Keynote lecture

International Symposium on:

“Emergence of Extensive Antimicrobial Resistance:

Current Status and Future Prospects”

National Institute for Biotechnology and Genetic Engineering

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THE MENACE OF ANTIMICROBIAL DRUG RESISTANCE AND STRATEGIES TO COMBAT IT

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WHAT ARE ANTIMICROBIALS?

- An **antimicrobial** is a substance that **kills or inhibits the growth of microorganisms** such as bacteria, fungi, or protozoa.

- Antimicrobial drugs either **kill microbes (microbiocidal)** or **prevent the growth of microbes (microbiostatic).**

- Antimicrobials include **not just antibiotics**, but synthetically formed compounds as well.

IMPORTANCE OF ANTIMICROBIALS

- The importance and value of antimicrobials **cannot be overestimated.**

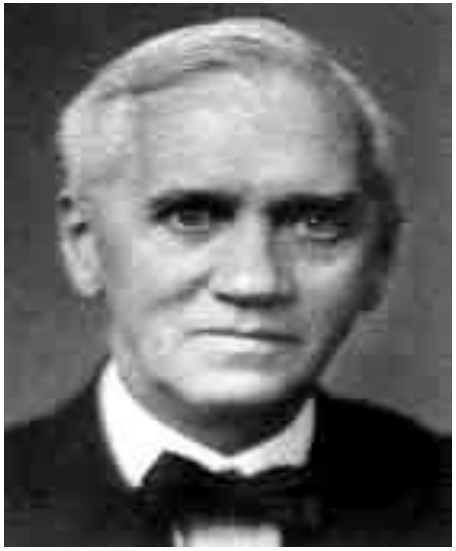
- We are **totally dependent** on antimicrobials for the **treatment of infectious diseases.**

- **In addition** to their use in the treatment of infectious diseases, antimicrobials are **critical to the success of advanced surgical procedures**, including organ and prosthetic transplants to prevent sepsis.

- It is estimated that **millions of lives are saved every year** due to the use of antimicrobial drugs.

Start of Antibiotic era

The first antibiotic, penicillin, was discovered in **1929** by **Sir Alexander Fleming**, who observed inhibition of staphylococci on an agar plate contaminated by a *Penicillium* mold.



Several years later, in **1939, Ernst Chain and Howard Florey developed a way to isolate penicillin** and used it to treat bacterial infections during the Second World War.

The new drug came into clinical usage in 1946 and made a huge impact on public health.

For these discoveries **Fleming, Chain and Florey were awarded the Nobel prize in 1945.**

Todar's online textbook book of bacteriology 2012

DEVELOPMENT AND SPREAD OF ANTIMICROBIAL DRUG RESISTANCE IN MICROBES

What is antimicrobial drug resistance?

Antimicrobial drug resistance is the ability of the microorganisms to withstand the dose of antimicrobial that was effective in the past, due to the repeated exposure of a microbe to a particular drug.

- At the time of discovery of antibiotics, it was calculated that **normal mutation rate (10^{-8})** would be too slow to develop resistance.

- In a few years, it became apparent that bacteria held a distinct advantage because of **very fast evolution rate** due to relatively **simple and small genome** and **short generation time**.

- But it was **not the only reason**.

- Scientists were also unaware of other dynamic powers of bacteria especially the mechanisms of **horizontal gene transfer** of resistant genes were not known.

- The genes which cause resistance against antimicrobials are **ancient** and **ubiquitous**.

- Bacteria from intestines of **Arctic explorers who froze to death in 1845** were grown and subjected to various modern antibiotic medications.

- Many of the bacterial colonies grown were found to be **resistant to modern antimicrobials**, proving that this resistance **did not evolve over just the past 70 years or so.**

McQuire. R. 29th Dec 1988. Medical Tribune pp. 1- 23.

- However, studies with collections of bacterial pathogens **isolated before the "antimicrobial era"** showed that **plasmids were common but resistance (r) genes were rare.**

- **Horizontal gene transfer** made the **rapid spread and evolution of these genes possible** when bacteria were **challenged with antimicrobials**.

- Now we know the existence of more than **20,000 potential r genes of nearly 400 different types.**

- Rate of development of resistance against various drugs **may vary by 100 times.**

Davies J., and Davies, D. 2010.Microbiol Mol Biol Reviews. 74: 417-433

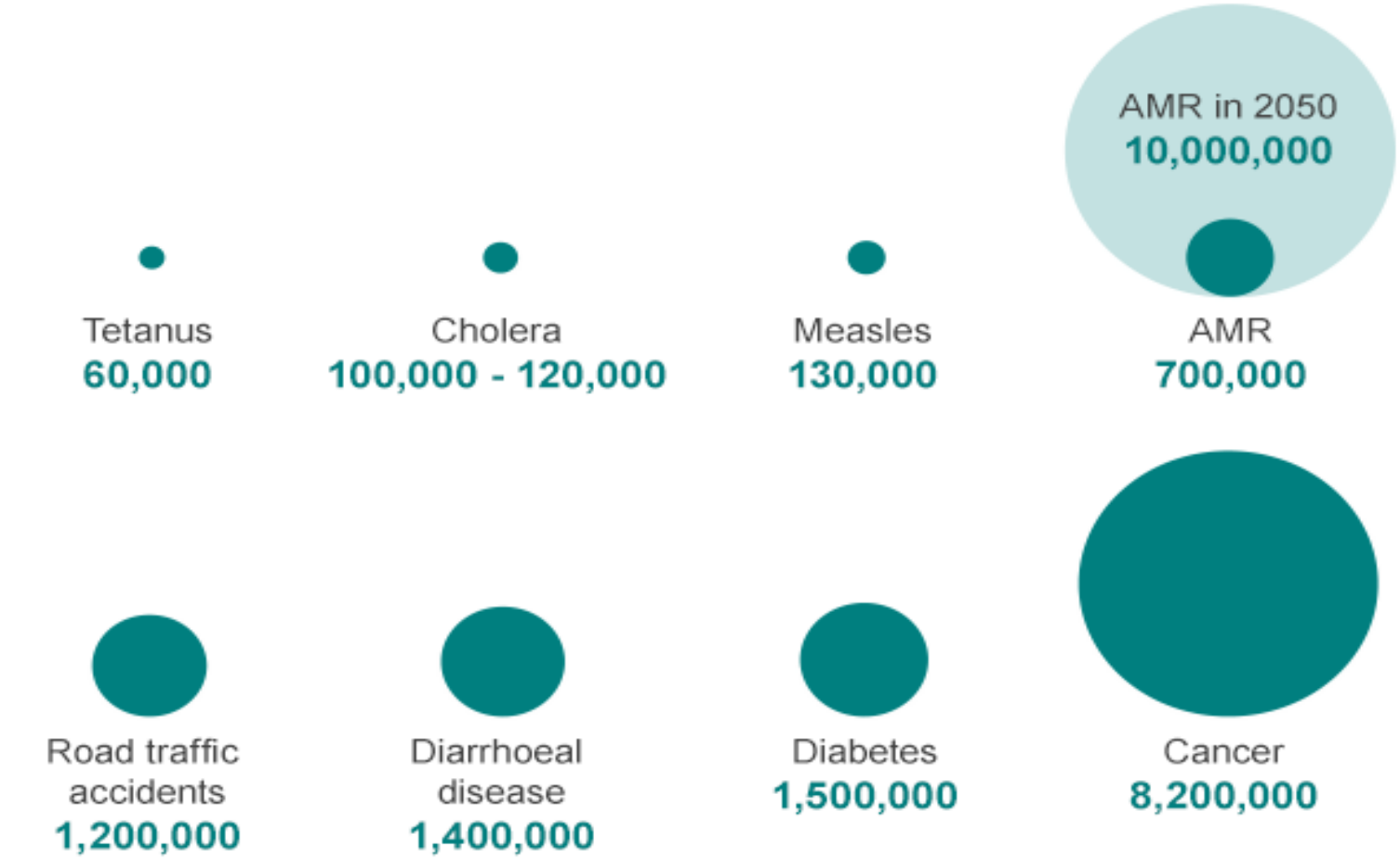
- This phenomenon of horizontal gene transfer has led to the **emergence of "superbugs"** and superbug infections that are **almost incurable.**

- It is feared that mankind **will soon enter the "post-antibiotic era" in response to the current situation.**

- It is estimated that **bacterial AMR** was **directly responsible for 1.27 million global deaths in 2019** and contributed to **4.95 million deaths** overall**.**

Kwon J.H., and Powderly W.G. Science. 2021;373:471.

Deaths attributable to antimicrobial resistance every year compared to other major causes of death



Source: Review on Antimicrobial Resistance 2014

Wellcome Trust Report 2014

MECHANISMS OF ANTIMICROBIAL DRUG RESISTANCE

Antimicrobial drug resistance can be attained through:

1. **Intrinsic mechanisms**
2. **Mutation acquired mechanisms**
3. **Extrachromosomal or acquired resistance**
4. **Selective pressure**
5. **Biochemical mechanisms**
6. **Drug efflux pumps**
7. **Physical barriers such as biofilm formation**

1. Intrinsic Mechanisms

- **Intrinsic resistance** may naturally occur as a result of the bacteria's **genetic makeup**.

