# *Challenges in Novel Antibacterial Drug Discovery*

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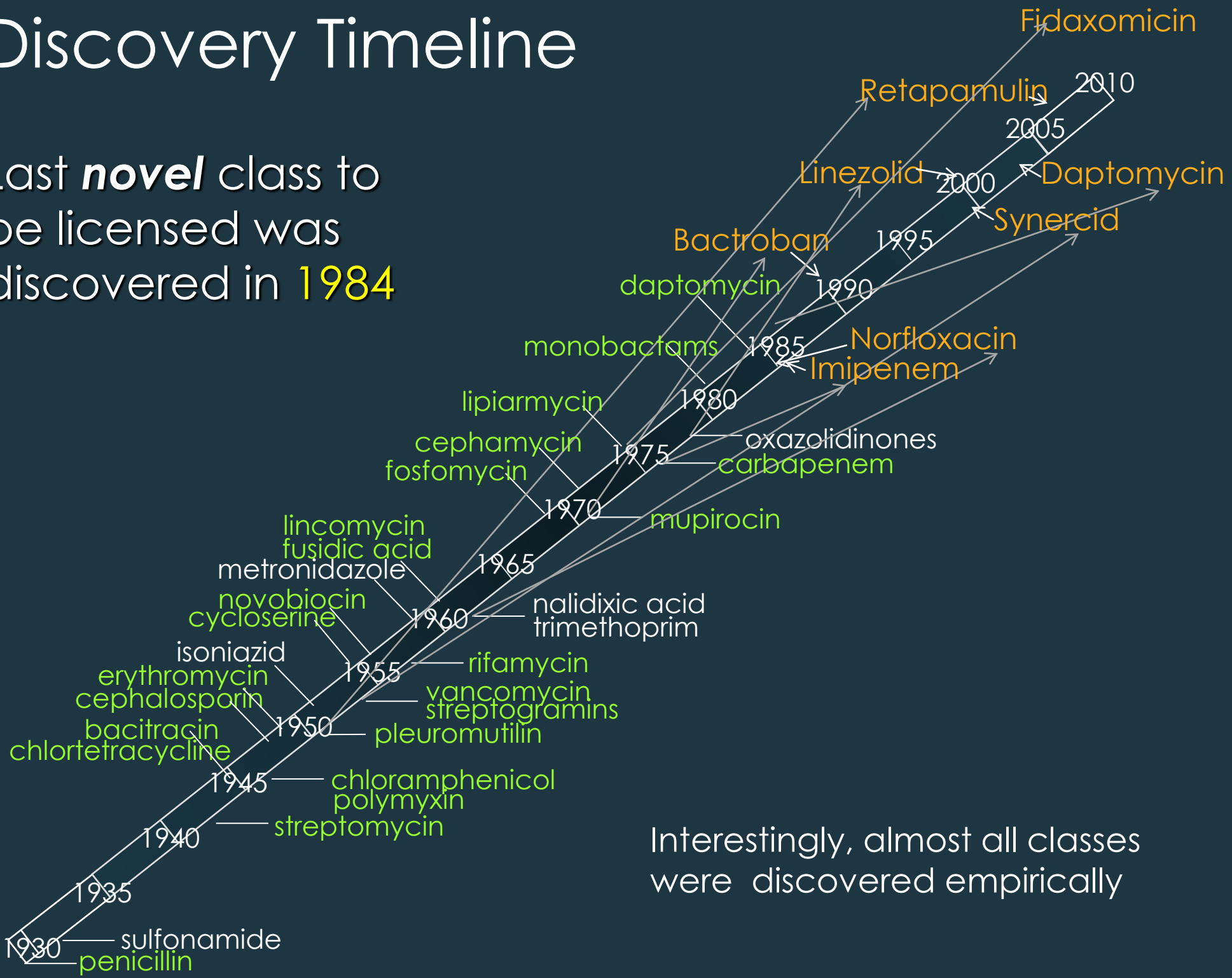
# Because NOVEL antibacterial discovery has been very difficult…

- I give talks on *Challenges in Antibacterial Discovery*
  - Apologizing for the pharmaceutical industry
  - Making excuses for my own shortcomings
  - As a Luddite rant
    - Technology has led us astray
  - To illustrate the problems so that others may be aware of them
  - To explain the rate limiting steps

- The Discovery Timeline
- What went wrong
- The rate limiting steps
- What to do about it?

