Texas Bioscience Institute
TEMPLE COLLEGE

# Resist the Resistance: Antimicrobial peptides to fight infections

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# About me….

- That's me
- I did my Bachelor's and Masters in Pharmaceutical Technology back in India.
- I worked in building nanoparticles for cancer drug delivery.
- I am doing my PhD from Baylor University at the Biology Department.
- And I am of the many certified nerds working there.

# How we began…

Ignaz Semmelweis

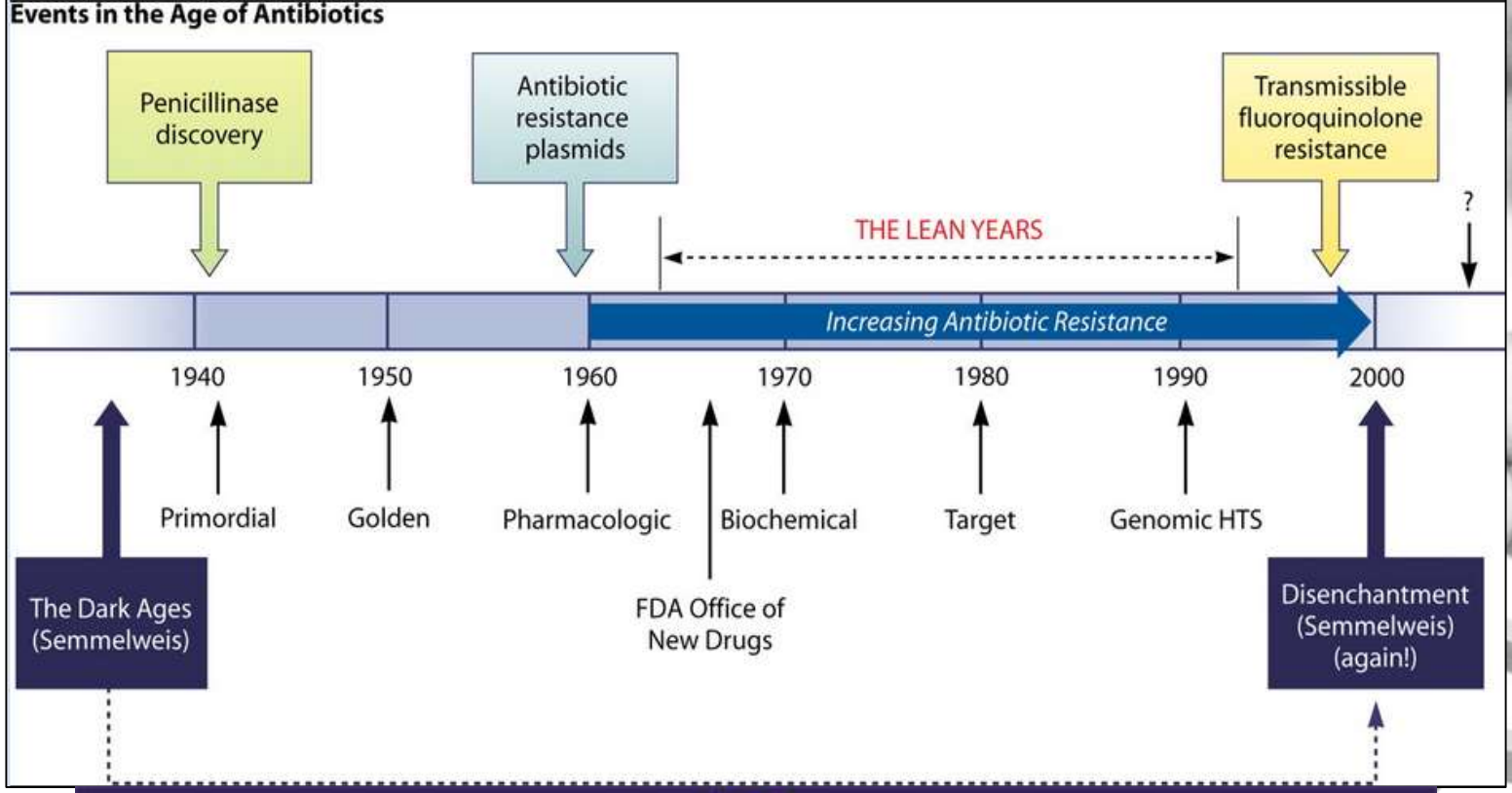
Louis Pasteur

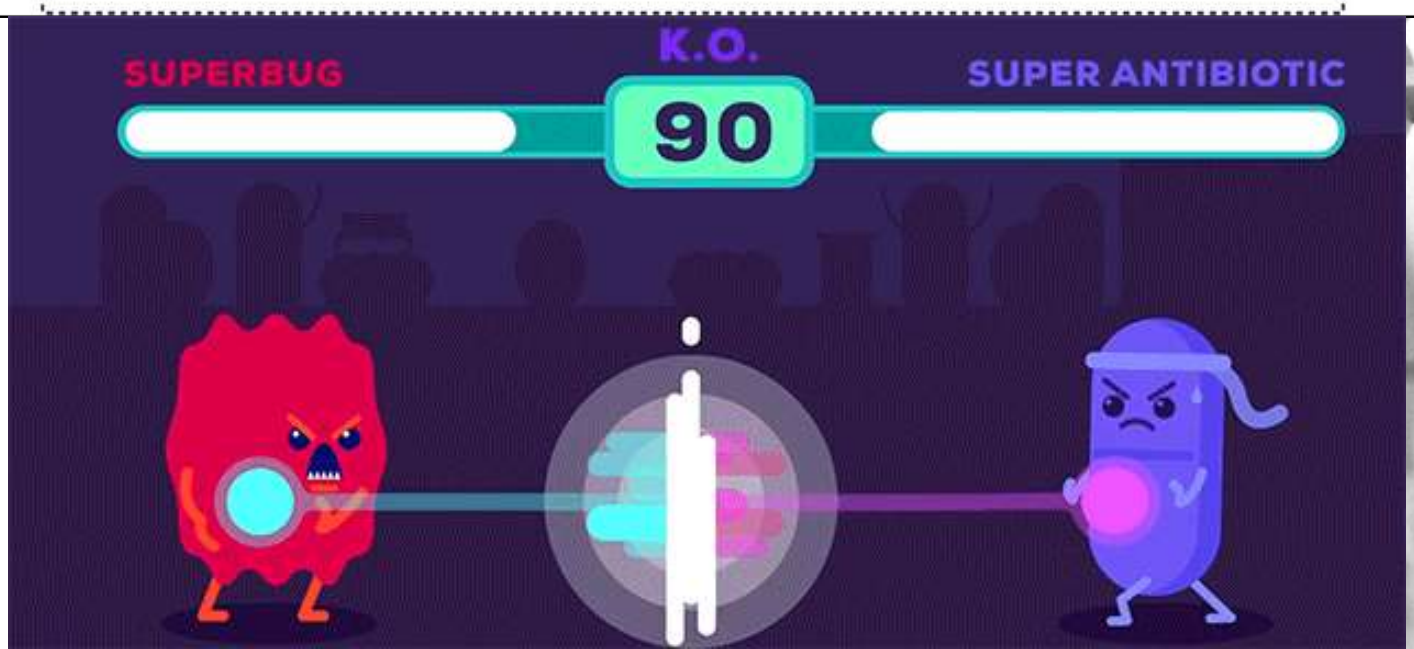
Joseph Lister

# Drugs for Bacterial infection

- It all began in the late 1880s with **Paul Ehrlich** and his “anti-biotic” dyes.
- In 1928, **Alexander Fleming** discovered **Penicillin** from the mold ***Penicillium notatum***

| Antibiotic class; example | Year of discovery | Year of introduction | Year resistance observed |
|---|---|---|---|
| Sulfadrugs; prontosil | 1932 | 1936 | 1942 |
| β-lactams; penicillin | 1928 | 1938 | 1945 |
| Aminoglycosides; streptomycin | 1943 | 1946 | 1946 |
| Chloramphenicols; chloramphenicol | 1946 | 1948 | 1950 |
| Macrolides; erythromycin | 1948 | 1951 | 1955 |
| Tetracyclines; chlortetracycline | 1944 | 1952 | 1950 |
| Rifamycins; rifampicin | 1957 | 1958 | 1962 |
| Glycopeptides; vancomycin | 1953 | 1958 | 1960 |
| Quinolones; ciprofloxacin | 1961 | 1968 | 1968 |


Events in the Age of Antibiotics
Penicillinase discovery
Antibiotic resistance plasmids
Transmissible fluoroquinolone resistance
?
THE LEAN YEARS
Increasing Antibiotic Resistance
1940
1950
1960
1970
1980
1990
2000
Primordial
Golden
Pharmacologic
Biochemical
Target
Genomic HTS
The Dark Ages (Semmelweis)
FDA Office of New Drugs
Disenchantment (Semmelweis) (again!)


SUPERBUG
K.O.
90
SUPER ANTIBIOTIC

# What happens?

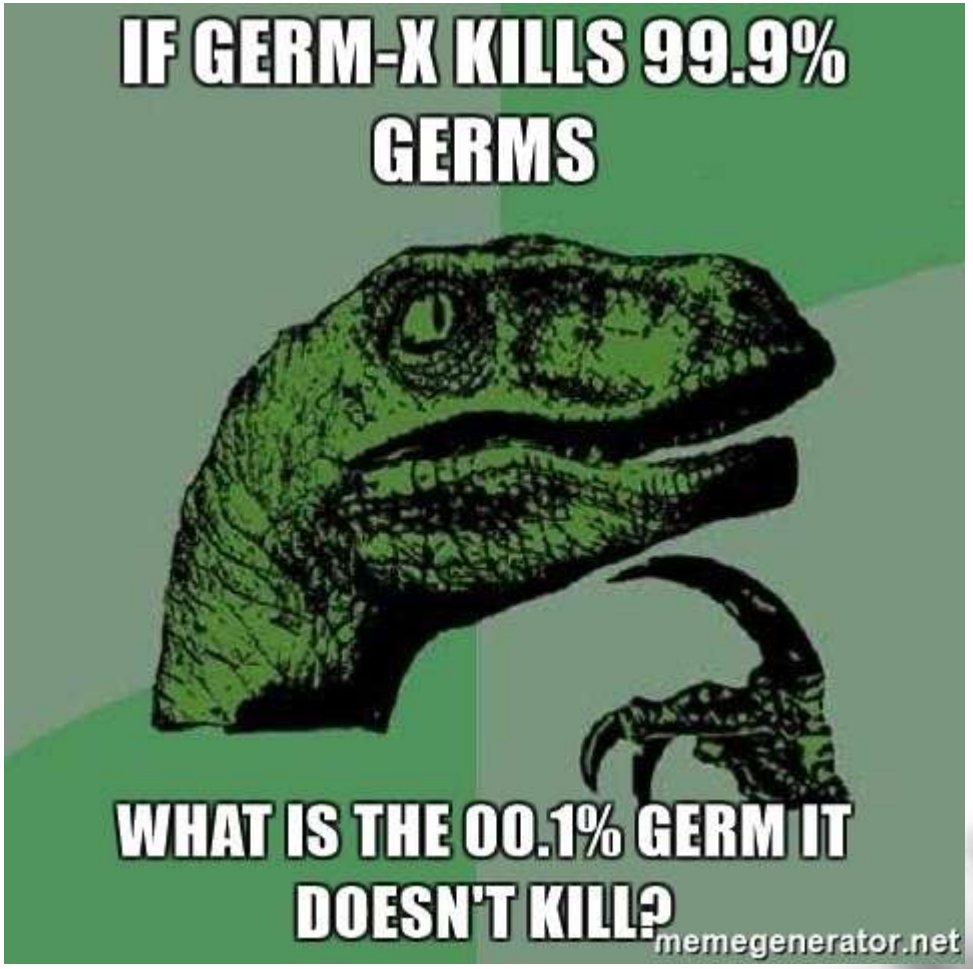
- Antibiotics work in two ways:

Bacteriostatics: They will stop the bacteria from growing.

Bacteriocidal: They will destroy them for good.

Both are equally effective/ ineffective in staving of infections.


# What happens?



# How does an antibiotic act?

- Several mechanisms,
- Can be classified broadly into the following:

**Inhibit cell wall synthesis of the bacteria eg-Penicillin, Ampicillin**

# How does an antibiotic act?

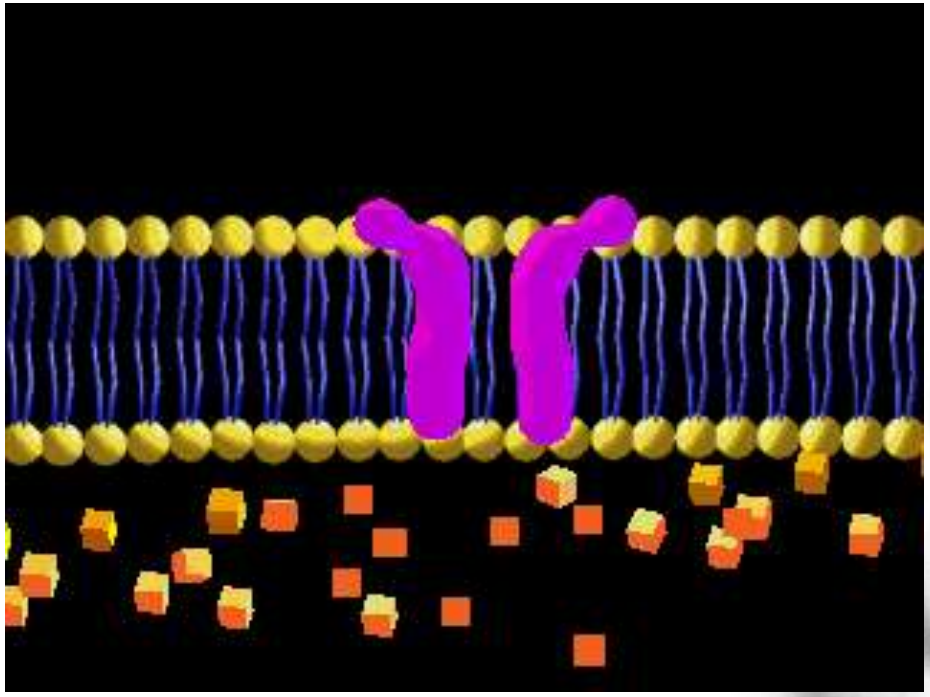
- Several mechanisms,
- Can be classified broadly into the following:

**Inhibit protein synthesis of bacteria eg- Streptomycin , vancomycin**

# How does an antibiotic act?

- Several mechanisms,
- Can be classified broadly into the following:

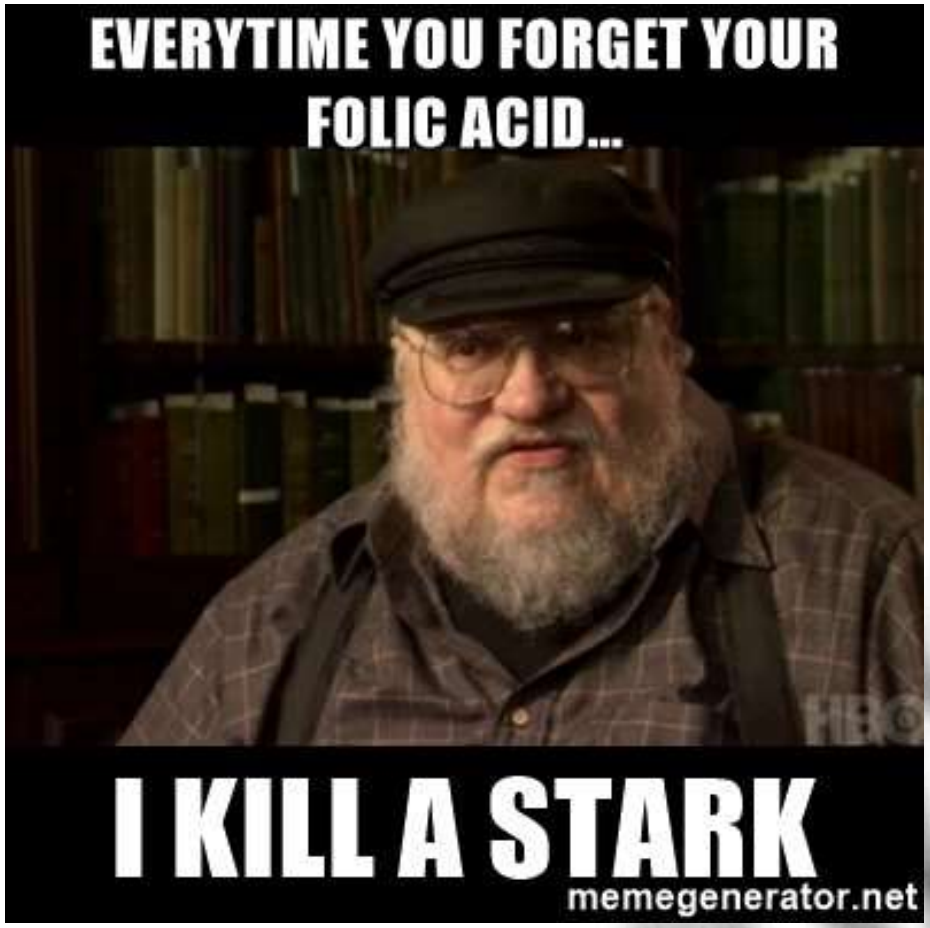
**Inhibits the bacterial membrane function**



