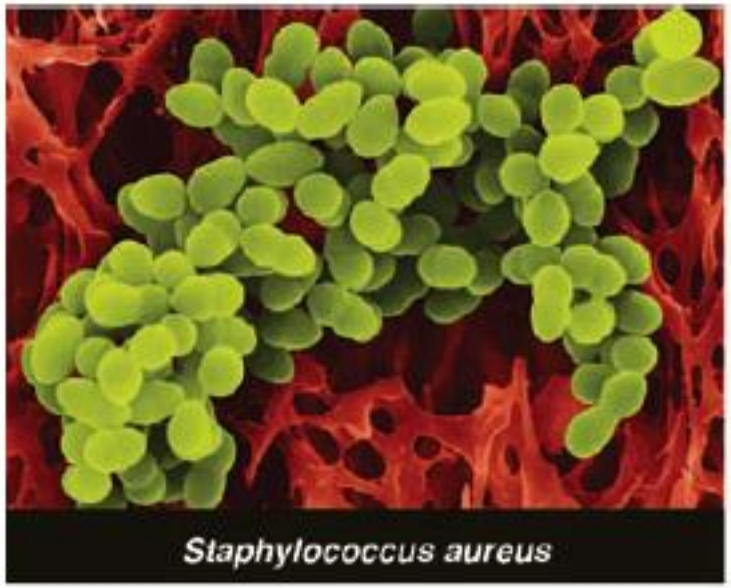
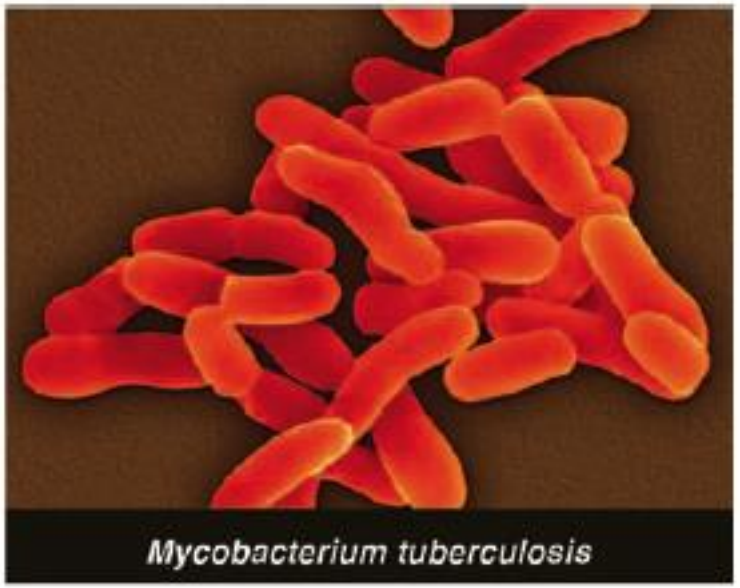
# Using Synergy to Potentiate Antibiotics and to Study Mechanisms of Antibiotic Action

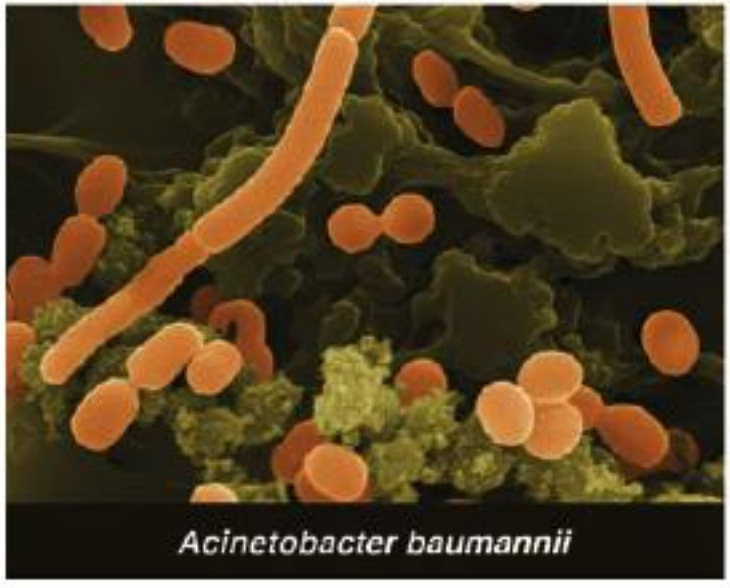
Jeffrey H. Miller Lab
UCLA



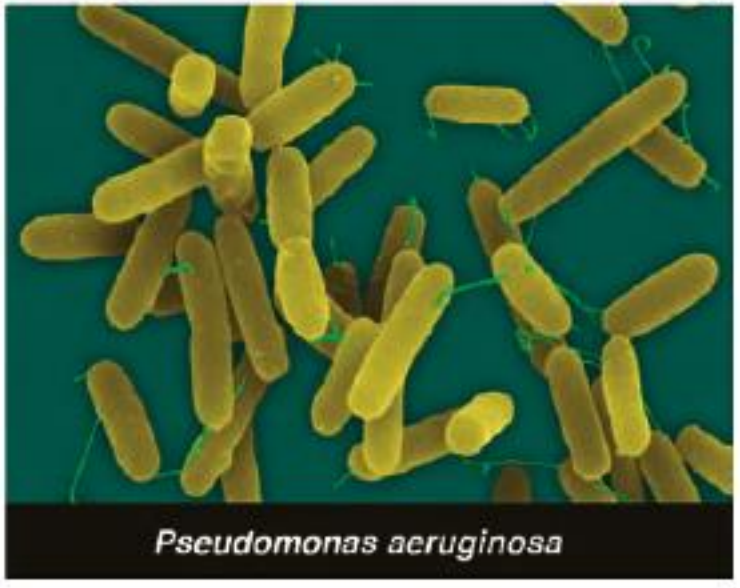
*Staphylococcus aureus*



*Mycobacterium tuberculosis*



*Acinetobacter baumannii*



*Pseudomonas aeruginosa*

# Brazilian amputee model dead at 20



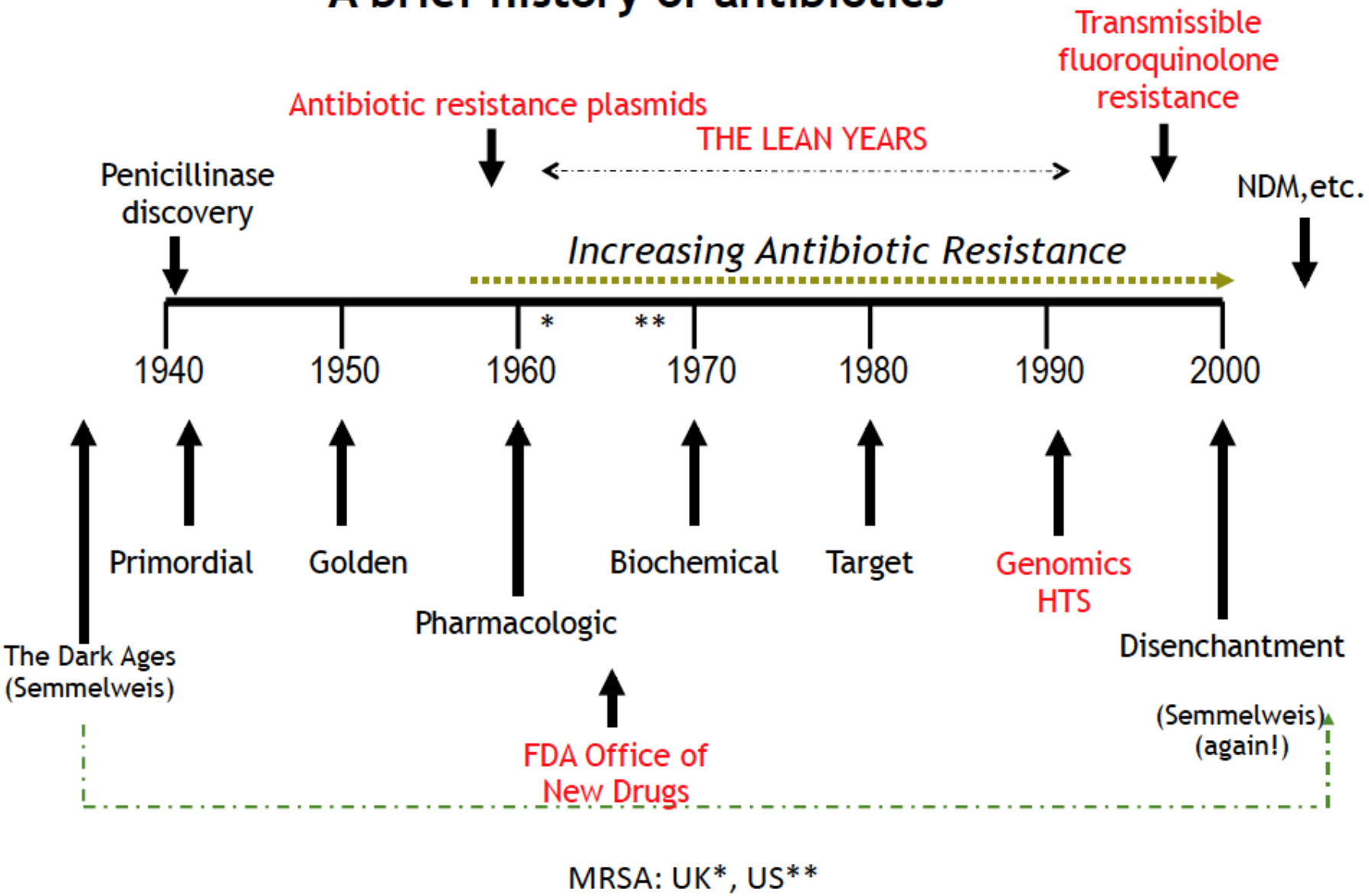

**·STORY HIGHLIGHTS**

·**NEW:** Brazilian amputee model Mariana Bridi da Costa died early Saturday

·Da Costa's hands, feet were amputated after she contracted septicemia

·Da Costa placed sixth in the Miss Bikini International competition in China

# A brief history of antibiotics



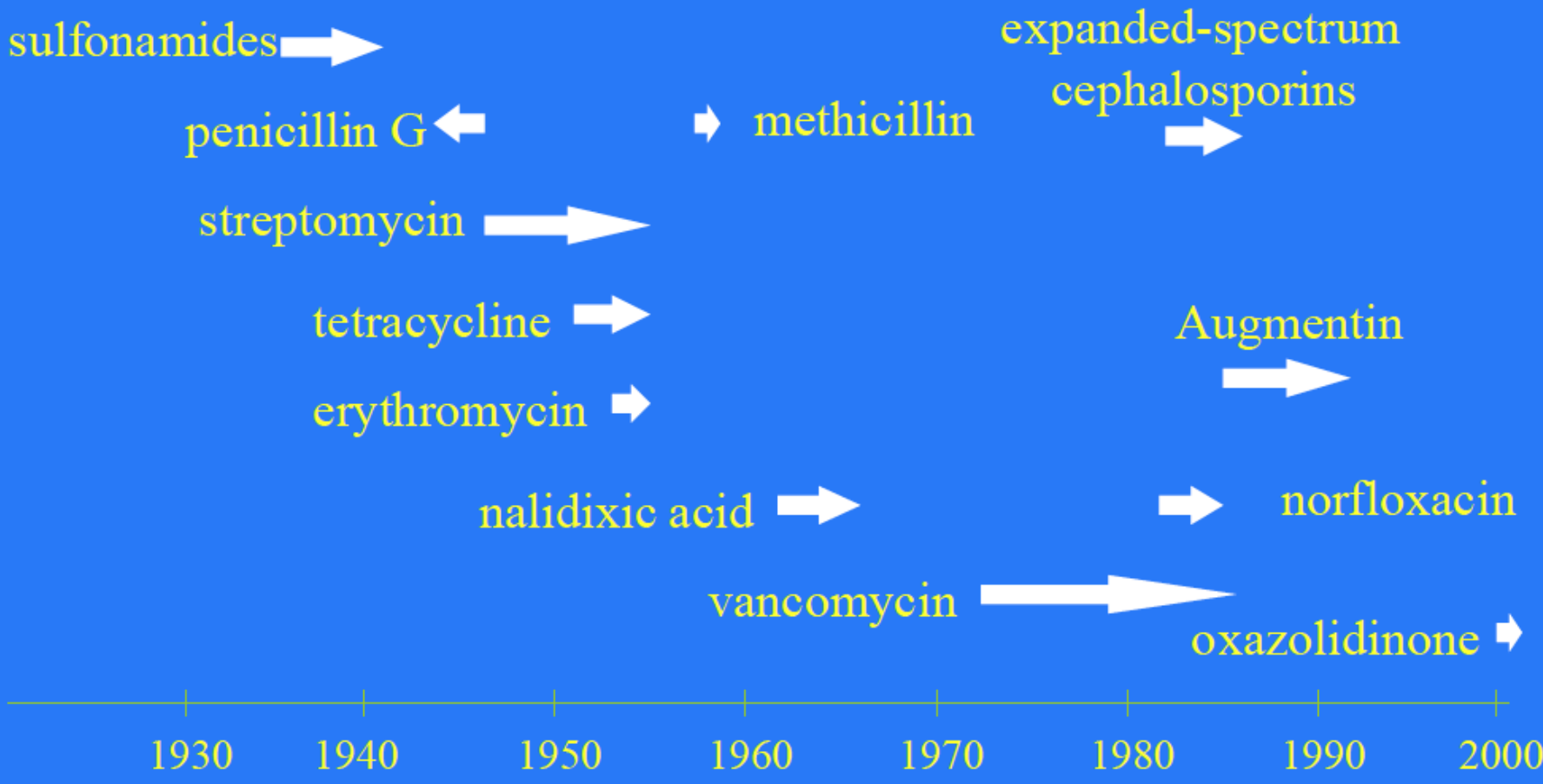

MRSA: UK*, US**

"It is time to close the book on infectious disease"