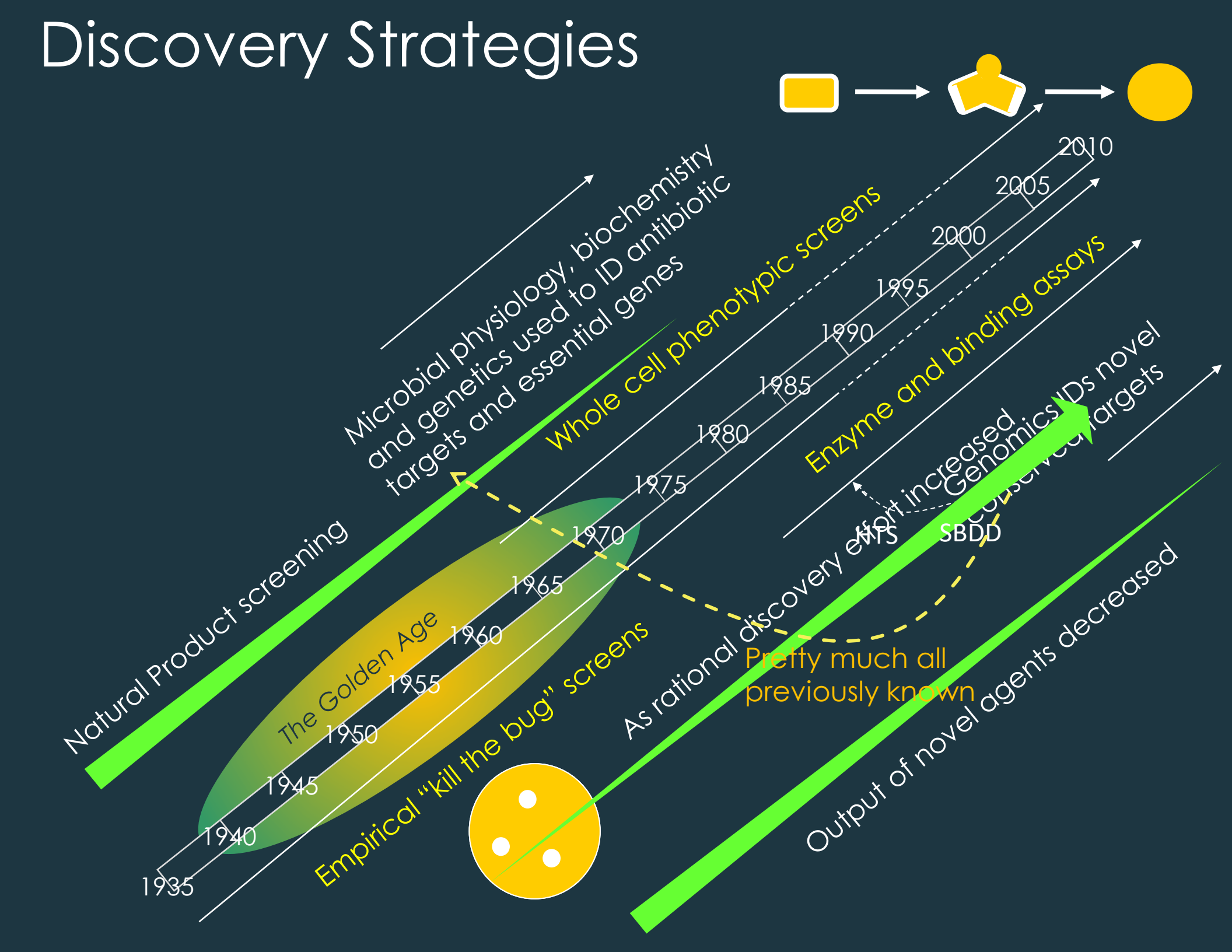
# Discovery Timeline

Last ***novel*** class to be licensed was discovered in 1984

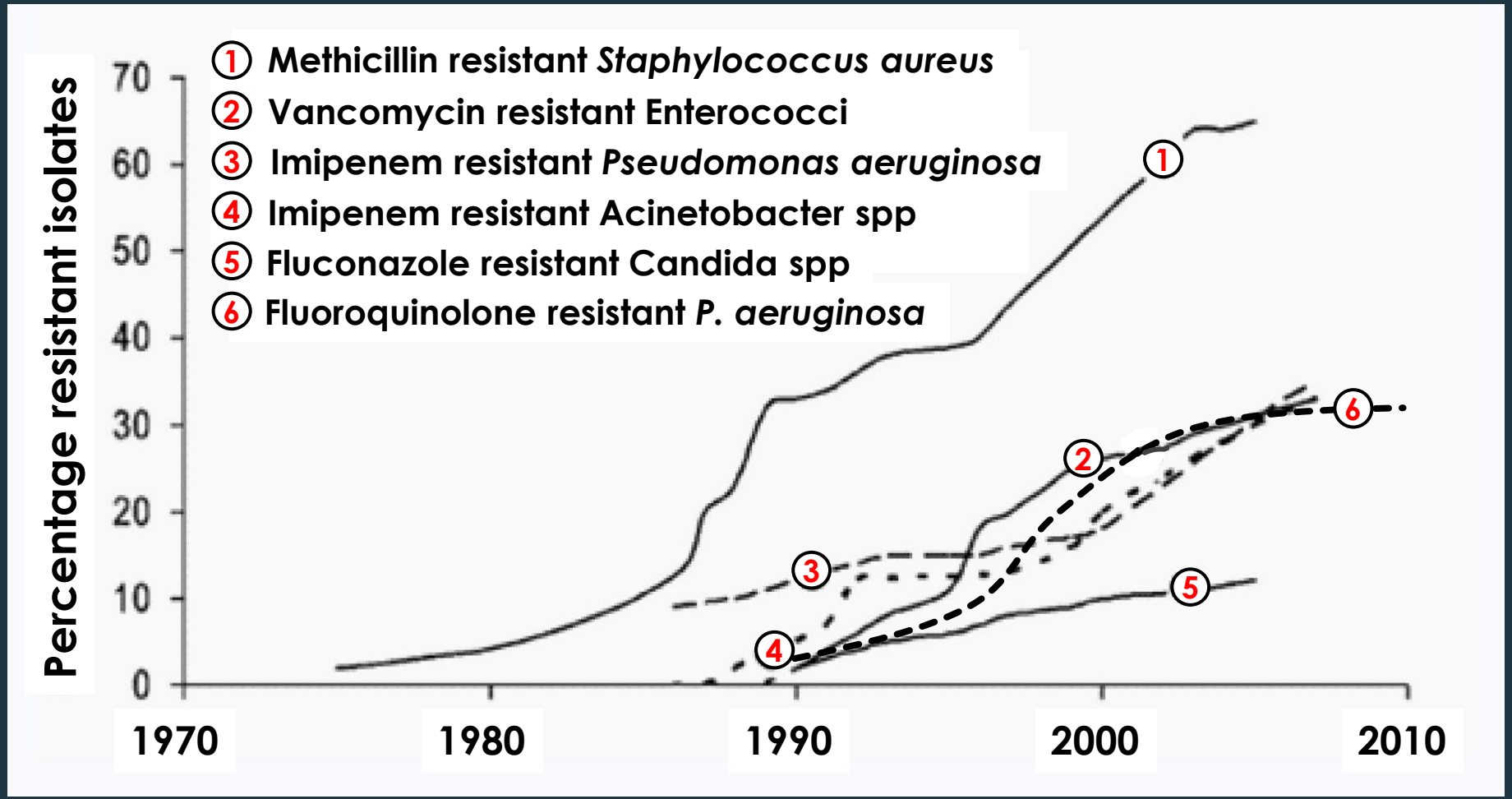



Interestingly, almost all classes were discovered empirically

# Discovery Strategies



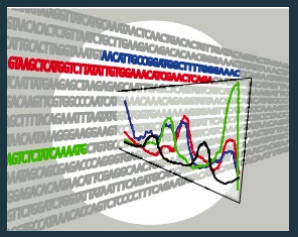
# Meanwhile, over the past 30 years, antimicrobial resistance has increased

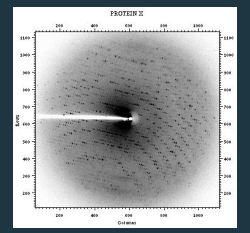


# Why has discovery of novel developable compounds failed thus far????

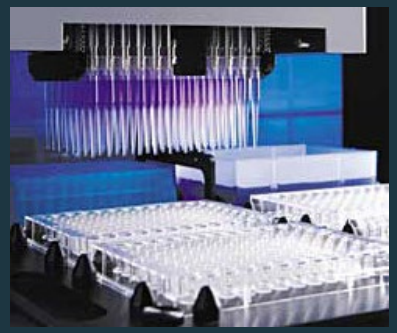
Silver LL. 2011. Challenges of antibacterial discovery. Clin. Microbiol. Rev. 24:71-109.

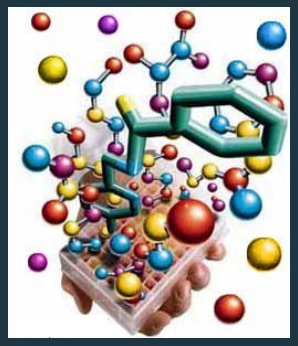
# What people seemed to think were the problems





- We don't find new compounds because we only look at a few targets –so find and inhibit new targets
  - Answer: Use Genomics, Crystallography, Bioinformatics
- We need to test more samples
  - Answer: High Throughput Screening (HTS)
- Natural product fermentation broths aren't amenable to HTS
  - Answer: extract and prefractionate
  - Answer: stop Natural Products screening
- Produce infinite chemicals rationally
  - Answer: Combinatorial Chemistry
- **Didn't work**



# In other words…

- When drug discovery output tailed off and drug resistance rose
  - New technologies were applied
  - Without analysis of the reasons for the decline
  - Failure to assess the rate-limiting steps of the process

# What is rate limiting?

## (the take home message)

- Selection of targets that are not subject to rapid resistance selection
- Chemistry appropriate for antibacterial discovery
  - We have no general rules, or even a rational approach, to getting things into Gram negative bacteria
  - Chemical collections favor physicochemical attributes not associated with antibacterials

# What makes a good antibacterial target?

- Received "wisdom"
  - No human homolog
  - Useful bacterial spectrum
  - Druggable
  - Essential
  - Low resistance potential
    - No cross resistance

- Added criteria
  - Location
  - Low resistance frequency
    - How low????

Insufficient

# To gain insight into characteristics of successful targets

- Investigate successful drugs
- Find patterns

L Silver and K Bostian. 1990. Eur J Clin Microbiol Inf Dis. 9:455-461.
LL Silver and KA Bostian. 1993 Antimicrob. Agents and Chemother 37:377-383.
LL Silver. 2007. Nature Rev Drug Discov. 6:41-52

# Targets of antibacterials used in systemic monotherapy

| ANTIBACTERIAL | TARGET |
|---|---|
| β-lactam | multiple penicillin binding proteins |
| glycopeptide | D-ala-D-ala of peptidoglycan substrate |
| tetracycline | rRNA of 30s ribosome subunit |
| gentamicin | rRNA of 30s ribosome subunit |
| macrolide | rRNA of 50s ribosome subunit |
| lincosamide | rRNA of 50s ribosome subunit |
| chloramphenicol | rRNA of 50s ribosome subunit |
| oxazolidinone | rRNA of 50s ribosome subunit |
| quinolone | bacterial topoisomerases (Gyr & Top IV) |
| metronidazole | DNA |
| daptomycin | membranes |

All have multiple targets or targets encoded by multiple genes

High-level target-based resistance to these compounds does not occur by single-step mutation (in standard pathogens)

# Single enzyme targets of antibiotics in clinical use

| ANTIBIOTIC | TARGET | USE |
|---|---|---|
| rifampicin | RNA polymerase | Multi-drugTB therapy |
| isoniazid | InhA | Multi-drug TB therapy |
| streptomycin | 30s ribosome/rpsL | Multi-drug TB therapy |
| trimethoprim | DHFR (FolA) | Combo w/ Sulfas |
| sulfamethoxazole | PABA synthase (FolP) | Combo w/ Trimethoprim |
| mupirocin | Ile tRNA-synthetase | Topical therapy |
| fosfomycin | MurA | UTI |
| fusidic acid | Elongation factor G | UTI |
| fidaxamicin | RNA Polymerase | Non-absorbed for *C. diff* |

All are subject to high-level single-step target-based resistance

# Based on existing antibacterial agents

- Successful monotherapeutic antibacterials
  - Are not subject to single mutation to high level resistance
  - Because they are multi-targeted
    - interact with multiple enzymes or are encoded by multiple genes
- Current drugs inhibiting single–enzymes
  - Are generally used in combination, where organismal load is low, or topically
  - Because they are subject to single step mutation to significant resistance

HYPOTHESIS:

"Multiple-targets" are preferable to single enzyme targets for systemic monotherapy

Single targeted agents will select rapidly for resistance – and may fail during therapy

# Resistance to single-targeted agents in other therapeutic areas

- Resistance to single- targeted drugs is generally due to pre-existing mutations in:
  - TB
  - HIV
  - HCV
  - Cancer
- The standard is or is becoming COMBINATION THERAPY
- But bacterial antibiotic resistance is thought of differently