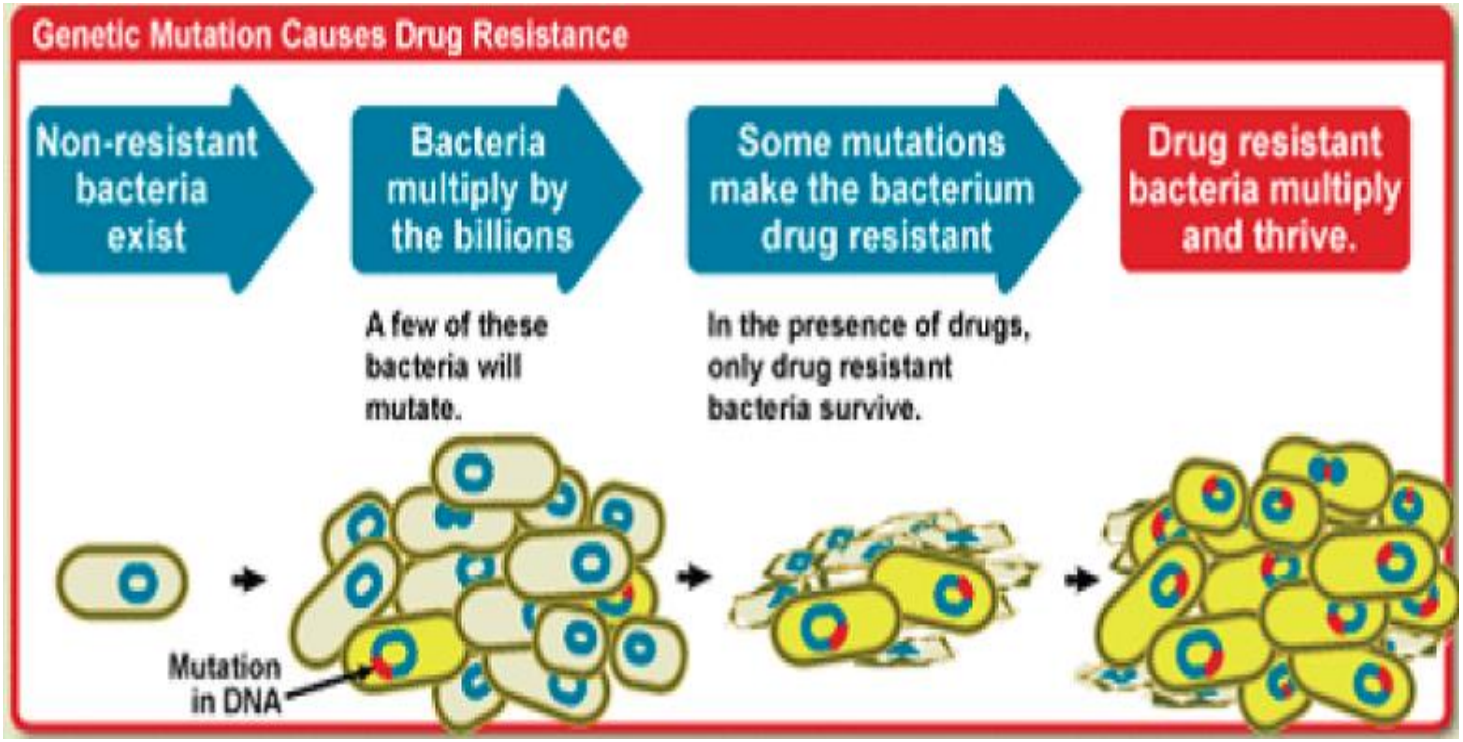
- It may be due to the **inaccessibility of the targets** by the drug, e.g., *E.coli* is intrinsically resistant to vancomycin.

- Drug resistance may also be due to **naturally occurring genes** found on the host's chromosome, such as, AmpC β-lactamase of Gram-negative bacteria and many genes for efflux systems.

2. Mutation acquired mechanisms

- Mutations in the genome results in the **target site modification** or **reduced permeability**.

- Mutations can also cause **metabolic bypass** or **derepression of multidrug efflux systems.**



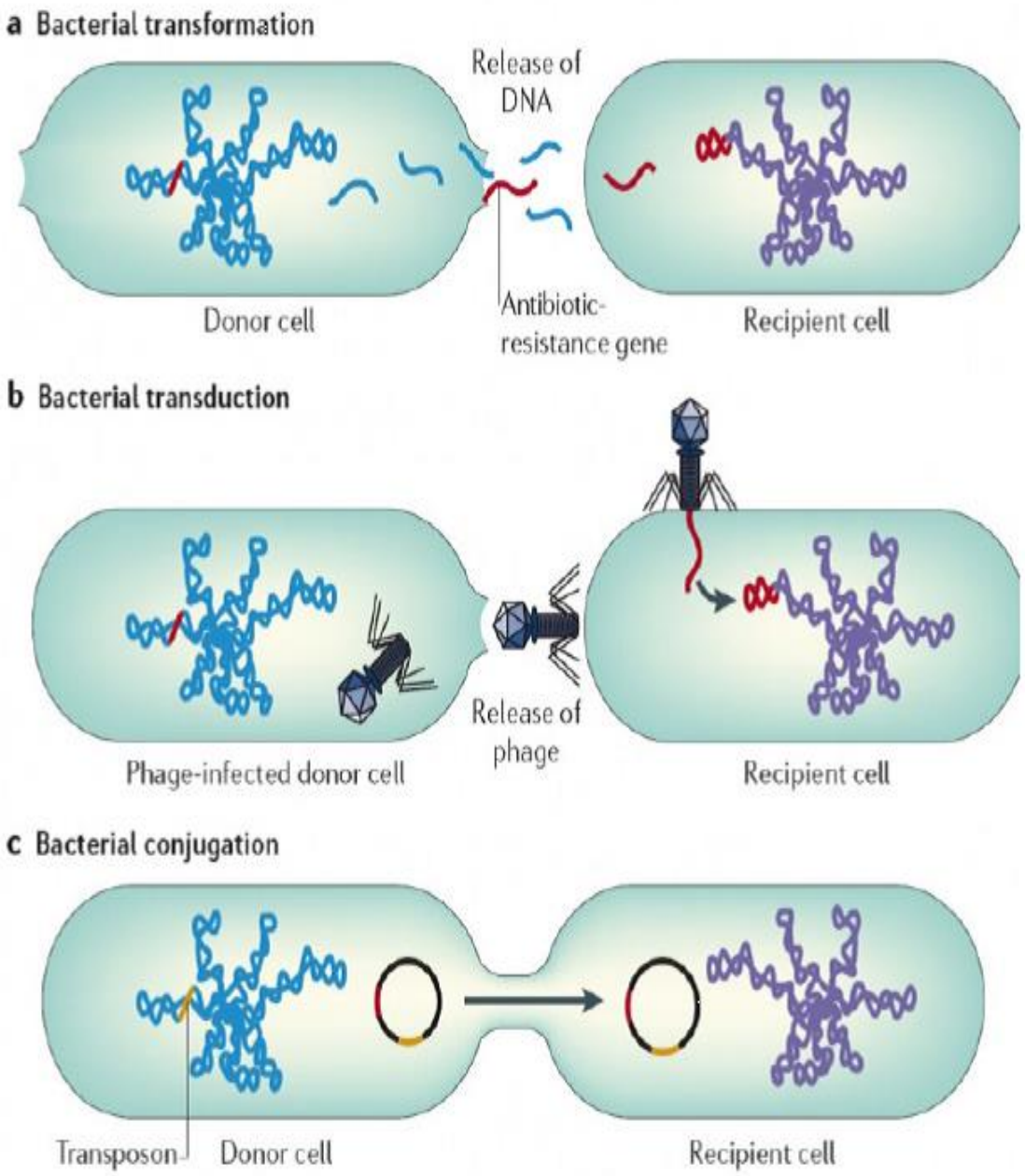
http://www.niaid.nih.gov/topics/antimicrobialResistance/Understanding/Pages/mutation.aspx.

3. Extrachromosomal or Acquired Resistance

- This type of drug resistance is **mainly disseminated by plasmids (through conjugation) or transposons** resulting in either drug inactivation, drug efflux, target site modification or metabolic bypass.

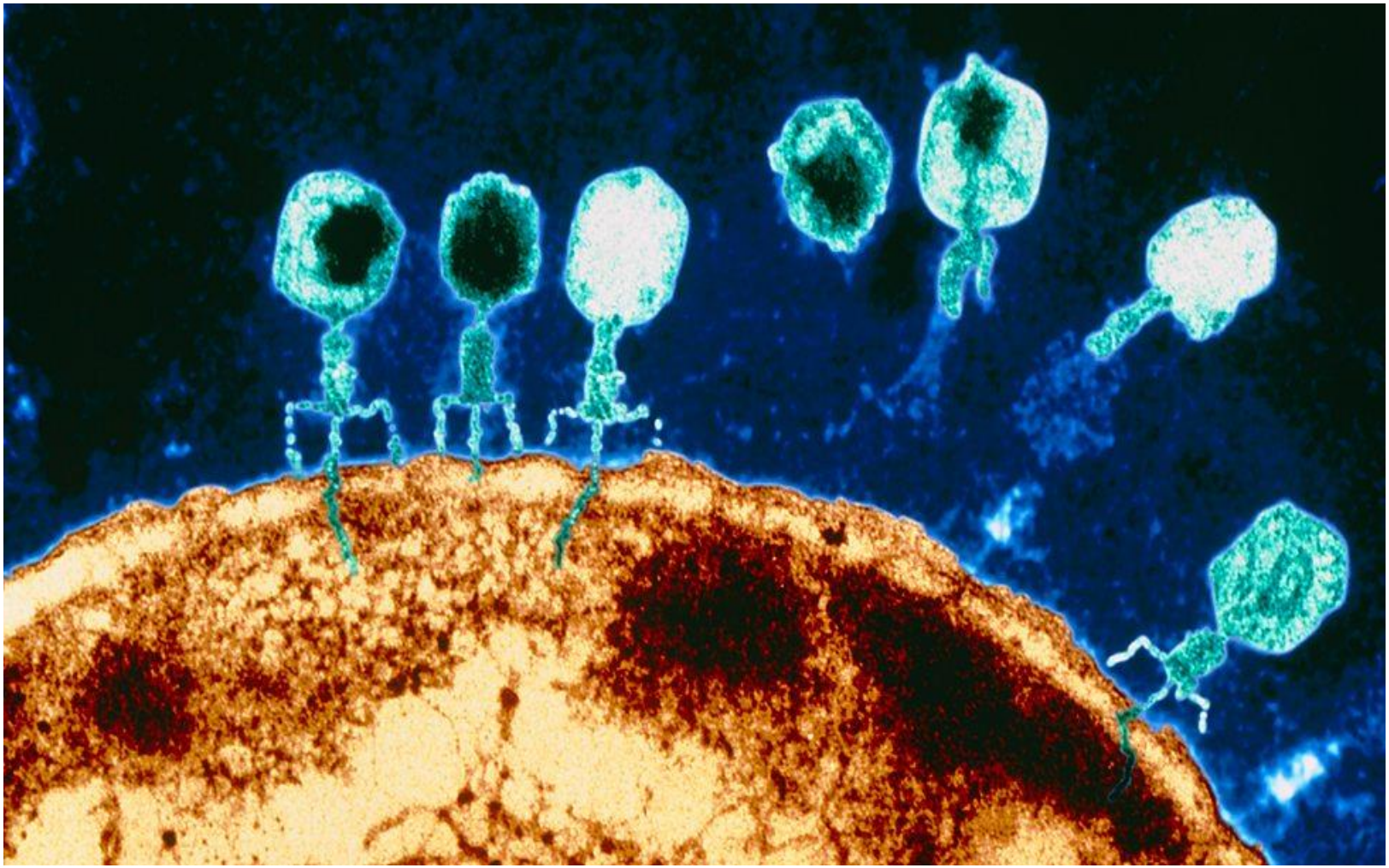
- Among these **horizontal gene transfer mechanisms**, **conjugation** is thought to play the **most significant role** in the spread of resistance genes.