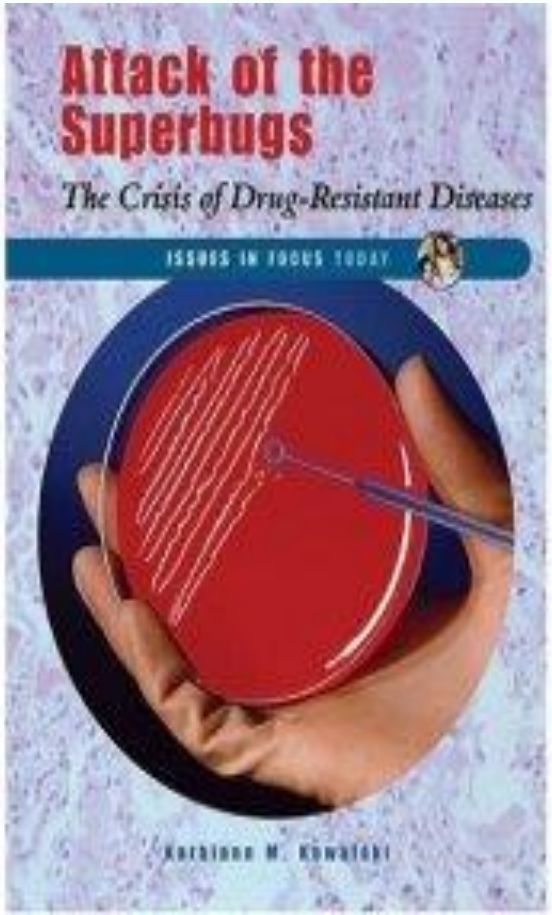
William H. Stewart, U.S. Surgeon General
Testimony before the U.S. Congress, 1969

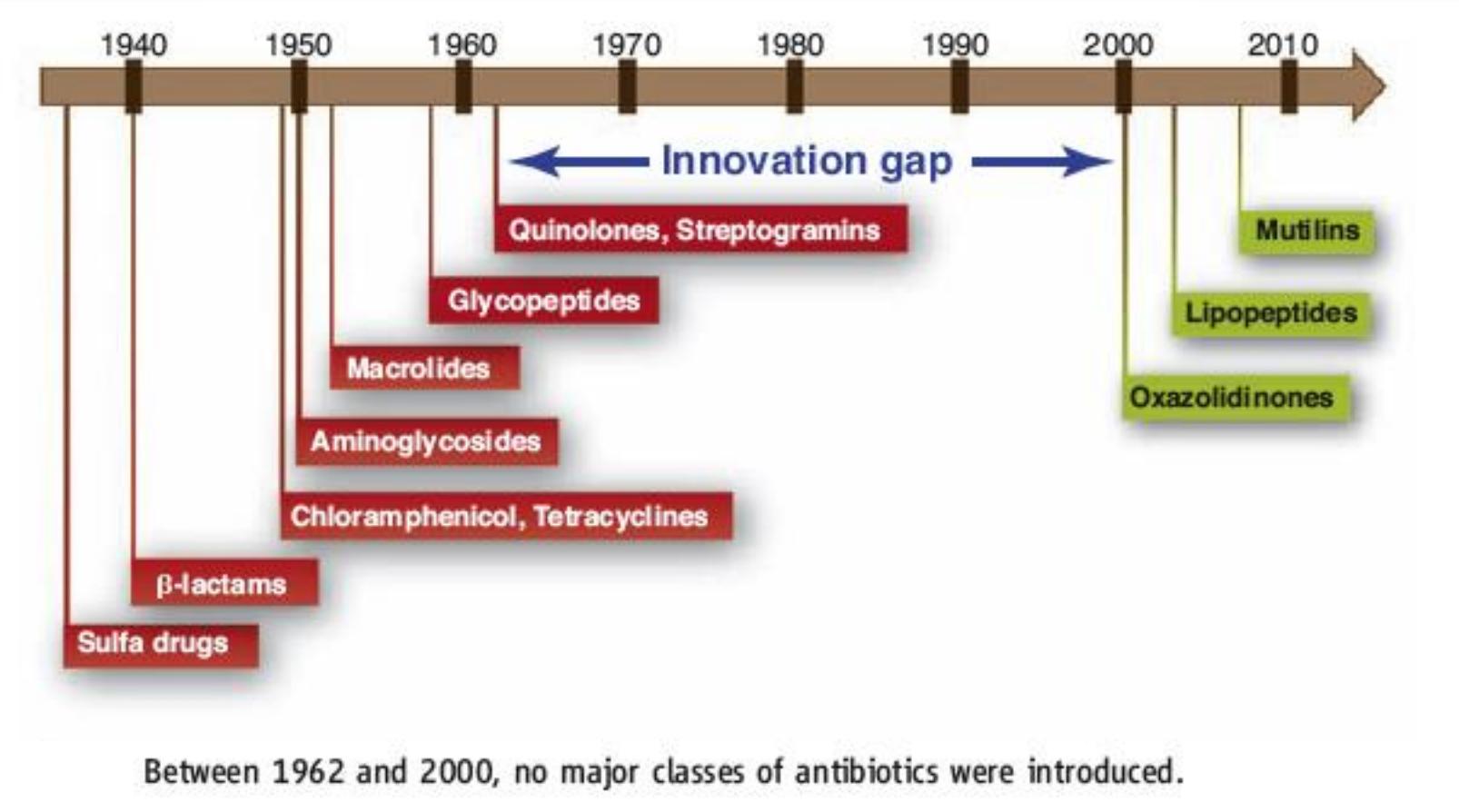
# Resistance to Antibiotics Develops Rapidly



# Some Statistics

- more than 70 percent of the bacteria that cause hospital-acquired infections are resistant to at least one of the antibiotics most commonly used to treat them.
- Many infectious diseases are increasingly difficult to treat.
- Over 2 million fall prey to microbes once they get in the hospital, in this country alone. Some 90,000 die. About 70 percent of those are infected by drug-resistant bacteria. Costs for treatment of these infections approach $5 billion a year. Overall, the yearly toll exacted by drug-resistant infections in the United States is estimated to exceed $30 billion.

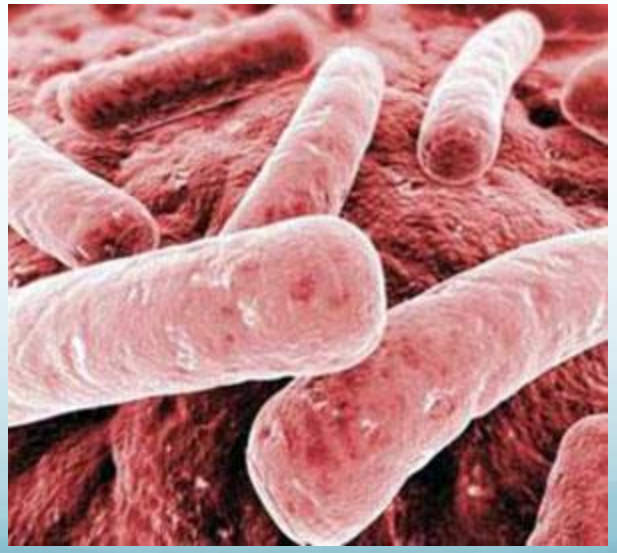





Between 1962 and 2000, no major classes of antibiotics were introduced.

# Gram-Negative Bacteria

- Multi-drug resistant bacteria are now a serious threat
  - Hospital-acquired *Klebsiella pneumoniae*
    - In a 2011 outbreak at the U.S. National Institutes of Health Clinical Center, 11 of 18 affected patients died (Snitkin et al. 2012)



# Approaches/Methods

- Find new antibiotics from natural sources
- Make chemical derivatives of existing antibiotics
- Find new targets in cell
- Find new combinations of existing drugs
- Find co-drug targets and co-drugs (potentiators)



# Recent Approaches

1. Finding natural products derived from nonconventional sources (plants, marine microorganisms, insects)

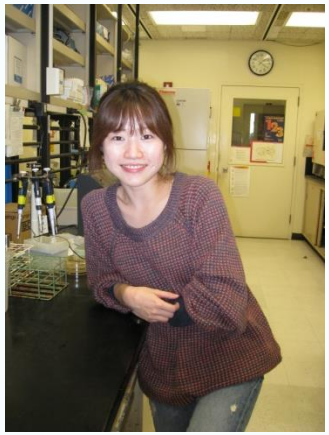
2. Using rational design to make new chemical derivatives of existing antibiotics from 3D structures