# How people think about antibiotic resistance…

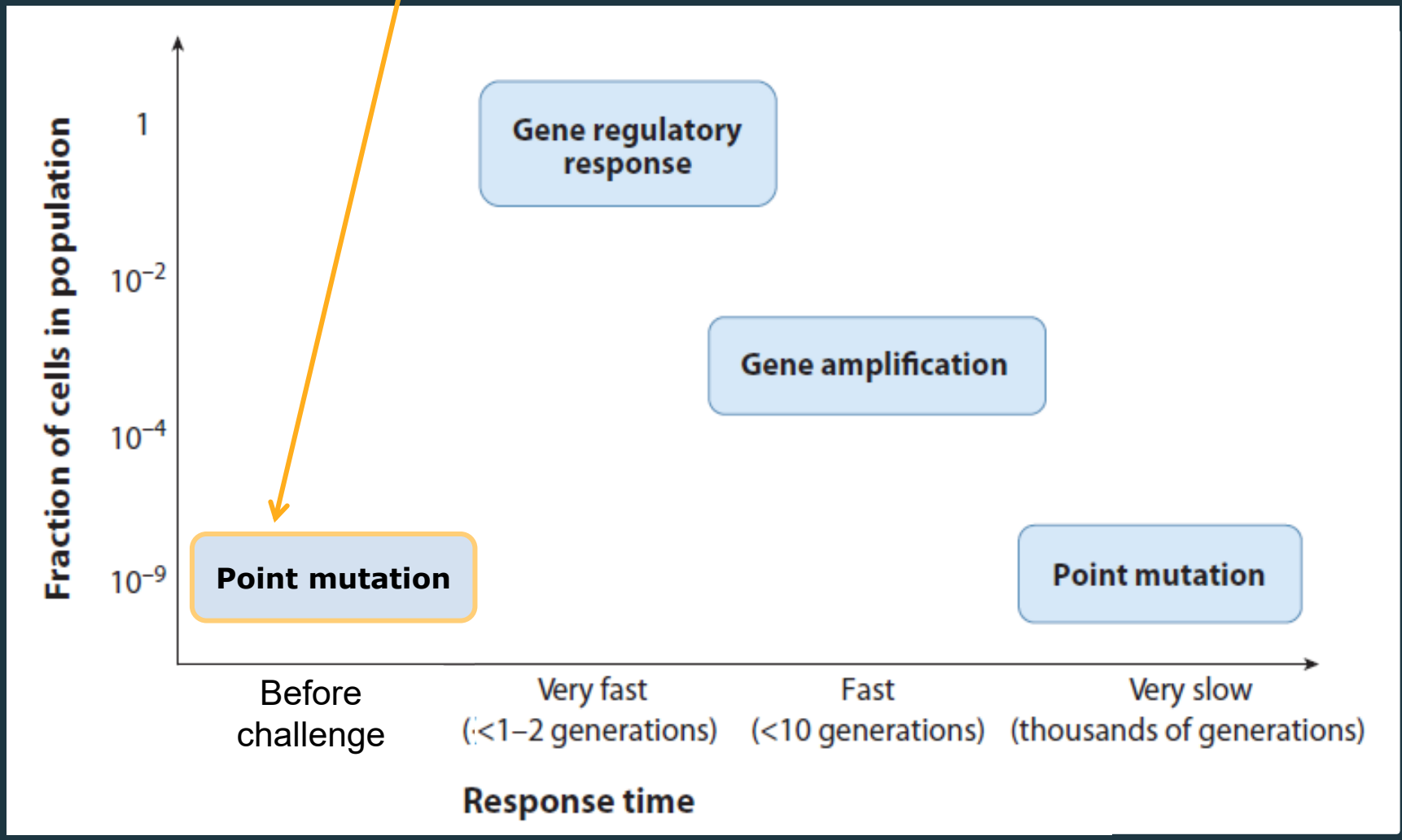
- Concepts of antibacterial resistance are generally based on experience with the multitargeted monotherapeutic systemic agents
- Most of this type of resistance is due to
  - Horizontal genetic transfer [years]
  - Endogenous mutations [sooner]

But for the new wave of single-targeted genomics-driven agents

# Adaptive [endogenous] resistance

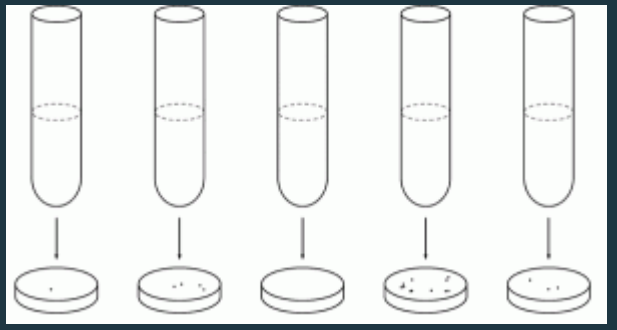
# Pre-existing spontaneous resistance

To multitargeted agents




Andersson, D. I., and D. Hughes. 2009. Gene amplification and adaptive evolution in bacteria. Annu Rev Genet 43:167-95.

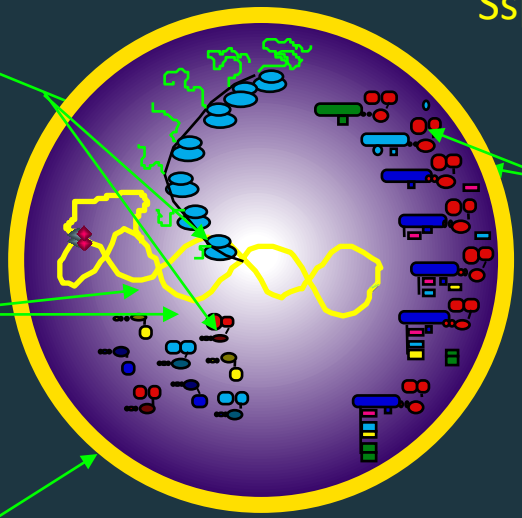
# Pre-existing mutations: back to Luria-Delbrück



- Spontaneous mutations occur in the absence of selection at a measurable rate per generation
- Rate and frequency can be determined in vitro
- Whether resistors survive in vivo depends upon fitness, rate of compensatory mutations, and continued drug presence
- Require standardized in vitro and high inoculum animal models to correlate laboratory resistance rates with in vivo outcomes

Luria SE, Delbrück M. 1943. Mutations of bacteria from virus sensitivity to virus resistance Genetics 28:491-511.
Luria S. 1946. Spontaneous bacterial mutations to resistance to antibacterial agents, p. 130-138, vol. 11. CSHSQB

# Enzyme targets with validated inhibitors [MIC is due solely to inhibition of target]



RNA & Protein Synthesis

RpoB/C
Ef-Tu Ef-G
Pdf Map
MetRS PheRS TrpRS
LeuRS IleRS ThrRS
ProRS

DNA Synthesis & Substrates

PolC DnaE
(DnaB) DnaG LigA
Gyrase A/B
Topo IV A/B
Ndk DHFR FolP TMK

Lipid and Membrane Synthesis

AccAD AccC
CoaA Fab B/F/H
Fab I/K
LpxC IspE KdsB

Proteases and Signal Peptidases

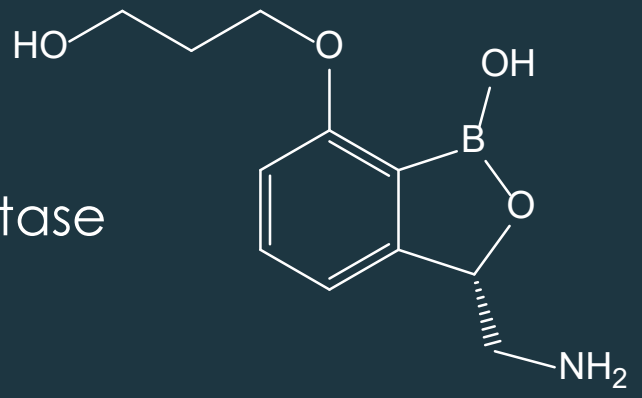
ClpXP
LspA
SspB

Cell Wall Synthesis & Cell Division

MurA MurI MraY PBPs
Alr/Ddl
GlmS GlmU GadA
SAV1754 MreB FtsZ
TarG

- Most are subject to single step mutation to resistance due to target alteration or bypass
- Some are "multitargets" of monotherapeutic systemic agents
- Certain single target inhibitors are drugs
  - Used in combinations, topically, UTI
- New single target inhibitors – what will happen?

