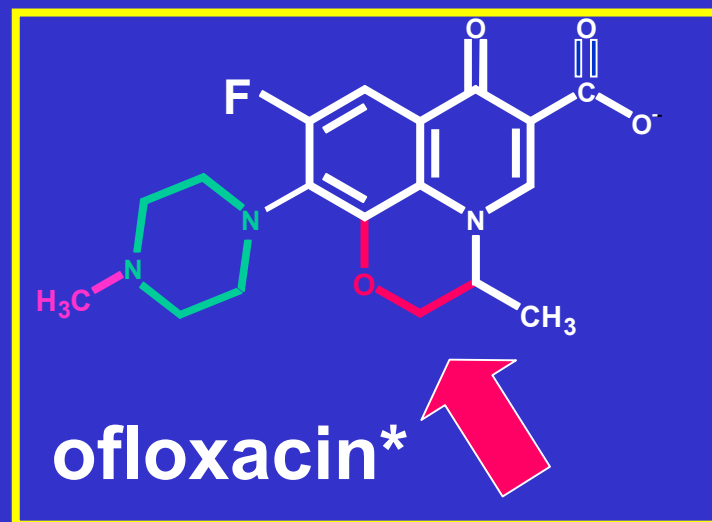
norfloxacin



tricyclic compound  
(as in flumequine but  
morpholine ring)