3. Linking peptides to drugs to facilitate entry through cell membranes

# Our Approach

Investigate synergy with new combinations of already approved antibiotics

# Thank you to…



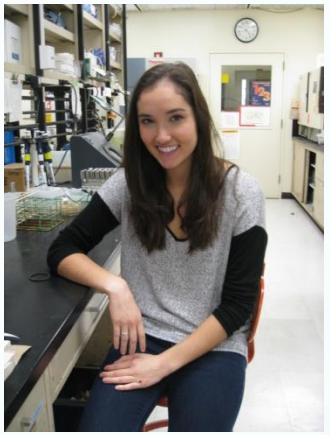
Tina Kang



Jessica Yuan



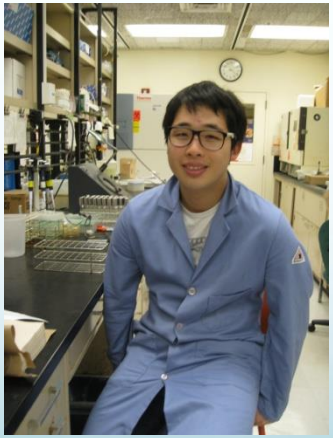
Alice Zhou



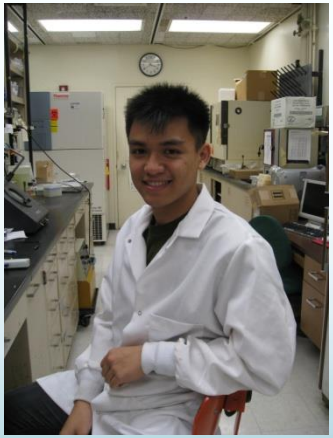
Casey Beppler



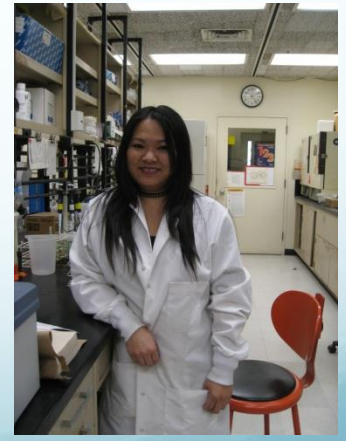
Caroline Nguyen



Jack Mao

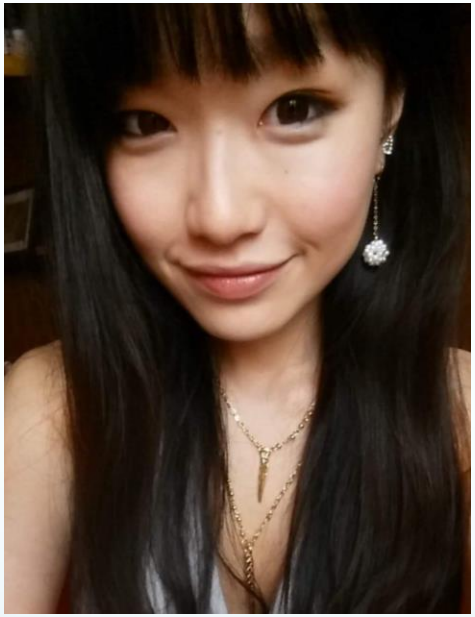


Minh Quan Nguyen

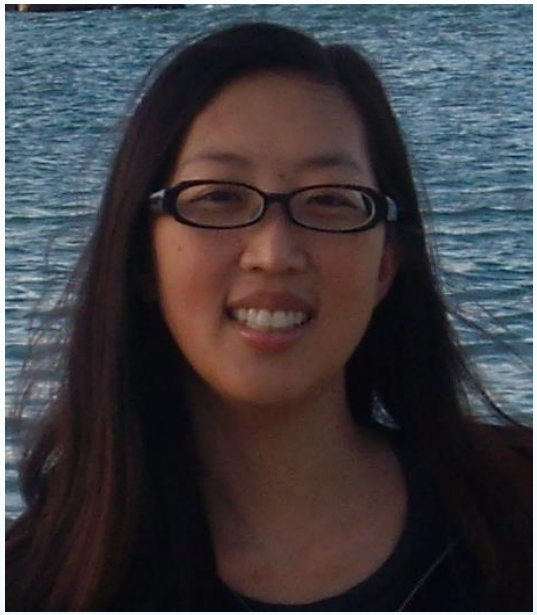


Chris Vu

# Thank you to…



Lisa Song



Pamela Yeh

# Pairwise Interactions of Antibiotics

- Pamela Yeh, et al. 2006

- Classified drugs according to similar interactions with other antibiotics

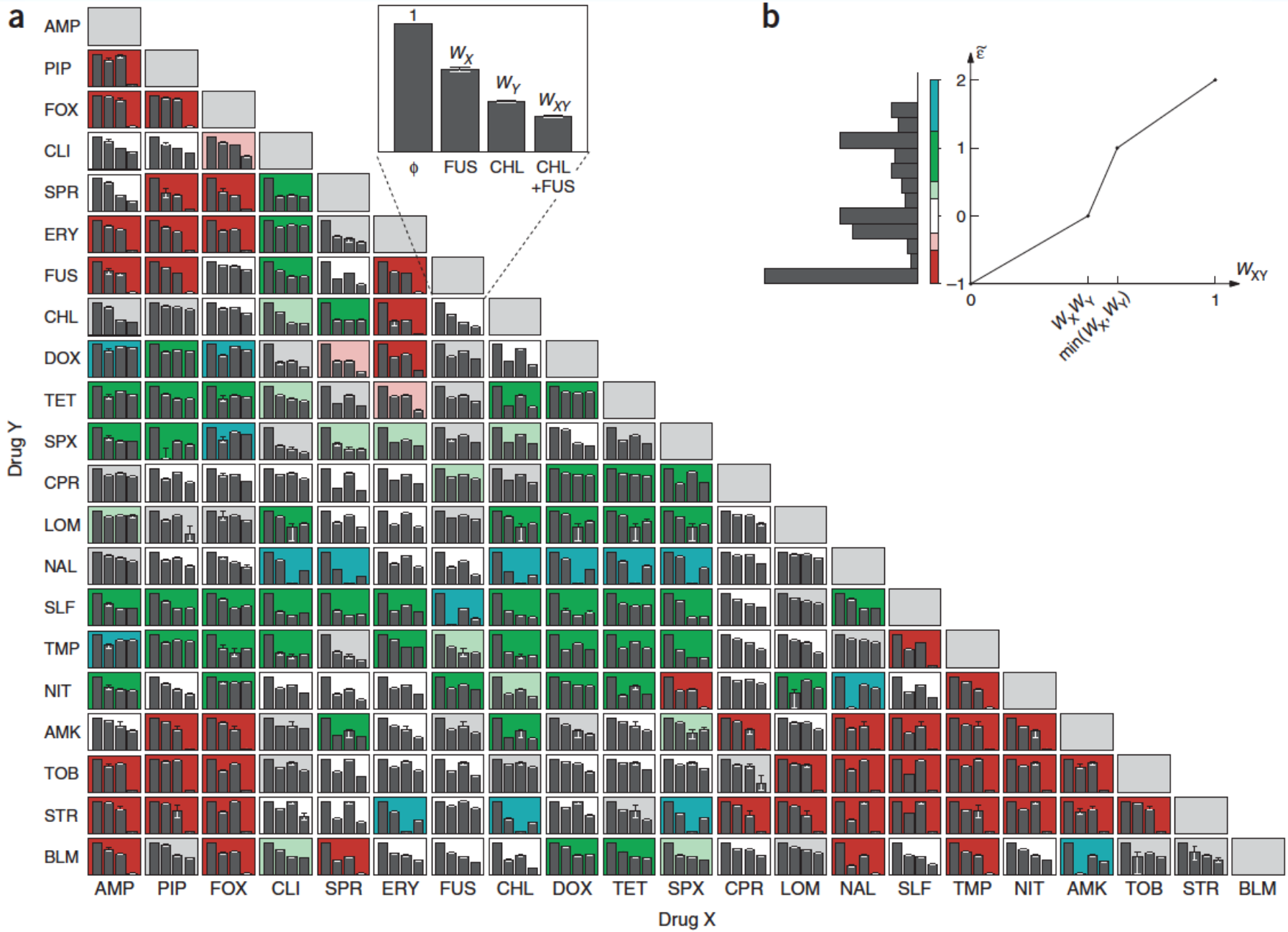
- Groups formed according to biochemical function

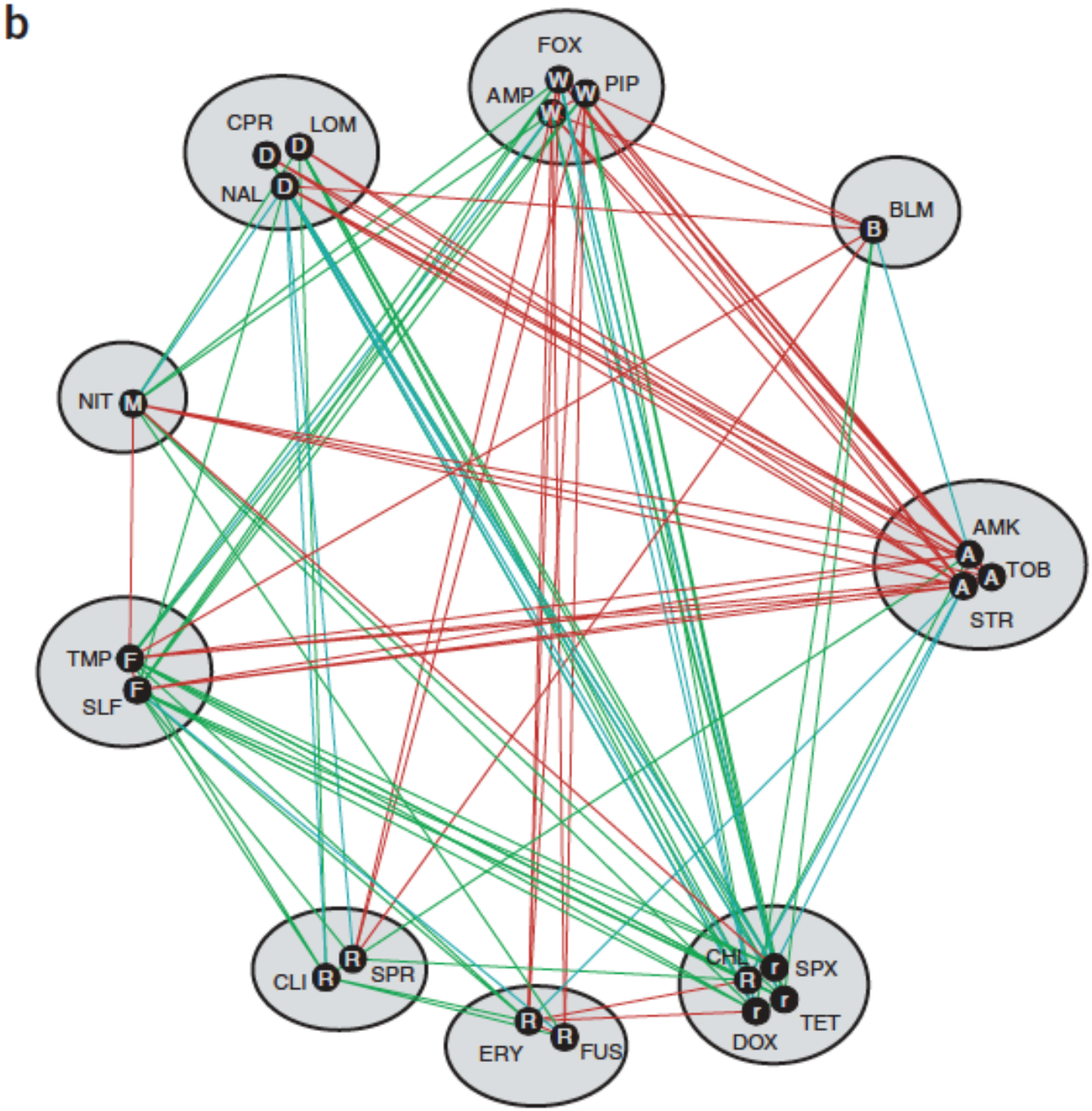
# Pairwise Interactions of Antibiotics

- General Procedure:
  1) Define sub-lethal concentrations for each antibiotic
     Concentration that doesn't kill, but leads to about 50-90% growth

  2) Test no-drug control, drugs individually, and drugs in combination

  3) Define interaction as synergistic, additive, antagonistic or suppressive

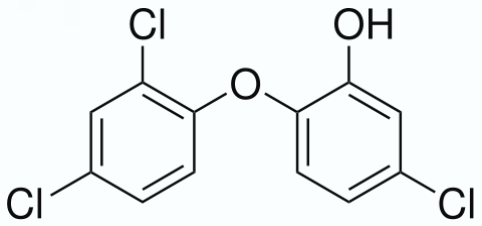

a
b
AMP
PIP
FOX
CLI
SPR
ERY
FUS
CHL
DOX
TET
SPX
CPR
LOM
NAL
SLF
TMP
NIT
AMK
TOB
STR
BLM
Drug Y
Drug X
1
$W_X$
$W_Y$
$W_{XY}$
ϕ
FUS
CHL
CHL +FUS
$\tilde{\varepsilon}$
2
1
0
−1
$W_{XY}$
$W_X W_Y$
$\min(W_X, W_Y)$


b
FOX
AMP
PIP
CPR
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SPX
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Questions

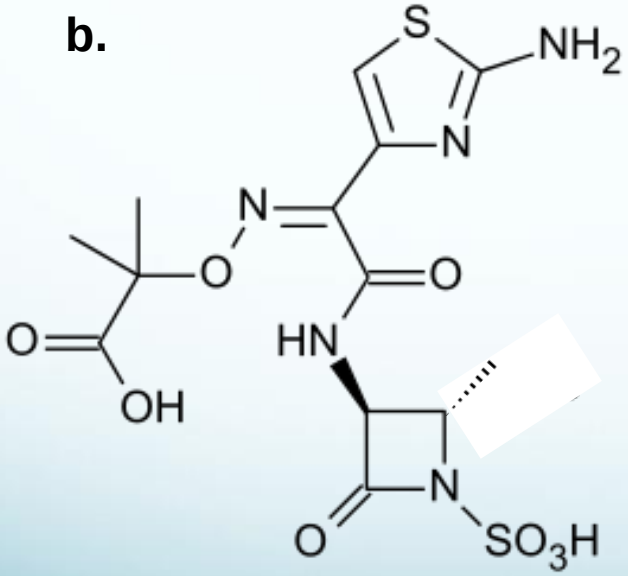
1. Can we expand the drug interaction network?
2. Can we use synergy to potentiate antibiotics?
3. What is the mechanism of synergy?
4. How do bactericidal antibiotics kill cells?
5. Can we use synergy to test models of killing?