# Case in point: GSK052 (AN3365)

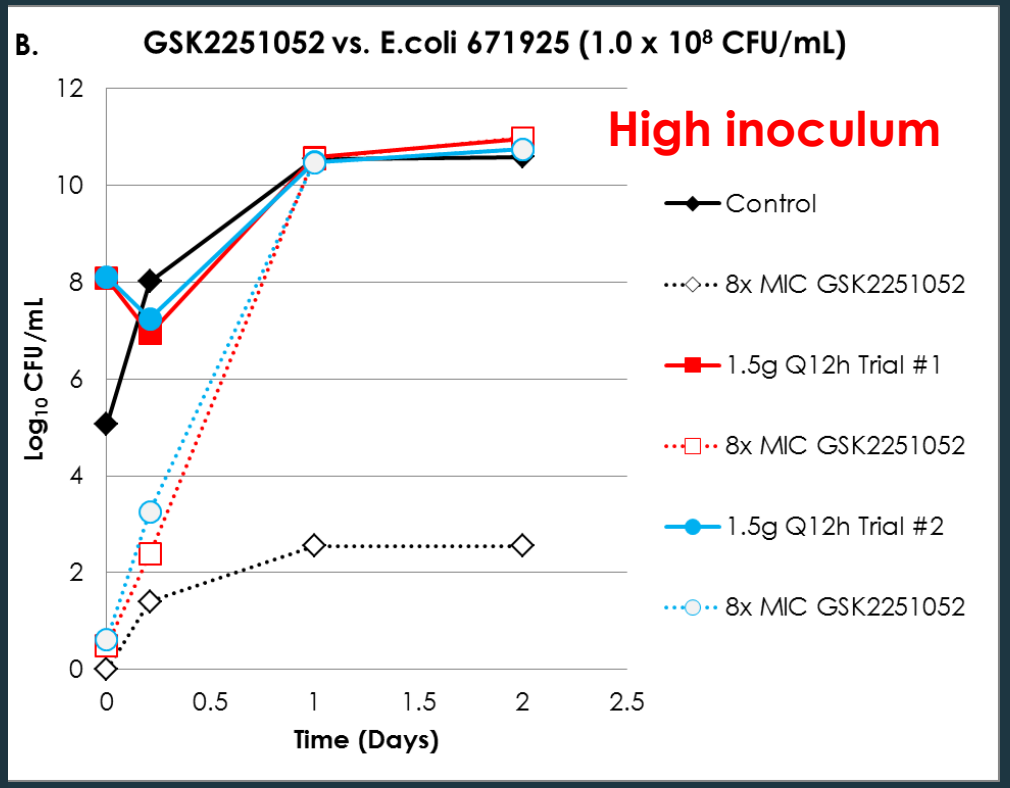



- Oxaborole inhibitor of Leucyl tRNA Synthetase
- Excellent Gram-negative spectrum
- In vitro resistance frequencies of ~$10^{-8}$
- In Phase 2b cUTI study, resistance occurred in 4 of 14 patients after one day of treatment
- Study was terminated in February 2011
- Mutants were highly fit and MICs raised >1000 fold
- This should have been predictable

Hernandez, V.,et al.. 2013. Antimicrob. Agents Chemother. 57:1394-1403.
Twynholm, M., et al. 2013. Poster -1251 at 53rd ICAAC, Denver

# Hollow fiber (in vitro) resistance study of GSK052

- GSK052 dosed vs *E. coli* at low ($10^5$/ml) and high ($10^8$/ml) inocula
- **Resistant mutants take over the population in one day at high inoculum**



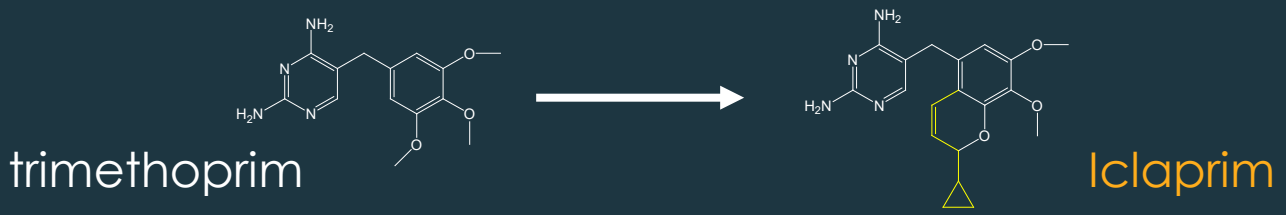

VanScoy, B. D., et al. 2013. Poster A-016 at 53rd ICAAC, Denver.

# How to deal with resistance

- If resistance frequency is high with initial leads, optimize to reduce it
- Discover more multitargeted inhibitors
- Change clinical practice to accommodate single-target inhibitors

# Optimize single target inhibitors to reduce resistance

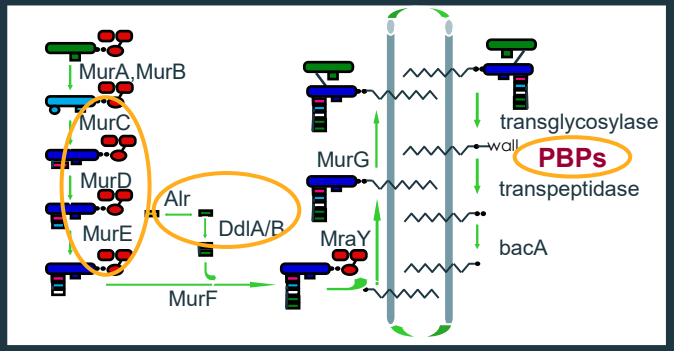
- Trimethoprim targets dihydrofolate reductase (DHFR)
- Arpida developed an analog that can bind to $Trm^{res}$ DHFR – by adding extra ligand-enzyme binding sites



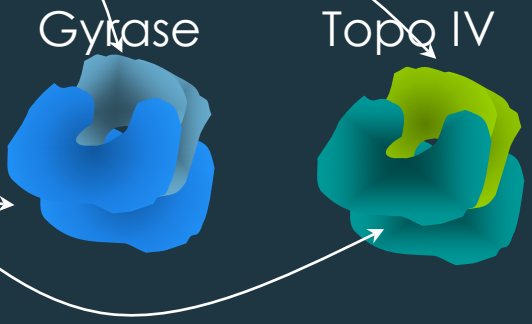

- Requires iterative resistance determination and optimization
- May limit spectrum (by tailoring to a single species)

# Multiple-target inhibitors: Homologous active site motifs of ≥2 enzymes

- Cell wall enzymes
  - PBPs [β-lactams]
  - Mur CDE [no antibacterial inhibitors]
  - Alr and DdlA/B [cycloserine]




- Gyrase and Topoisomerase IV
  - Quinolones hit GyrA and ParC
  - New compounds hit GyrB and ParE
    - Many programs on dual inhibitors of both enzymes

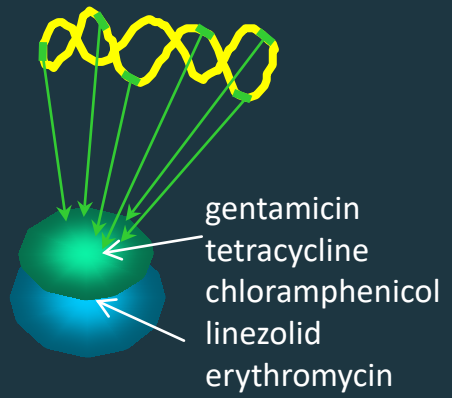



- Other enzymes sharing active sites
  - FabH and FabF [platencin, thiolactomycin]
  - DNA Polymerases PolC and DnaE [7-morpholinobutyl-DCBG]
  - *B. anthracis* DHFR and DHPS [5-nitro-6-methylamino-isocytosine]

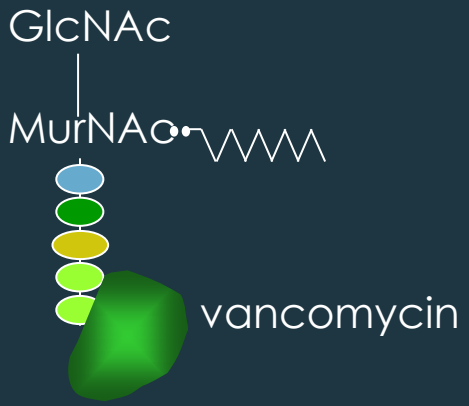
# Multiple-target inhibitors:

## Multi copy gene products or complex structures

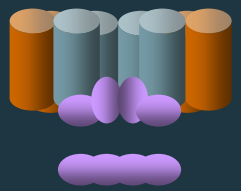
- rRNA – inhibitors of protein synthesis
  - New ones by SBDD?
  - And other genes where resistance is recessive




- Peptidoglycan precursor – eg., Lipid II




- Cytoplasmic membrane
  - Daptomycin target
  - AMPs



# How to find other multitargets

- Explore
  - Additional pathways with similar active sites/ligands
    - Multiple tRNA synthetases
    - Purine pathway?
  - Cofactors

- Chemi- and bio-informatics
  - Focus on the site of ligand-target interactions independent of homologies between entire proteins
  - Identify families of targets by their interaction with similar ligands

# Clinical approaches:

- Dose HIGHER
  - If possible, dose to reach the MPC
  - MPC (mutation prevention concentration)

    The concentration of drug above which single step mutations to resistance are not selected

- Use COMBINATIONS
  - Are combinations the answer for single targeted antibacterials?
    - Must have paired PK – so that each drug is always in excess of its MIC
    - How to do clinical trials if the single drugs are subject to resistance at some low but significant rate?