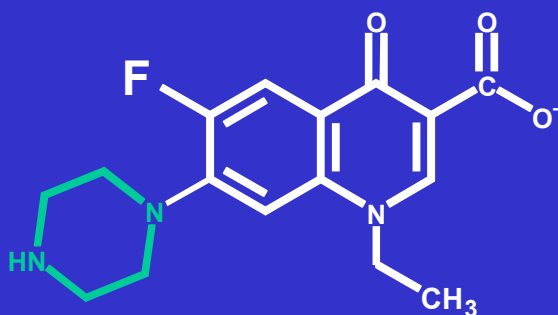
pefloxacin





# From norfloxacin to the other 1st generation fluoroquinolones: ciprofloxacin

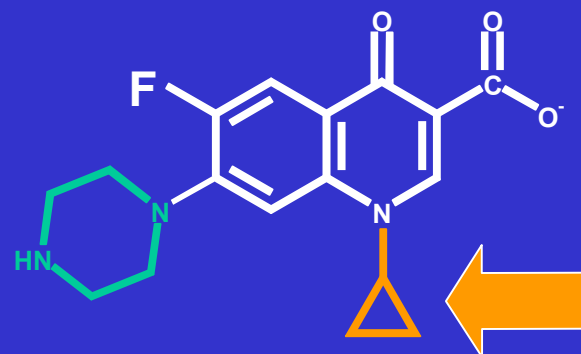
norfloxacin



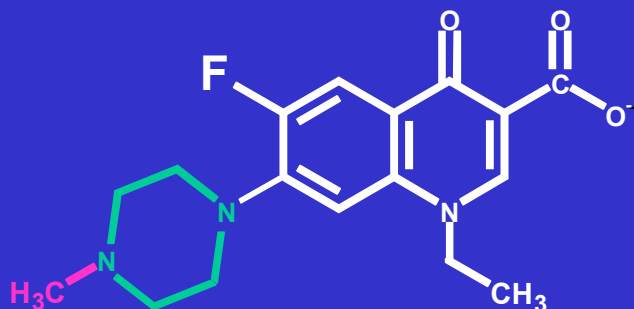
cyclopropyl to  
increase potency



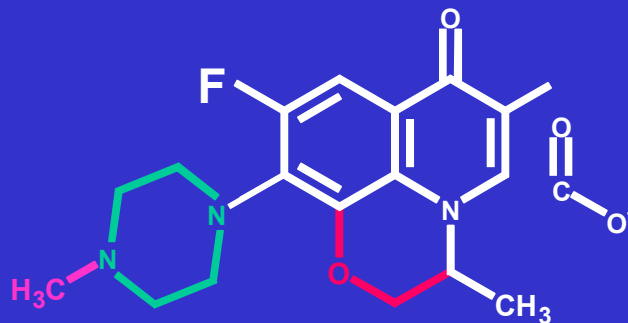
ciprofloxacin \*



pefloxacin



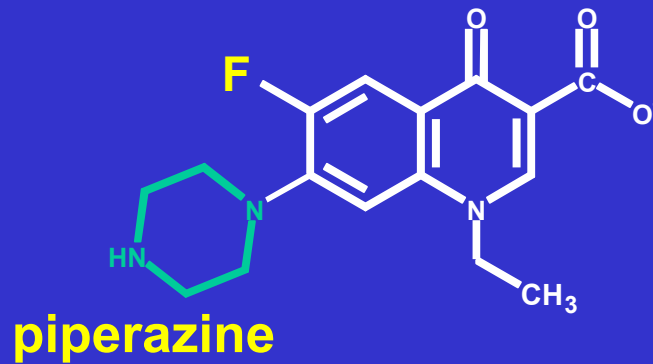
ofloxacin



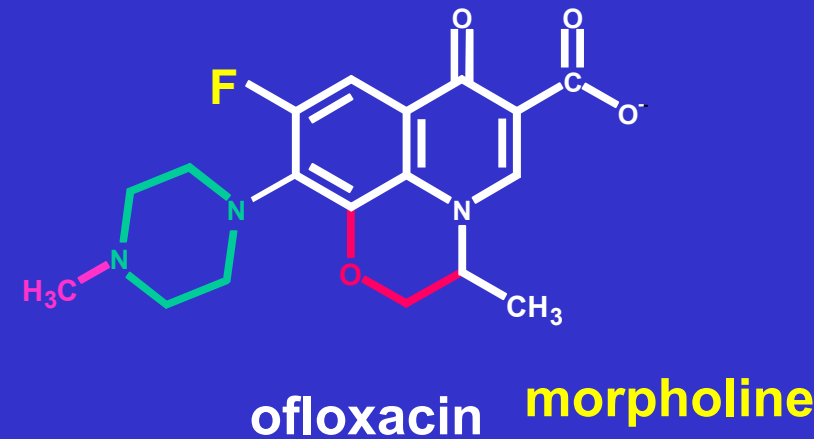
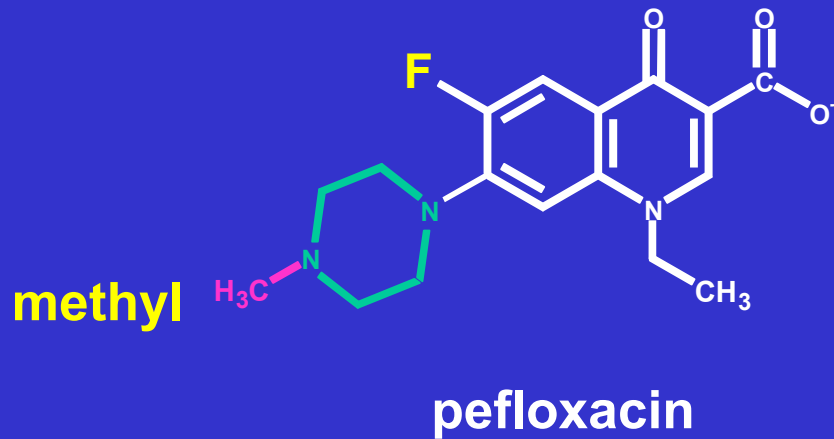
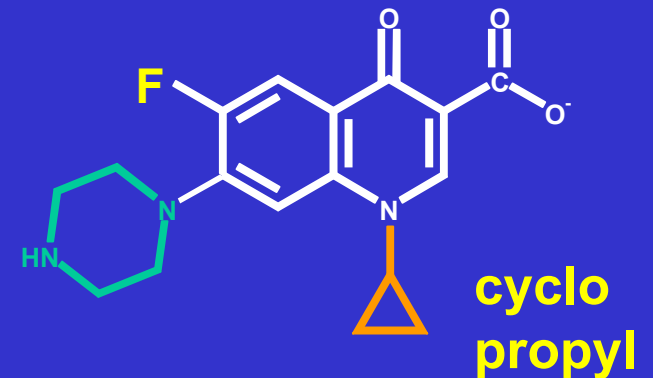
\* Ger. pat. 3,142,854 to Bayer AG, 1983

# "1st generation" fluoroquinolones

**norfloxacin**



**ciprofloxacin**



# The "first generation" of fluoroquinolones

1960

1970

1980

- Nalidixic acid
- Oxolinic acid
- Cinoxacin
- Pipemidic acid

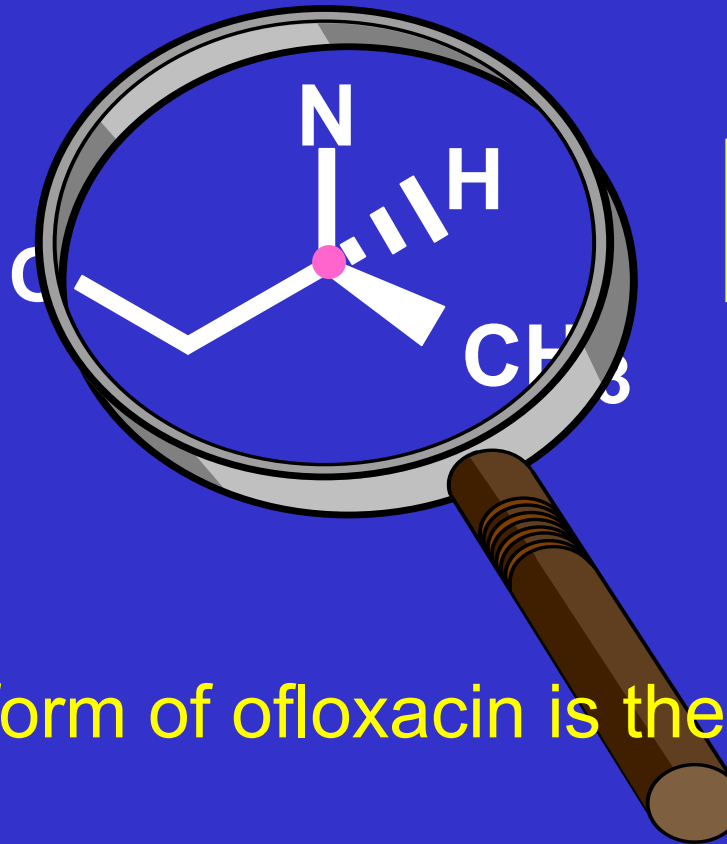
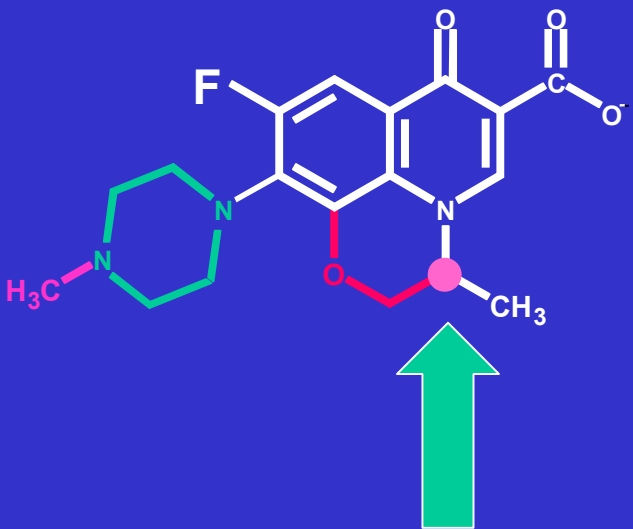
- Norfloxacin
- Pefloxacin
- Ofloxacin
- Ciprofloxacin
- Fleroxacin
- Rufloxacin