**a.**



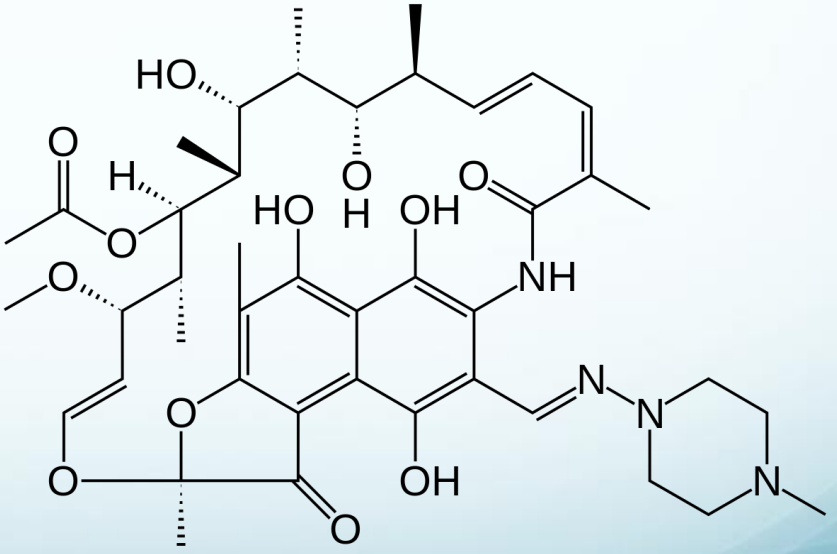

Triclosan

**b.**

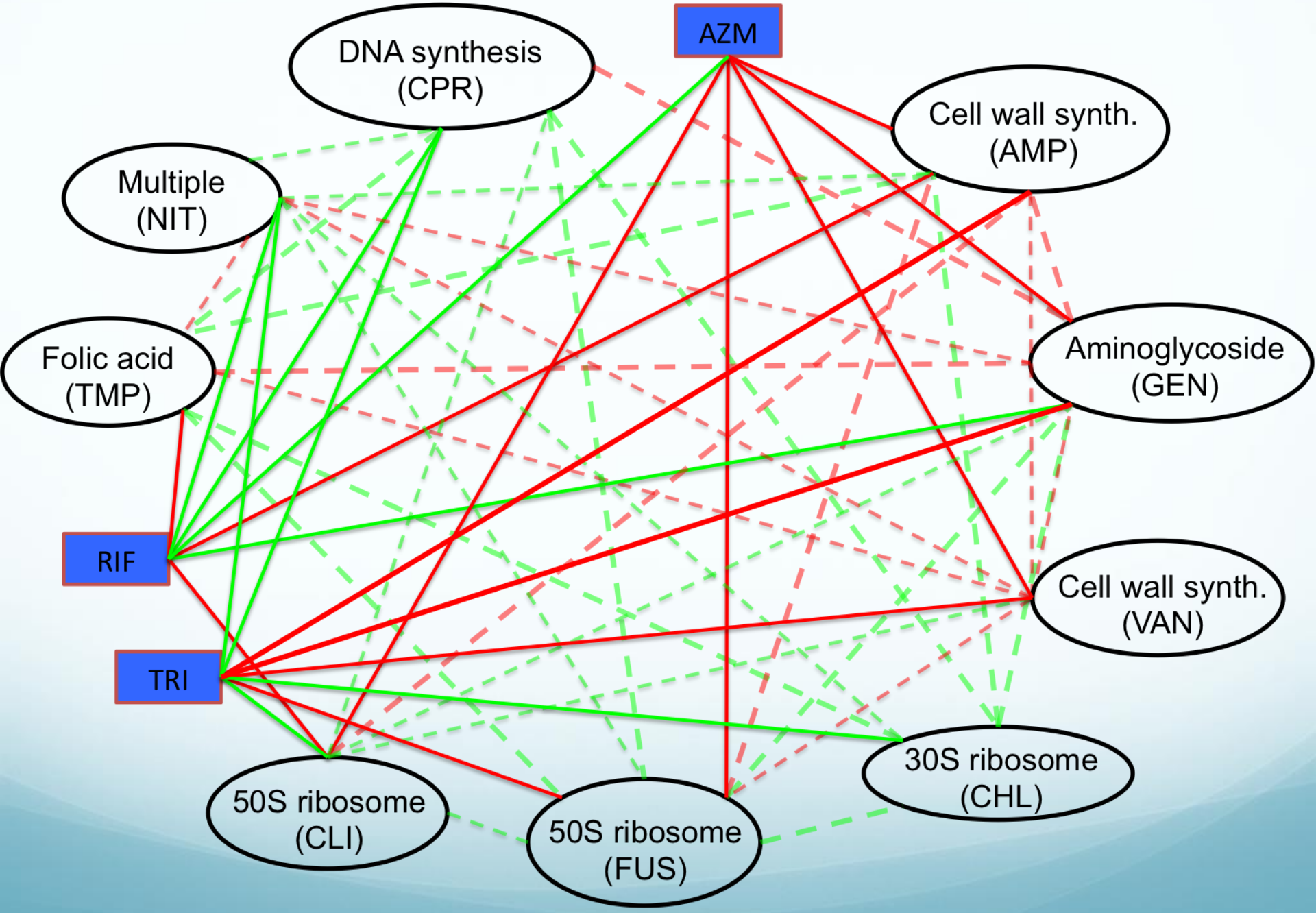



Aztreonam

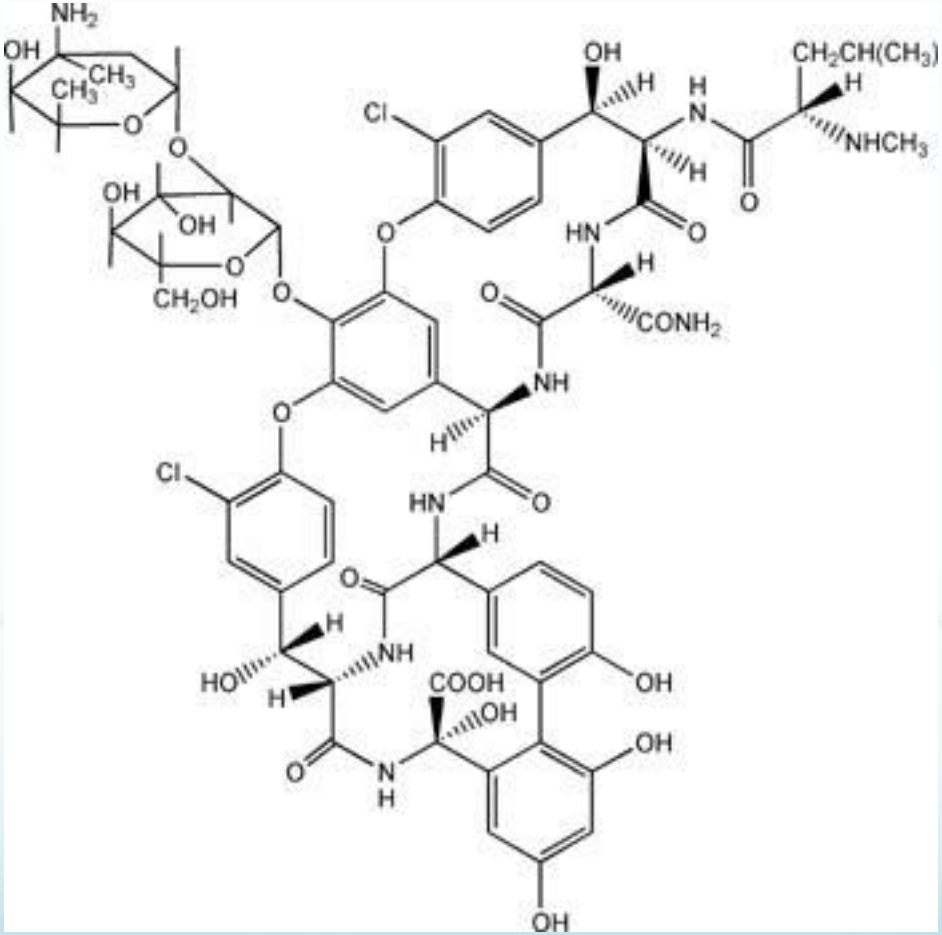
**c.**




Rifampicin


AZM
DNA synthesis (CPR)
Cell wall synth. (AMP)
Multiple (NIT)
Folic acid (TMP)
Aminoglycoside (GEN)
RIF
Cell wall synth. (VAN)
TRI
30S ribosome (CHL)
50S ribosome (CLI)
50S ribosome (FUS)

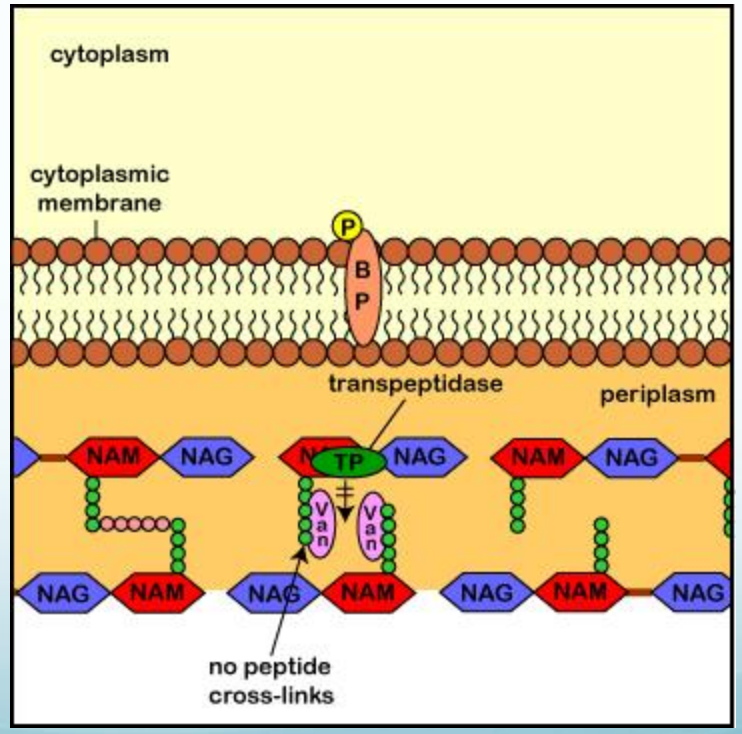
# Vancomycin

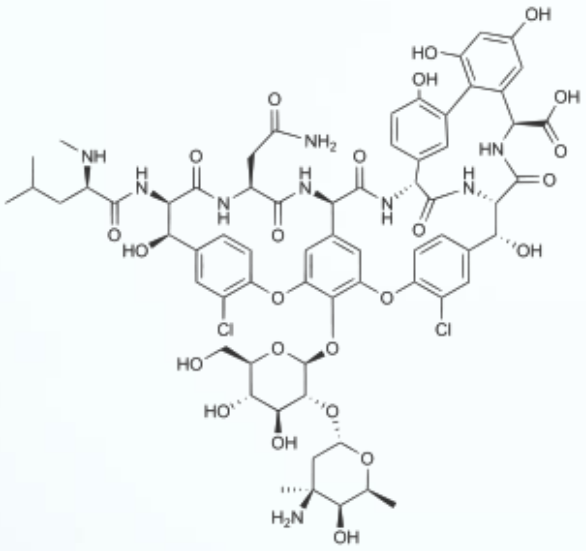

NH2
OH
CH3
CH3
O
O
OH
OH
O
CH2OH
O
Cl
OH
H
H
N
CH2CH(CH3)
H
NHCH3
H
O
O
HN
O
H
O
CONH2
NH
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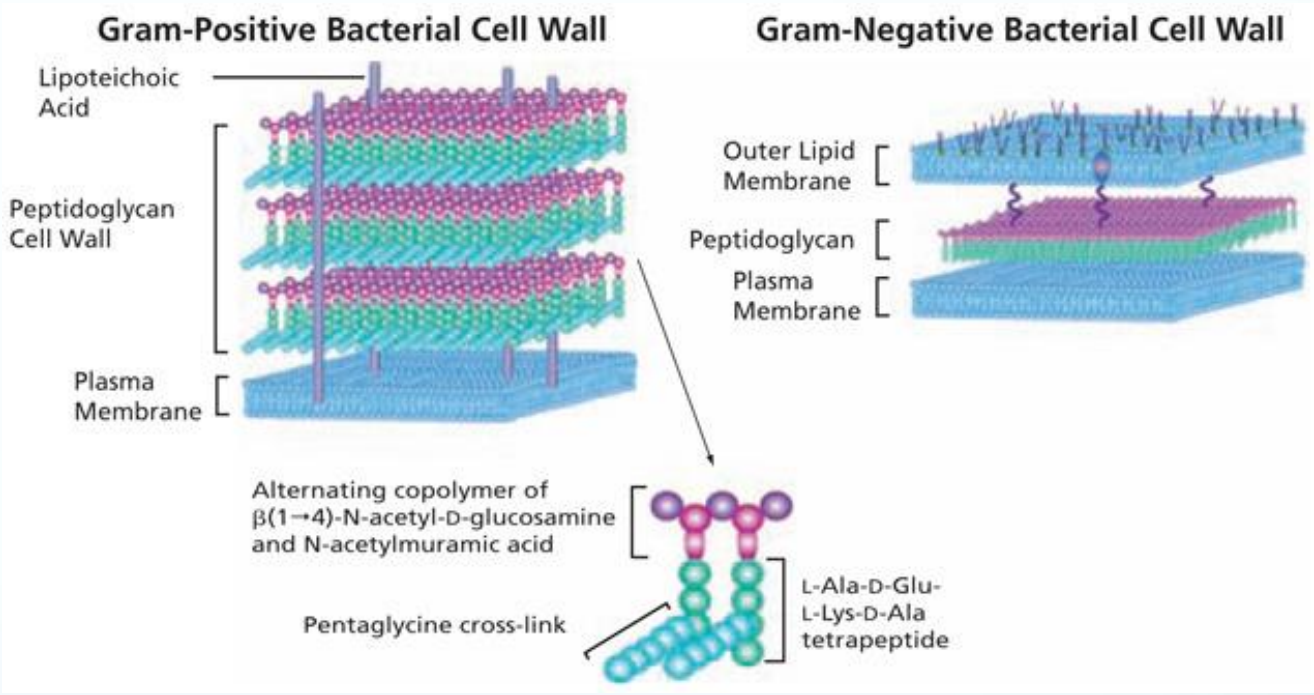
# Why vancomycin?