But even good targets are useless if you can't reach them

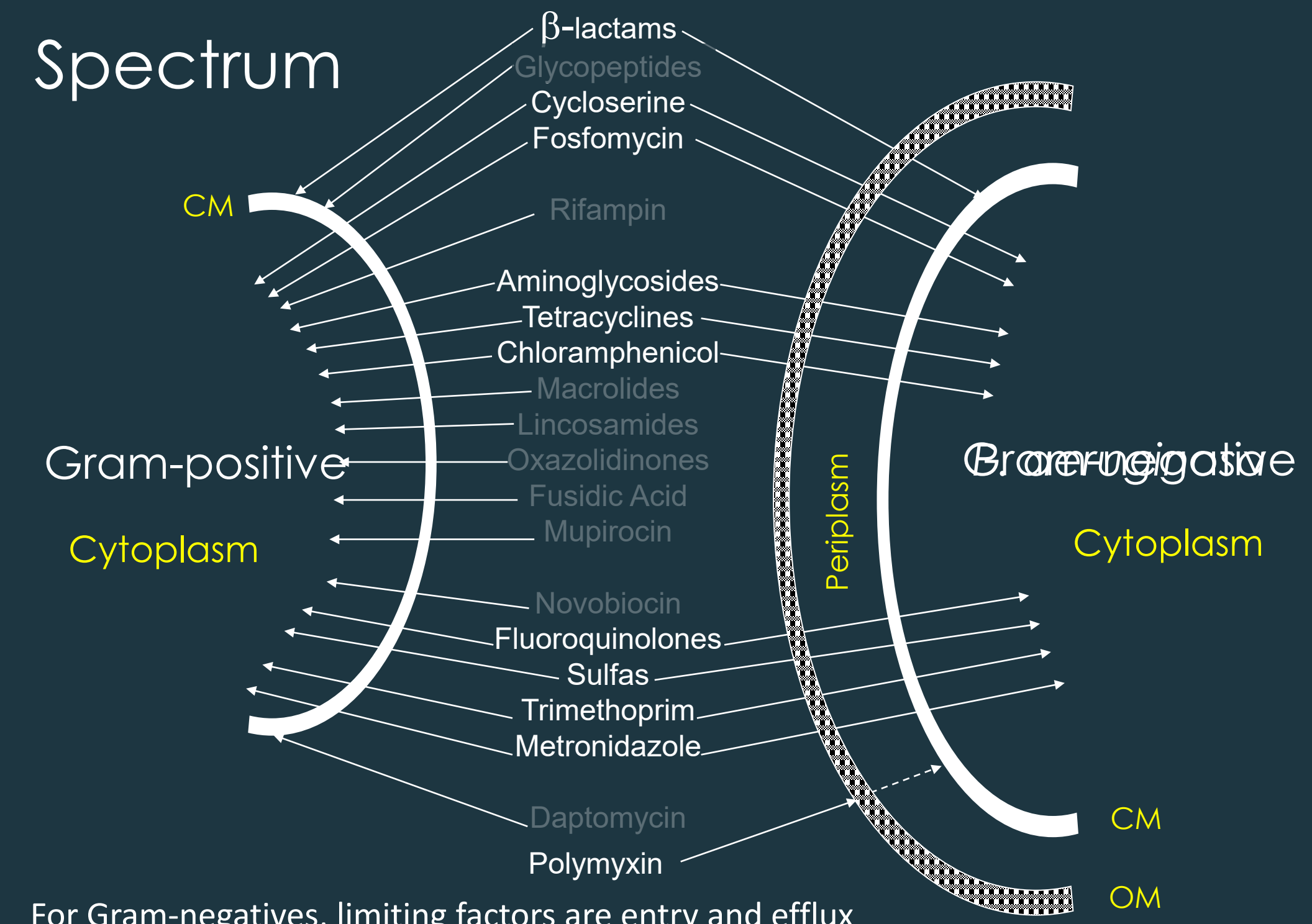
…location, location, location

# What is rate limiting?

- Selection of targets that are not subject to rapid resistance selection

- Chemistry appropriate for antibacterial discovery
  - We have no general rules, or even a rational approach, to getting things into Gram negative bacteria
  - Chemical collections favor physicochemical attributes not associated with antibacterials

# Spectrum

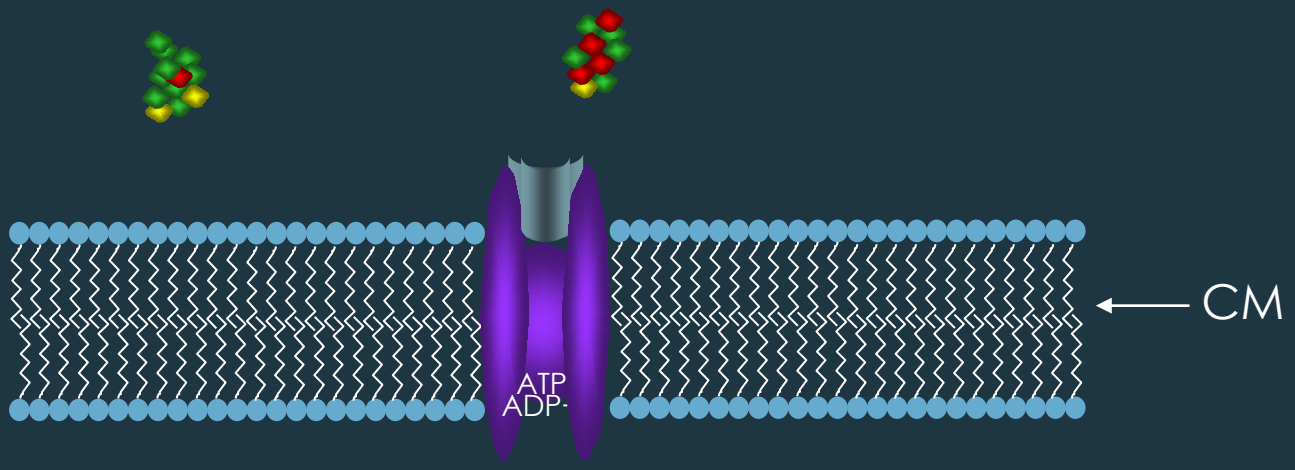



For Gram-negatives, limiting factors are entry and efflux

Gram negative membranes have orthogonal sieving properties

# Cytoplasmic membrane (CM) barrier

- For diffusion through the cytoplasmic membrane (CM) require uncharged, lipophilic species

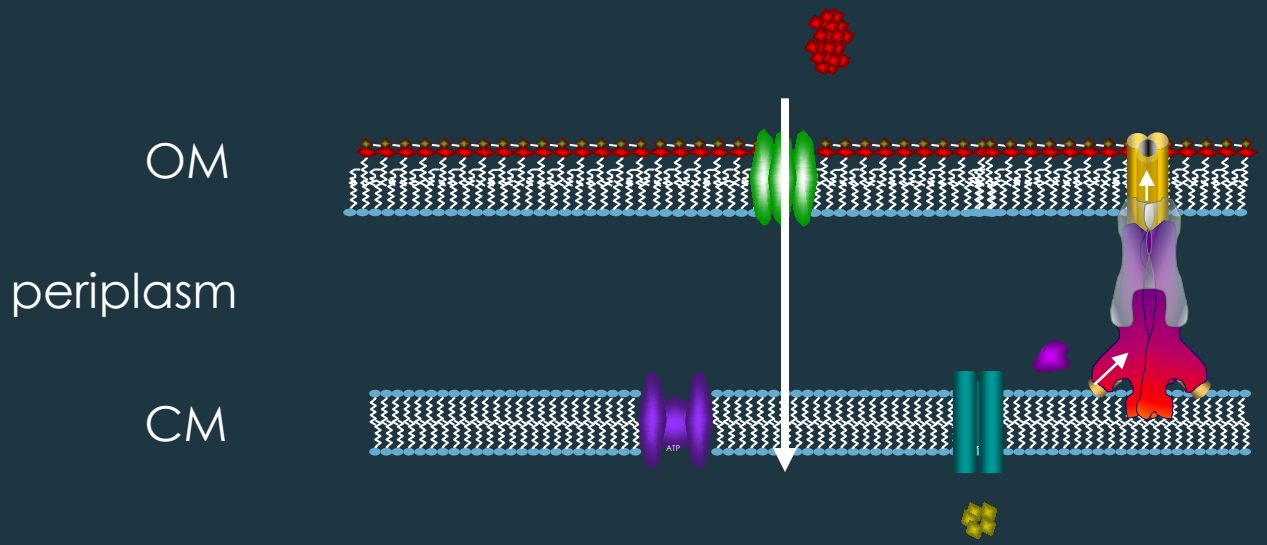



- Polar, hydrophilic, highly charged compounds require active transport

  However, active transport permeases have not been found for most marketed antibacterials