improved  
anti Gram (-)  
activity

t <sub>1/2</sub>	activity
3-4 h	++
11 h	+
6 h	++
3-4 h	+++

# From ofloxacin to levofloxacin...

Ofloxacin is a racemic mixture



Levofloxacin is the pure (-) S isomer \*

The active form of ofloxacin is the (-) S isomer

\* Eur. pat. 206,283 to Daiichi, 1987

# The present "first generation" of fluoroquinolones ...

1960

1970

1980

- Nalidixic acid
- Oxolinic acid
- Flumequine
- Pipemidic acid

- Norfloxacin
- Pefloxacin
- Ofloxacin
- Ciprofloxacin
- Fleroxacin
- Rufloxacin

improved  
anti Gram (-)  
activity

$t_{1/2}$  activity

3-4 h ++

11 h +

6 h ++

3-4 h +++

- Levofloxacin

6 h ++++

twice  
as active as  
ofloxacin per g

# How to improve the chemotherapeutic usefulness of the "first generation" fluoroquinolones

**1. Maintain broad Gram(-) activity**

**2. Improve Gram(+) activity**

**3. Acquire activity against anaerobes**

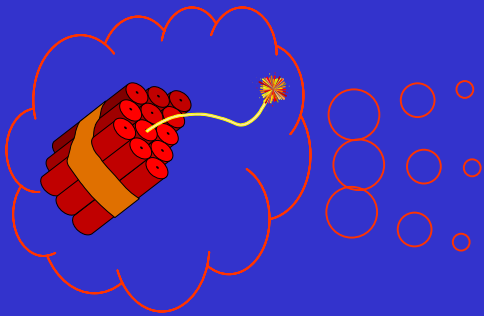


"2d generation"



"3d generation"

# The “second generation” fluoroquinolones



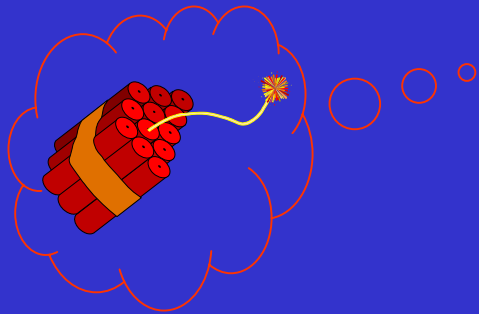
- **Temafloxacin**<sup>a</sup>
- **Sparfloxacin**<sup>b</sup>
- **Grepafloxacin**<sup>c</sup>
- **Gatifloxacin**<sup>d</sup>

- **Gram (-);**
- **improved Gram (+)**

anti-anaerobe

a: Toyama, 1988 (?) ; b: Dainippon, 1985-1987; c: Otskuda, 1989; d: Kyorin, 1988

# The “third generation” fluoroquinolones



- **Clinafloxacin** <sup>a</sup>
- **Trovaflaxacin** <sup>b</sup>
- **Moxifloxacin** <sup>c</sup>
- **Gemifloxacin** <sup>d</sup>

anti-Gram (-)  
anti-Gram (+)  
anti-anaerobe

a:Kyorin, 1987; b: Pfizer, 1993; c: Bayer, 1994; d: LG Chemical Ltd., S. Korea, 1994-98