# How does an antibiotic act?

- Several mechanisms,
- Can be classified broadly into the following:

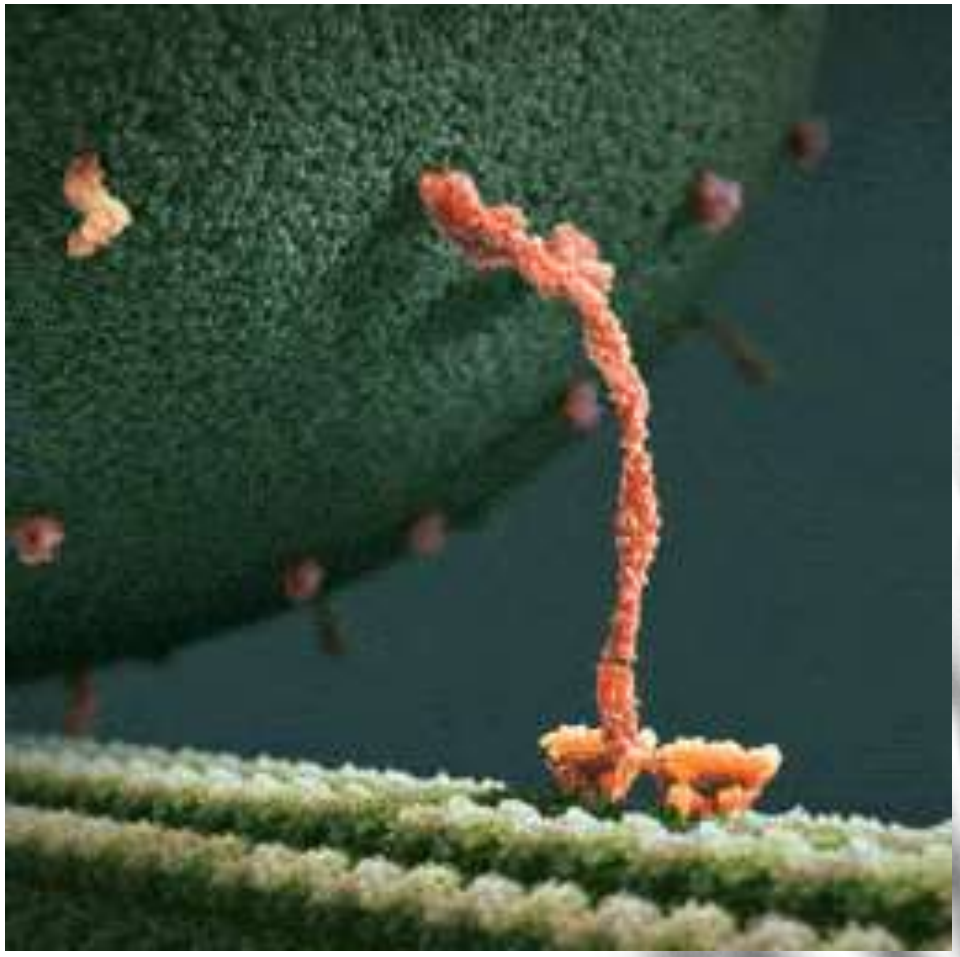
**Inhibit metabolite synthesis like Folic acid eg- Sulfonamides**



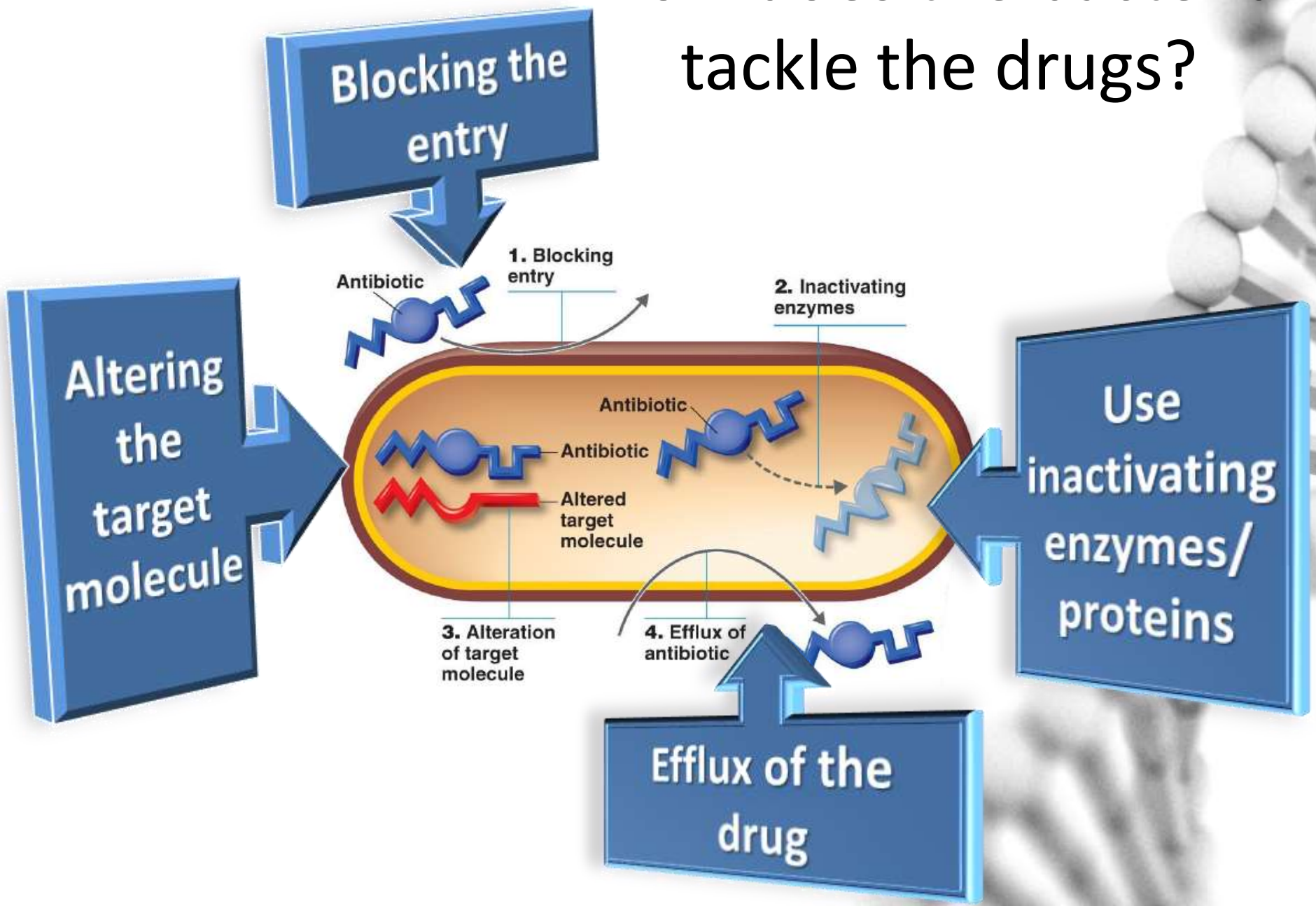
# How does an antibiotic act?

- Several mechanisms,
- Can be classified broadly into the following:

**Inhibitor of bacterial nucleic acid synthesis/ arrangement eg- Ciprofloxacin**



# How does the bacteria tackle the drugs?



# Who's responsible for it?

**EVOLUTION!!!**

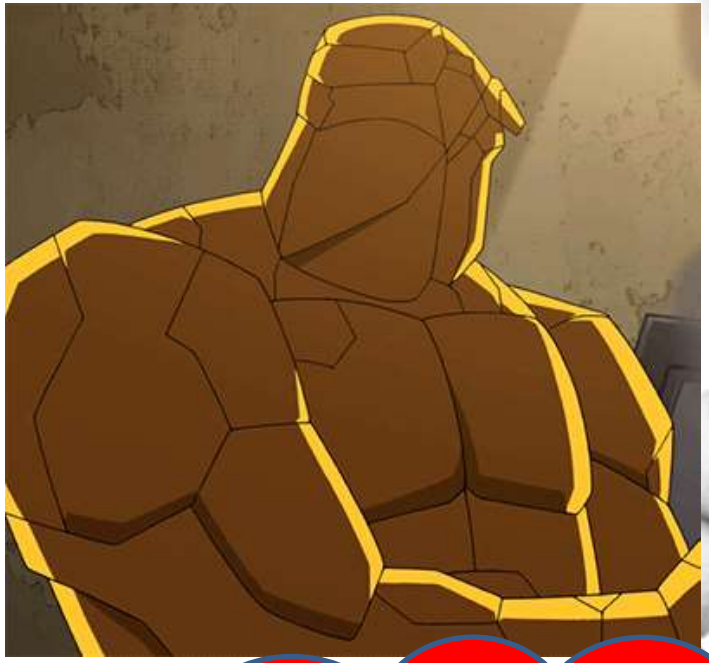
# Natural Selection and "Hopeful Monsters"

Background mutations happen all the time in all organisms.

A very few of the mutations are helpful or harmful while almost all of them are neutral.

Their lifestyle is costly for the body to maintain and hence they are pretty rare.

Mutated "monsters" remain mostly in the background of the population.

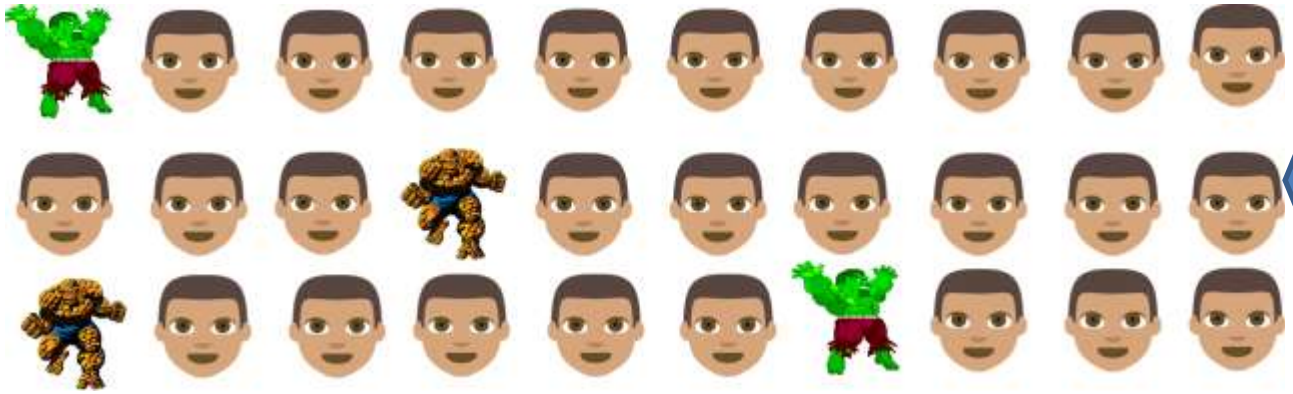


**Pros**

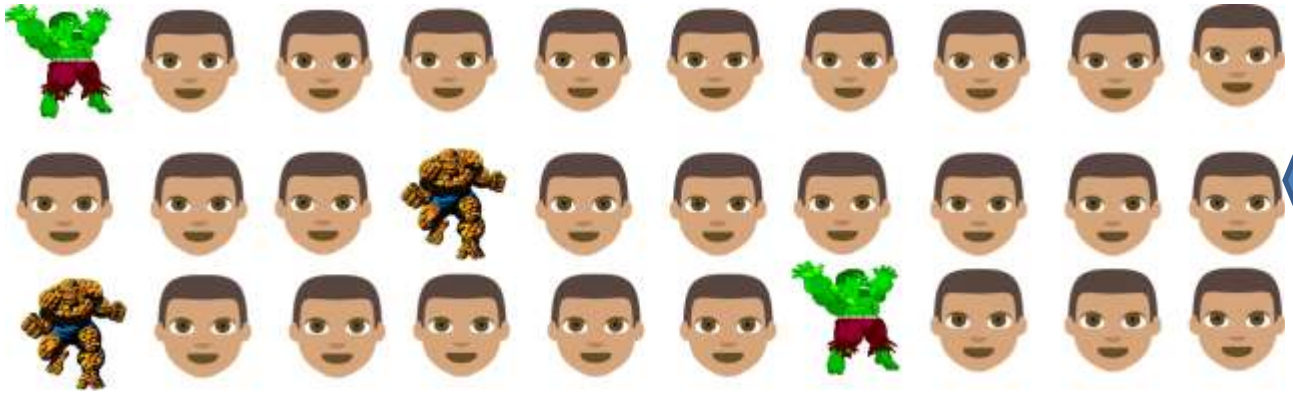
- Superhuman strength
- Indestructible body