- But other mechanisms including **transduction** (via bacteriophages) and **transformation** (via incorporation of chromosomal DNA, plasmids, and transfer of DNAs from dying organisms into the chromosome) are also important.



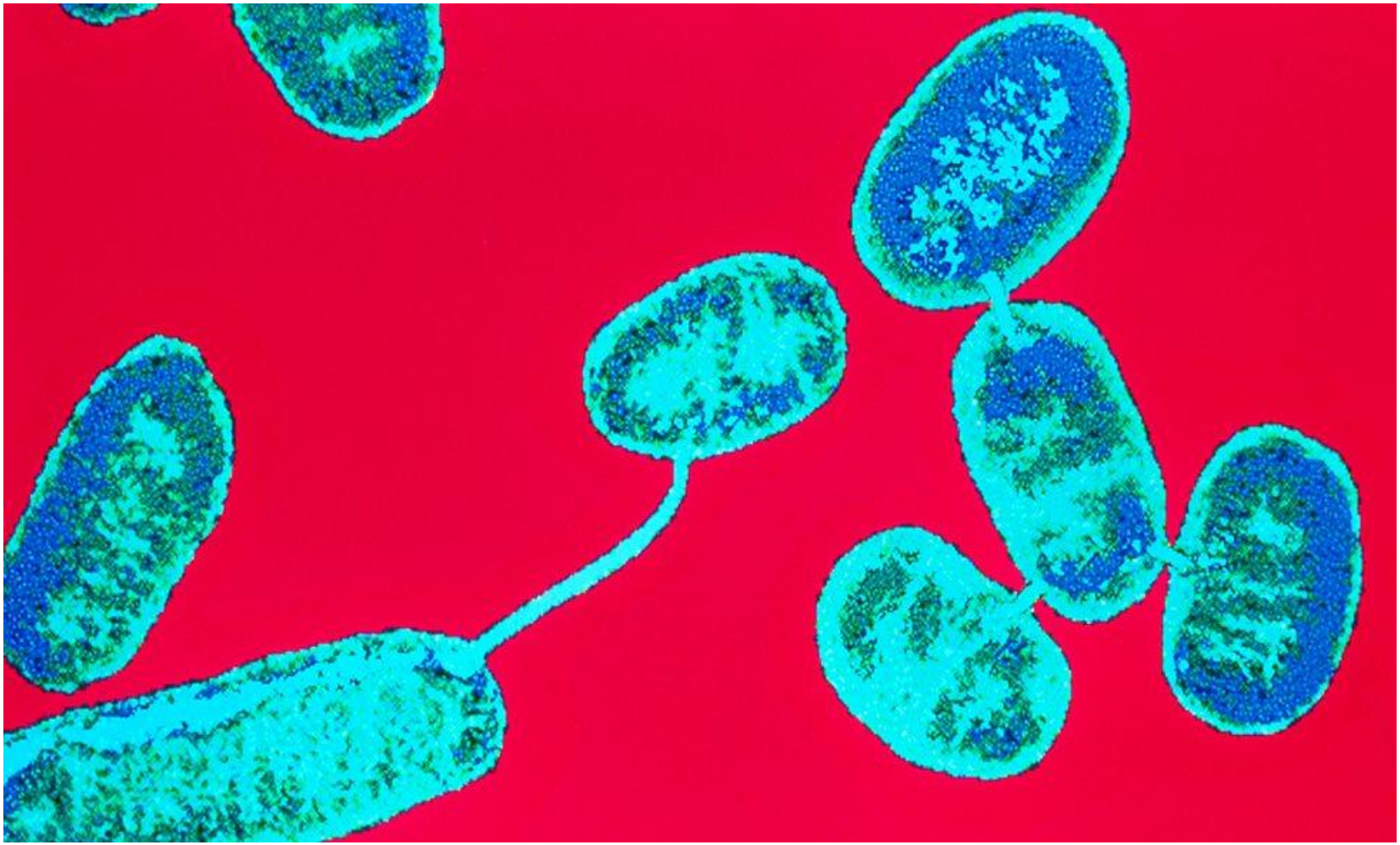
Furuya EY., Lowy FD. 2006. Nat Rev Microbiol. 4(1): 36-45.

Transduction



Bonin L, 26th Sept 2014 iWonder BBC News

CONJUGATION



Dr Linda Stannard, UCT/SPL 26th Sept 2014 iWonder BBC News

4. Selective Pressure

- In the presence of an antimicrobial, microbes are either killed or, **if they carry resistance genes, they survive.**

- Many **surviving bacteria** adopt **the "viable but non-culturable (VBNC)" state** under stress.

- In this state, bacterial growth and metabolism are **very slow or may even nearly cease.**

- Most antibacterial drugs **only kill metabolically active bacteria**; therefore, these bacteria can **escape the action of antibiotics** and enter the latent phase.

Zhu L., et. al. J. Hazard. Mater. 2022;423:127228.

5. Biochemical Mechanisms of Drug Resistance

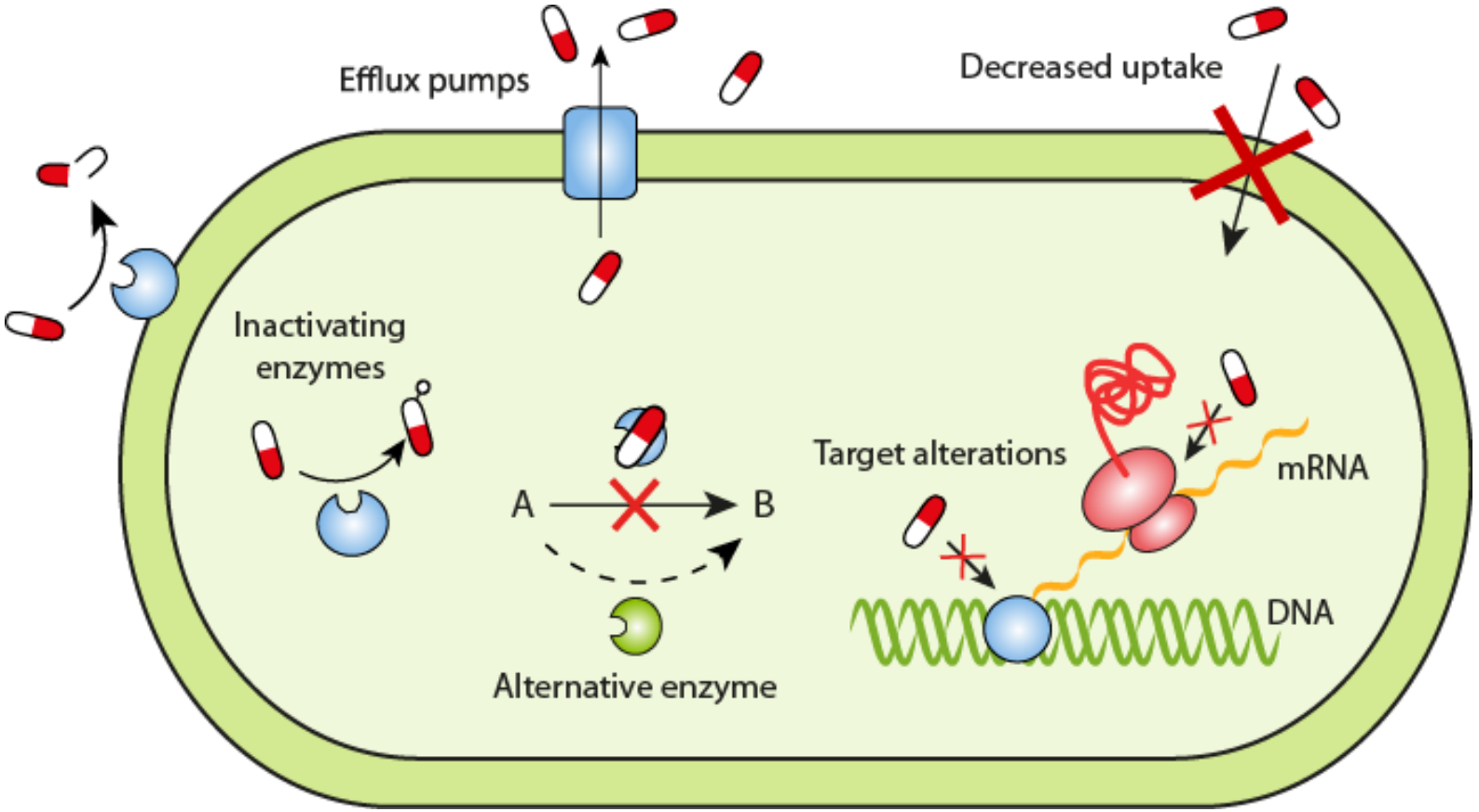
Antimicrobial agents are rendered inactive by different mechanisms that include:

-- **Enzymatic drug modification and destruction**

-- **Mutational alteration of the target protein**

-- **Substitution and protection of drug targets**

-- **Reduced drug accumulation due to efflux systems, porins and outer membrane proteins.**


Efflux pumps
Decreased uptake
Inactivating enzymes
A
B
Alternative enzyme
Target alterations
mRNA
DNA

6. Drug Efflux Pumps

- **Efflux pumps**, found in bacteria and other eukaryotes, **are transport proteins involved in the expulsion of toxic substrates (including drugs)** into the external environment from inside of cells.