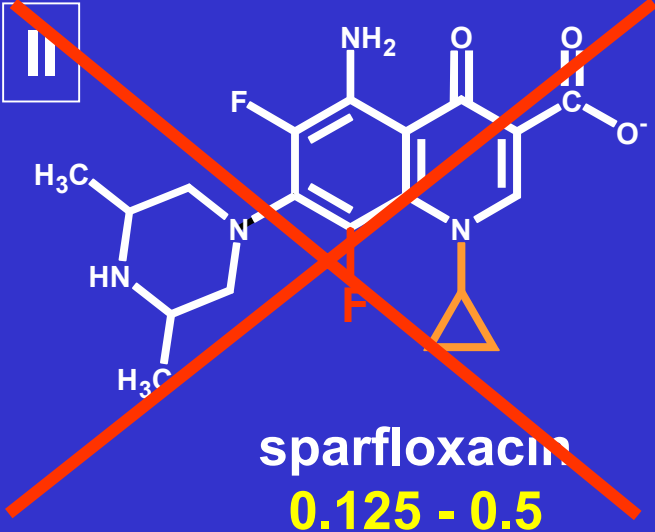
# Activity against *S. pneumoniae*

I

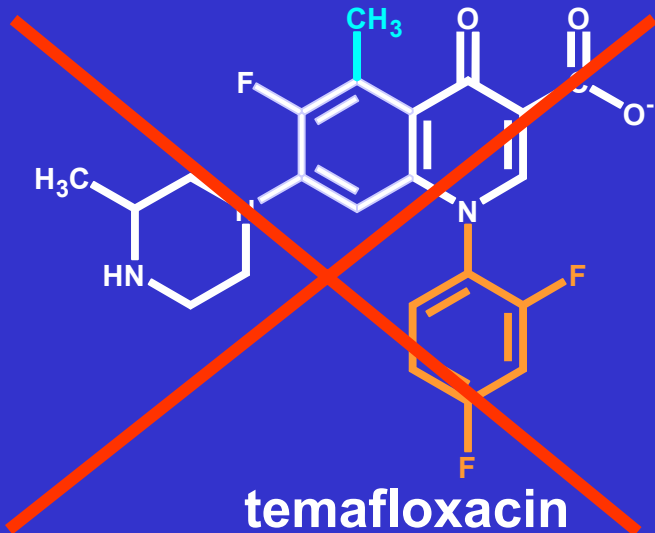


**ciprofloxacin**  
**0.5 - 2**

II

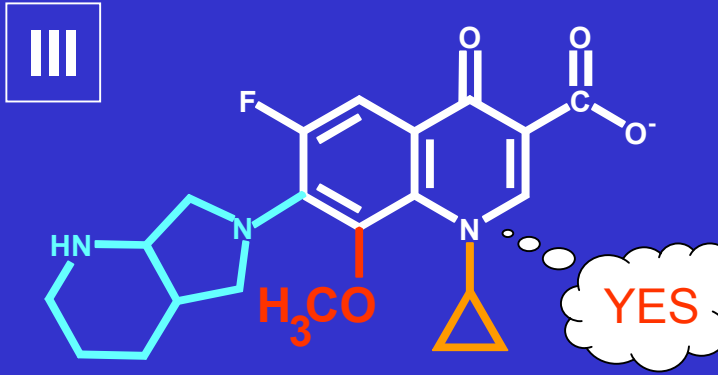


**sparfloxacin**  
**0.125 - 0.5**

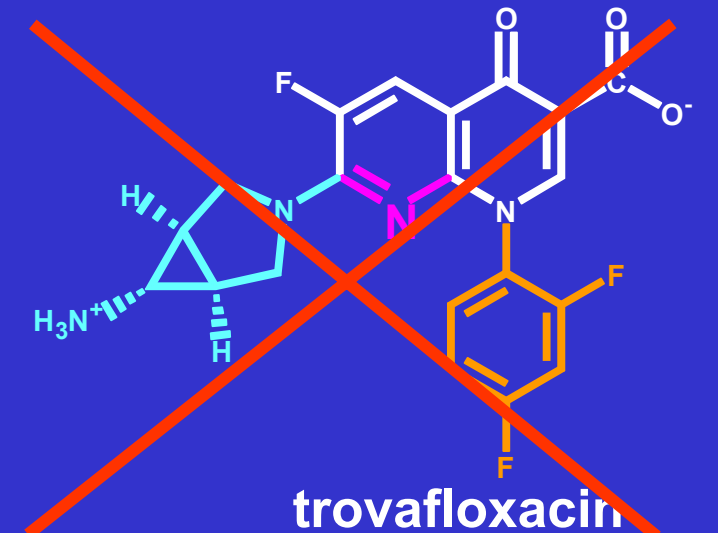


**temafloxacin**  
**0.5 - 1**

III