**Cons**

- Too big a body to maintain
- Can do not much else



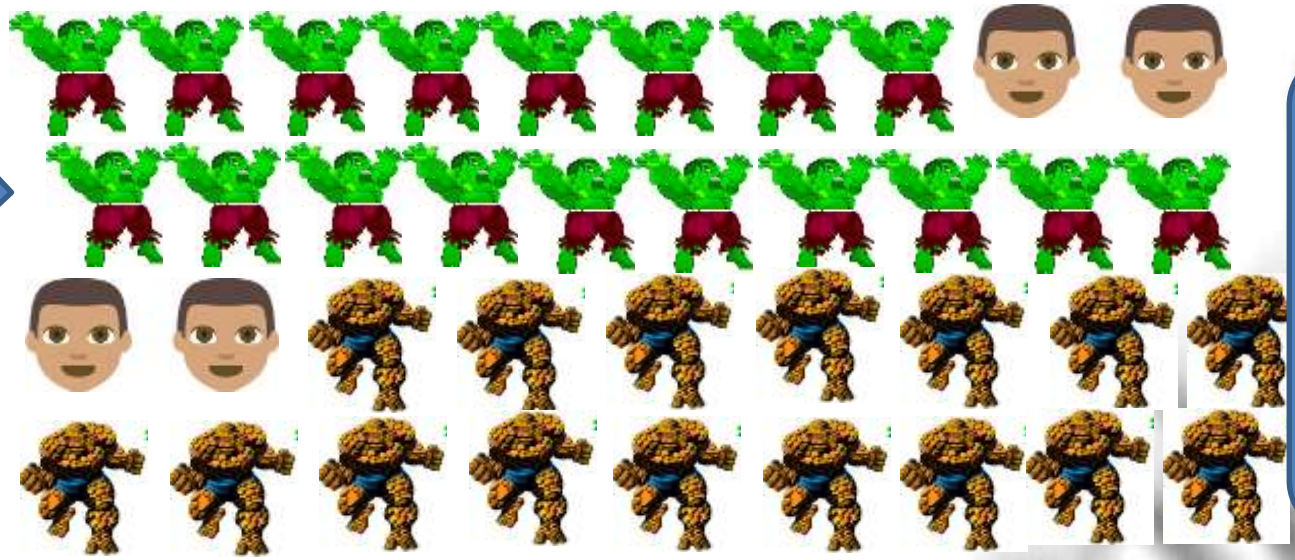
Normal Population with rare mutants



Normal Population with rare mutants

Population bottleneck

Mutants survive better

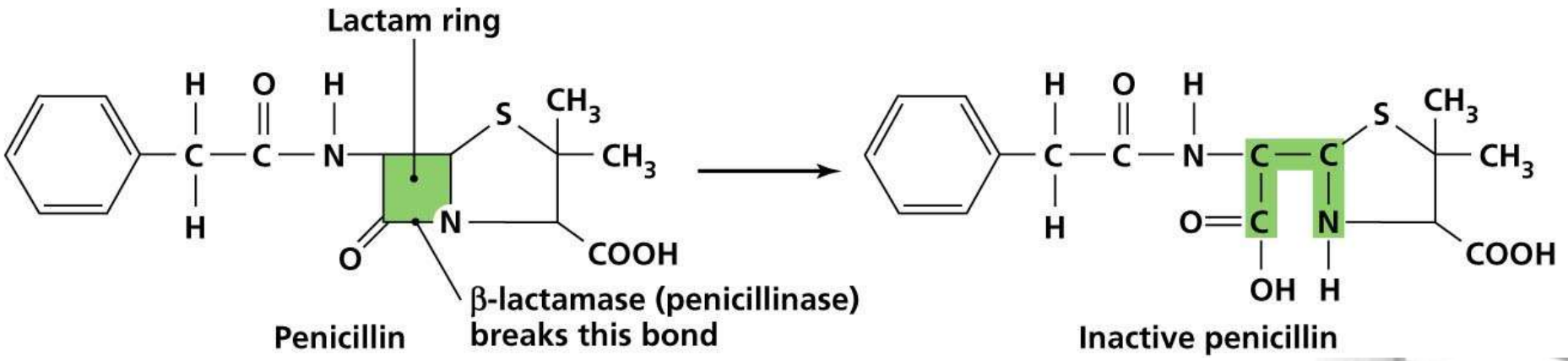


Multiply

New population with the mutants being dominant

# How scary it is in real life?

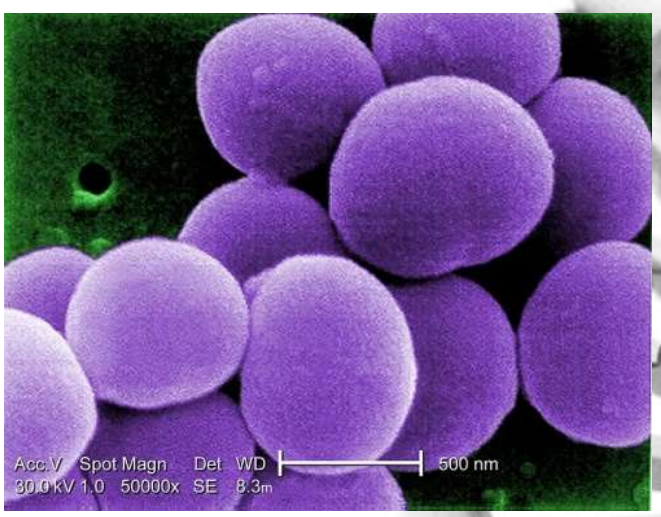
# Common resistances that we face…



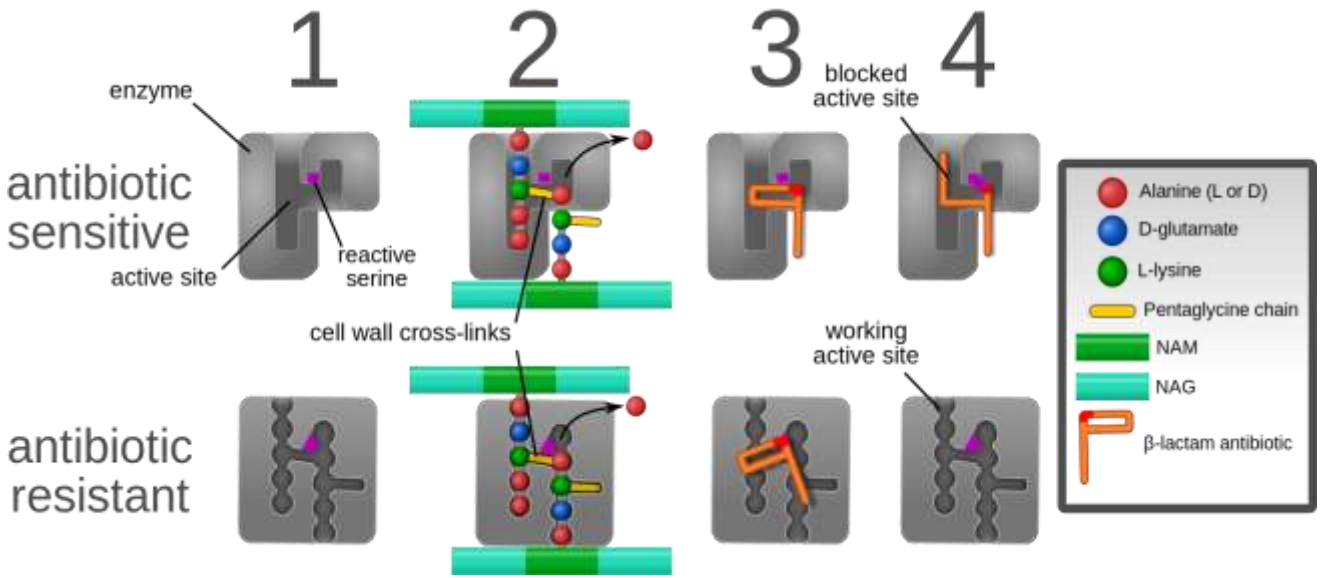

**Penicillin** Family is resisted by β-lactamase (penicillinase) enzyme

# Common resistances that we face…

**Vancomycin** Resistant S. aureus (VRSA) produces thicker cell wall to block **Vancomycin** entry

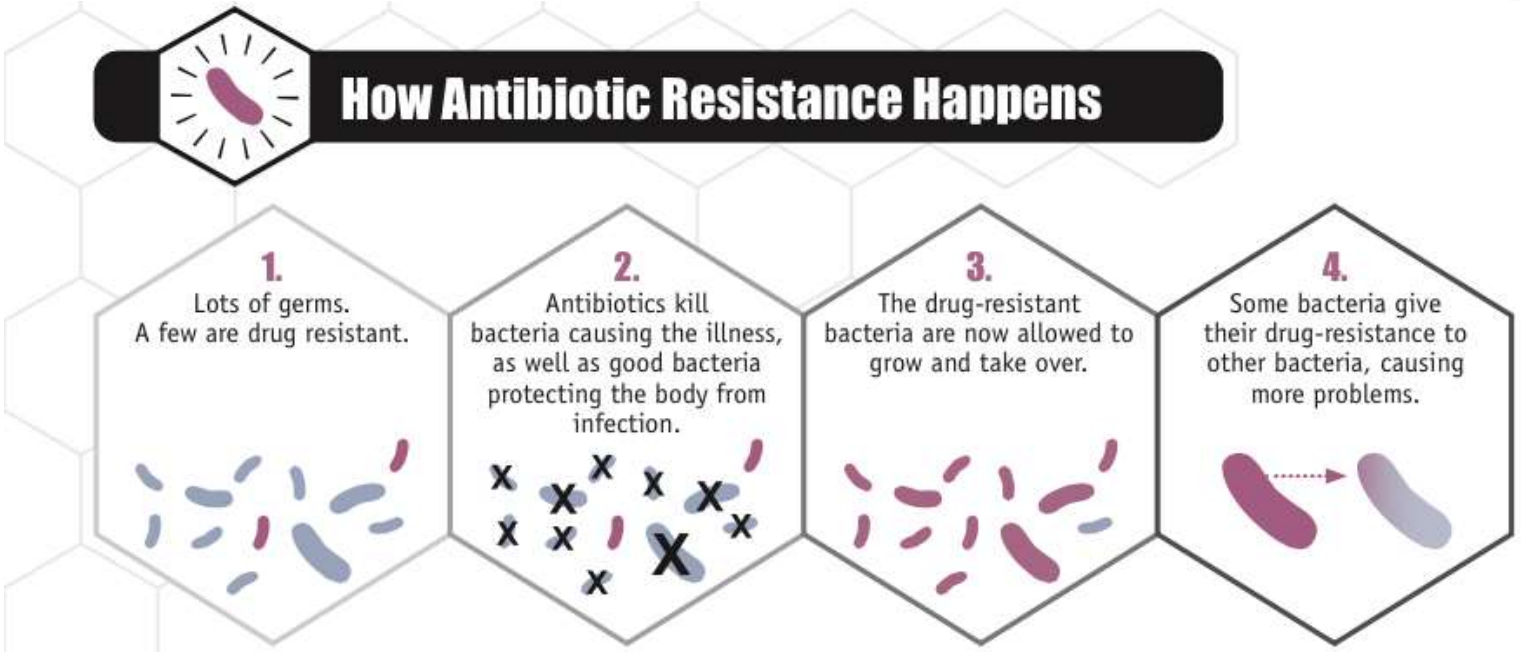


# Common resistances that we face…

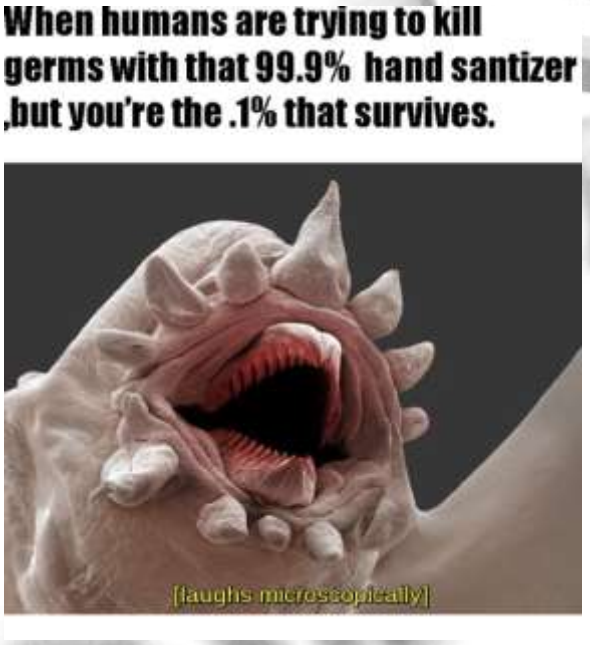



Active site in the cell wall changes conformation to block antibiotic actions e.g. Methicillin Resistant S. aureus (MRSA)

Multidrug Resistance genes encode **ATP Binding Casette (ABC) transporters** that efflux drug out of the cells. *e.g.* **Multidrug Resistant *A. baumannii* (MRAB).**




- Along with **Evolutionary mutations**, other methods of attaining drug resistance by the bacteria include-
  - **Horizontal Gene Transfer**
  - **Viral transduction**

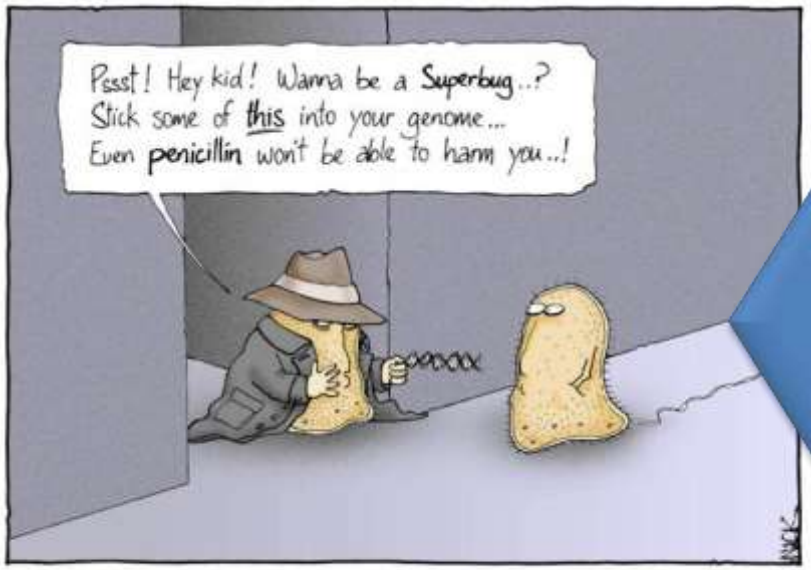


Naturally existing resistant bacteria may not always be **virulent**

The resistance gene is very rare because only a small portion of bacterial population has it

Excess antibiotics kill the other bacteria not meant to be killed and allow the resistant strain to grow unchecked and without competition.

This turns a benign population of bacteria pathogenic in a span of years

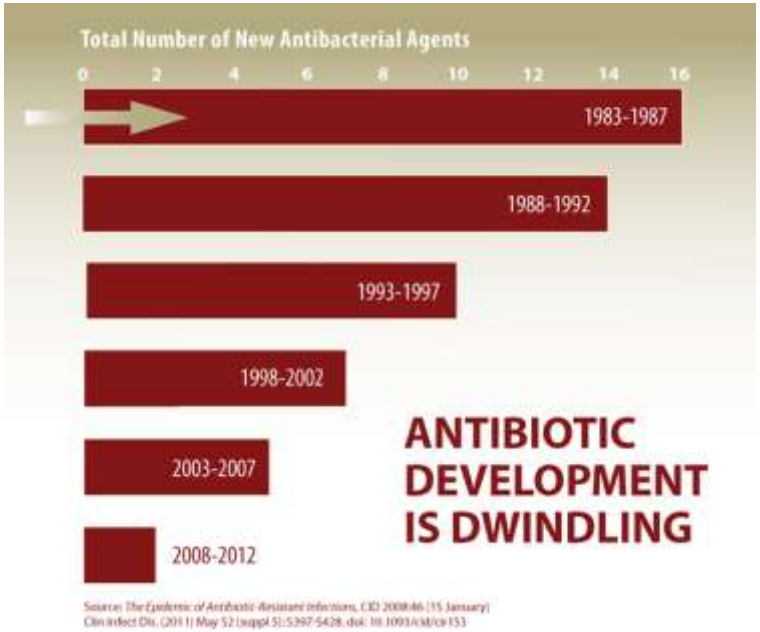


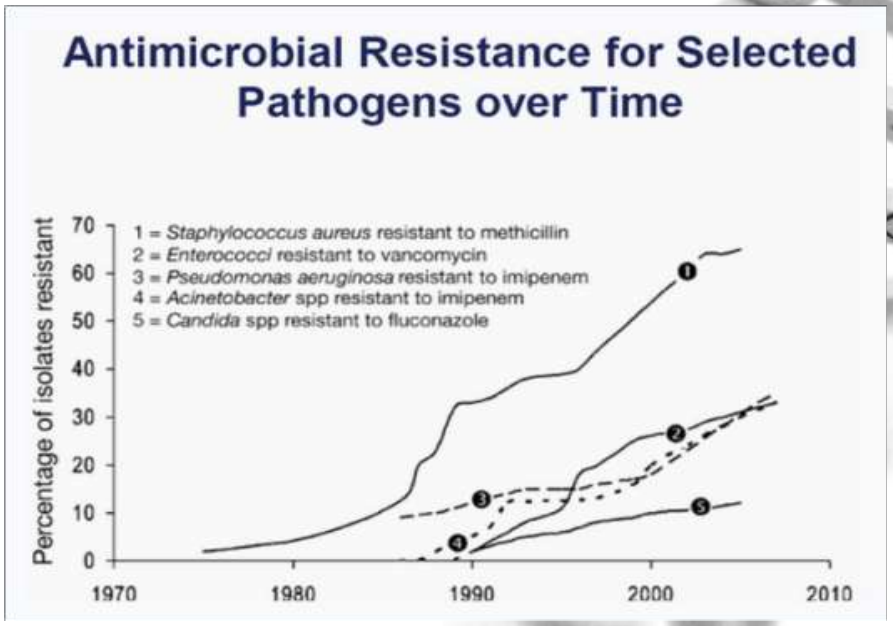

It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

Especially in environments like hospitals, all strains of bacteria get exposed to all kind of antibiotics which creates virulent strains from the most benign species, spreading **nosocomial diseases**

# What should we do?

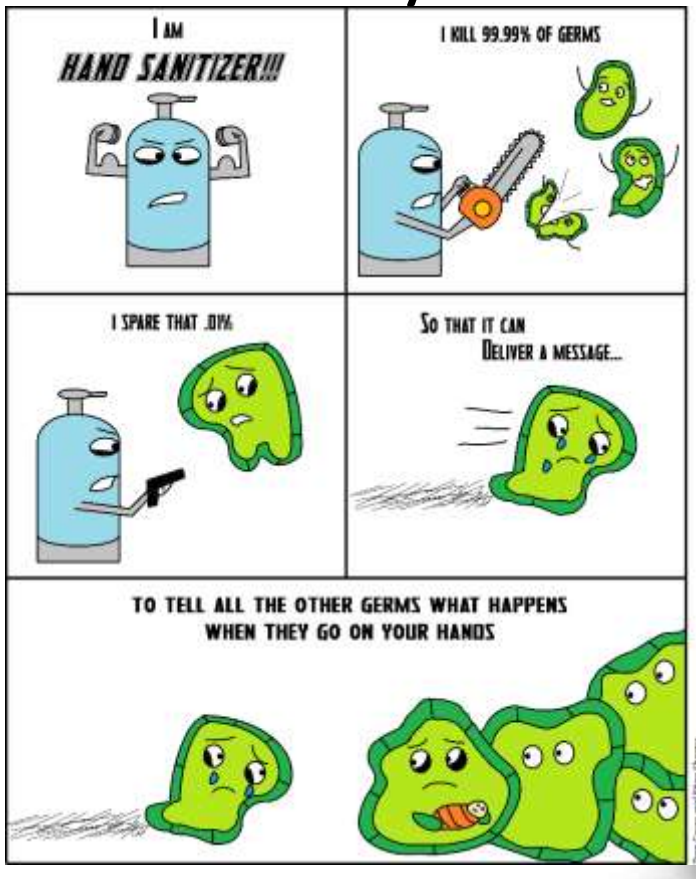
- Discover new drugs that the bacteria has not been tested against.



# What should we do?

- Use antibiotic judiciously, complete the course so that few bacteria may survive.