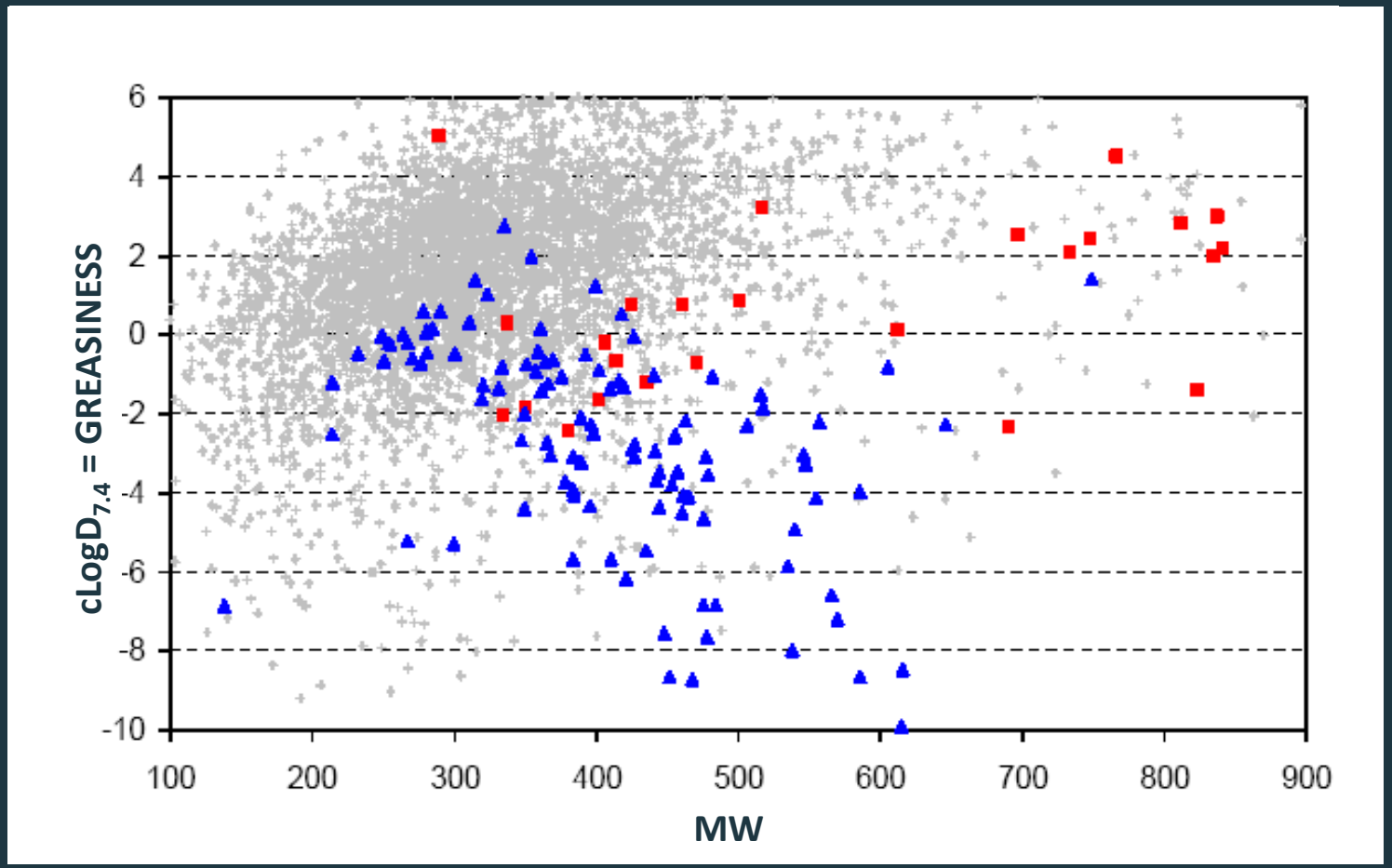
# Gram negative barriers

- The Outer Membrane (OM) of gram negatives adds an orthogonal barrier to that of the cytoplasmic membrane



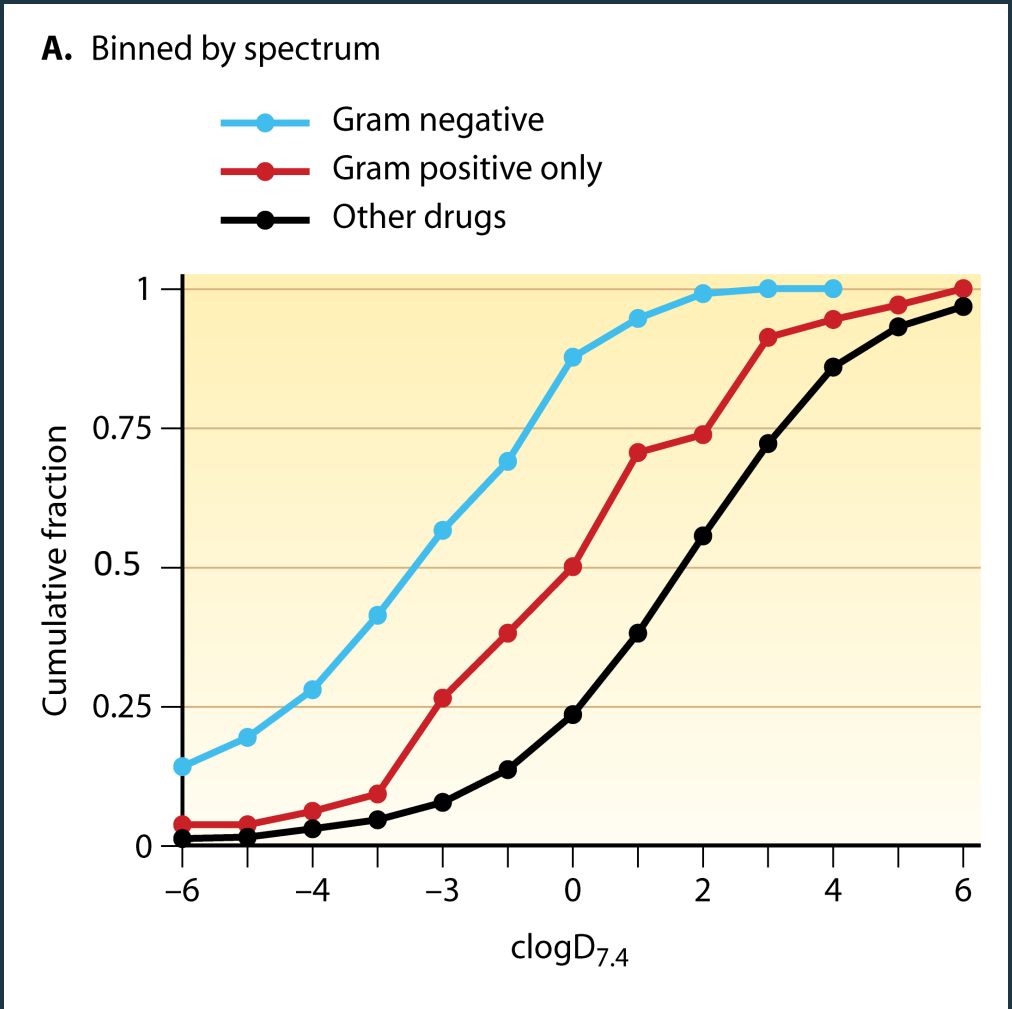

- Penetration of the OM – through porins – prefers small (<600 MW) hydrophilic, charged compounds
- But highly charged molecules can't penetrate the CM (unless actively transported)
- Molecules that do penetrate can be effluxed from the cytoplasm – or periplasm
- What kind of molecules can enter the gram negative cytoplasm?