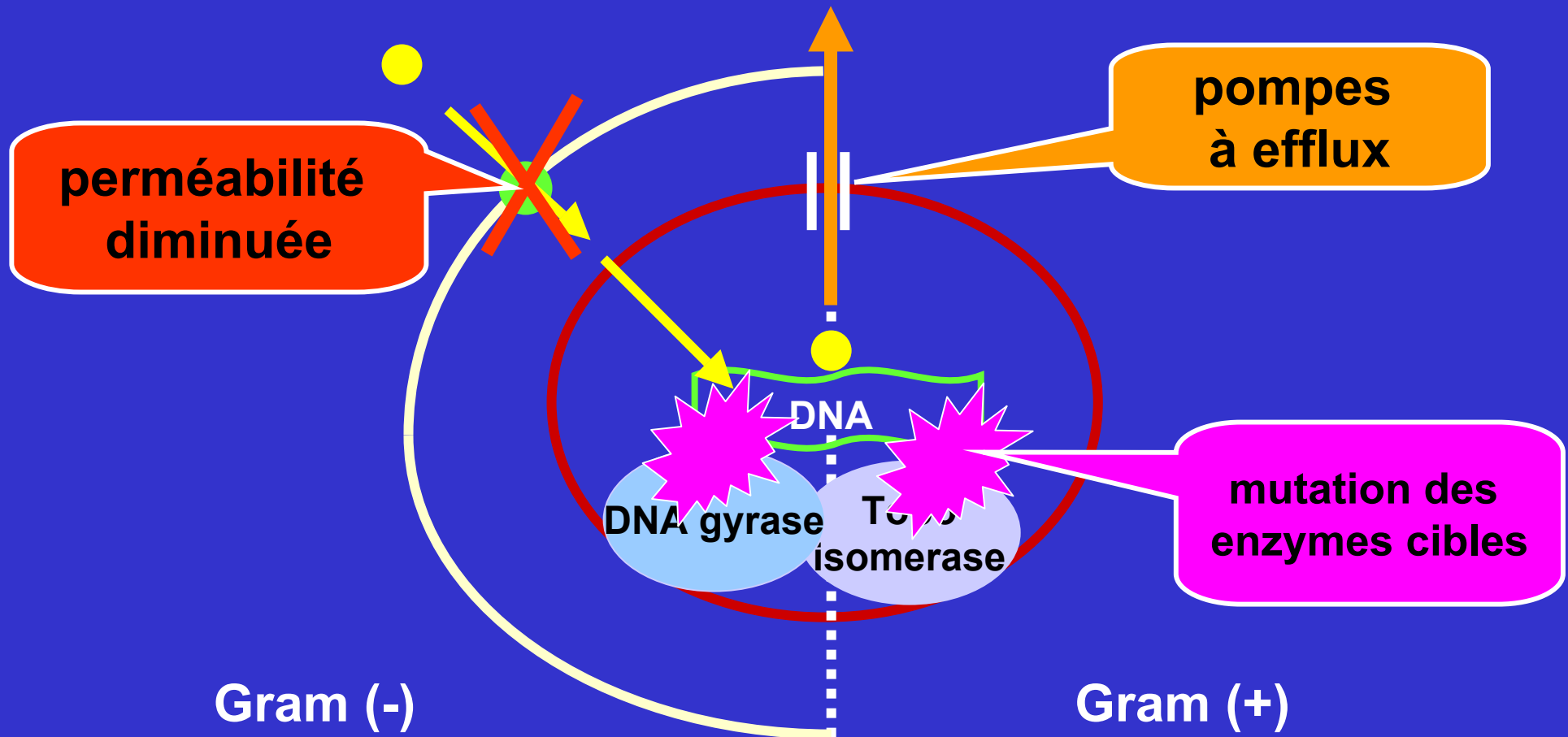


**moxifloxacin**  
**0.01 - 0.5**



**trovafloxacin**  
**0.007 - 0.25**

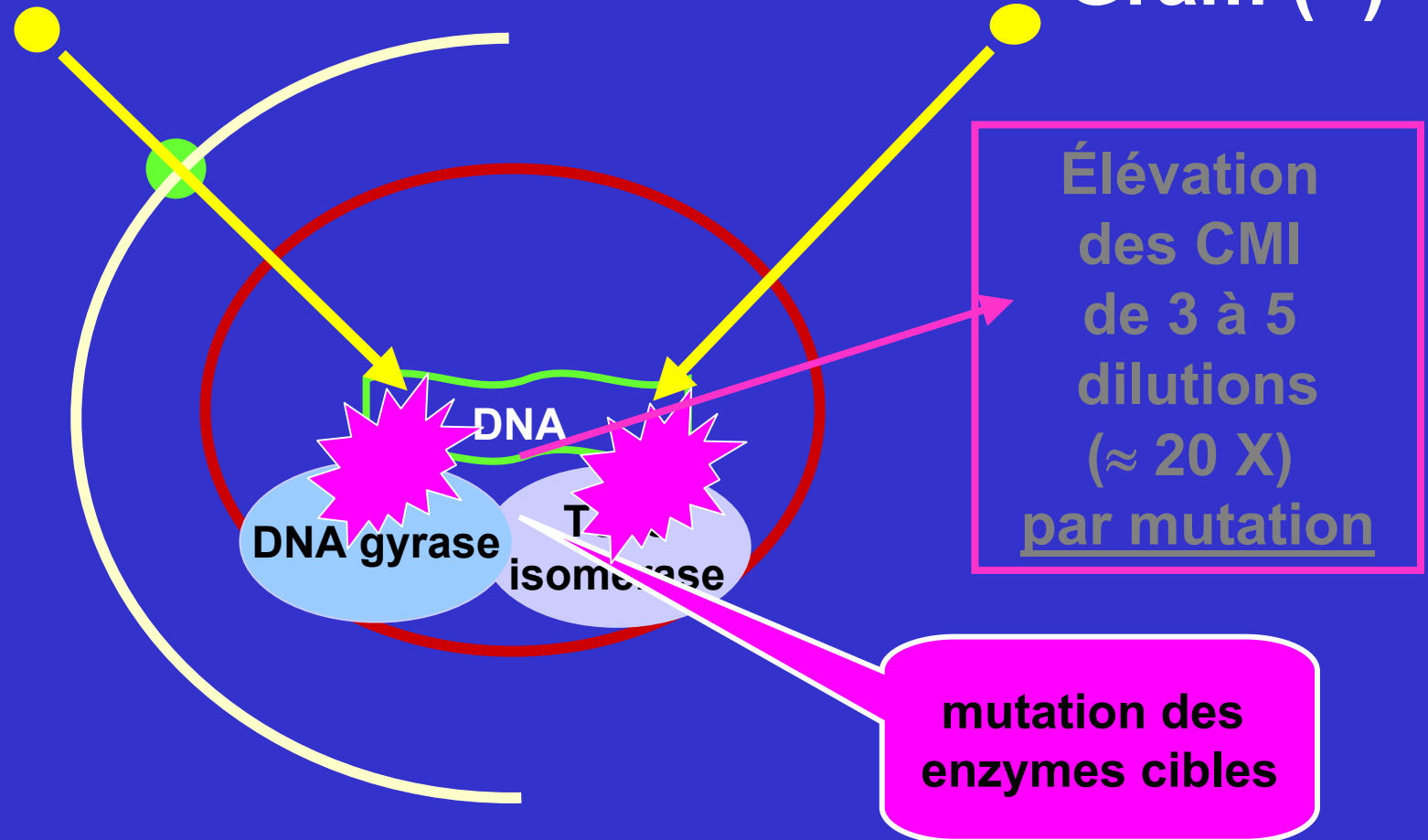
# Resistance au fluoroquinolones : les mécanismes de base ...



# Resistance au fluoroquinolones : rôle des mutations au niveau de la cible

Gram (-)

Gram (+)