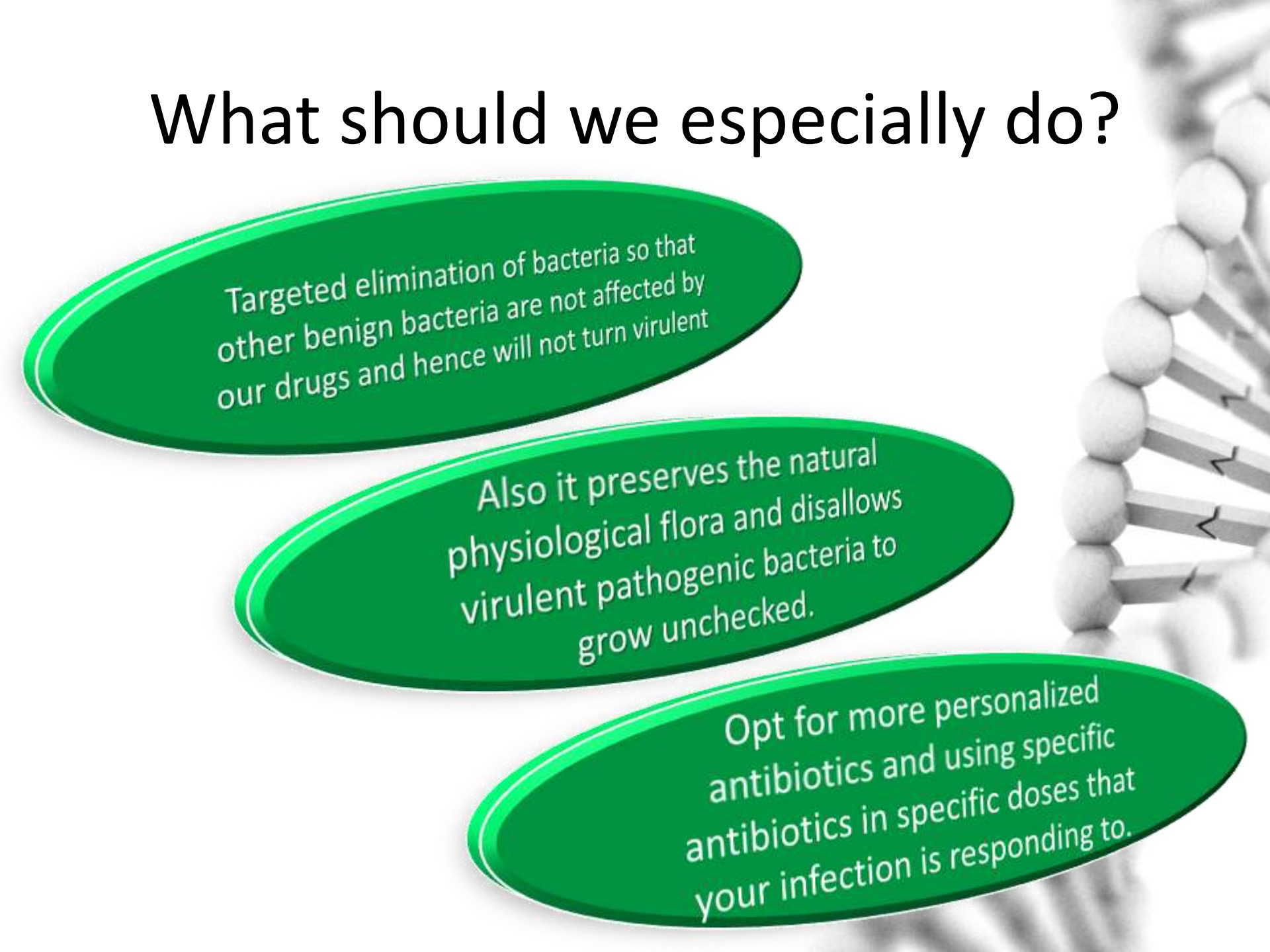


# What should we especially do?

Targeted elimination of bacteria so that other benign bacteria are not affected by our drugs and hence will not turn virulent

Also it preserves the natural physiological flora and disallows virulent pathogenic bacteria to grow unchecked.

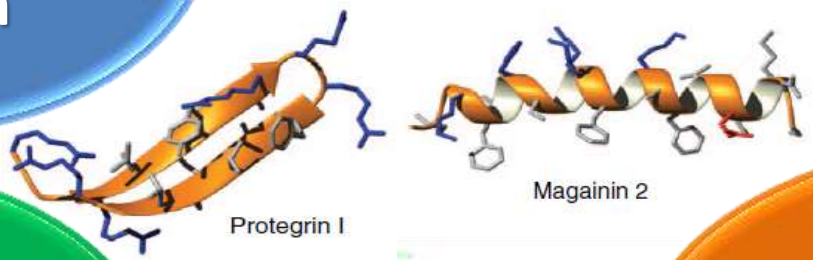
Opt for more personalized antibiotics and using specific antibiotics in specific doses that your infection is responding to.



# What do I do?

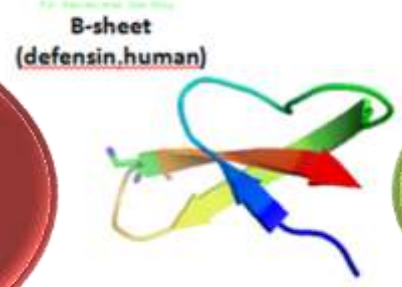
Antimicrobial peptides (AMPs) are found in nature

Wide variety of complex structures




Part of the innate immune system of almost all organisms

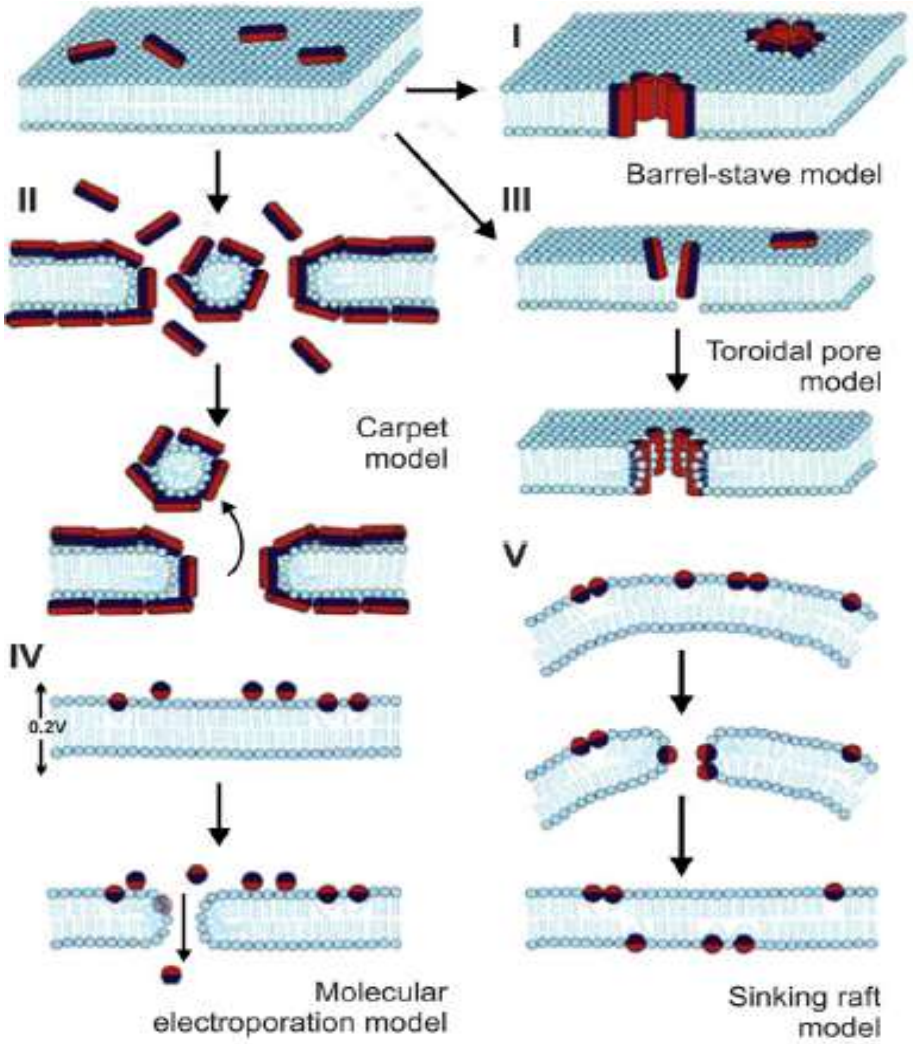

Fundamentally different MOA against eukaryotic and prokaryotic targets

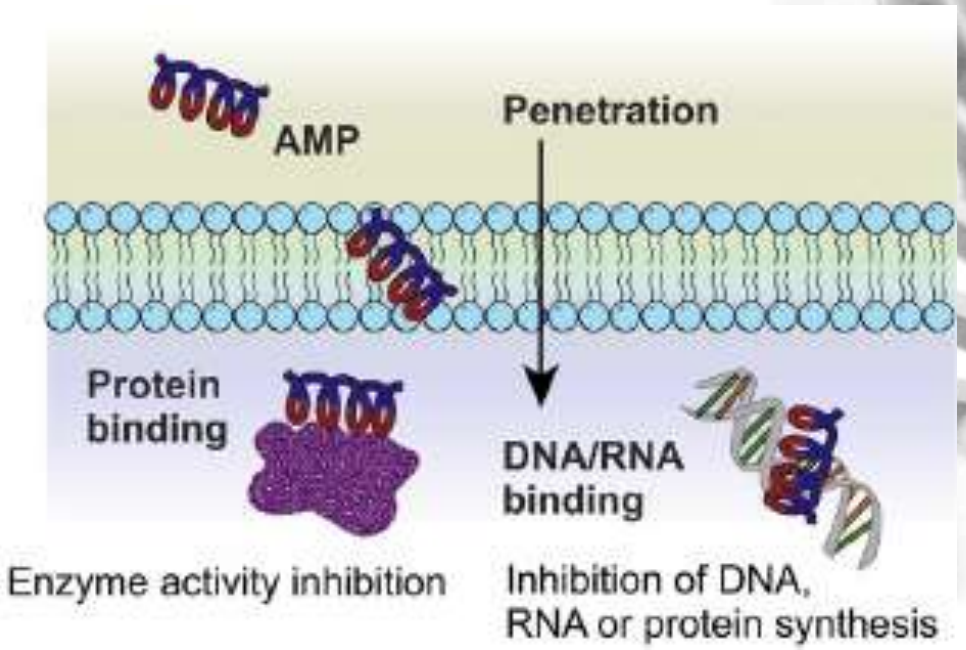



Broad spectrum of action against bacteria, fungi, viruses and even cancerous cells

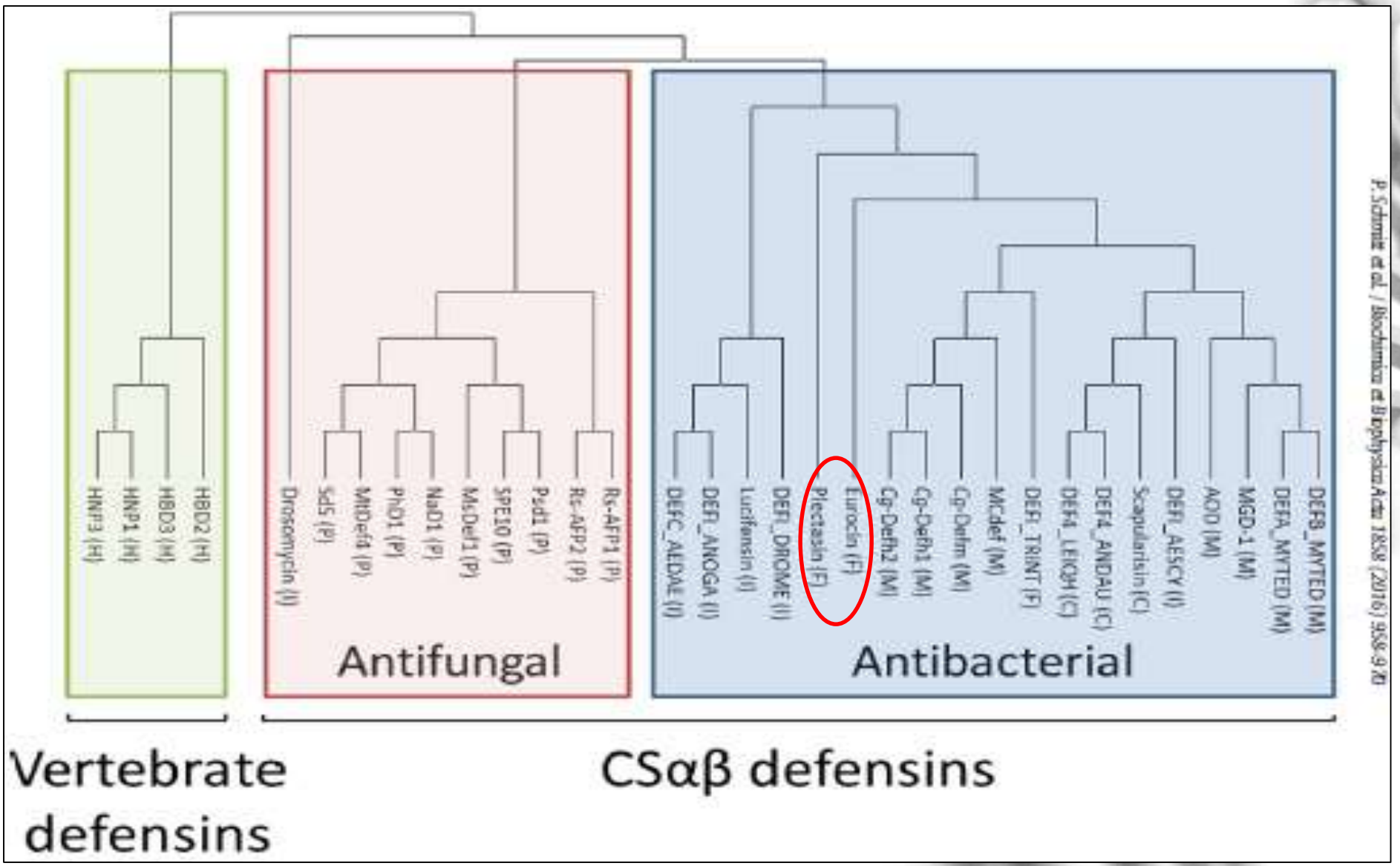
Hard to develop resistance against due to minimum exposure

# AMPs act mainly by disrupting bacterial cell membrane



# Plectasin and Eurocin



# Plectasin

# Eurocin

Isolated from fungus *Pseudoplectania nigrella*

Around 40 amino acids long

Another fungal defensin, isolated from *Eurotium amstelodami*

- Loosely defined MOA.
- May target and bind with Lipid II
- Lipid II is required for cell wall formation

Especially active against gram-positive bacteria like multidrug resistant strains of *Streptococcus pneumoniae* and *S. aureus*.