# Antibacterials Are Chemically Unlike other Drugs



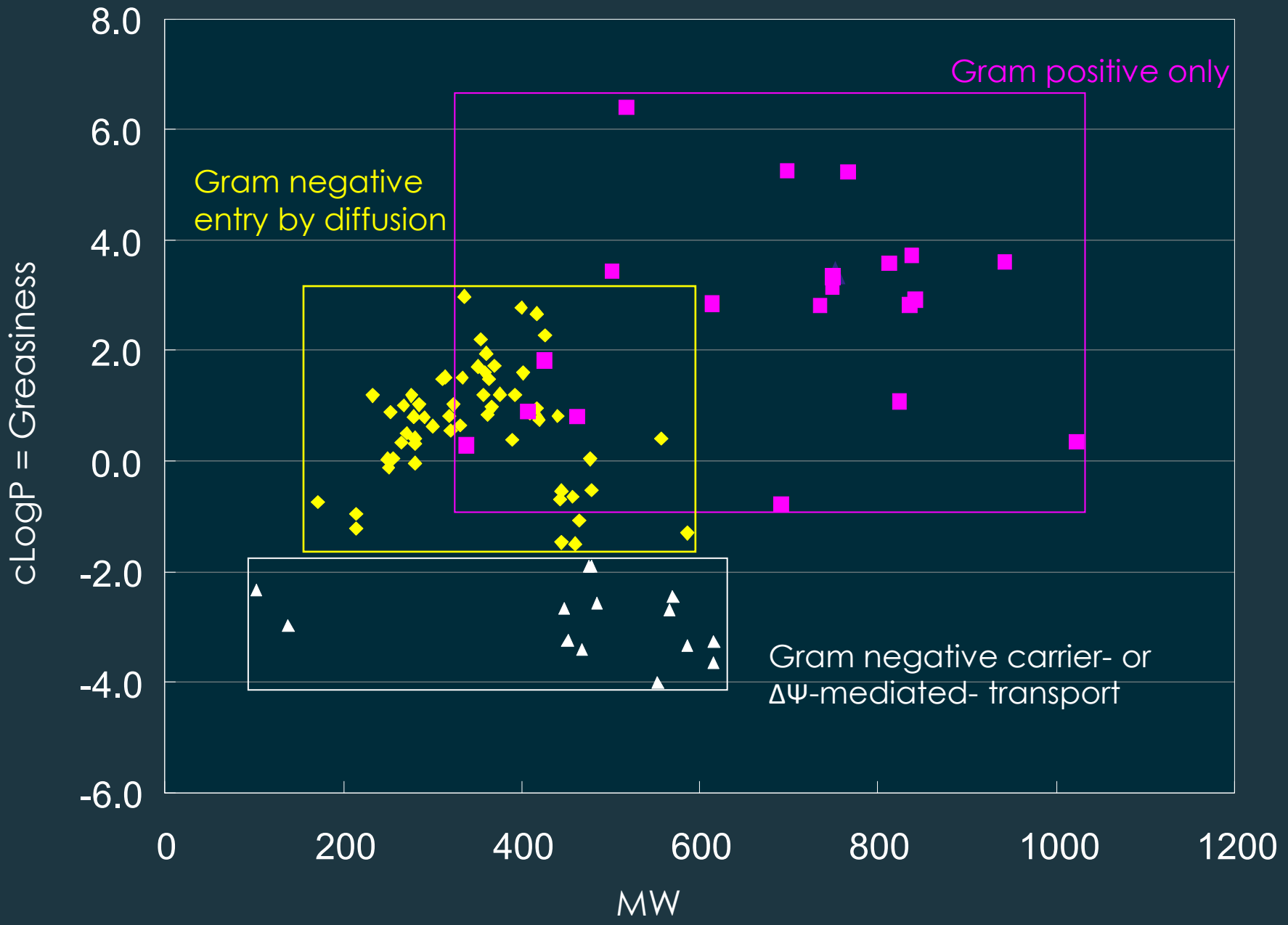

O'Shea, R. O. and H. E. Moser (2008). J. Med. Chem. 51: 2871-2878.

# Characterizing Antibacterials




Silver, L. L. (2011). Clin. Microbiol. Rev. 24(1): 71-109 based on data from O'Shea, R. O. and H. E. Moser (2008).
.

# Cytoplasm-targeted antibacterials




Silver, L. L. (2008). Exp. Opin. Drug Disc. 3(5): 487-500.

# Are there rules for G- entry by diffusion?

- Can a set of rules be arrived at
  - With sufficient data from many more chemotypes
  - Measurement of entry not dependent on activity

- Chemical descriptors
  - cLogD at pH 6.5 through 8
  - MW
  - pKa / charge
  - Radius
  - PSA
  - etc

| | |
|---|---|
| $cLogD_{7.4}$ | -1 to +2 |
| MW | < 500 |
| Charge pH 7.5 | -1 to 0 |

# But some compounds use "tricks"

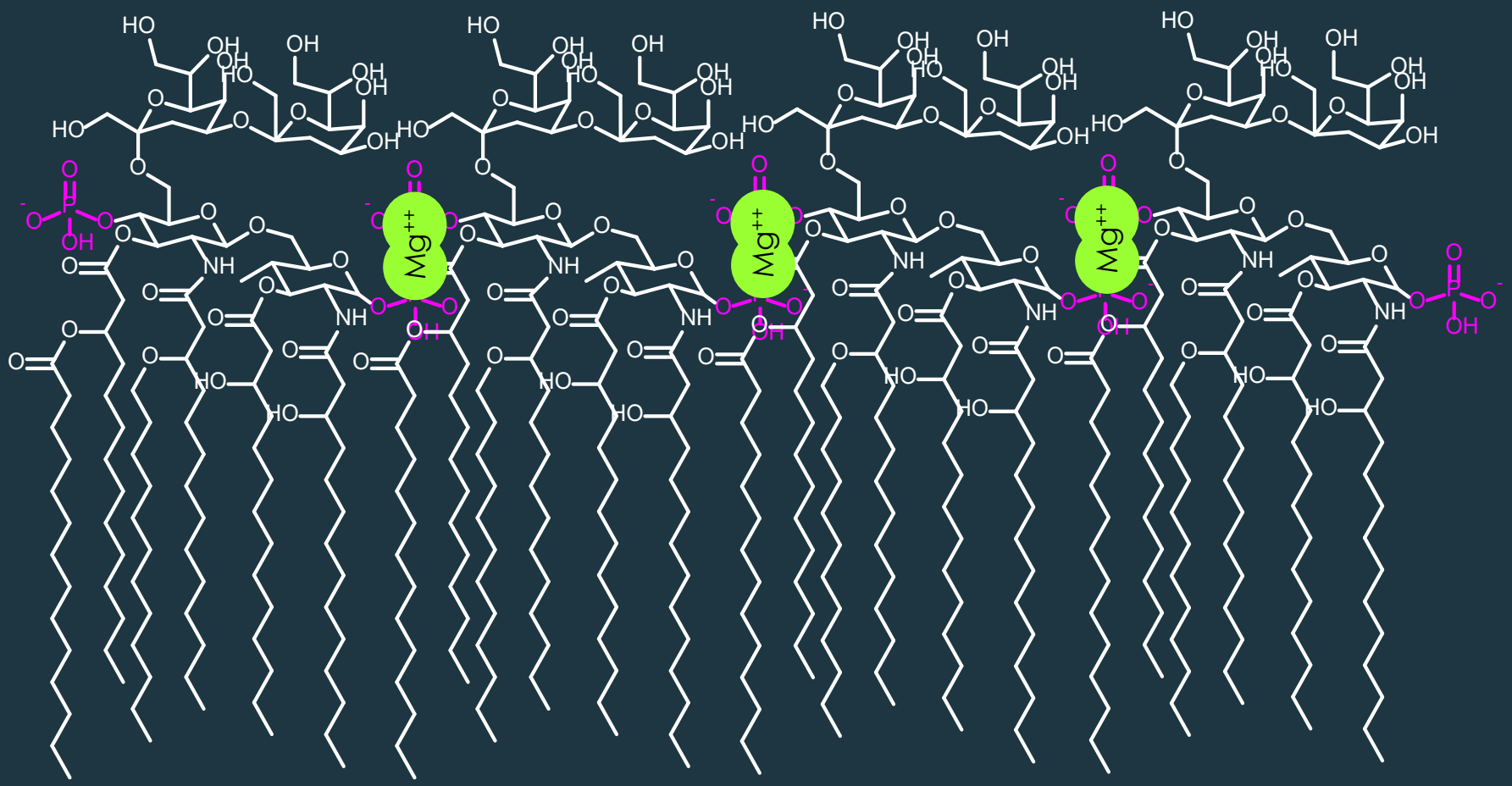
Eg: To cross the outer membrane, cations (or polycations) can use "self-promoted uptake"

- Locally disrupt the outer membrane to enter the periplasm
- Efflux will still play a role
- Crossing the cytoplasmic membrane may be PMF-dependent ($\Delta\Psi$)
- Proposed by Bob Hancock in 1984

Hancock REW. 1984. Ann. Rev. Microbiol. 38:237-264.