- Highly potent drug
- Mechanism of action: Inhibits cell wall synthesis in Gram-positive bacteria

Peptidoglycan








- But…
  - Extremely large molecule
  - Unable to penetrate the outer membrane of Gram-negative bacteria

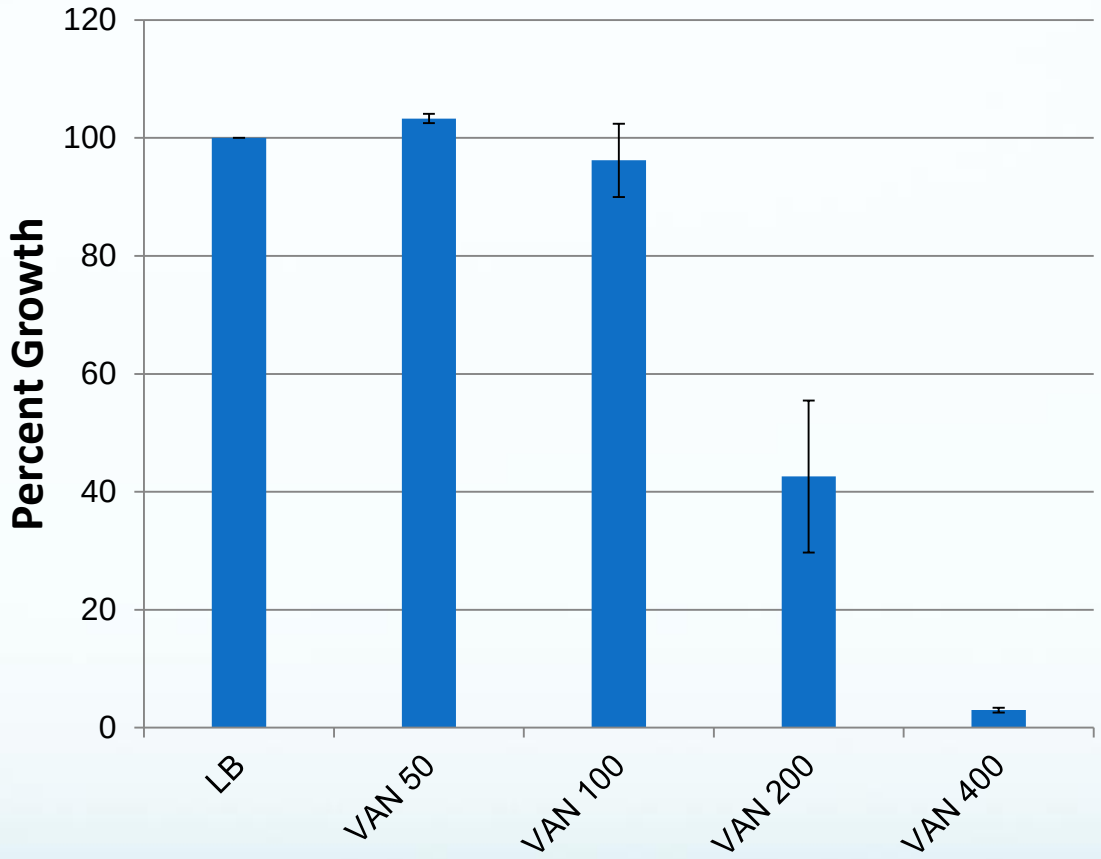
# Why use *Escherichia coli?*

- Avoid working with pathogenic *K. pneumoniae*
- Also a Gram-negative bacteria
  - Specifically gammaproteobacteria
  - Behaves similarly to *K. pneumoniae*
    - Enzymes
    - Metabolism
    - Permeability
    - Drug interactions



# Can we find a way to use vancomycin against Gram-negative bacteria?

- Clinically relevant doses in wild-type *E. coli* are ineffective
- Highly synergistic interactions with vancomycin could potentiate its effects
- Study drug interactions to develop combinatorial drug therapies




Vancomycin (µg/ml) susceptibility of WT in LB medium

# Pairwise Interactions of Antibiotics

- Yeh, et al. did not include vancomycin
- The inefficiency of vancomycin in wild-type *E. coli* makes it a poor candidate for initial studies of drug interactions.
- Instead, we can take advantage of weakened backgrounds to see how vancomycin might interact with other drugs in *E. coli.*
  - SurA-deficient

# SurA-Deficient *E. coli*

- peptidyl-prolyl cis-trans isomerase (SurA)
- Periplasmic molecular chaperone that facilitates the folding and assemblage of outer-membrane proteins in Gram-negative bacteria
- SurA-deficient *E. coli m*ore

  sensitive to certain antibiotics

# Vancomycin MIC (μg/ml)

| | |
|---|---|
| WT | 500 |
| *surA* | 4 |
| *smpA* | 70 |
| *surA smpA* | 1.5 |

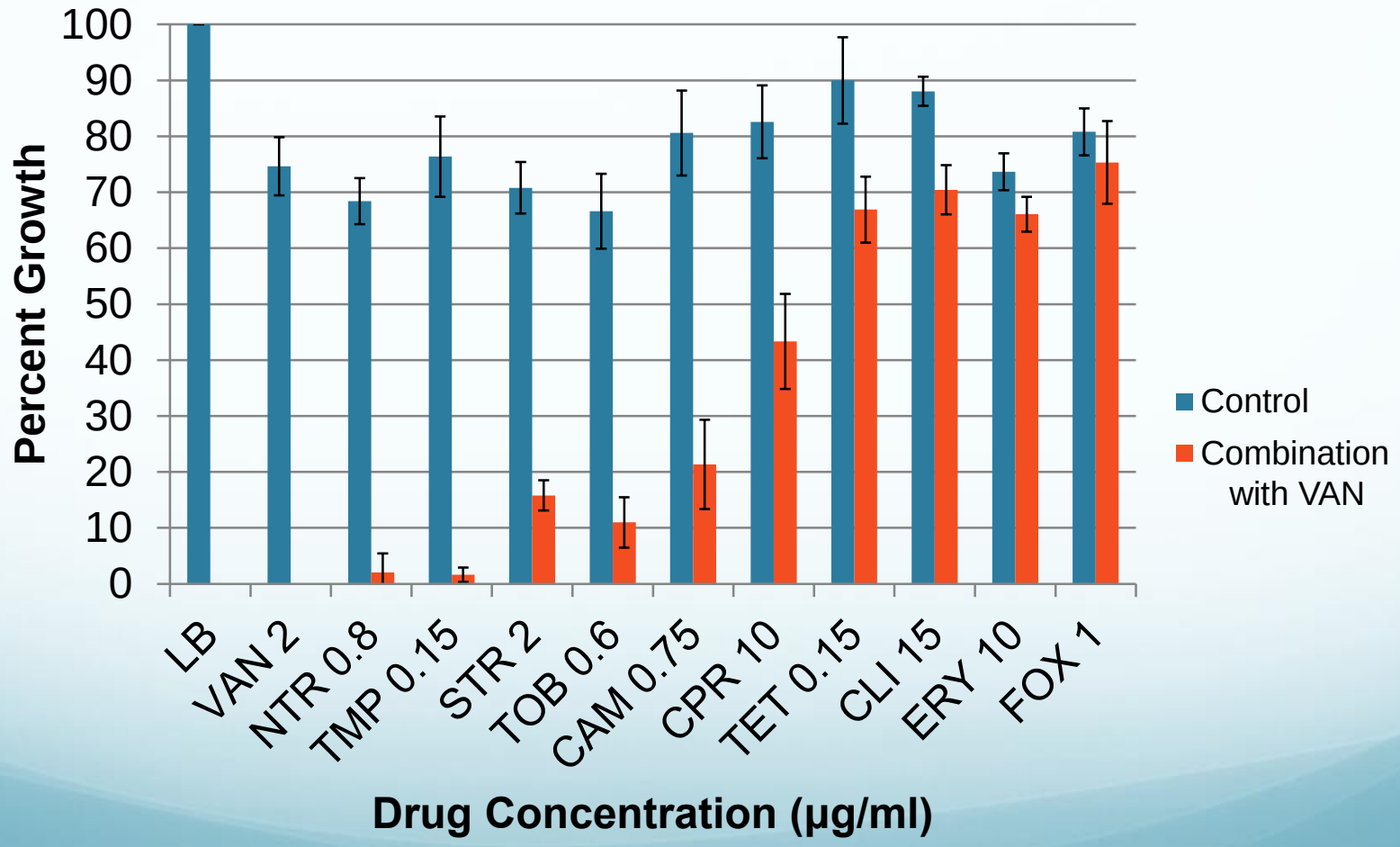
# Experimental Design

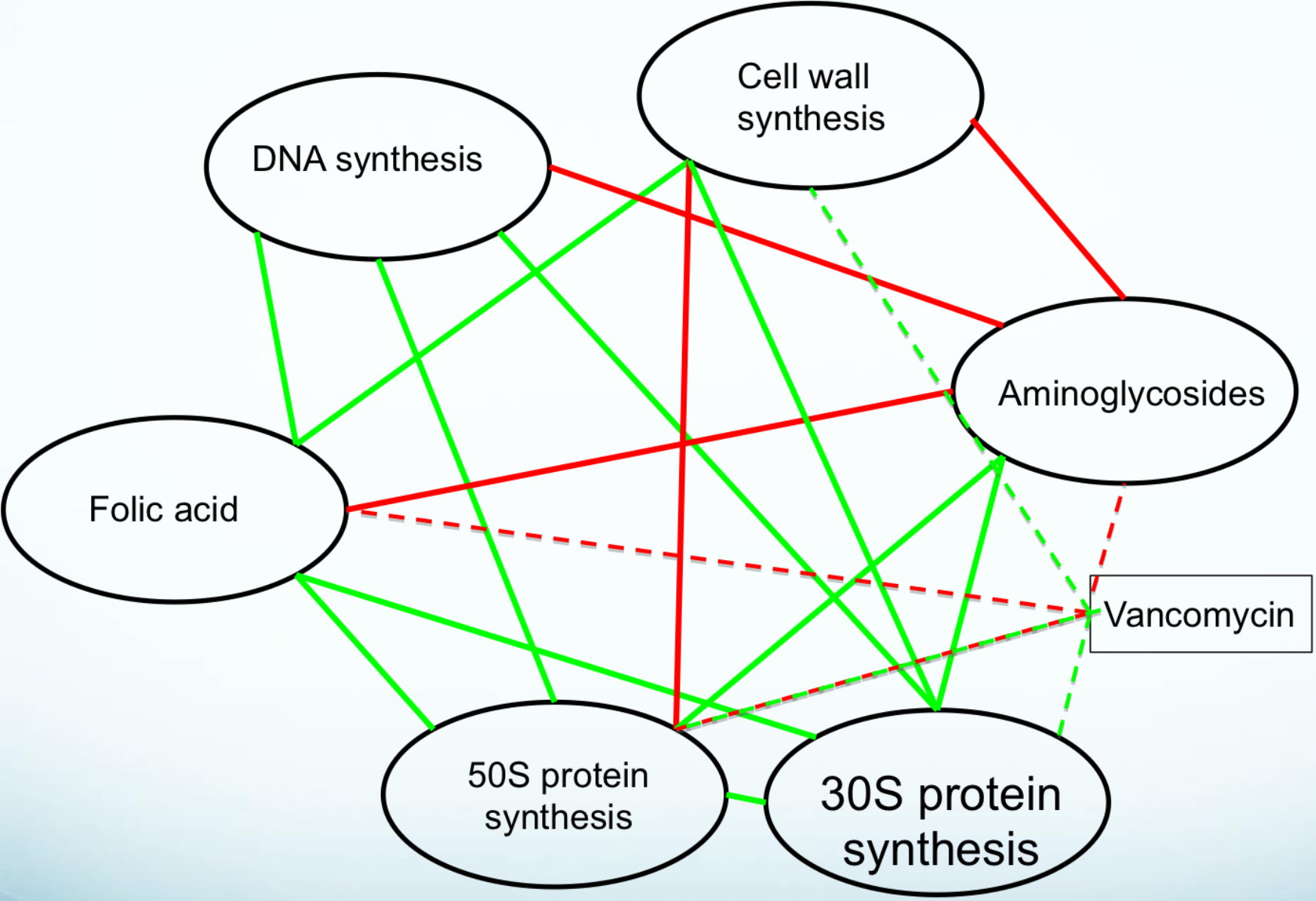
1) Define sub-lethal dose for each background.
2) Test for synergy in the SurA-deficient background.
3) Test promising synergistic combinations in the wild-type background.