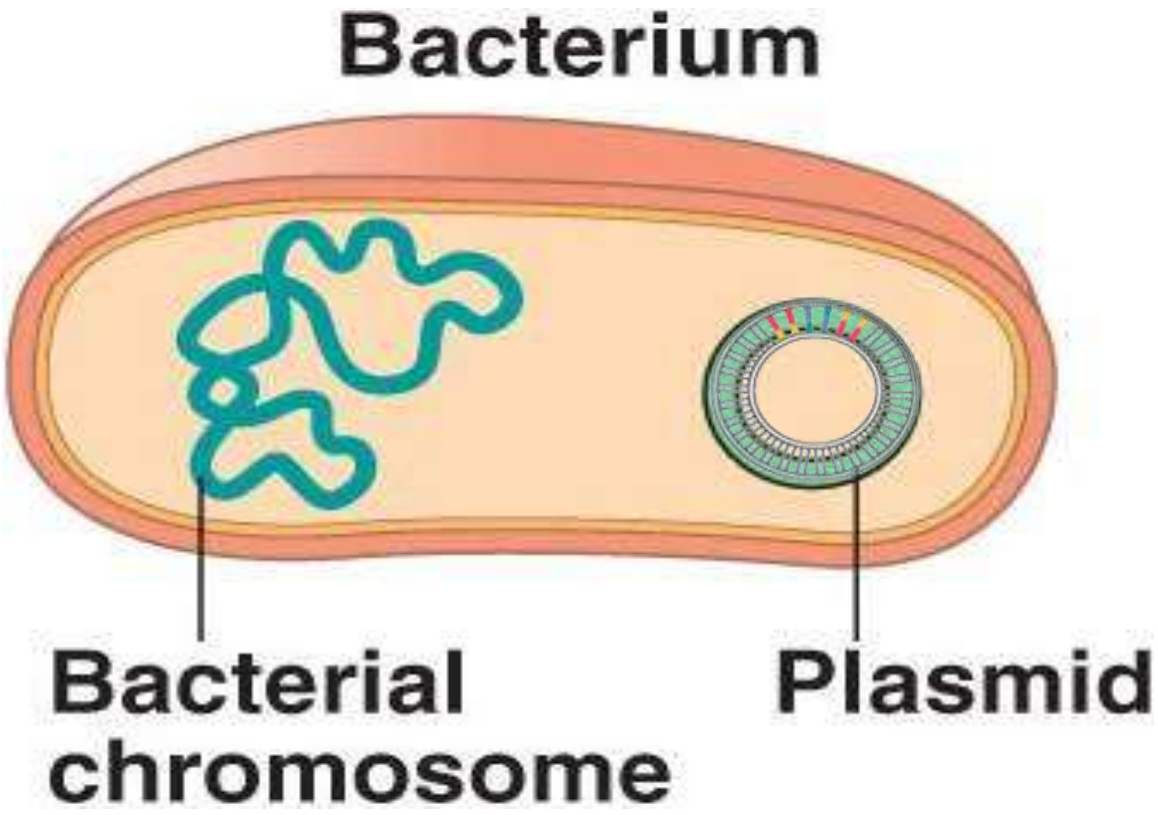
# Recombinant expression of AMPs

**Create plasmids containing our desired AMP.**

**Clone the plasmid in competent *E. coli* cells**

**Produce, extract and purify the AMP from the *E. coli* cells**

# Some essential deets…..



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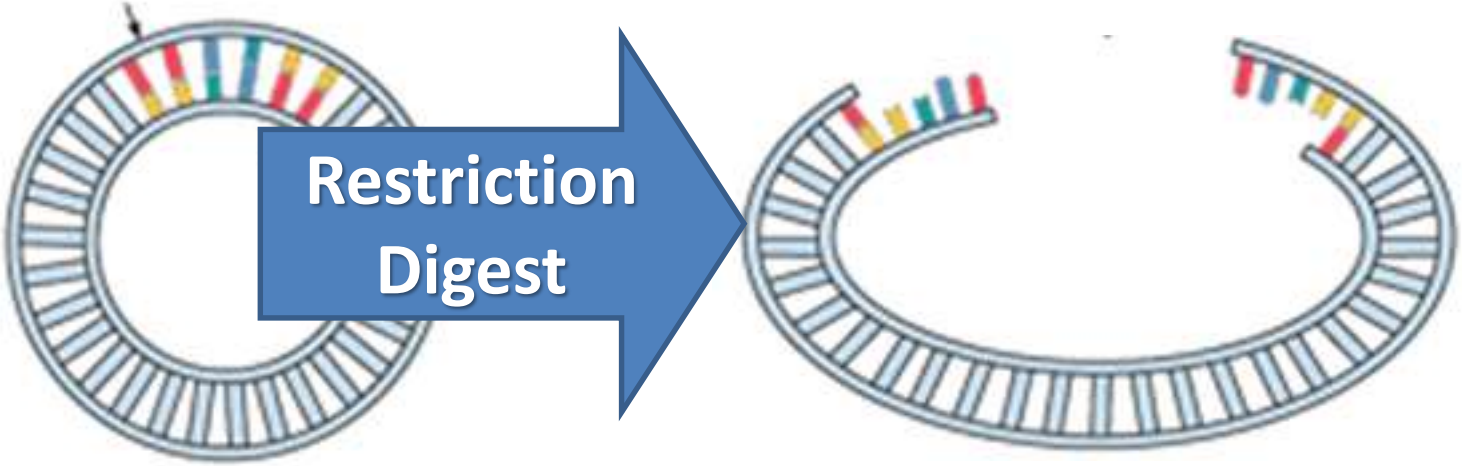


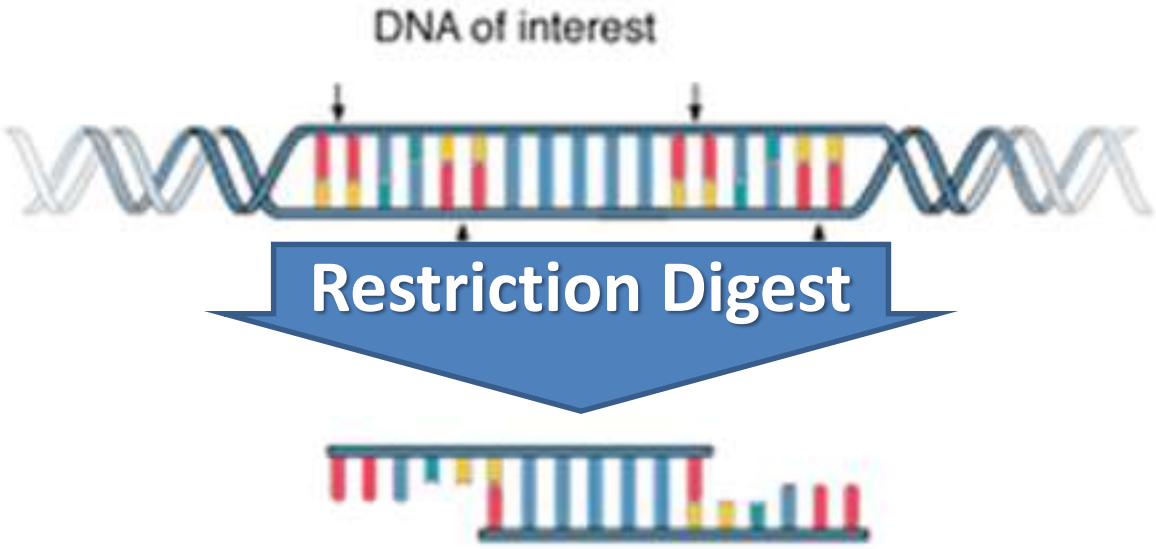

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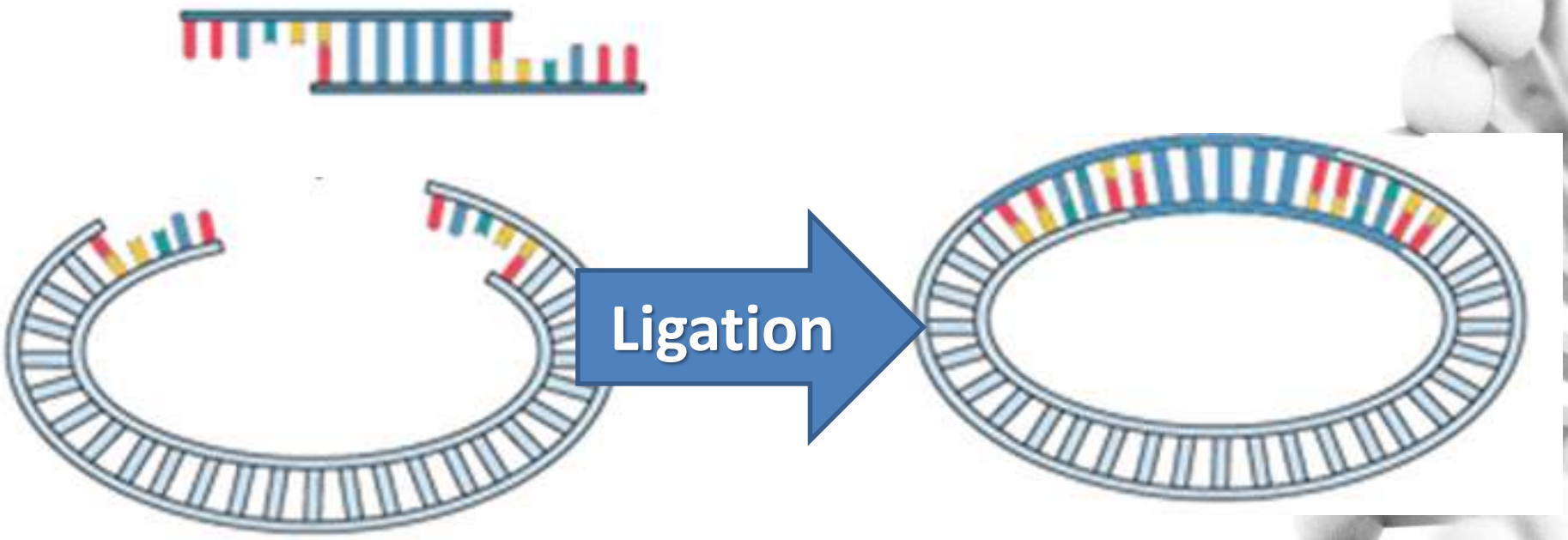

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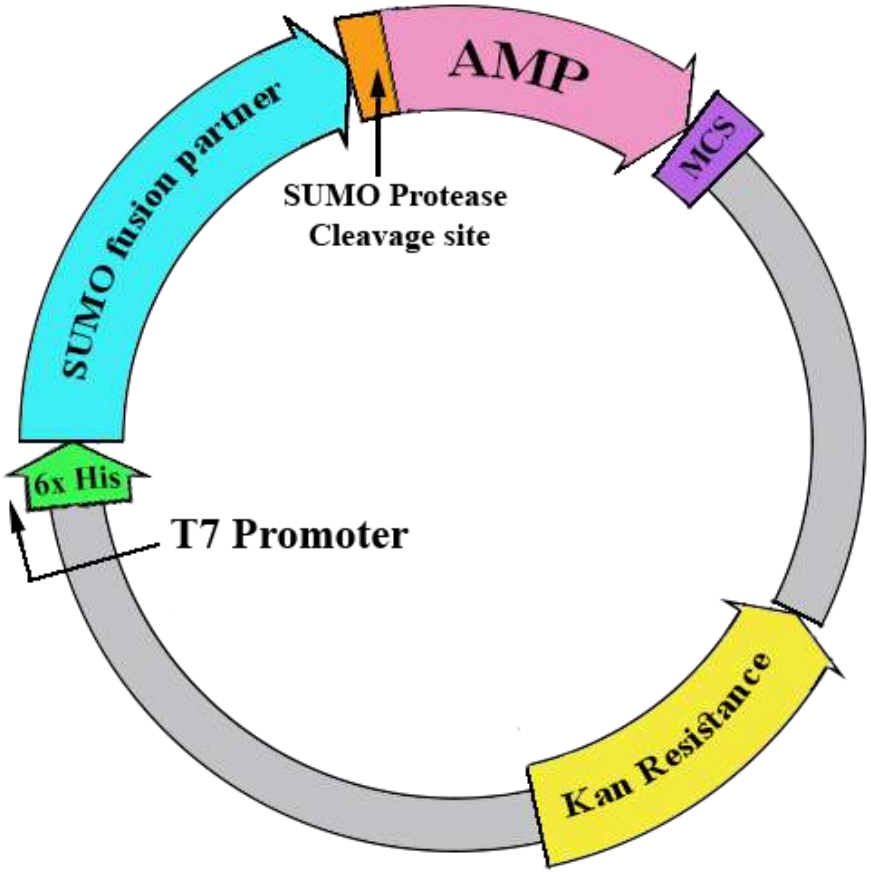
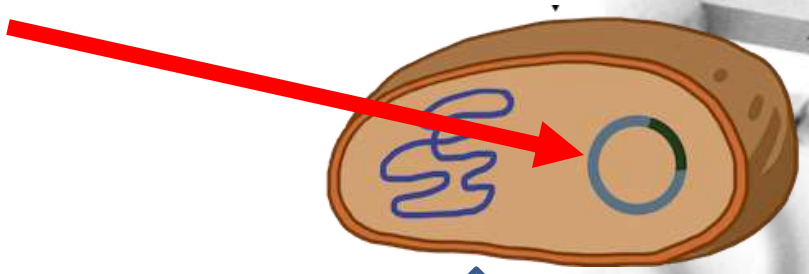


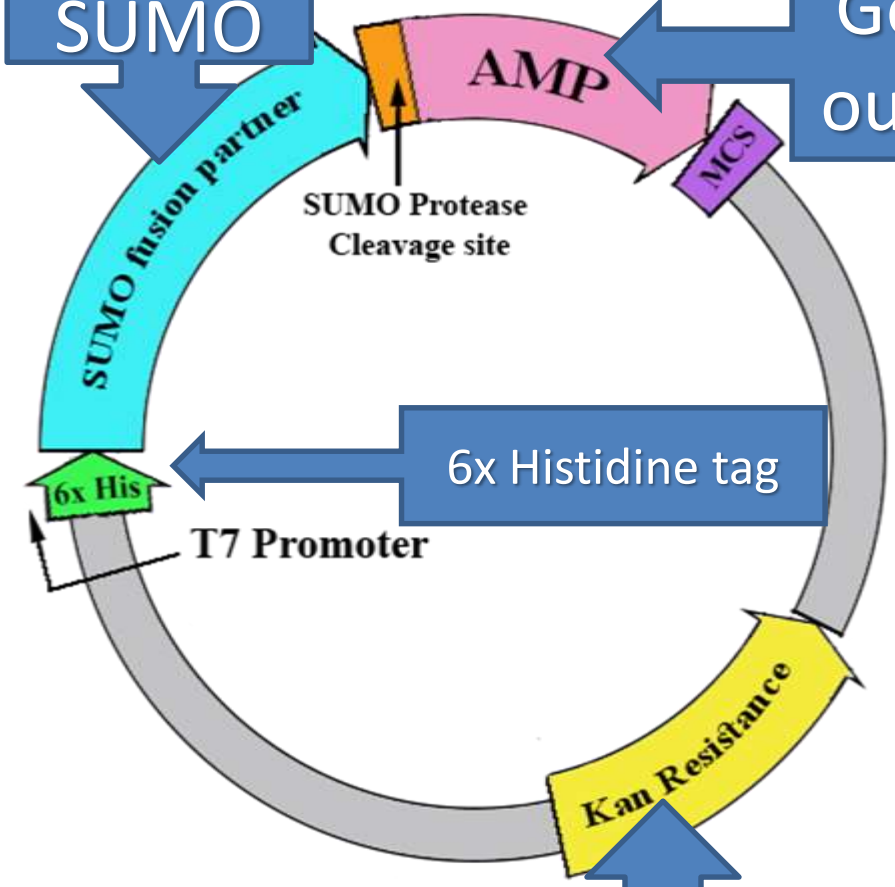



**Transformed bacteria with our AMP gene**

# More essential deets…..

Fusion partner SUMO

Gene of our AMP



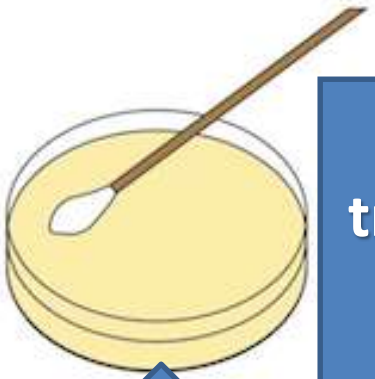

6x Histidine tag

Kanamycin Resistance gene

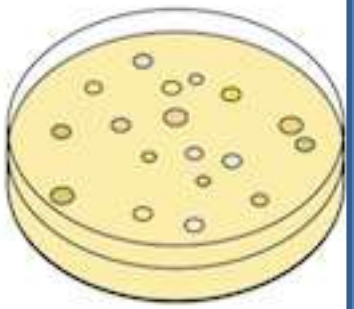
**SUMO** helps in amplifying protein production and makes the protein soluble

**Histidine** tag helps in purifying the protein using Nickel column

Kanamycin resistance protein helps in screening the bacteria on a plate with antibiotic Kanamycin