# Is there a SAR for emergence of resistance ?

## The "*Mutant Prevention Concentration*" \*



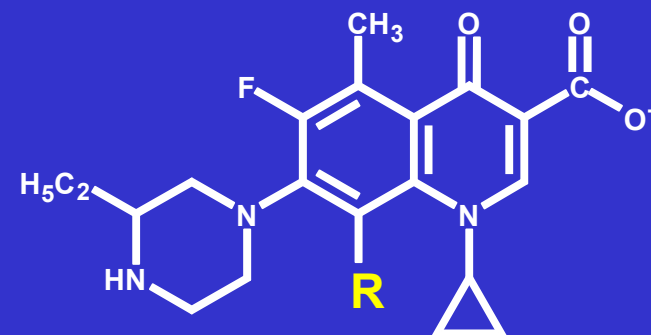
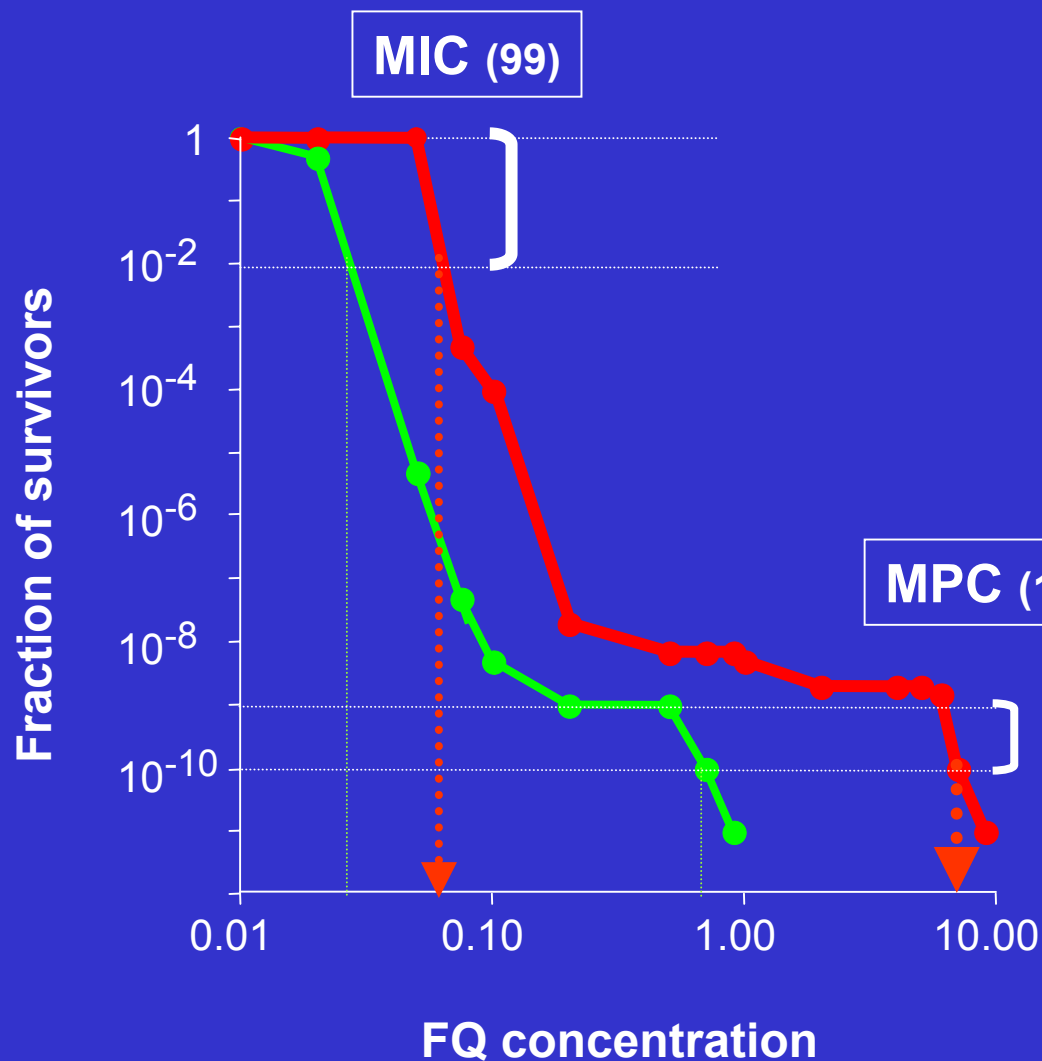
"When *Mycobacterium bovis* BCG and *Staphylococcus aureus* were plated on agar containing increasing concentrations of fluoroquinolone, colony numbers exhibited a sharp drop, followed by a plateau and a second sharp drop.

The plateau region correlated, with the presence of first-step resistant mutants. Mutants were not recovered at concentrations above those required for the second sharp drop, thereby defining a **mutant prevention concentration (MPC)**.

A **C8-methoxy group** lowered the MPC for an N-1-cyclopropyl fluoroquinolone"

# Is there a SAR for emergence of resistance ?

## Bactericidal activity of FQs against *Mycobacterium bovis*



PD160793

PD161148

R = OCH<sub>3</sub>

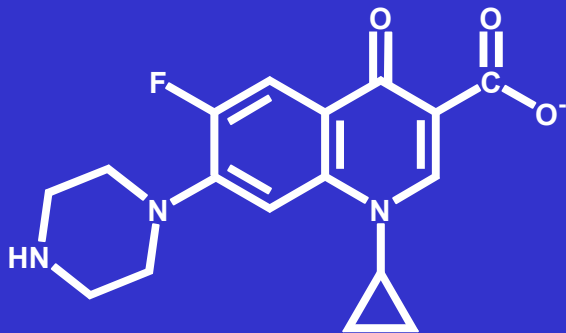
R = H

MIC 99	0.25	0.8
MPC 10	0.9	9
MPC/MIC	3.6	12

Dong et al; AAC 43:1756-1758

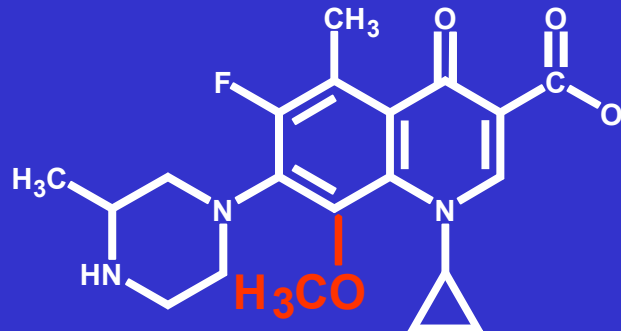
# Fluoroquinolones with a C8-methoxy

I



ciprofloxacin

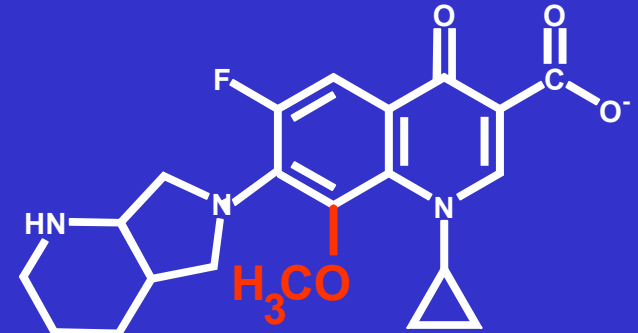
II



gatifloxacin

Not in  
Belgium

III



moxifloxacin

Yes

# Toxicity



This is where all may fail...