# Drugs and Mechanism of Action

| Antibiotic | Abbreviation | Primary Target |
|---|---|---|
| Cefoxitin | FOX | Cell wall |
| Chloramphenicol | CAM | Protein synthesis, 50S |
| Ciprofloxacin | CPR | DNA gyrase |
| Clindamycin | CLI | Protein synthesis, 50S |
| Erythromycin | ERY | Protein synthesis, 50S |
| Nitrofurantonin | NTR | DNA |
| Streptomycin | STR | Protein synthesis, 30S |
| Tetracycline | TET | Protein synthesis, 30S |
| Tobramycin | TOB | Protein synthesis, 30S |
| Trimethoprim | TMP | Folic acid biosynthesis |
| Vancomycin | VAN | Cell wall synthesis |

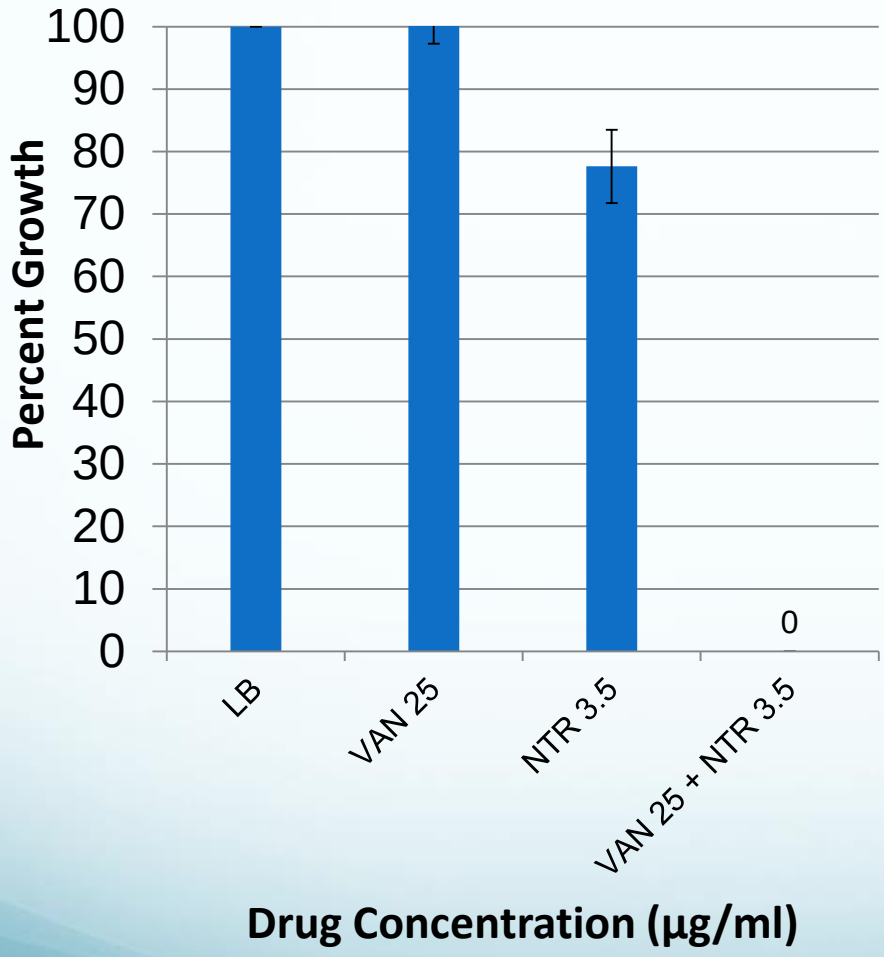
# Results: SurA-deficient background

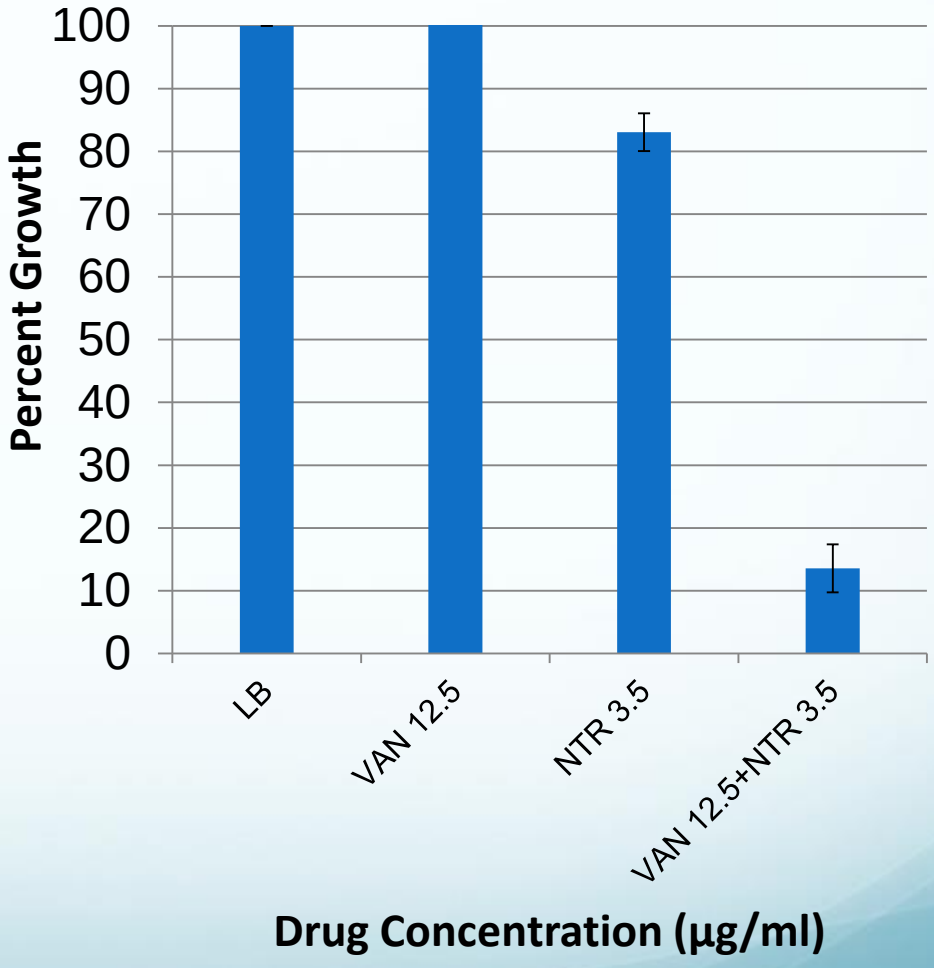




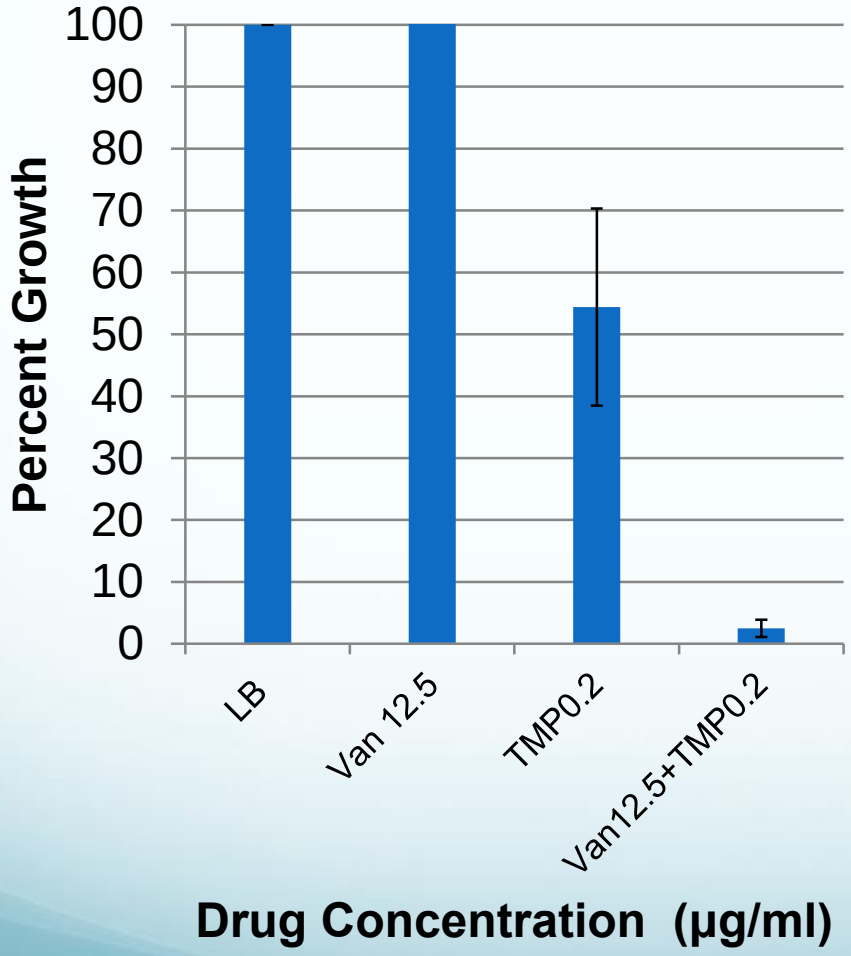

Vancomycin interactions with other drug classes in *surA* background

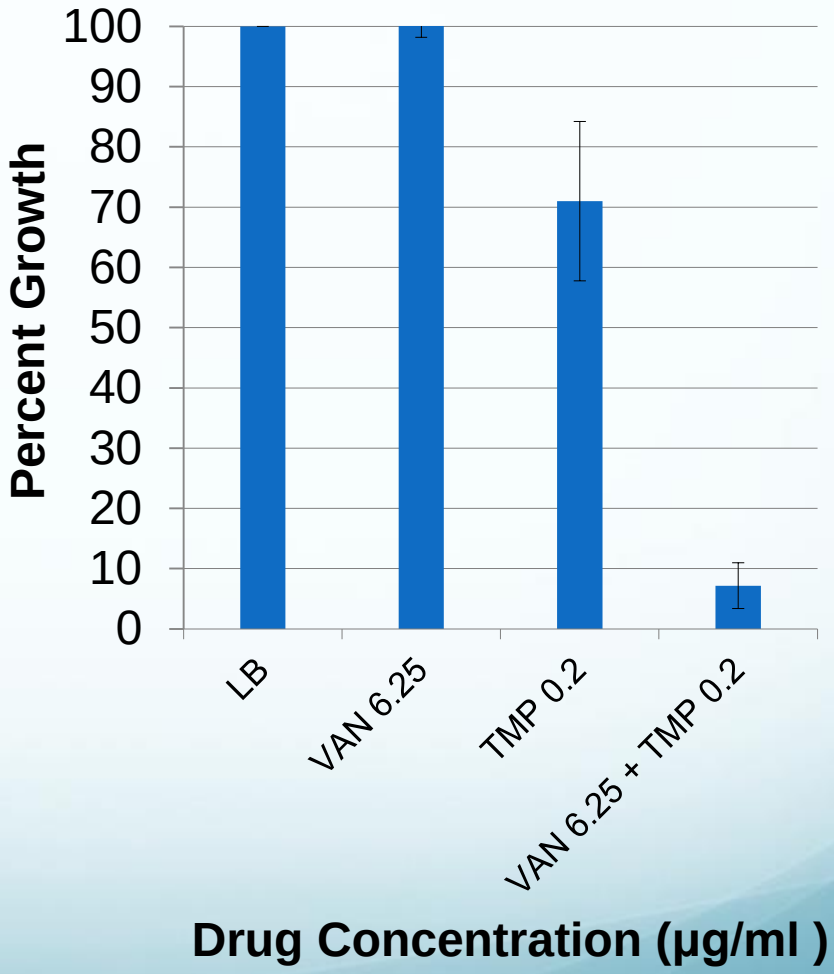
# Wild-type background



# Wild-type background



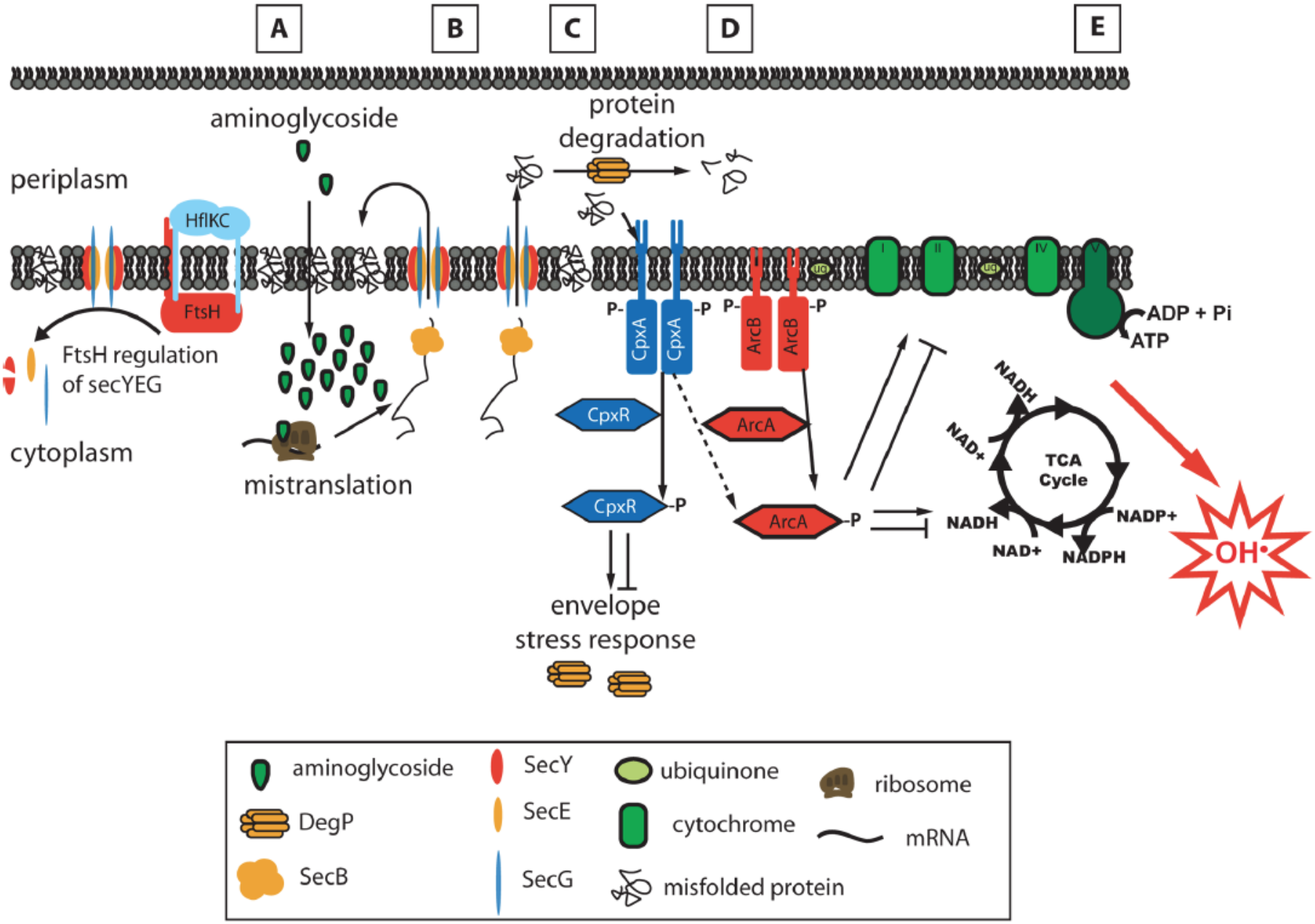
# Questions

- How do bactericidal antibiotics kill cells?
- Do each have a unique mechanism based on their initial target?
- Do they all kill via a common mechanism?
- Are there multiple mechanisms operating at the same time?

# A Common Mechanism of Cellular Death Induced by Bactericidal Antibiotics

Michael A. Kohanski,[1,2,5,6] Daniel J. Dwyer,[1,3,6] Boris Hayete,[1,4] Carolyn A. Lawrence,[1,2] and James J. Collins[1,2,3,4,*]

[1] Center for BioDynamics and Center for Advanced Biotechnology
[2] Department of Biomedical Engineering
[3] Program in Molecular Biology, Cell Biology, and Biochemistry
[4] Bioinformatics Program
Boston University, Boston, MA 02215, USA
[5] Boston University School of Medicine, Boston, MA 02118, USA
[6] These authors contributed equally to this work.
*Correspondence: jcollins@bu.edu
DOI 10.1016/j.cell.2007.06.049


A
B
C
D
E
aminoglycoside
periplasm
HflKC