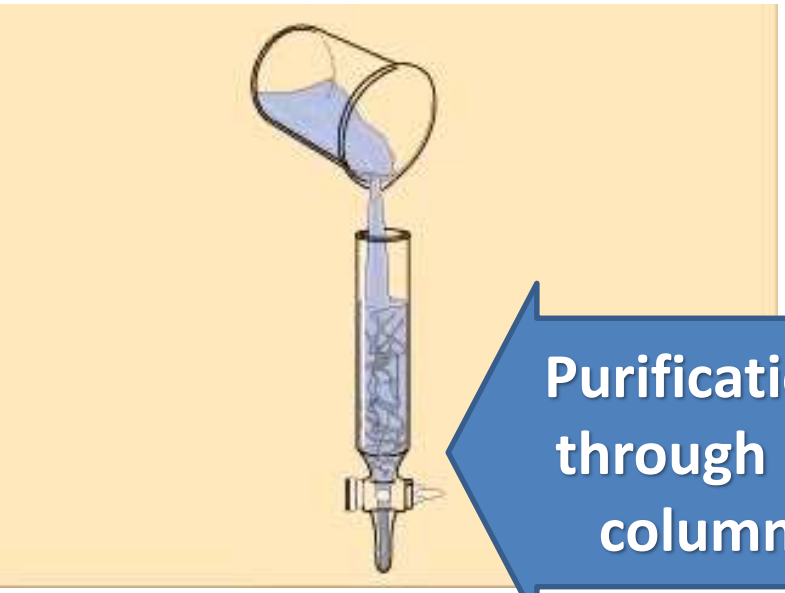
Only transformed bacteria grows

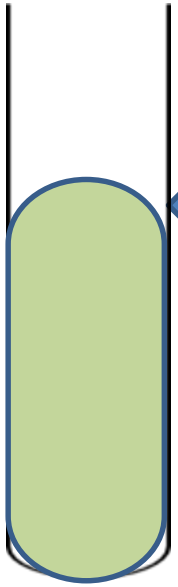


Bacteria are cultured in liquid media

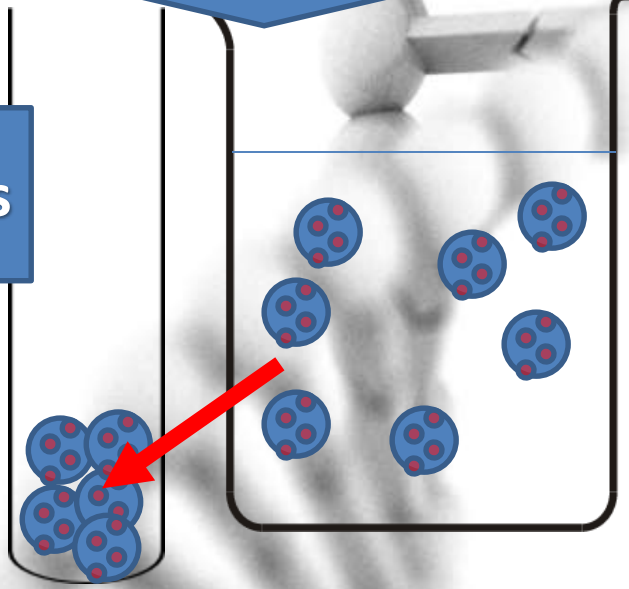
Kanamycin Agar plate

The cells are precipitated by centrifugation



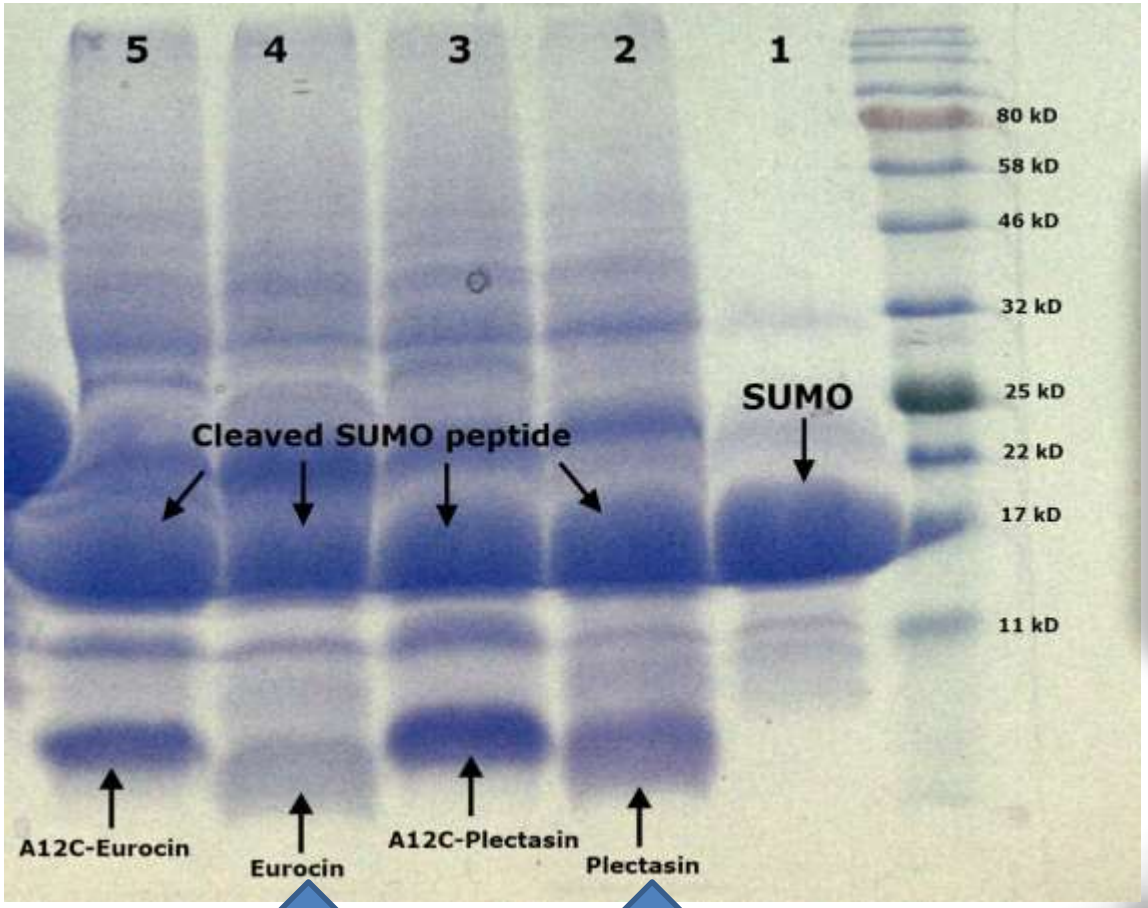


Lysis



Purification through Ni column

# How do the proteins look?




**Eurocin**

**Plectasin**

**SDS PAGE of proteins**

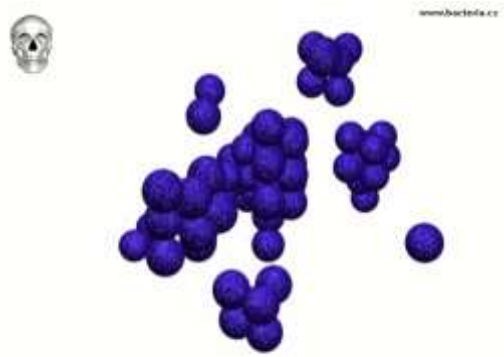
# Is this enough?

# Shouldn’t newer molecules like AMP solve everything?

# But why?

**Say we have 3 bacterial species**

**Species A is pathogenic**



**Species B is non-pathogenic but opportunistic**

**Species C is commensal**

We apply a Broad Spectrum antibiotic i.e., it kills a wide variety of bacteria- both pathogenic and non-pathogenic


It kills Species A but also Species C which was part of the natural microflora

# But why?


It may however spare a few colonies of Species B

In absence of Species C, Species B will grow unchecked

Being opportunistic, Species B may evolve to become pathogenic

# So….


Whatever we try its not enough…

# Or is it?

**We need to look for approaches that can make our AMPs kill only the kind of bacteria we need**

# Or is it?

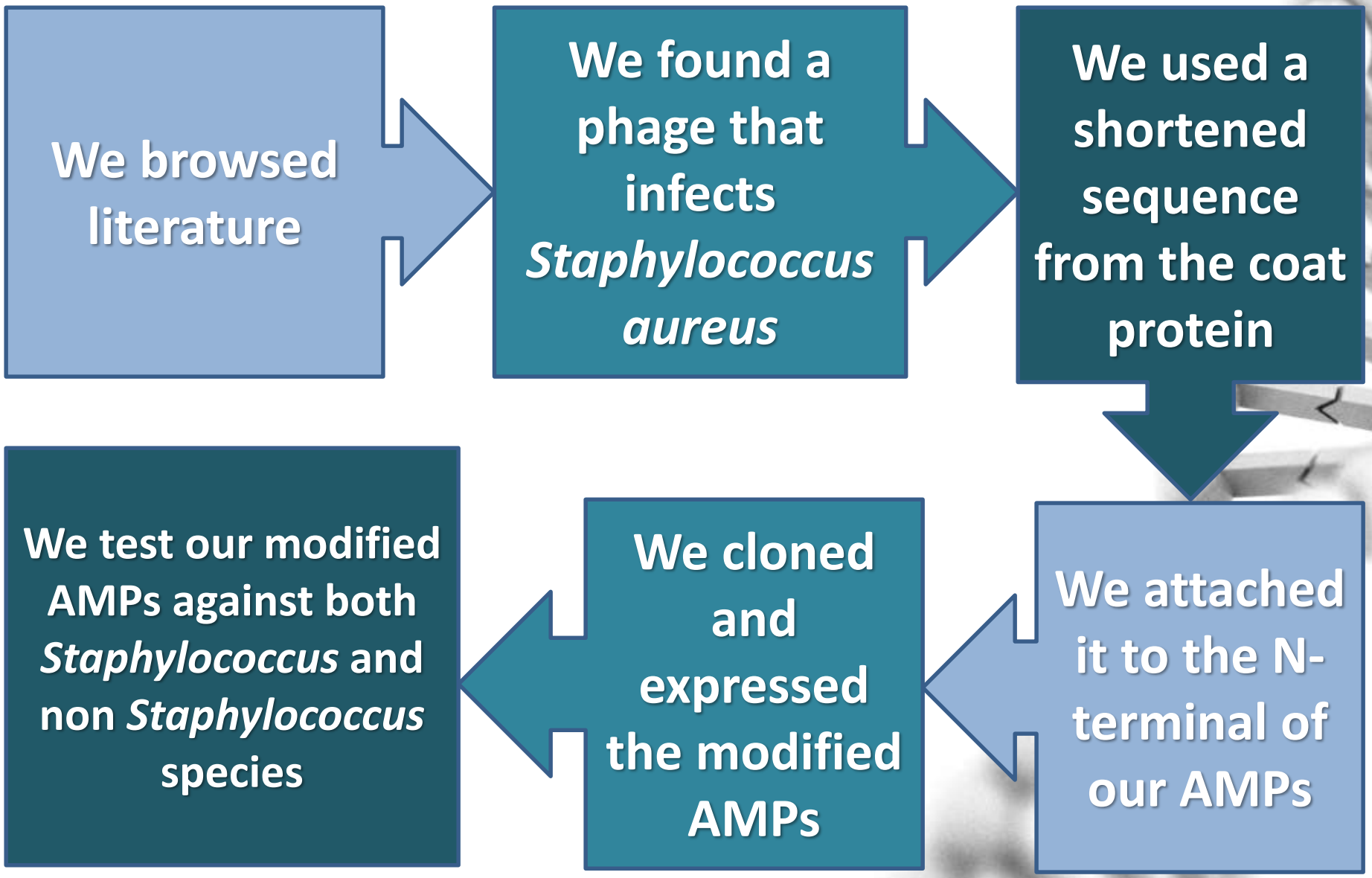
**VOILA!!!**

**Bacteriophages are highly specific**

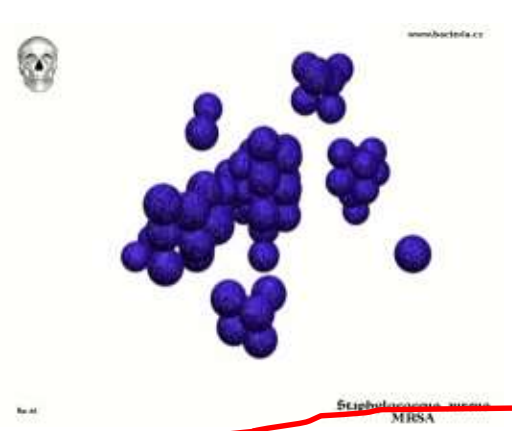
**They bind on the bacterial cell surface**