- They may be **specific for one substrate or may transport a range of structurally dissimilar compounds**; such pumps can be **associated with multiple drug resistance (MDR).**

- Drug efflux pumps are now recognized as **significant contributors to bacterial resistance** because of the **very broad variety of substrates they recognize.**

Van Bambeke F., et al. 2000. Biochem Pharm 60(4):457-70.

7. Biofilms - The Physical Barriers

- A biofilm is an **aggregate of microorganisms in which cells adhere to each other and to a surface and are embedded within an extracellular polymeric substance (EPS).**

- These are **highly developed colonies** with **bacteria at different stages of life placed in separate segments** with **intercommunication facilities *via* water channels**.

- Biofilms have been found to be involved in **nearly 80% human bacterial infections** including urinary, ear, dental infections, endocarditis, and cutaneous wound infections.

1. Karatan E., et. al. 2009. Microbiol Mol Biol Rev. 73(2): 310-47.

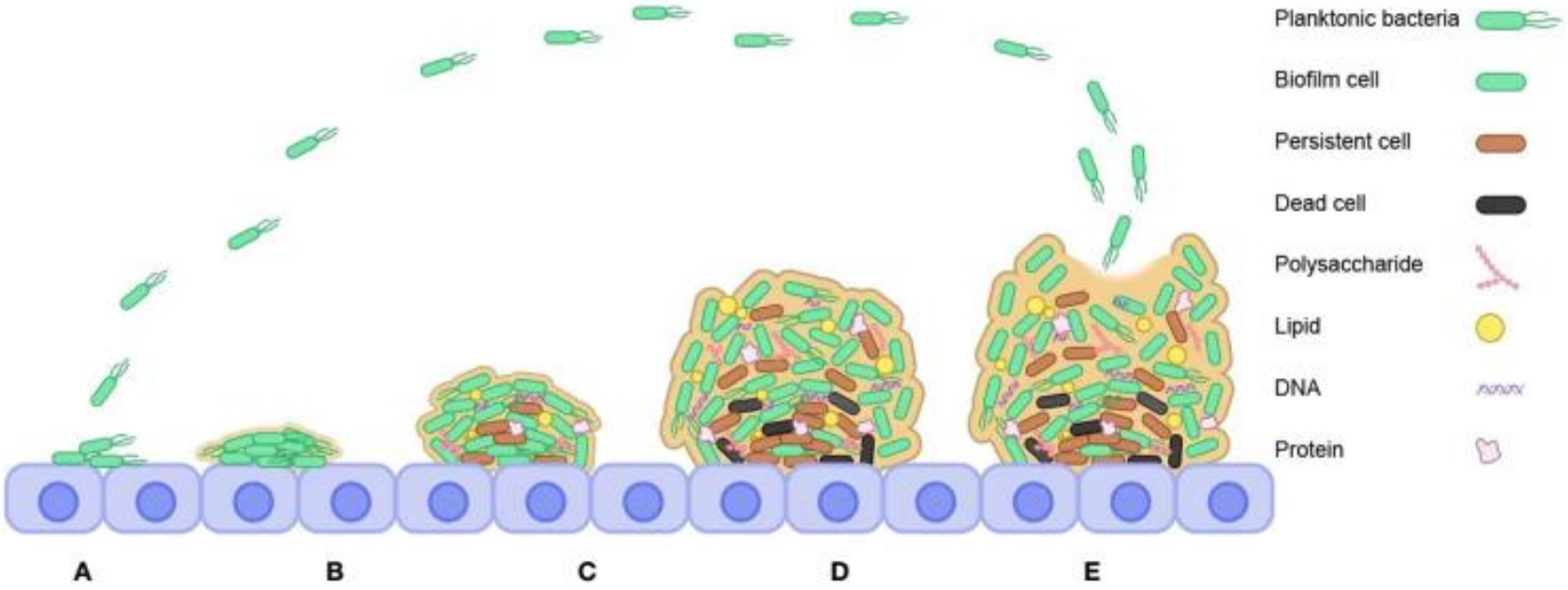


FIGURE 1
Steps to bacterial biofilm formation. **(A)** Reversible attachment. **(B)** Irreversible attachment. **(C)** Bacterial cells synthesize and secrete EPS. **(D)** Maturation. **(E)** Dispersal.

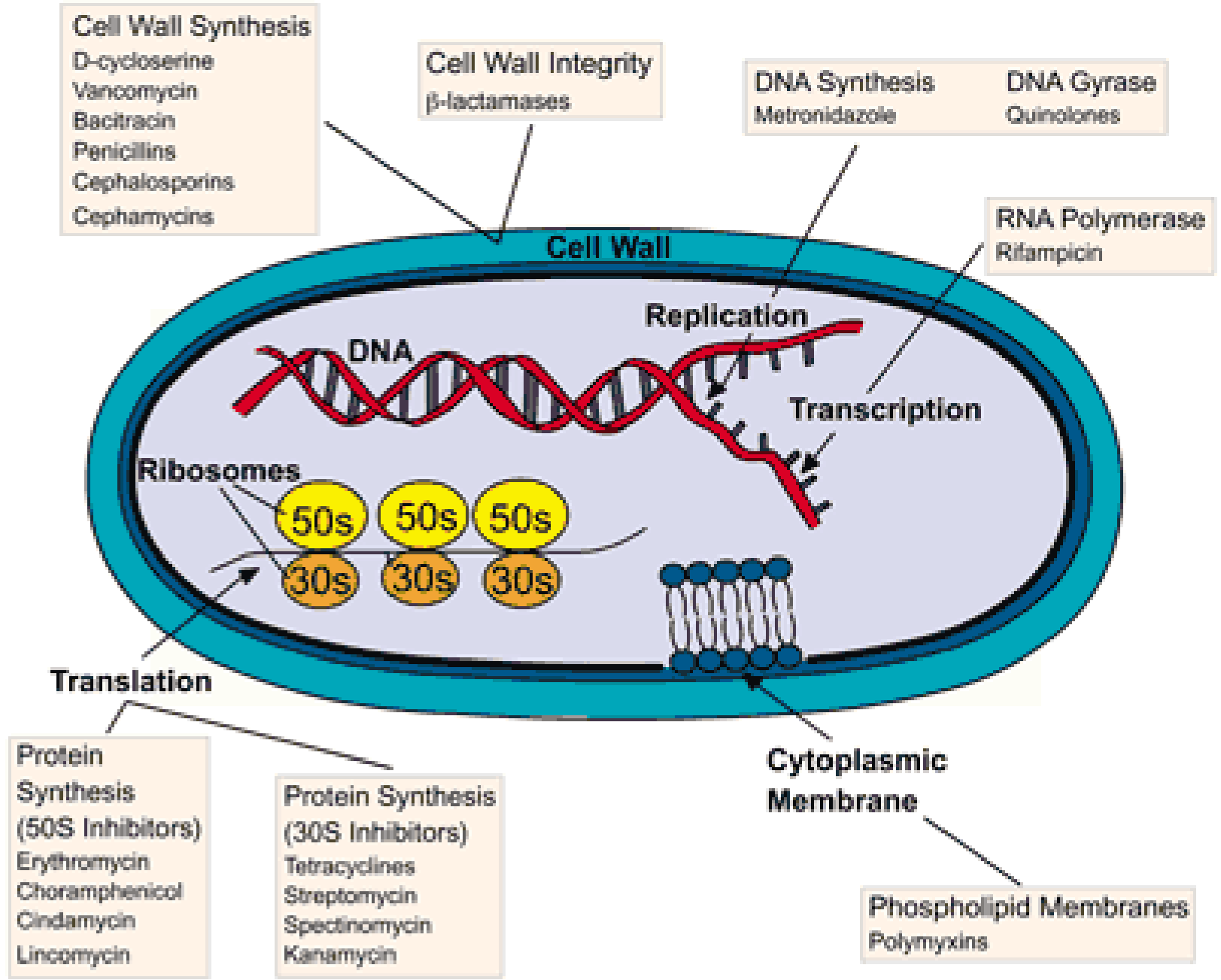
MODES OF ACTION OF ANTIMICROBIALS

The antimicrobials act on **different targets** in a bacterial cell.

The mechanisms of action mainly include:

1. **Inhibition of the bacterial cell wall synthesis**
2. **Alteration of the permeability of bacterial cell membranes**
3. **Inhibition of nucleic acid synthesis**
4. **Inhibition of protein synthesis**

Reygaert W.C. AIMS Microbiol. 2018;4:482–501. doi: 10.3934/microbiol.2018.3.482.



Mulvey MR., and Simor AE. 2009. Canadian Medical Association Journal 180(4): 408-15.

1. Inhibition of bacterial cell wall synthesis

- It is the main action mechanism of **β-lactam and glycopeptide antibiotics.**

- The β-lactam antibiotics work by **binding through the β-lactam ring to the bacterial penicillin-binding protein (PBP),** which acts to synthesize and remodel bacterial peptidoglycans, thus **inhibiting the transpeptidation effect**.

- **Vancomycin**, a glycopeptide antibiotic, **interferes with the peptidoglycan layer maturation process** and thereby prevent cell wall synthesis.

Kohanski M.A., et al. Nat. Rev. Microbiol. 2010;8:423–435.