FtsH
FtsH regulation
of secYEG
cytoplasm
mistranslation
protein
degradation
CpxA
CpxA
ArcB
ArcB
P-
-P
CpxR
CpxR
ArcA
ArcA
envelope
stress response
ADP + Pi
ATP
NADH
NAD+
TCA
Cycle
NADH
NADP+
NAD+
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cytochrome
misfolded protein
ribosome
mRNA

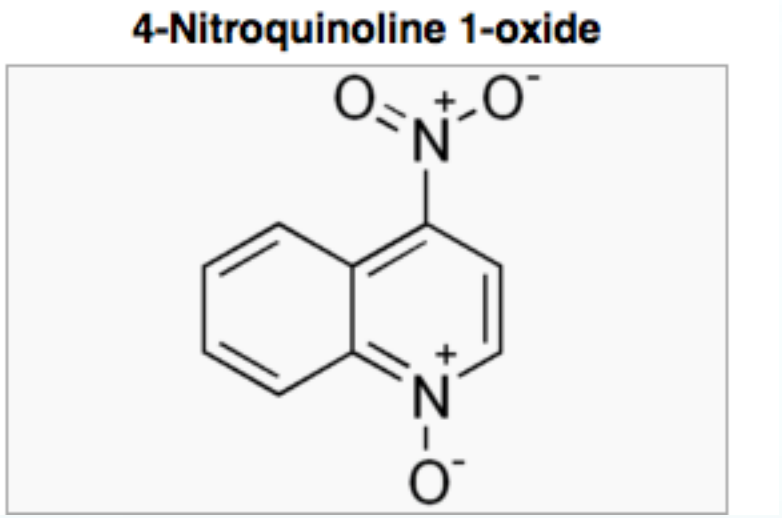
# Oxidative damage killing pathway

- Hydroxyl radicals lead to damaged DNA bases and double strand breaks
- The RecABC system repairs the breaks
- When the damage exceeds the repair capacity, cell death results

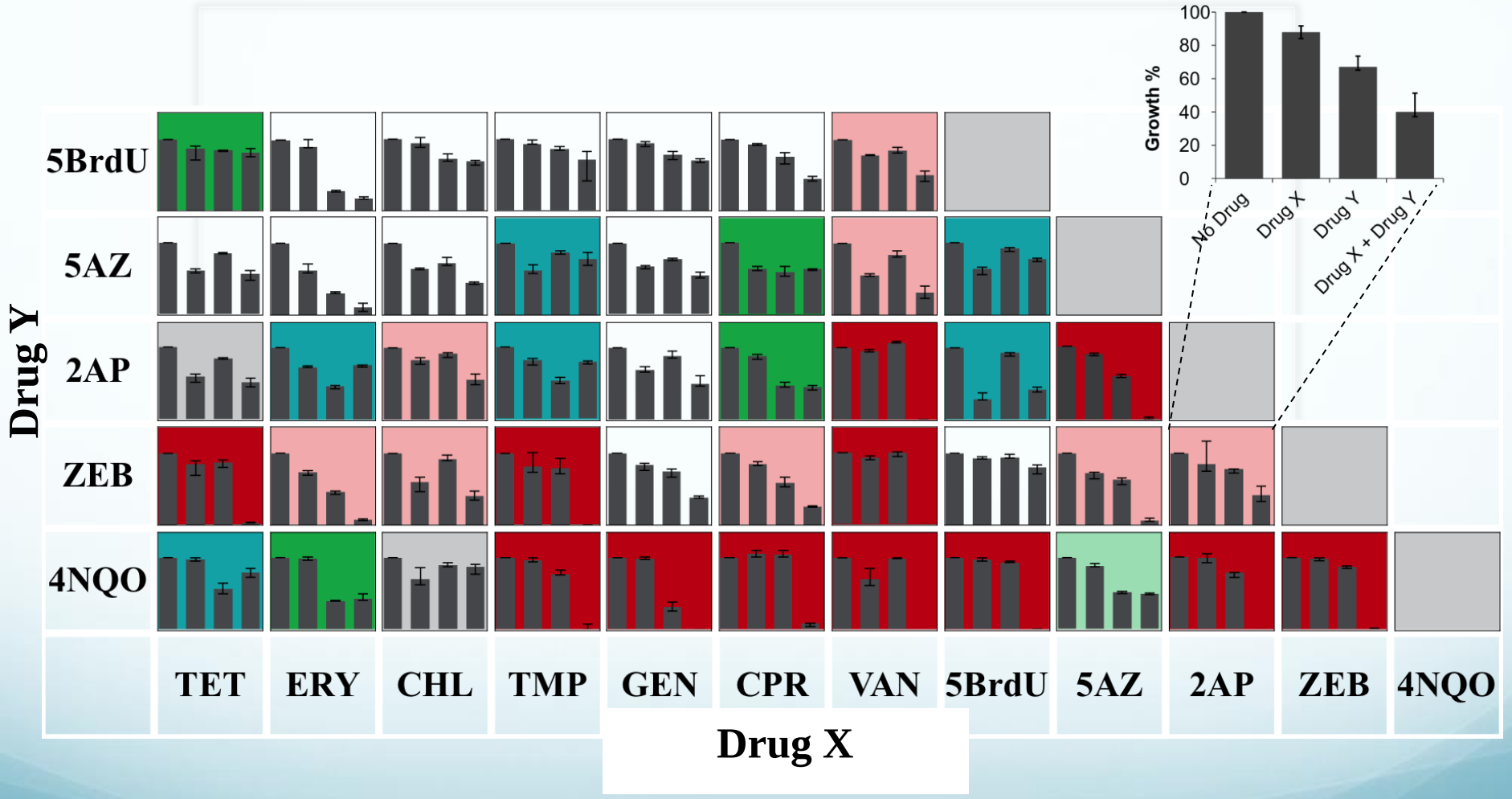
# Hypothesis

- Synergy between bactericidal drugs can result from each drug contributing damage that results in double strand breaks.
- Can test this by using known compounds that cause double strand breaks via known mechanisms, and thus act as antimicrobials.
- These compounds include commonly used mutagens.

4-Nitroquinoline 1-oxide (4NQO) oxidizes guanines in the DNA, leading to double strand breaks

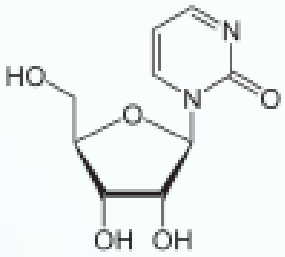



4NQO should therefore show strong synergies with bactericidal antibiotics, but not bacteriostatic drugs.

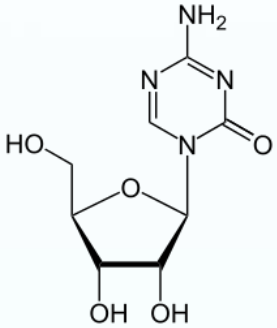

Drug Y
5BrdU
5AZ
2AP
ZEB
4NQO
TET
ERY
CHL
TMP
GEN
CPR
VAN
5BrdU
5AZ
2AP
ZEB
4NQO
Drug X
Growth %
100
80
60
40
20
0
No Drug
Drug X
Drug Y
Drug X + Drug Y

| | | | | | |
|---|---|---|---|---|---|
| 4NQO | | | | | |
| ZEB | S | | | | |
| 2AP | S | WS | | | |
| 5AZ | B (strong) | WS | S | | |
| 5BrdU | S | A | SUP | SUP | |
| VAN | S | S | S | S | WS |
| CPR | S | S | B (strong) | A | A |
| GEN | S | WS or A (repeat) | A | A | A |
| TMP | S | S | SUP (repeat) | SUP | A |
| CHL | Strong B | WS | WS | A | A |
| ERY | Strong B | WS | SUP | A | A |
| TET | SUP | S | A or Strong B (repeat) | A or B (repeat!) | Strong B |
| | 4NQO | ZEB | 2AP | 5AZ | 5BrdU |