**Binding happens using coat /surface proteins**

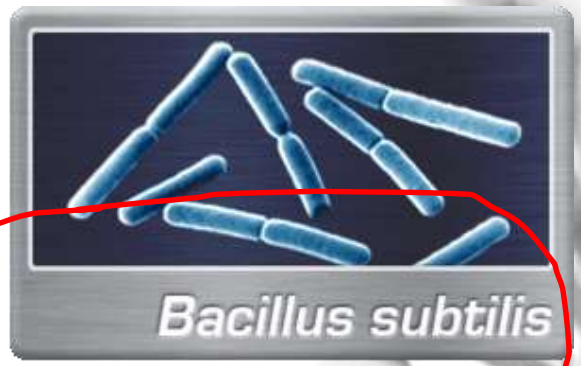
# So we borrowed Phage's technique



# Testing the AMPs





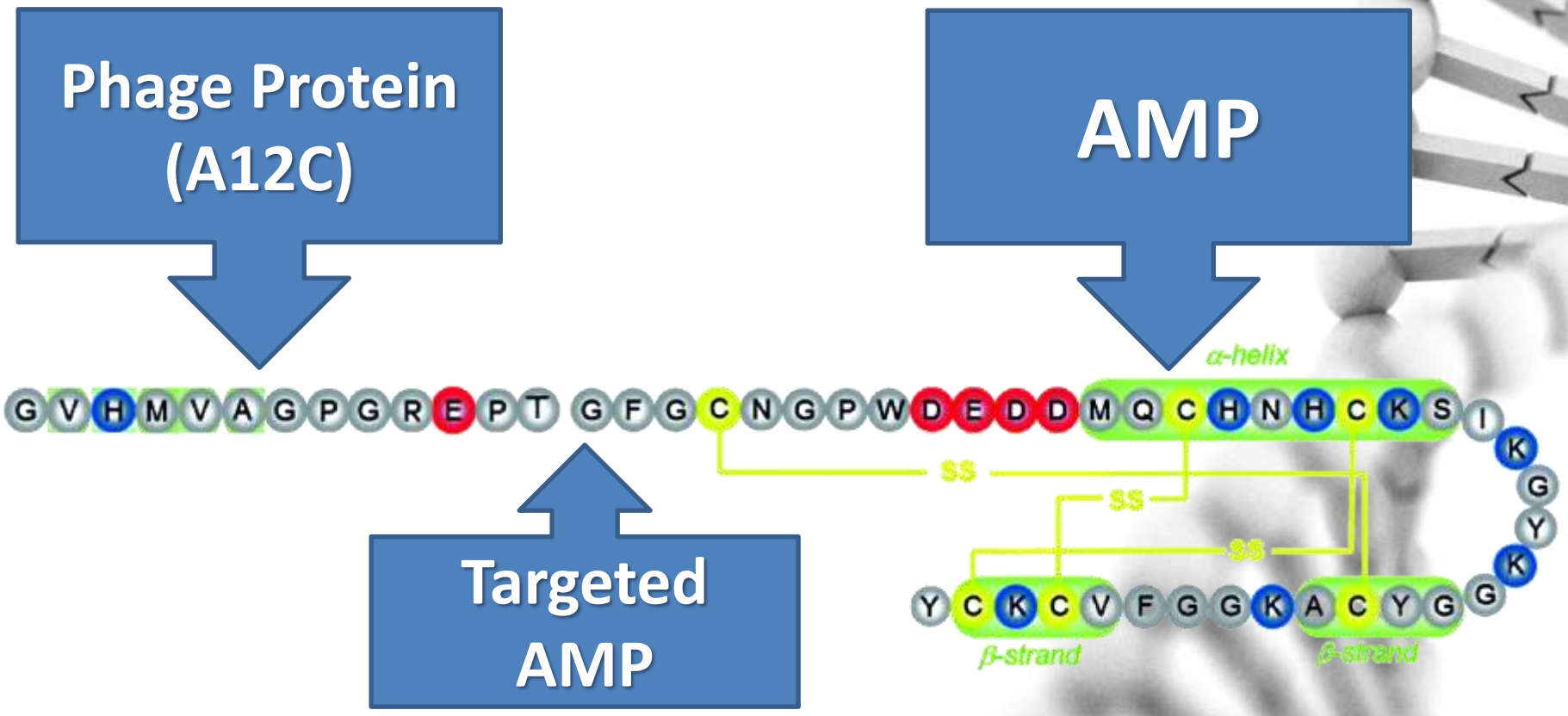



***Staphylococcus aureus***

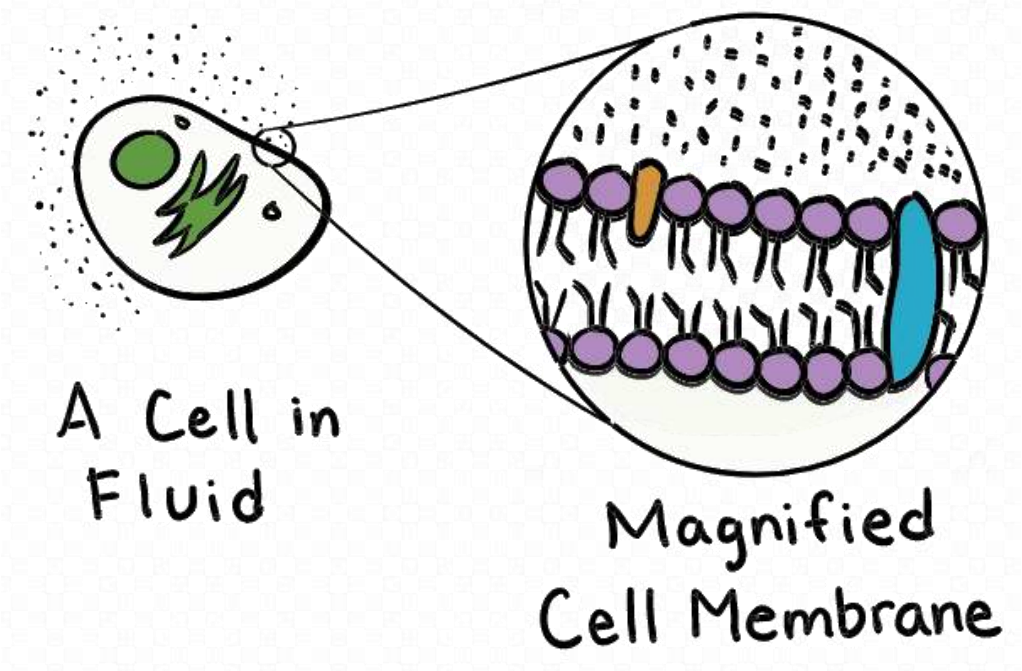
***Staphylococcus epidermidis***

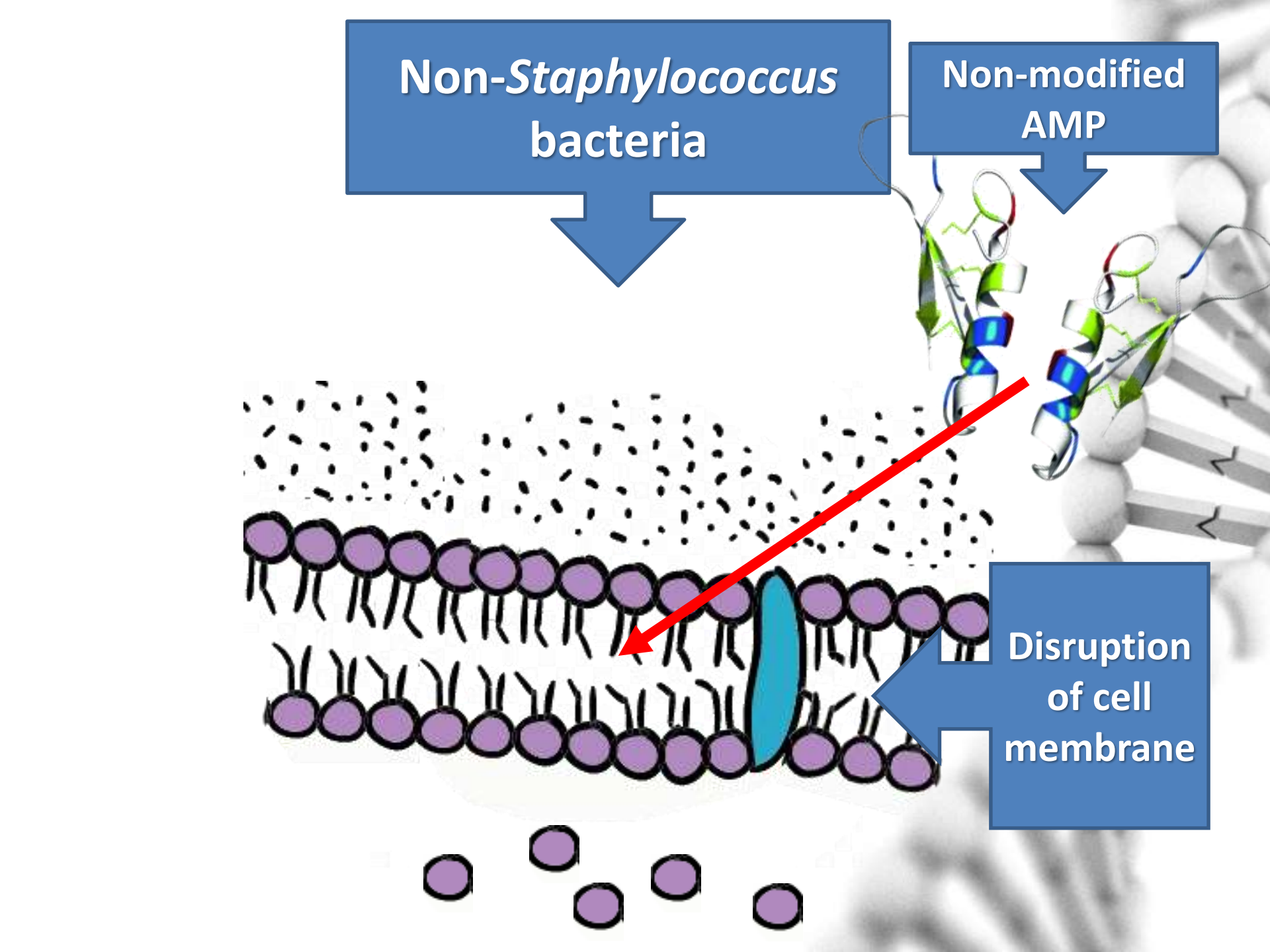
***Enterococcus faecalis***

# Testing the AMPs

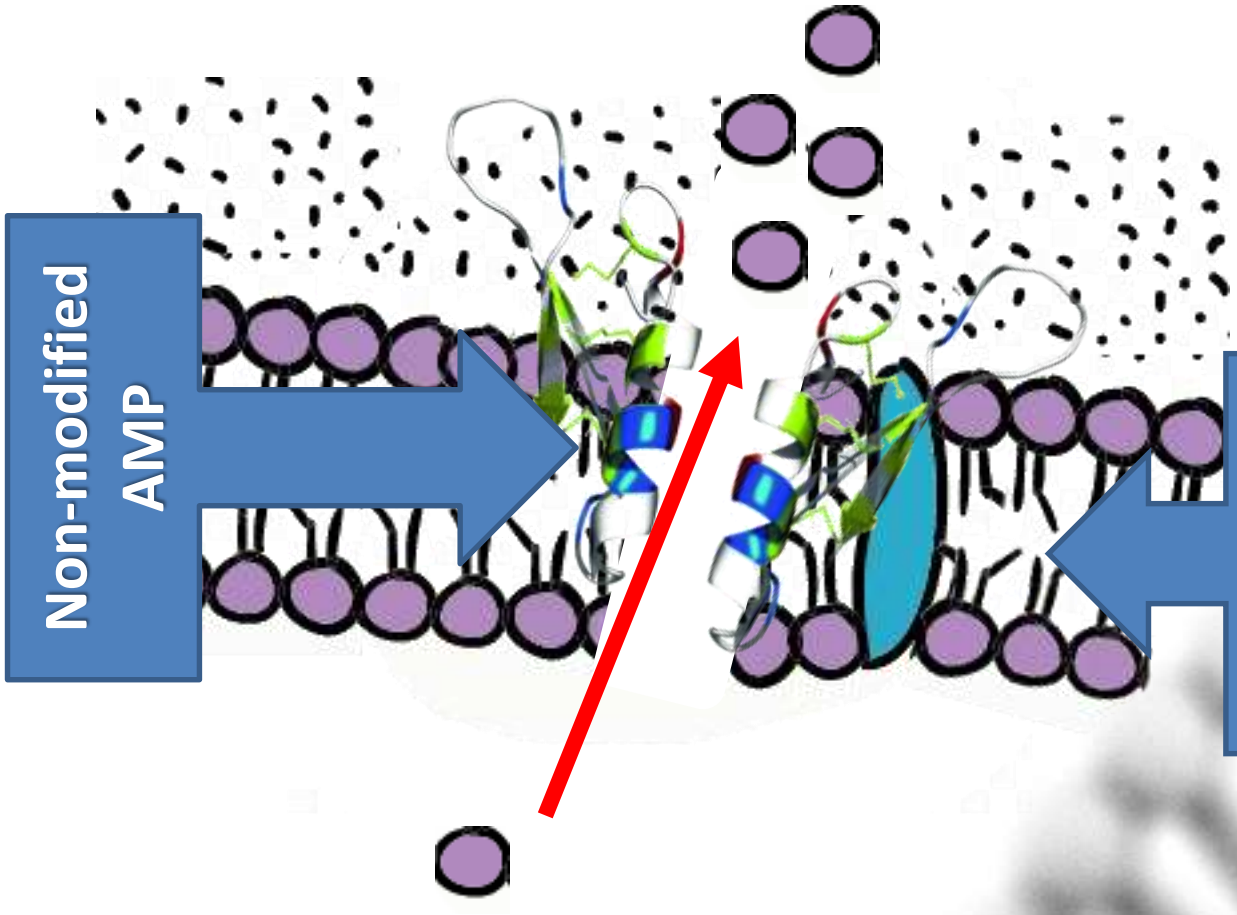


# How does this work?




Non-*Staphylococcus* bacteria
Non-modified AMP
Disruption of cell membrane

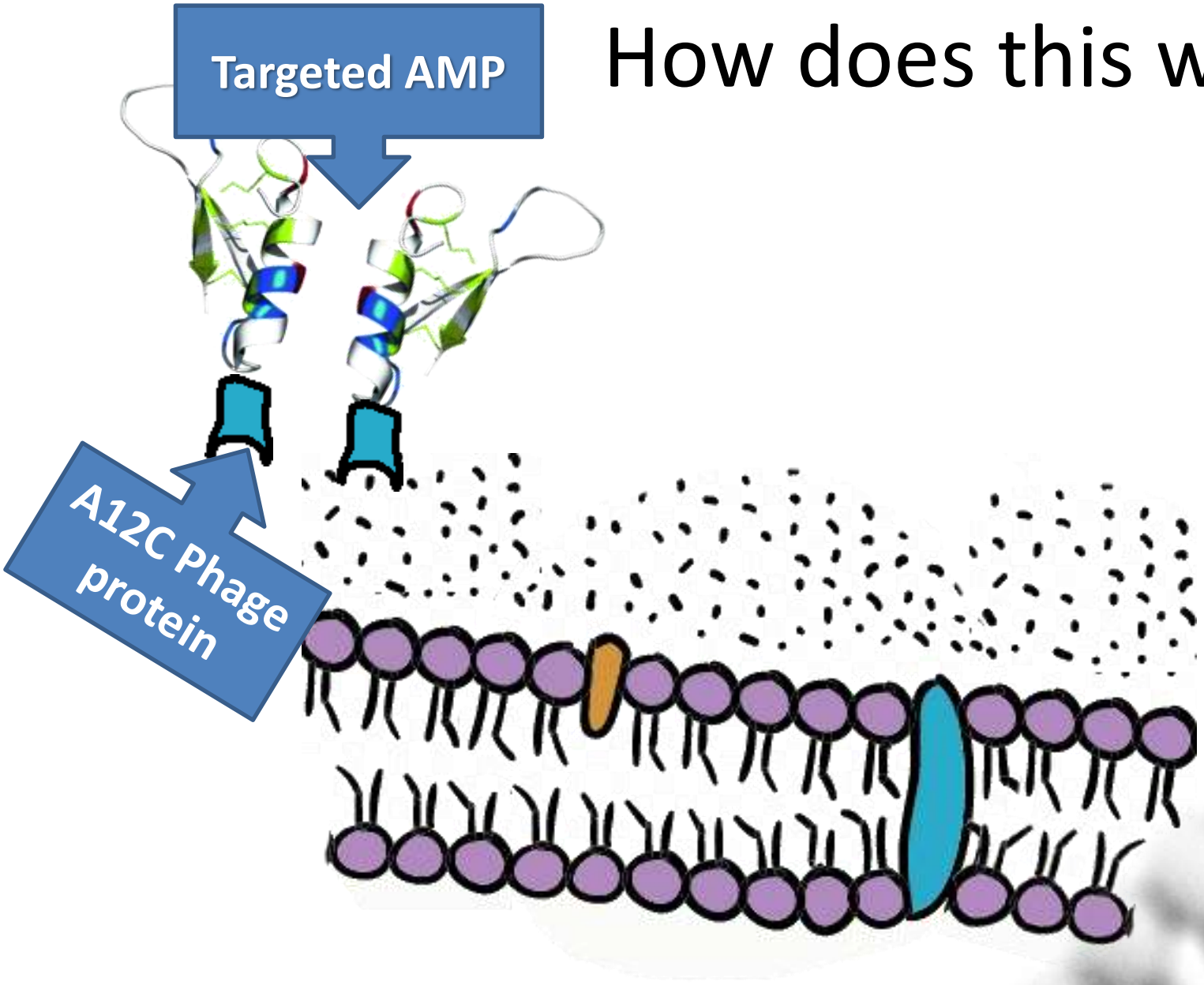
Non-*Staphylococcus* bacteria



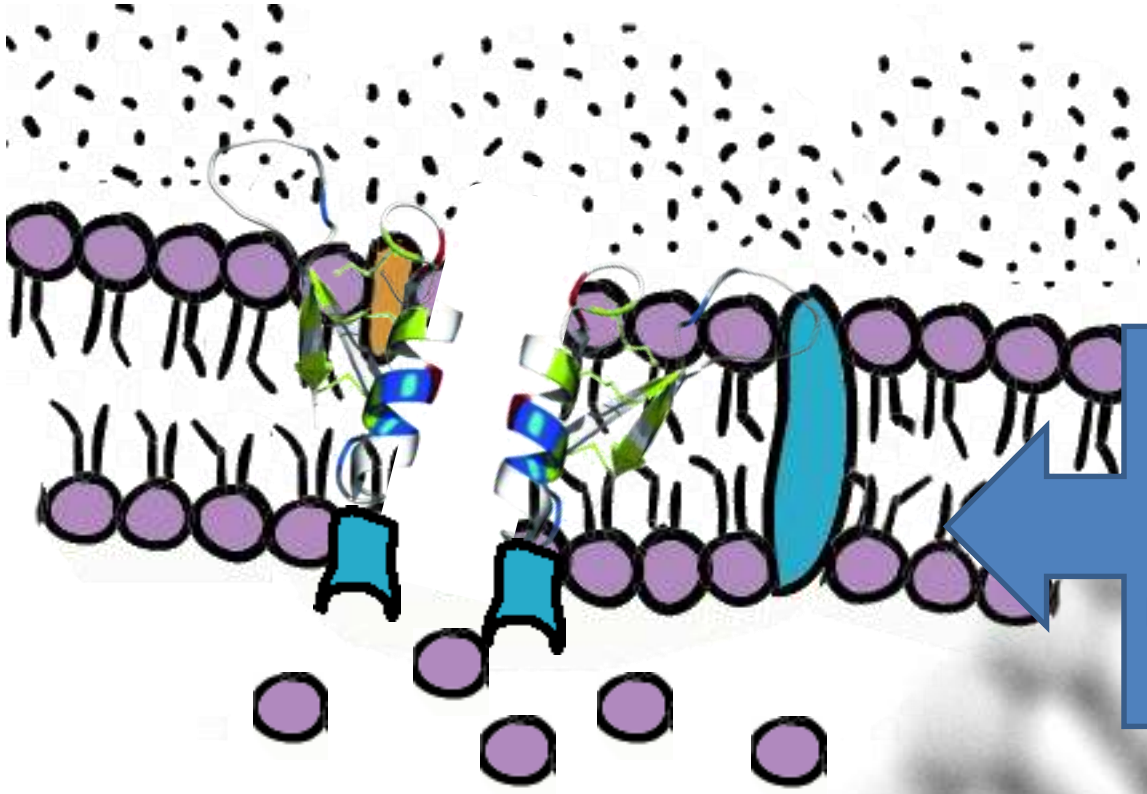
Non-modified AMP

Disruption of cell membrane

# How does this work?

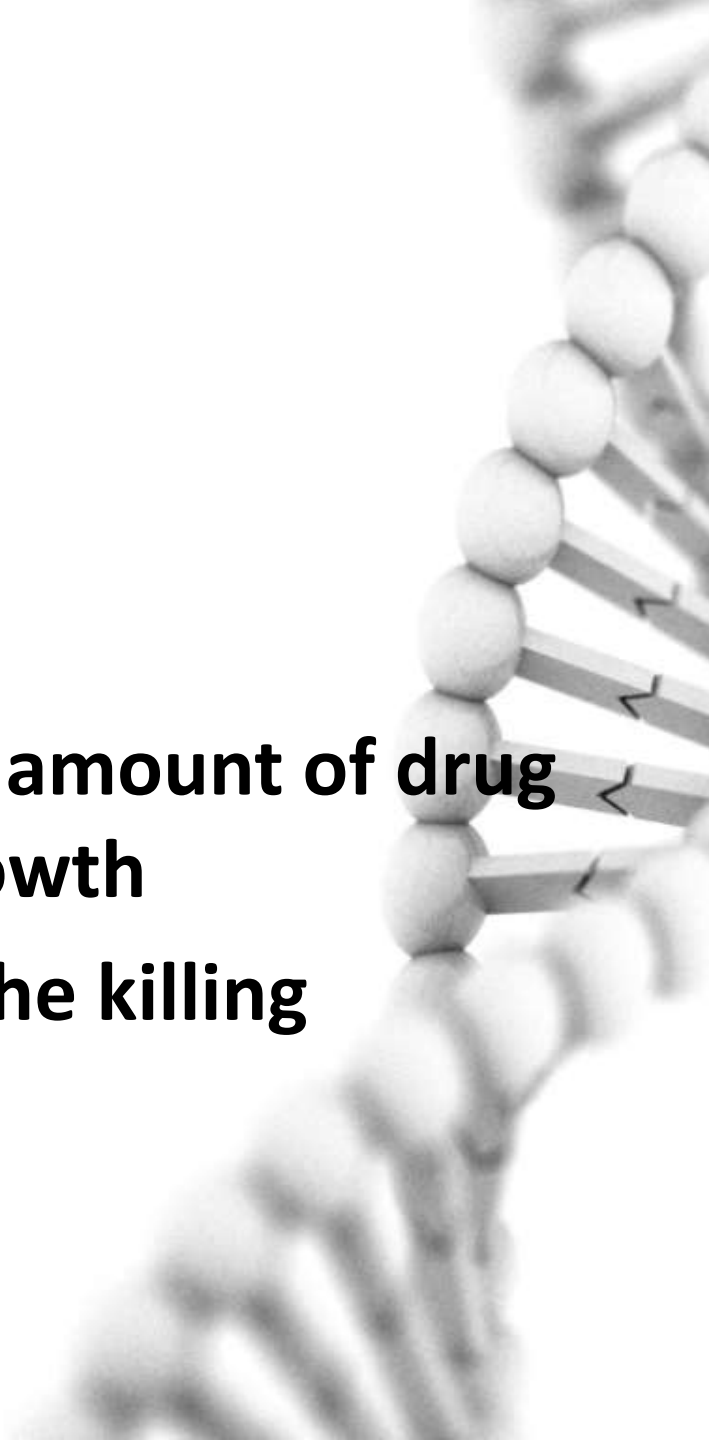


# How does this work?

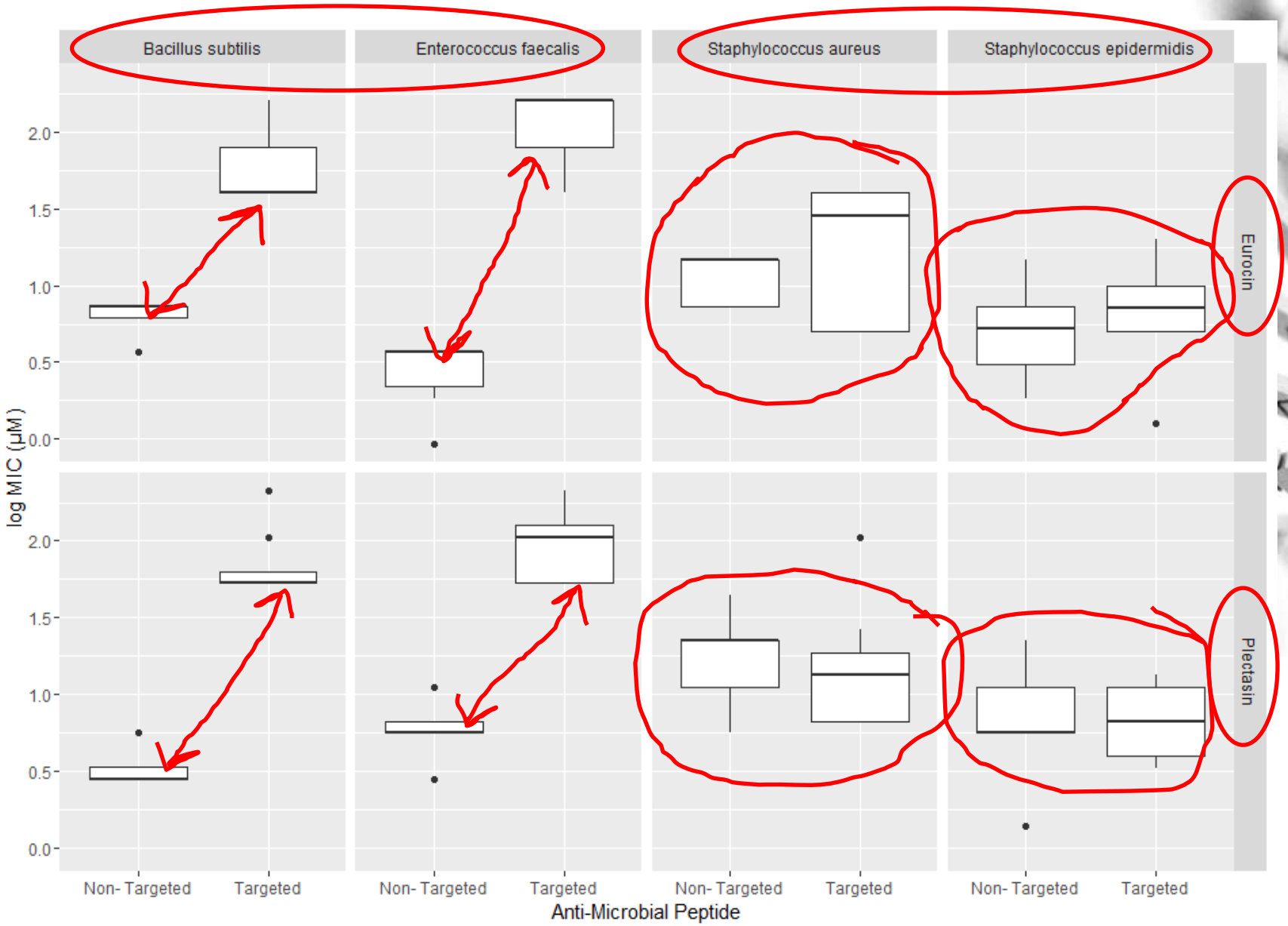


# MIC

- **M**inimum
- **I**nbitory
- **C**oncentration
- **This is measured as the least amount of drug that inhibits the bacterial growth**
- **This is what we measure as the killing potential of antibiotics.**
- **Lower MIC-> better killing**