2. Alteration of the permeability of bacterial cell membranes

- Some antibiotics **target and interact with cell membranes to change their permeability.**

- Representative drugs are **polymyxin antibiotics.**

- They act on the **lipopolysaccharide on the outer membrane of Gram-negative bacteria** and enter the periplasm after binding to the phosphate group of lipid A in the lipopolysaccharide.

- Polymyxins are commonly used in the **treatment of infections** caused by **carbapenem-resistant bacteria** such as *A. baumannii*, *E. coli* and *P. aeruginosa*.

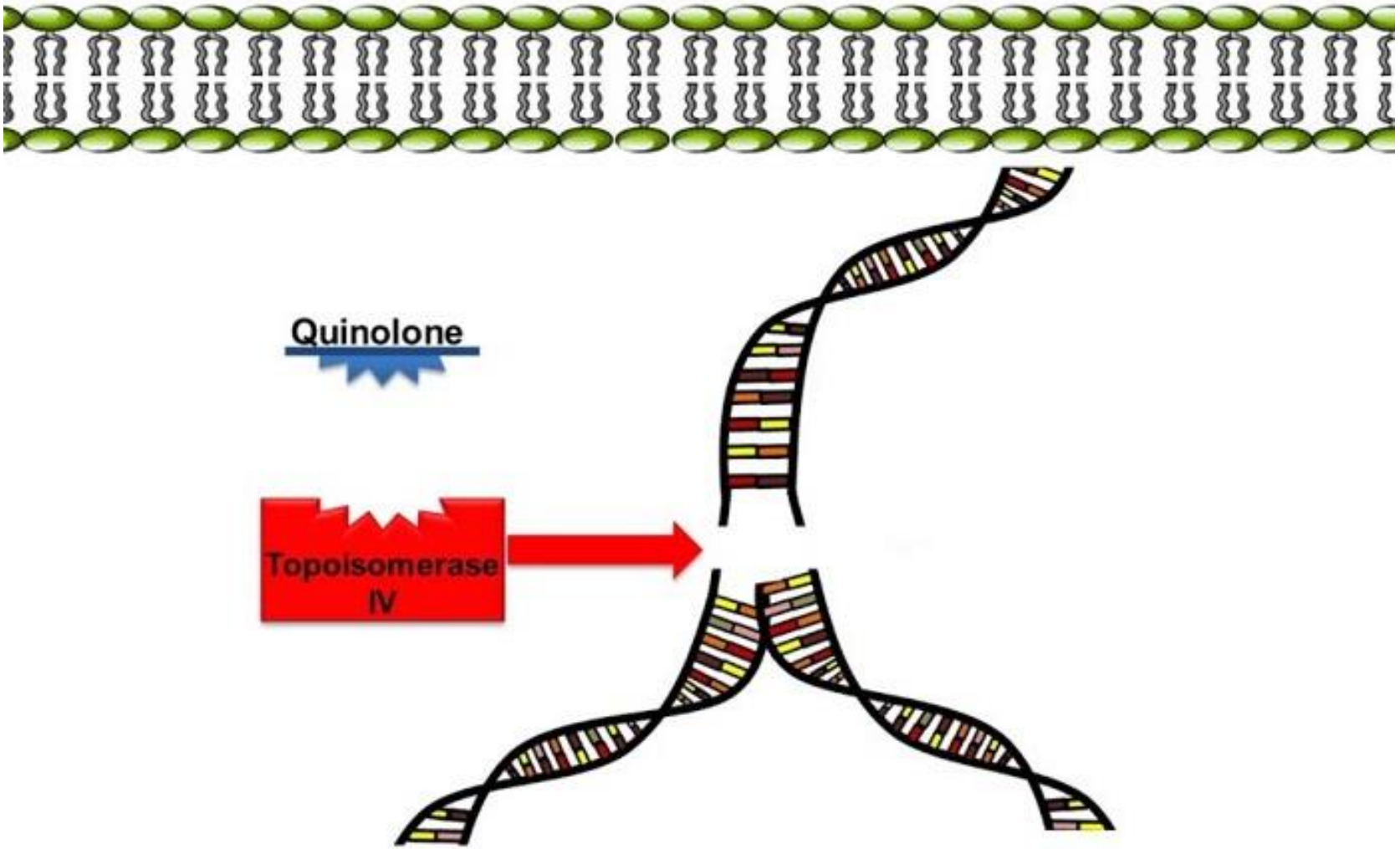
3. Inhibition of nucleic acid synthesis

- These drugs **inhibit RNA and DNA synthesis**, and **DNA replication.**

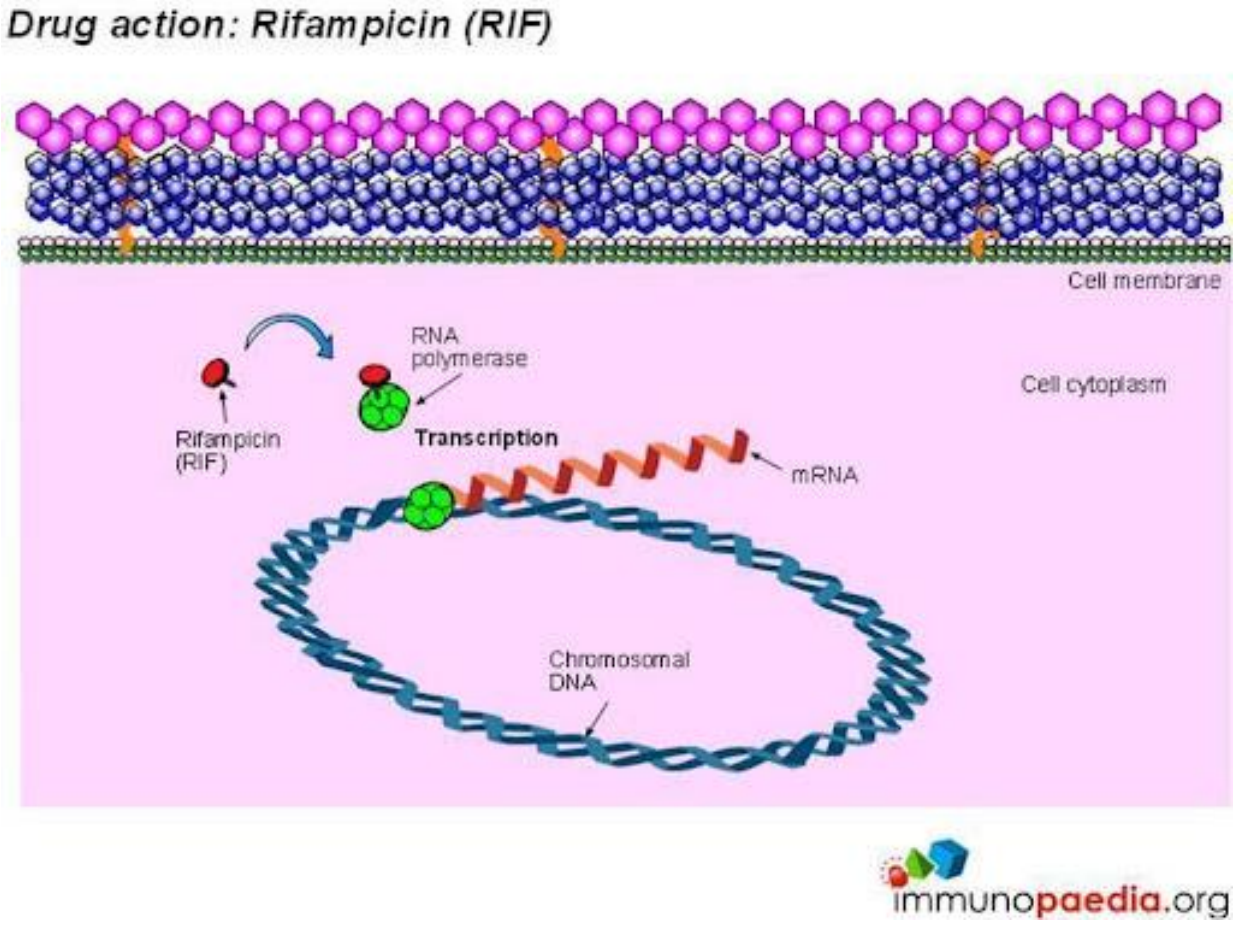
- Prototypes are **rifamycin** and **quinolone** antibiotics.

- **Quinolone** antibiotics **interfere with DNA superhelical changes by binding to topoisomerase II or topoisomerase IV**, leading to double-stranded DNA breaks that induce cell death.

Drlica K., et. al. Antimicrob. Agents Chemother. 2008;52:385–392.


Quinolone
Topoisomerase IV

- **Rifamycin inhibits the activity of RNA polymerase** in the bacterium by specifically binding to the DNA-dependent β-subunit of RNA polymerase, thereby impeding mRNA synthesis.