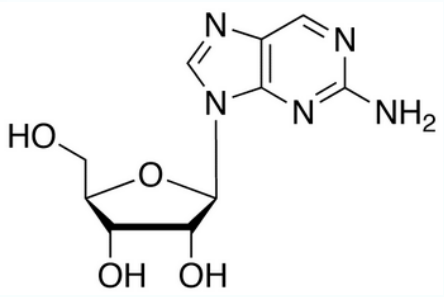
# Other Mutagens



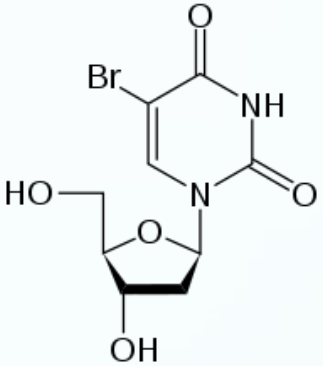

Zebularine



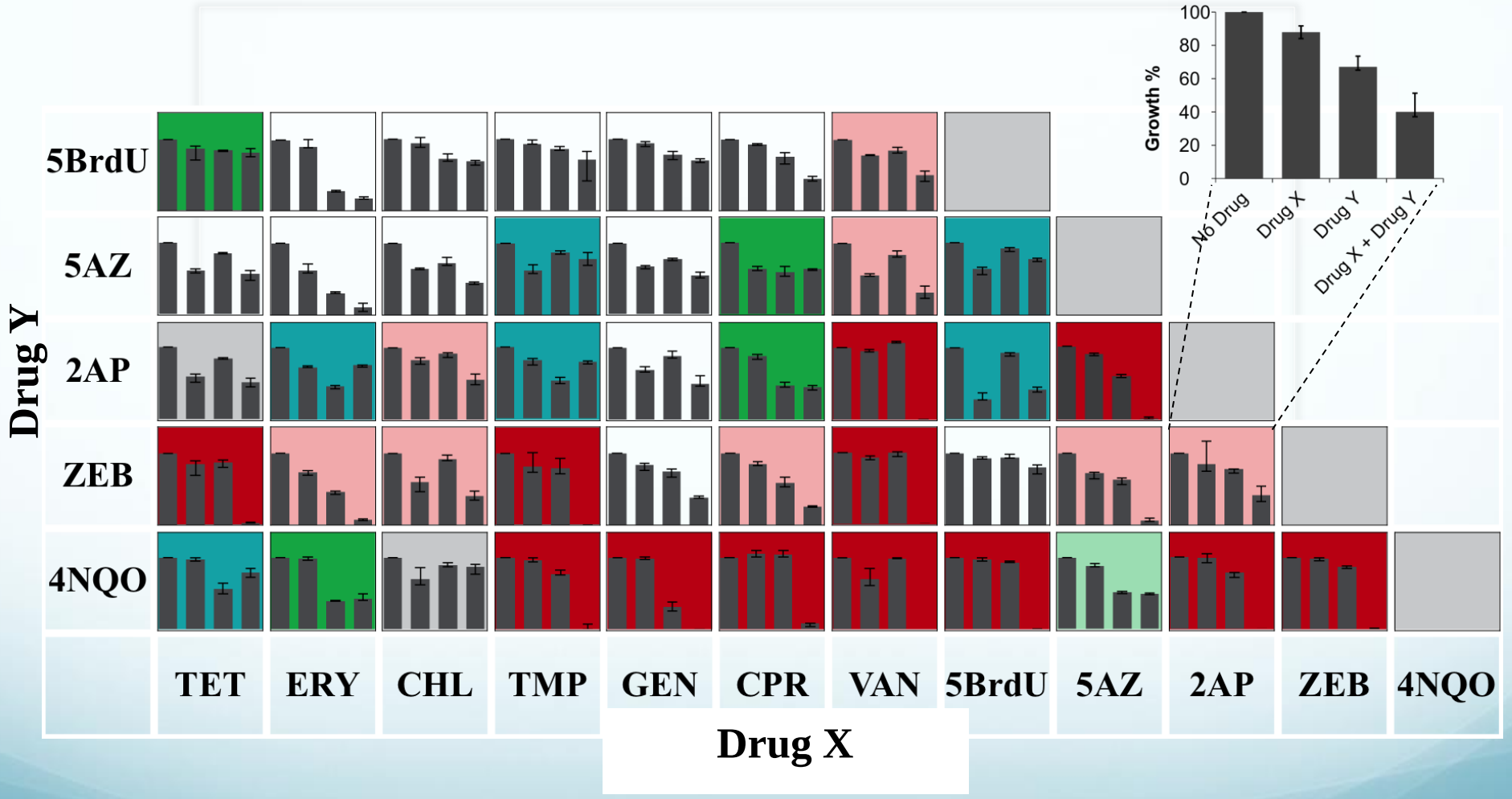

5-Azacytidine



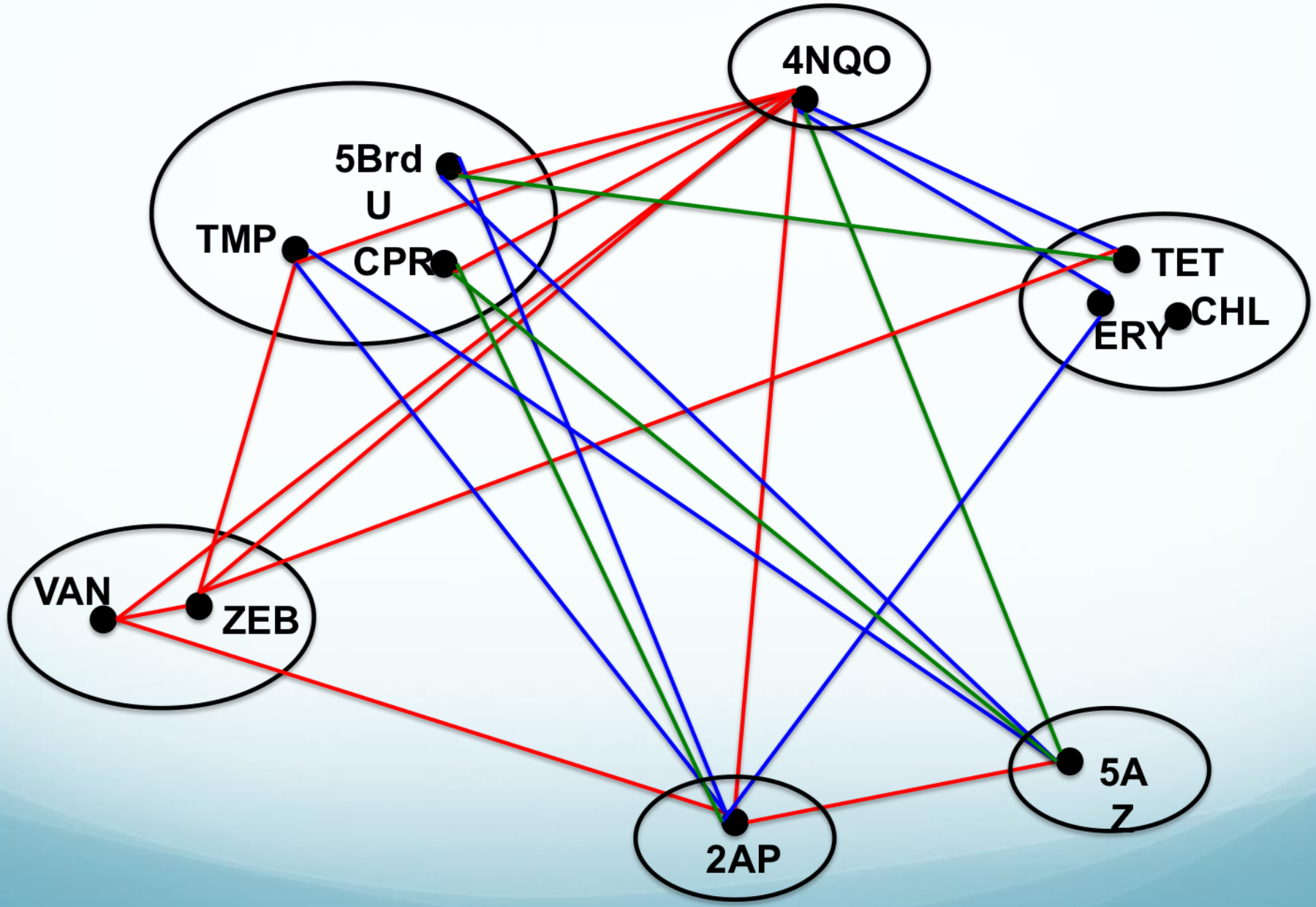

2-Aminopurine

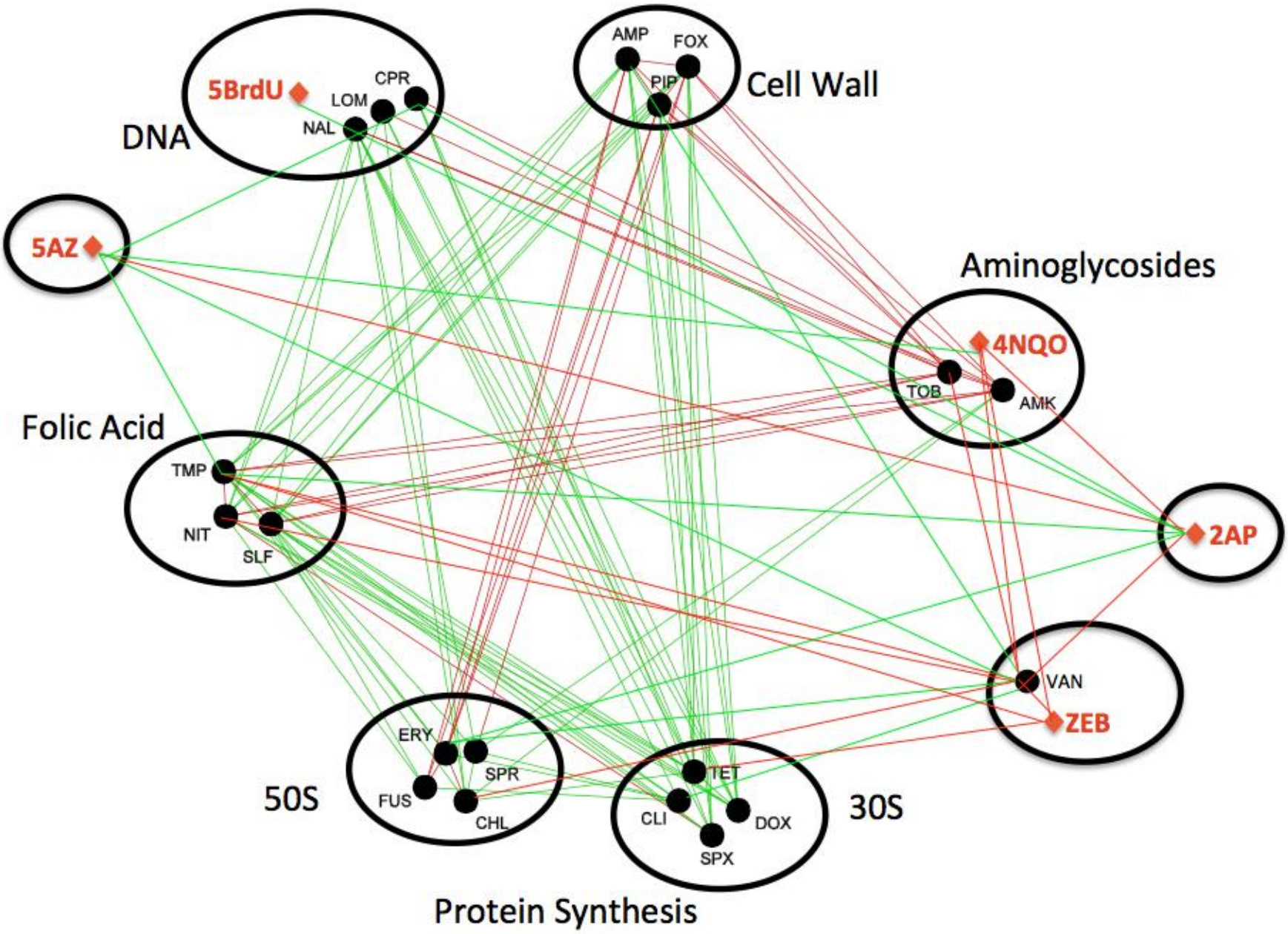



5-Bromodeoxyuridine


Drug Y
5BrdU
5AZ
2AP
ZEB
4NQO
TET
ERY
CHL
TMP
GEN
CPR
VAN
5BrdU
5AZ
2AP
ZEB
4NQO
Drug X
Growth %
100
80
60
40
20
0
No Drug
Drug X
Drug Y
Drug X + Drug Y

| | | | | | |
|---|---|---|---|---|---|
| 4NQO | | | | | |
| ZEB | S | | | | |
| 2AP | S | WS | | | |
| 5AZ | B (strong) | WS | S | | |
| 5BrdU | S | A | SUP | SUP | |
| VAN | S | S | S | S | WS |
| CPR | S | S | B (strong) | A | A |
| GEN | S | WS or A (repeat) | A | A | A |
| TMP | S | S | SUP (repeat) | SUP | A |
| CHL | Strong B | WS | WS | A | A |
| ERY | Strong B | WS | SUP | A | A |
| TET | SUP | S | A or Strong B (repeat) | A or B (repeat!) | Strong B |
| | 4NQO | ZEB | 2AP | 5AZ | 5BrdU |


4NQO
5Brd
U
TMP
CPR
TET
CHL
ERY
VAN
ZEB
5A
Z
2AP


DNA
5BrdU
CPR
LOM
NAL
Cell Wall
AMP
FOX
PIP
5AZ
Aminoglycosides
4NQO
TOB
AMK
Folic Acid
TMP
NIT
SLF
2AP
VAN
ZEB
50S
ERY
SPR
FUS
CHL
30S
TET
CLI
DOX
SPX
Protein Synthesis

# Conclusions

- Vancomycin exhibits strong synergistic interactions with at least nitrofurantoin and trimethoprim.
- Synergy experiments validate the oxidative damage route to cell killing.
- Synergy can be explained in many cases as a dose effect of double strand breaks, occurring in later step (ultimate) targets.

# Future Directions

- Mutagenic Fingerprints of antibiotics