# EDTA chelates $Mg^{++}$, disrupts LPS


EDTA at 1 mM



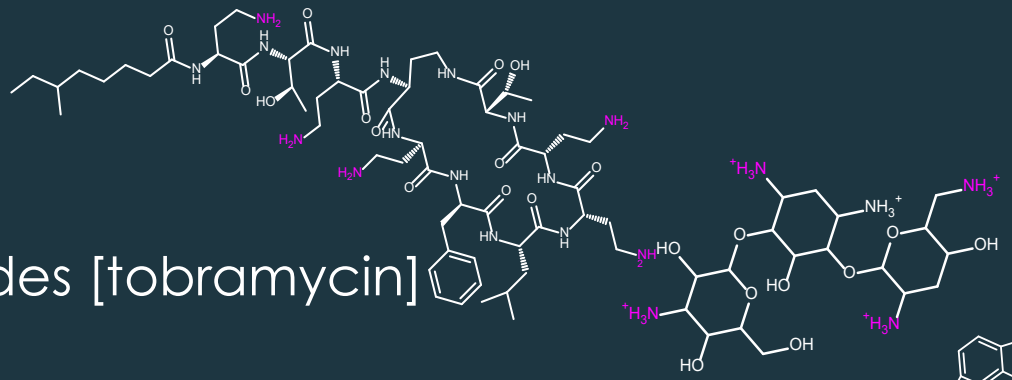

Lipid A

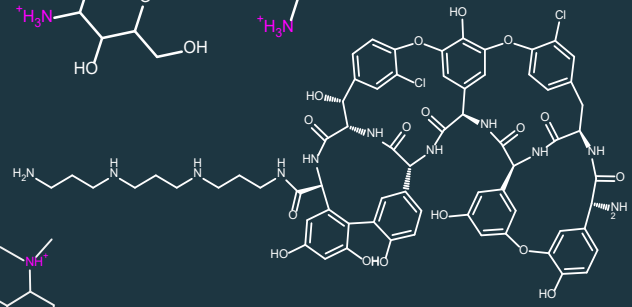
**LPS** Outer leaflet of the OM

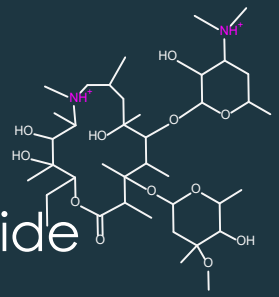
Leive L. 1965. Proc. Natl. Acad. Sci. 53:745-750.

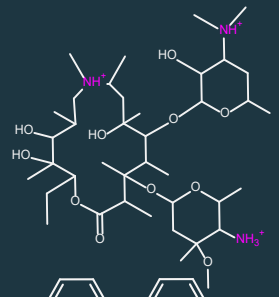
# Compounds proposed to cross the OM by Self-promoted uptake

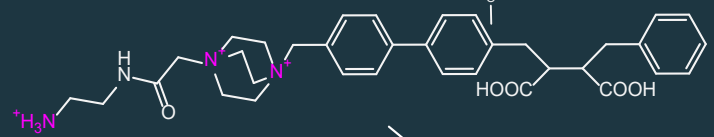
- Polymyxin B
- Aminoglycosides [tobramycin]
- Deglucoteicoplanin-polyamine
- Azithromycin
- Merck amino-azalide
- Merck IMP-inhibitor
- Trius GyrB/ParE?
- Rib-X  04 series?

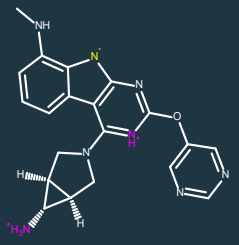


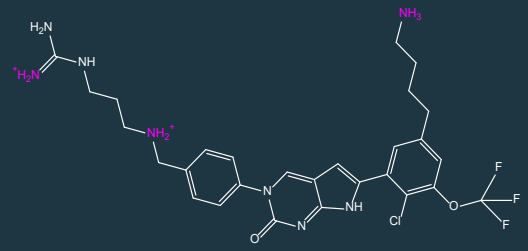






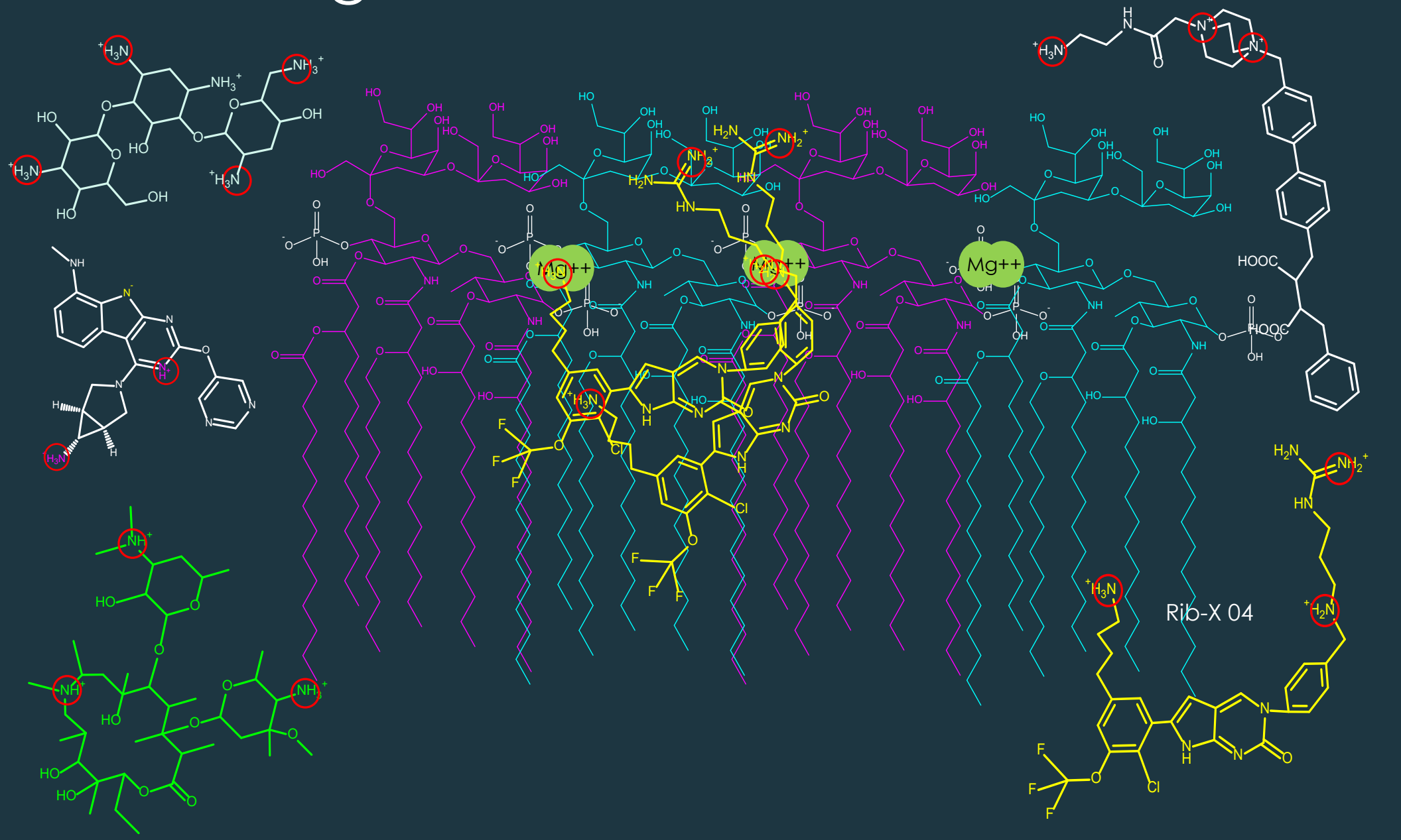






At pH 7.4 all are at least dibasic

# Speculation: Self-promoted uptake by dibasic agents?




Local disruption at ≤ µM concentrations

# Efflux

- Most important for *P. aeruginosa* and other lactose non-fermenters but also for enterics
- Structural information shows a "voluminous aromatic " binding site within the CM (AcrB) subunit
- Computer modeling predicts two general, rather promiscuous, binding sites.
- Will it be possible to find broad spectrum inhibitors?
- Or design compounds to avoid efflux?

# …to discover new antibacterials

- Recognize the nature of targets
  - Explore multiple-targets
  - Expect rapid resistance development w/ single targets
    - Attempt to avoid it by design
  - Explore the use of combinations to block resistance
- Solve the problems of bacterial entry & bad libraries
  - Formulate rules for entry/efflux avoidance
  - Adapt chemical libraries to new rules
  - Explore self-promoted uptake
  - Revive natural products screening
- Track entry and efflux as well as target inhibition, throughout optimization

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