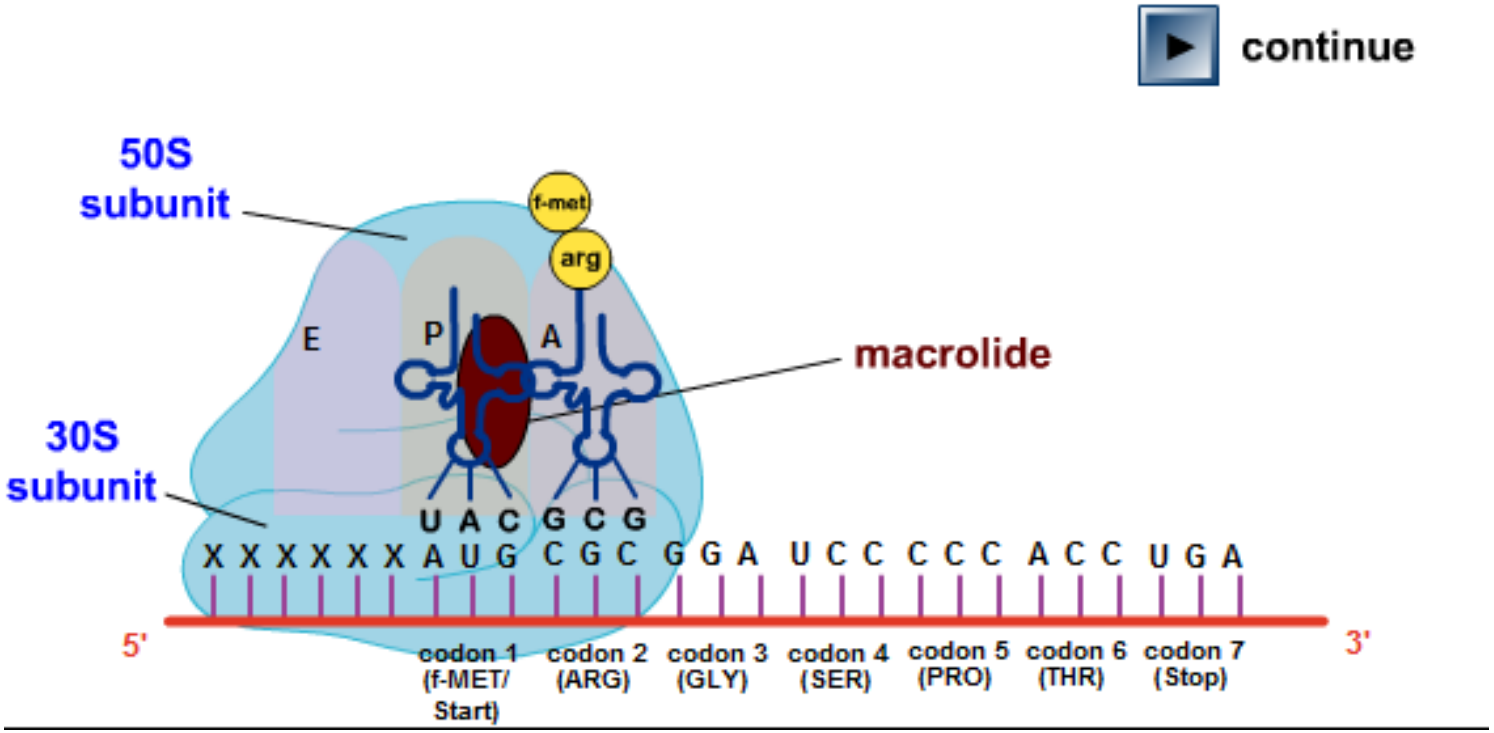
Mosaei H., and Zenkin N. EcoSal Plus. 2020;9.

4. Inhibition of Protein synthesis

- The drugs that inhibit bacterial growth by inhibiting protein synthesis are very extensive.

- These include **macrolides** (e.g., erythromycin), **lincosamides** (e.g., clindamycin), **aminoglycosides** (e.g., streptomycin and gentamicin), **amphetamines** (e.g., chloramphenicol), **oxazolidinones** (e.g., linezolid) and **tetracycline** antibiotics.

- The mechanism of action is mainly through the **physical blockade of protein translation initiation** or **translocation of peptidyl tRNAs** to interfere with the stability of peptidyl tRNA binding to ribosomes.



Kohanski M.A., Dwyer D.J., Collins J.J. Nat. Rev. Microbiol. 2010;8:423–435.

Future approaches to combat Antimicrobial Drug Resistance

In recent years, a research boom in **non-traditional antibiotic treatment strategies** combating bacterial resistance has emerged.

These include:

1. **Antimicrobial Peptides (AMPs)**
2. **Inhibitors as Adjuvants**
    A. Enzyme Inhibitors
    B. Efflux Pump Inhibitors
3. **Metal Nanomaterials**
4. **Cationic Polymers**
5. **Photo-Sensitive Materials**

6. Drug Delivery Systems

A. Carbon-Based Nano-Delivery Systems

B. Liposomal Drug Delivery Systems

C. Biomimetic Nano-Delivery Systems

   a. Erythrocytes

   b. Exosomes

D. Polymer-Based Antibiotic Delivery Systems (PADSs)

1. Antimicrobial Peptides (AMPs)

- AMPs are **powerful tools** against a range of MDR pathogens with minimal resistance due to the **specific bactericidal multi-modal action mechanisms**.

- **In addition**, AMPs can also act as **antibiotic adjuvants**, enhancing the efficacy of antibiotics through a synergistic strategy.

- Since the isolation of world's first animal AMP **cecropins** in 1980, a variety of antimicrobial peptides with antimicrobial activity have been discovered, designed and modified.

Li W., et al. Chem. Soc. Rev. 2021;50:4932–4973.

- AMP research has become a **hot spot** in the fight against bacterial resistance in recent years.

- For example, **Breij et al.** designed and synthesized a series of antimicrobial and antibiofilm peptides.

  Among these, **SAAP-148** successfully destroyed MDR pathogens, **blocked biofilm development** and **eliminated pre-existing biofilms** and **persister cells.**

De Breij A., et al. Sci. Transl. Med. 2018;10:eaan4044.

2. Inhibitors as Adjuvants

A. Enzyme Inhibitors

- For bacteria that render antibiotics ineffective by producing enzymes, enzyme inhibitors can be useful.

- **Clavulanic acid**, the first broad-spectrum β-lactamase inhibitor in clinical use, has **minimal antibiotic efficacy**. However, **in combination** with other antibiotics, it **enhances the efficacy of the antibiotics.**

  Clavulanic acid **penetrates the cell walls** of several bacteria more effectively (2 to 25 times) than other β-lactamase inhibitors.

Filby B.W., et al. ACS Appl. Bio. Mater. 2022;5:3826–3840.

- Similarly an antirheumatic drug **auranofin** has been found to **inactivate NDM-1, a β-lactamase** by replacing zinc in the active site.

  Aauranofin **substantially reduces the MIC of meropenem** in NDM-1-producing *E. coli*. , with a fractional inhibitory concentration index (FICI) of 0.156.

  For colistin resistant bacteria, the combination of auranofin and polymyxin **substantially reduces the MIC of polymyxin**, with a FICI of about 0.125.

- **Combination of auranofin with carbapenems** can also be very effective against superbugs.

Sun H., et. al. Nat. Commun. 2020;11:5263.

B. Efflux Pump Inhibitors (EPIs)

- Efflux pumps are important for **both intrinsic and acquired resistance** of bacteria.

- Over the past 20 years, significant efforts have been made to identify novel EPIs and their subsequent inhibition.

- **NorA** is a chromosomally encoded **multidrug efflux pump in MRSA** (Methicillin Resistant *Staph aureus*).

  Synthetic antigen-binding fragments Fabs (of antibodies) were inserted into the **substrate binding pocket of NorA** to inhibit antimicrobial drug pumping.

Grimsey E.M., et.al. mBio. 2020;11:e00465-20.

3. Metal Nanomaterials

- The **metal nanomaterials** such as **gold, silver** and **zinc** can be used directly for the **detection and treatment** of bacterial infections.

- Their antimicrobial effect depends on their **physicochemical properties** such as size, shape and composition of the formed nanoparticles.

- **Silver nanoparticles** have shown promising results in **drug delivery**, **imaging** and **biosensing**, and in **antimicrobial wound dressings**.

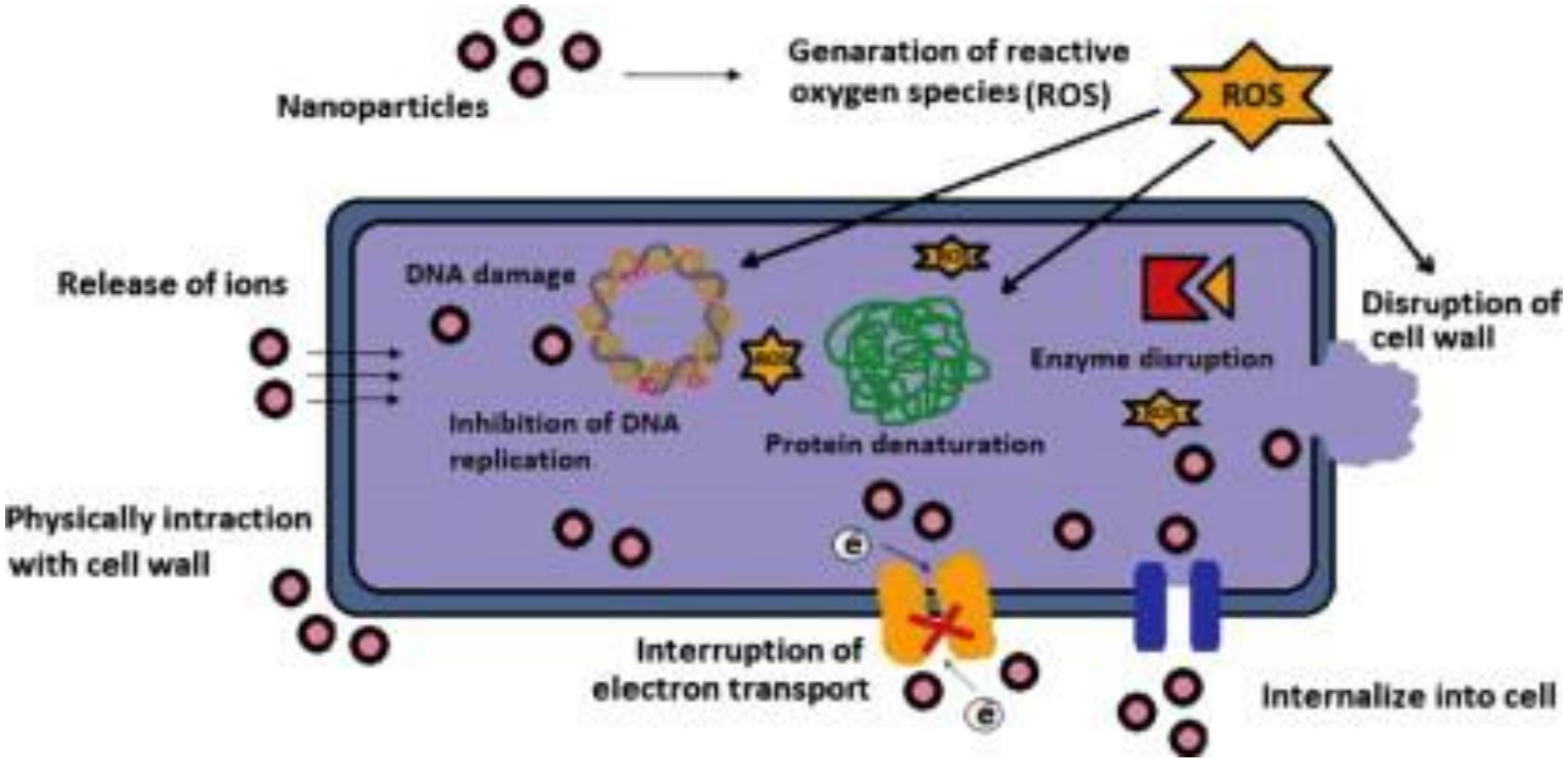
Abbas M., et al. J. Mater. Chem. B. 2021;9:4444–4458.