# What did we see?



# Conclusion

**Both AMPs- Plectasin and Eurocin, had significant activity against all 4 species of bacteria**

**When the AMPs had the targeting domain from the phage, it would not kill the non-*Staphylococci* species**

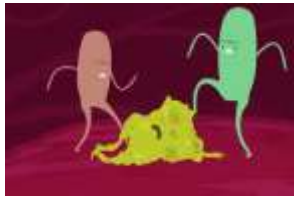
**But AMPs with targeting domain had same killing potential as the AMPs without it for the *Staphylococci* species**

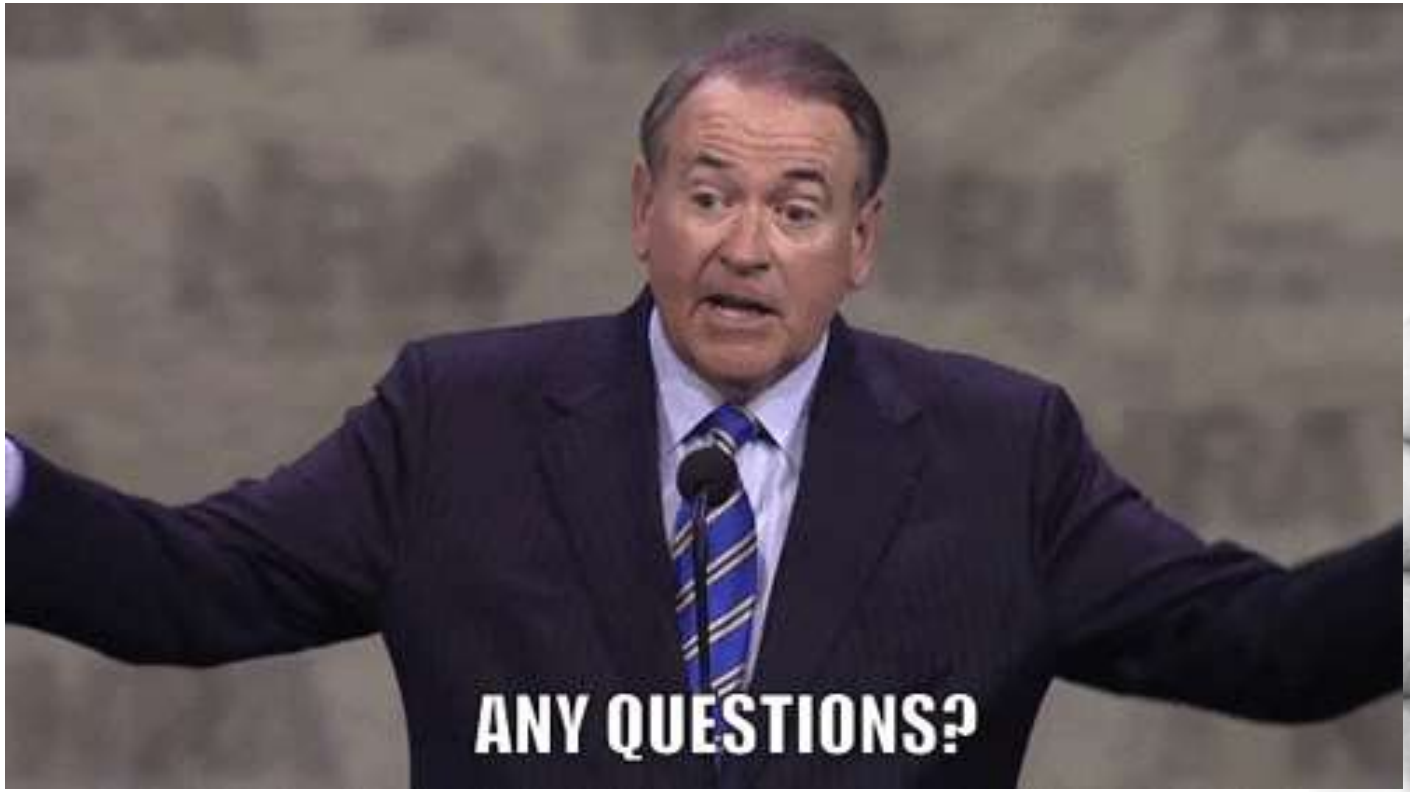
# Thus….

**By attaching the phage protein “A12C” we turned the AMP non-lethal to certain species of bacteria while killing others!!!!**

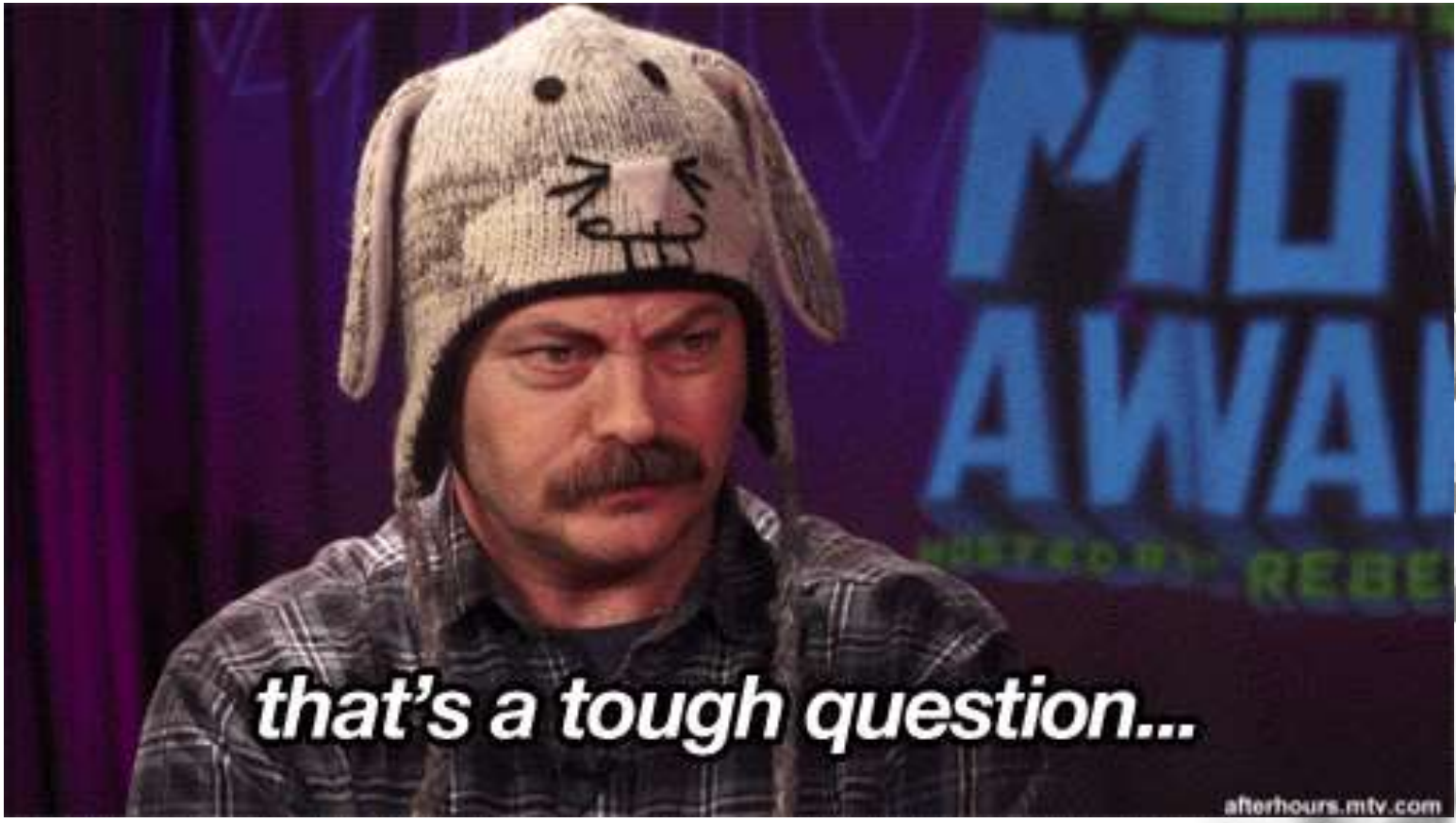
# Then…

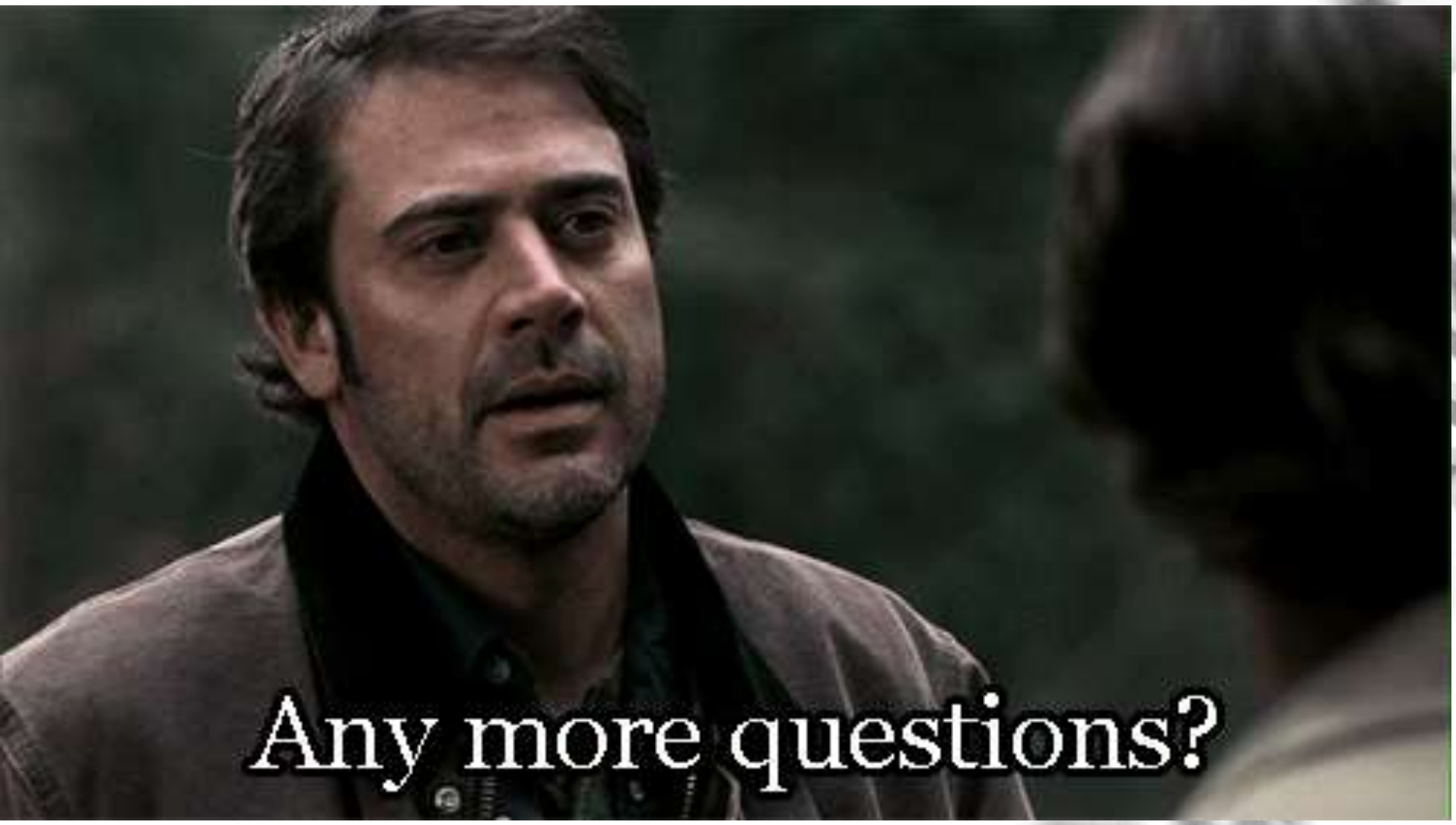
- More studies are required.
- Make the AMPs more suited to therapeutic use
- Figure out how to deliver proteins succesfully
- Work with other types of bacteria and targeting approach
- Be happy and get ice-cream!
- And of course…. get my PhD eventually




ANY QUESTIONS?


THAT IS AN EXCELLENT QUESTION


that's a tough question...
afterhours.mtv.com


Any more questions?

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