## Frequent side effects of fluoroquinolones: is there a SAR ?



COMPLEXATION WITH METALLIC IONS (Fe, Al, Mg, Ca)



PHOTOTOXICITY



DRUG INTERACTIONS: INHIBITION OF cyt P450 (1A2)



CNS TOXICITY (BINDING TO GABA RECEPTOR)



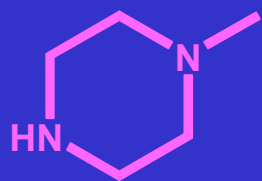
GASTRO-INTESTINAL DISCOMFORT



CARTILAGE and MUSCULOSQUELETAL TOXICITY



# SAR of frequent side effects



Binding to  
GABA receptor



Penetration  
in CNS

$\text{Ca}^{++}$ ,  $\text{Al}^{+++}$ ,  $\text{Fe}^{++}$   
complexation

All FQs

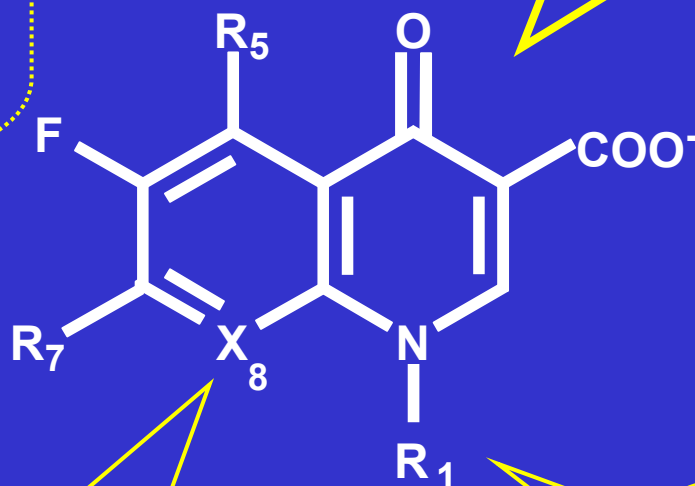
cipro,  
grepa ...

Inhibition of P450

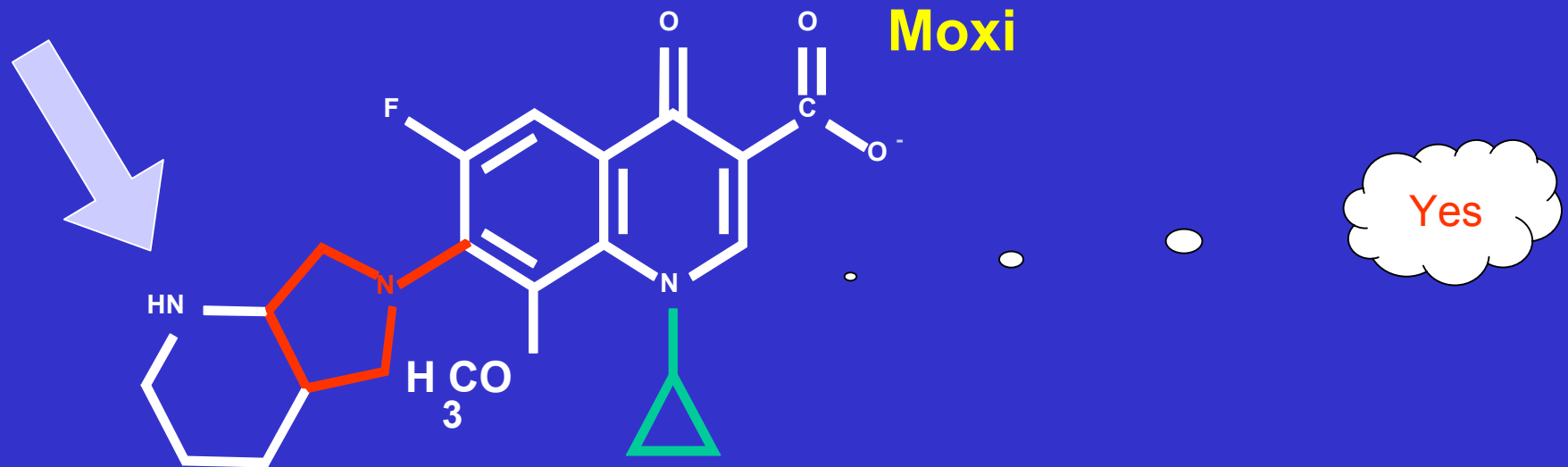
sparflo,  
flero,  
lomeflo

Phototoxicity

Inhibition of P450



# Fluoroquinolone with low or no drug interactions..



## Rare side effects of fluoroquinolones:



### RENAL TOXICITY

crystalluria, hematuria, interstitial nephritis, acute renal failure



### CARDIAC TOXICITY (QT prolongation, *Torsades de pointe*)



### HEPATOTOXICITY

temafloxacin syndrome / trovafloxacin syndrome

# Pharmacokinetics



This is where people start sleeping..

# SAR of pharmacokinetic parameters

Bulky  
substituent

$t_{1/2} \uparrow$

cipro  
gati  
moxi

peflo, oflo,  
gati,  
moxi



$V_d \uparrow$

# SAR of main pharmacokinetic parameters: how to get a long half life

	$t_{1/2}$ (h)	no. of daily administrations
	<b>oflo / lévo</b> 5 - 7	<b>2 x*</b>
	<b>peflo</b> 10	<b>2 x*</b>
	<b>flero</b> 9 - 13	<b>1 x</b>
	<b>grepa</b> 10 - 12	<b>1 x</b>
	<b>gati</b> 13	<b>1 x</b>
	<b>gemi</b> 8	<b>1 x</b>
	<b>trova</b> 10	<b>1 x</b>
	<b>moxi</b> 12	<b>1 x</b>
<b>other FQ</b>	<b>3 - 6</b>	<b>2 x</b>

\* higher MIC... 38

# Resistance: do not forget the correct dosing...

“Inadequate dosing of antibiotics is probably an important reason for misuse and subsequent risk of resistance. A recommendation on proper dosing regimens for different infections would be an important part of a comprehensive strategy. The possibility to produce such a dose recommendation based on **pharmacokinetic** and **pharmacodynamic** considerations will be further investigated in one of the CPMP working parties...”