- Similarly zinc nanoparticles have shown antibacterial activity against **both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*)** bacteria in antibacterial assay testing.

Pillai A.M., et, al. J. Mol. Struct. 2020;1211:128107.

- It was also found that bacteria treated with gold nanoparticles at a concentration of **33% of the MIC** remained susceptible after **30 days of continuous passages.**

Zheng Y., et. al. Bioconjug. Chem. 2018;29:3094–3103.


Nanoparticles
Genaration of reactive oxygen species (ROS)
ROS
Release of ions
DNA damage
Inhibition of DNA replication
Protein denaturation
Enzyme disruption
Disruption of cell wall
Physically intraction with cell wall
Interruption of electron transport
Internalize into cell

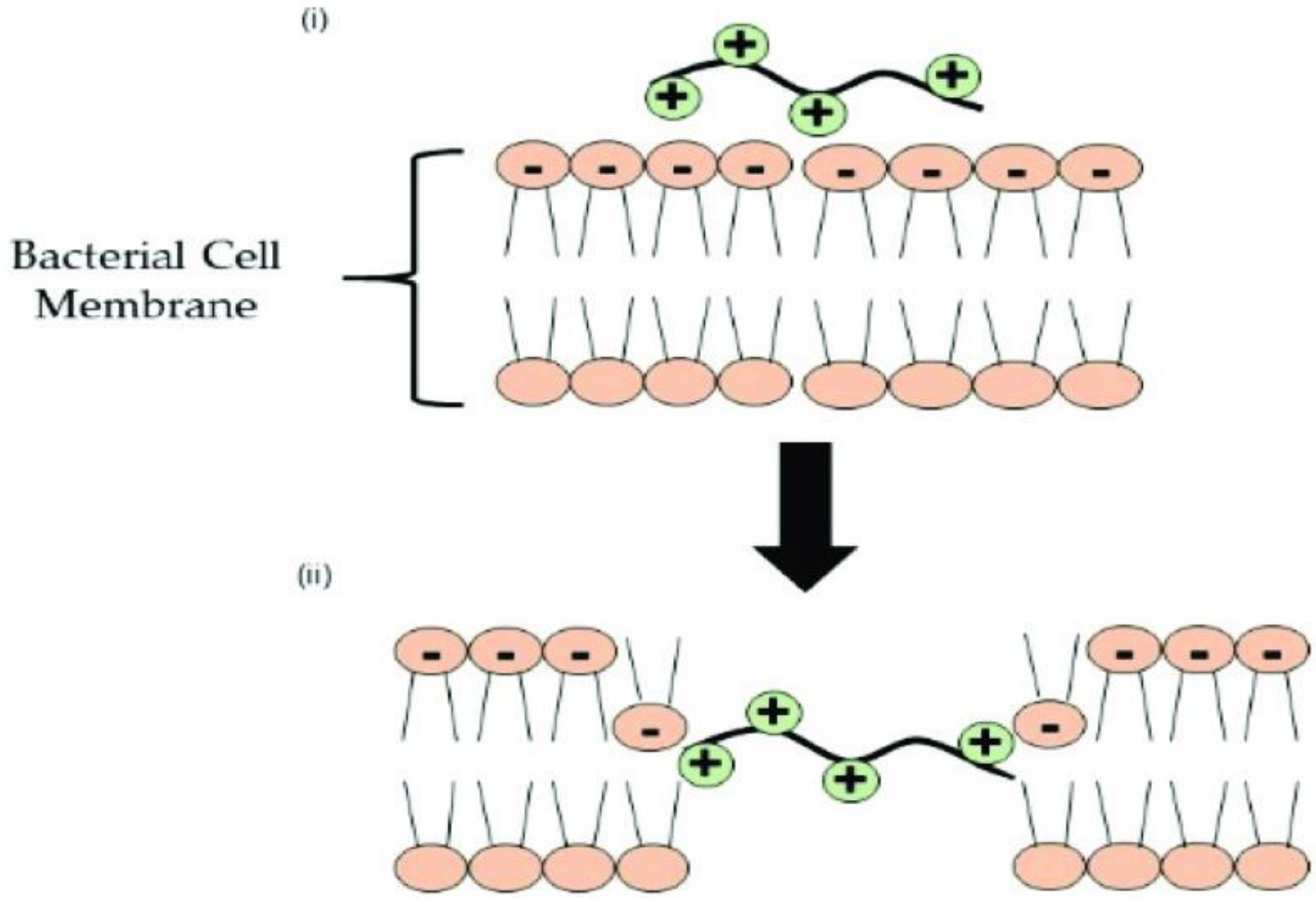
4. Cationic Polymers

- The **positive charge** of cationic polymers **interacts** with the **negative charge** of the bacterial surface, causing **destruction of the bacterial cell membrane or cell wall structure**, extravasation of cytoplasm and ultimately bacterial death.

Examples are **polyquaternary ammonium salts (PQASs), chitosan** (a natural polymer material), **polyethyleneimine (PEI)** and their derivatives.

NFM-6, one of the derivatives of PQASs showed a nearly **99% bacterial killing ratio** for both *E. coli* and *S. aureus*.

Lv C., et. al. J. Colloid Interface Sci. 2019;556:492–502.


(i)
Bacterial Cell Membrane
(ii)

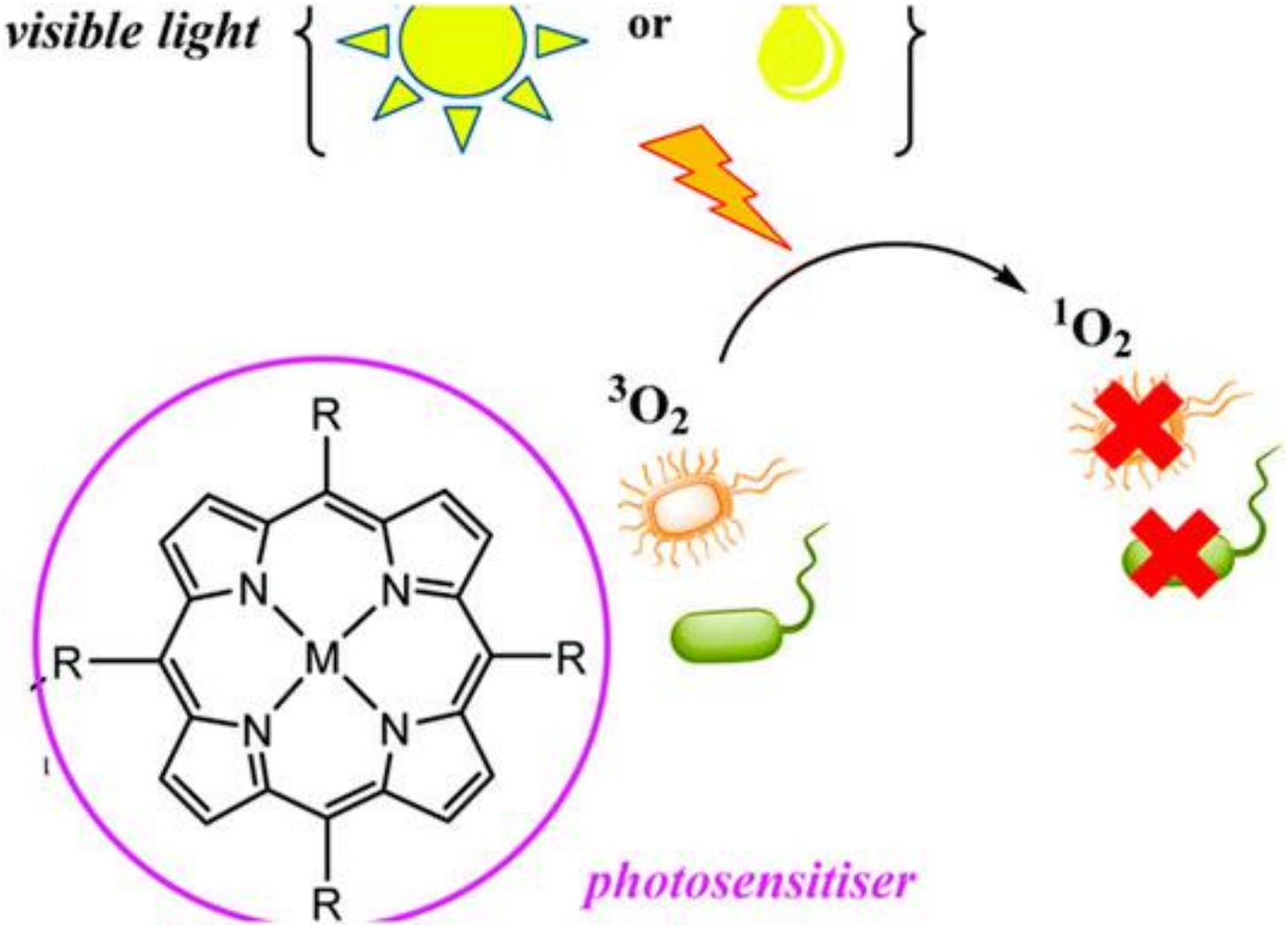
5. Photo-Sensitive Materials

- **Photo-sensitive materials** combined with **photodynamic therapy** can induce cellular and microbial inactivation, which is called **photodynamic antimicrobial chemotherapy (PACT).**

- **PACT** is an **oxidative damage mechanism** based on the **synergistic** action of three factors: **light, a photosensitizer** and **oxygen.**

- **PACT** has received widespread attention because of its **noninvasiveness**, **low incidence of drug resistance**, and **suitability for localized infection treatment.**

Park J.H., et. al. Biol. Pharm. Bull. 2012;35:509–514.


visible light
or
$^3O_2$
$^1O_2$
R
N
N
M
N
N
R
R
R
photosensitiser

6. Drug Delivery Systems

- **Drug delivery systems** (DDSs) enhance **biodistribution** and **bioavailability** of antibiotics thus **reducing the resistance** to antibiotics and **extending the lifespan** of novel antibiotics.