**European Agency of the Evaluation of  
Medicinal Products (London)**

*EMA discussion paper  
on Antimicrobial resistance  
3 January 1999 EMEA/9880/99*



# Pharmacokinetic parameters in relation with efficacy

	Dose (mg)	Cmax (mg/l)	MIC for pk/MIC=10	AUC (mg.h/l)	MIC for AUC=125
<b>norflo</b>	<b>400 (X2)</b>	<b>1.6</b>	<b>0.2</b>	<b>14</b>	<b>0.1</b>
<b>peflo</b>	<b>400 (X2)</b>	<b>4.6</b>	<b>0.4</b>	<b>108</b>	<b>1.0</b>
<b>cipro</b>	<b>500 (X2)</b>	<b>1.5</b>	<b>0.2</b>	<b>17</b>	<b>0.1</b>
<b>oflo</b>	<b>200 (X2)</b>	<b>3.1</b>	<b>0.4</b>	<b>66</b>	<b>0.4</b>
<b>levoflo</b>	<b>500</b>	<b>5.0</b>	<b>0.5</b>	<b>47</b>	<b>0.4</b>
<b>moxi</b>	<b>400</b>	<b>4.5</b>	<b>0.4</b>	<b>48</b>	<b>0.4</b>



# Optimizing dosage for fluoroquinolones

increase the amount administered,  
in order to optimize AUC/MIC

➡ should be  $> 125$   
➡ should be  $> 10$

and peak/MIC

Concentration

Peak/MIC

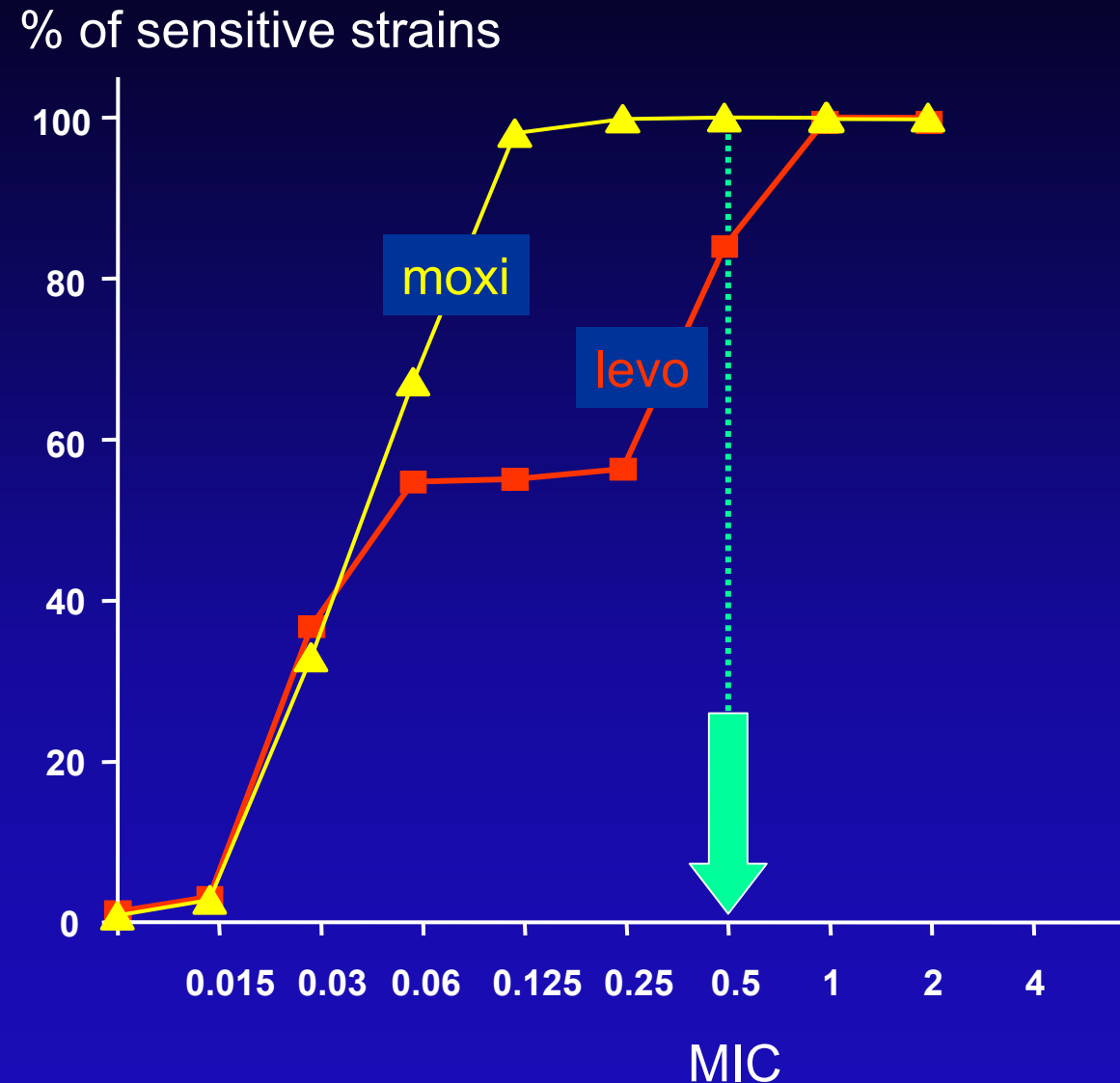
AUC/MIC

Get both a peak and a AUC !!

MIC

Time (h)

# How to apply this ?



<b>Levofloxacin</b>	500 mg	
	1X /day	2X /day
AUC [(mg/l)xh]	47	94
• peak [mg/l]	5	5
👉 MIC <sub>max</sub>	< 0.5	< 1

<b>Moxifloxacin</b>		400 mg
		1X/day
• AUC [(mg/l)xh]	48	
• peak [mg/l]	4.5	
👉 MIC <sub>max</sub>	< 0.5	

MIC data: J. Verhaegen et al., 2001

## Take home” message

- Dosage is key to success
- Dosage should match bacterial sensitivity
- peak, AUC/MIC are keys to success
- use a single, appropriate dose for long-life fluoroquinolones (moxifloxacin), or
- repeat the dose for short-lived fluoroquinolones (all others so far...)
- for fluoroquinolones, the limit is an **MIC of 0.5 µg/ml**