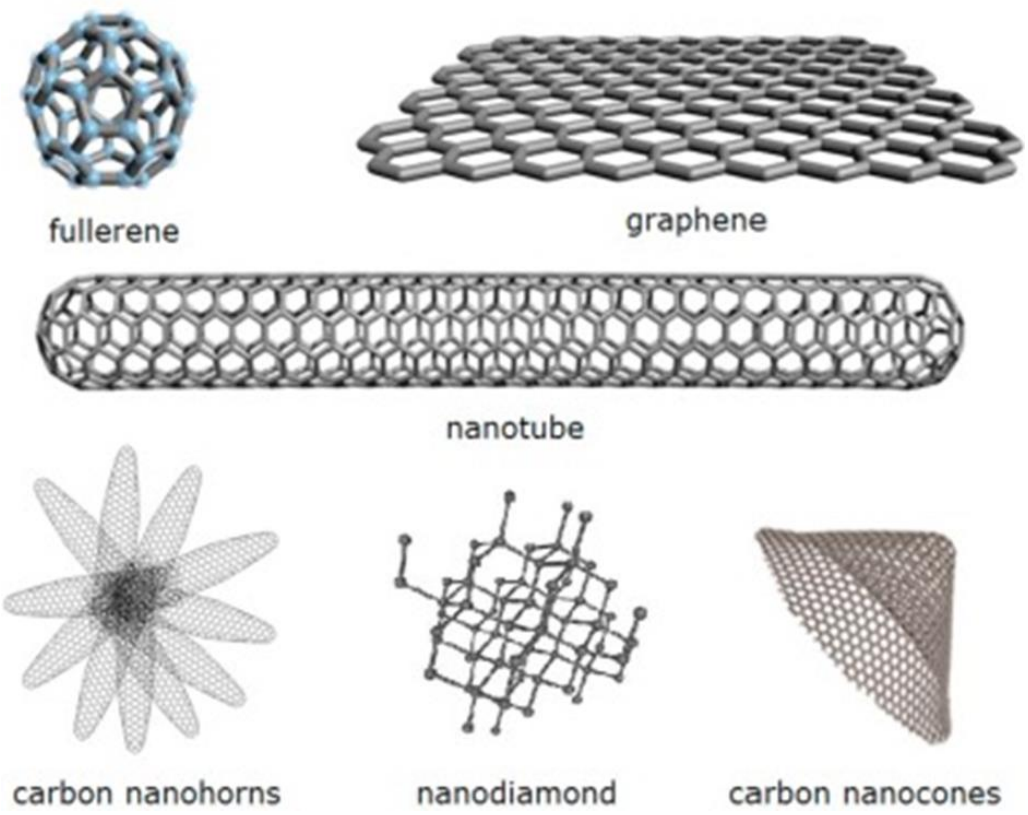
- **Antimicrobials** are **combined with different types of carriers** (e.g., liposomes, erythrocytes, exosomes, polymers and self-assembled peptides) to achieve efficient drug delivery.

- In addition, drug release can be achieved using DDSs by **targeting the unique microenvironment** associated with the infected tissue or by guidance from external stimuli.

Pham T.N., et. al. Med. Res. Rev. 2019;39:2343–2396.

A. Carbon-Based Nano-Delivery Systems

Carbon-based nanomaterials such as **fullerenes, carbon nanotubes (CNTs)** and **graphene oxide (GO) nanoparticles** have shown powerful antibacterial properties.



- They also have **good biocompatibility** and **environmental friendliness.**

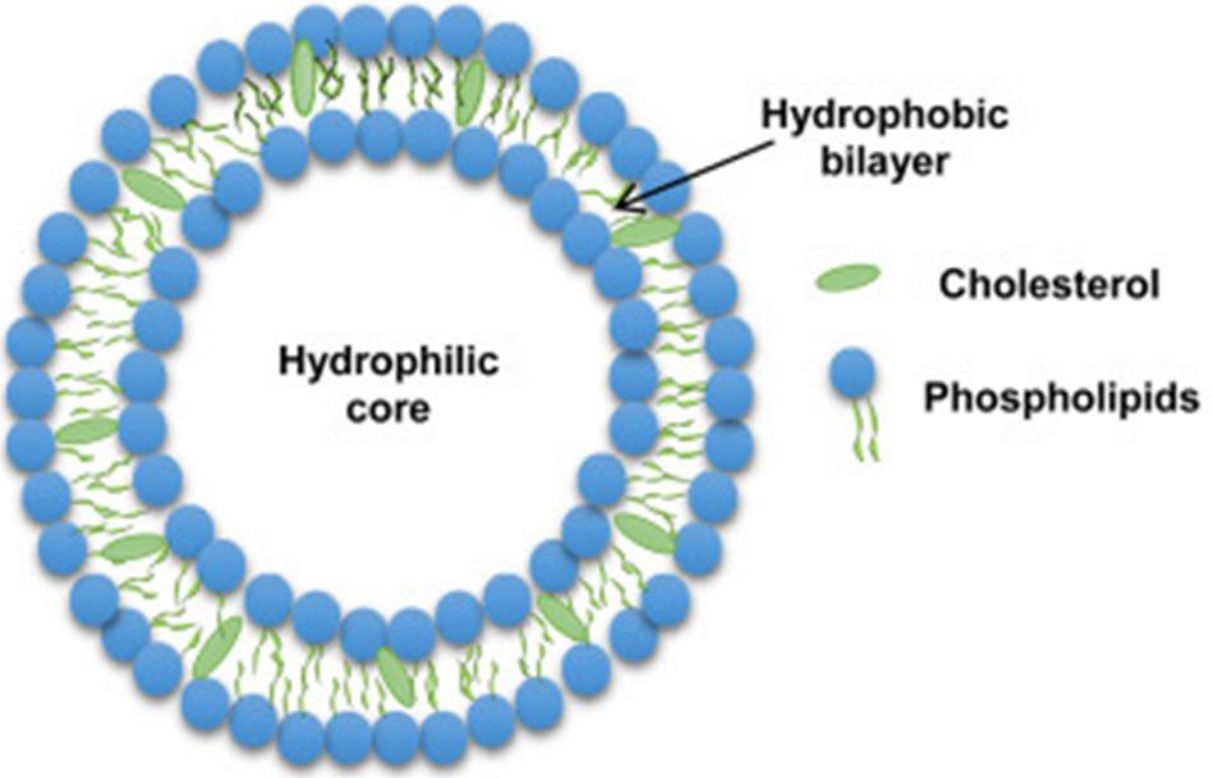
- They can come into **direct contact** with bacteria, thereby **disrupting their cell membrane integrity, metabolic processes** and **morphology.**

- Compared to other inorganic metallic nanomaterials, they have a **higher biosafety profile**, but their **toxicity is still one of the inevitable drawbacks.**

Xin Q., et. al. Adv. Mater. 2018;31:1804838.

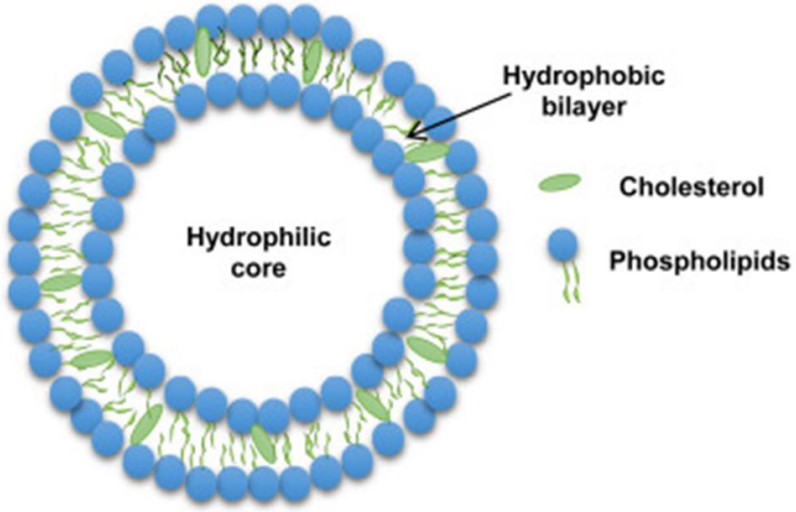
B. Liposomal Drug Delivery Systems

Liposomes are **spherical vesicles** formed by **self-assembly of amphiphilic lipid molecules** with a **bilayer structure**, with particle sizes ranging from **20 nm to 10 µm**.



- These are among the **most widely studied** nano-DDSs due to **good biocompatibility** and **modifiability**.

- **Hydrophilic** and **amphiphilic** molecules can be **encapsulated in the core**, whereas **hydrophobic molecules** can be **partitioned into lipid bilayers** for selective loading of drugs.



Schiffelers R., et.al. J. Antimicrob. Chemother. 2001;48:333–344.

- The **unique properties** of liposomes, such as **non-immunogenicity**, **low toxicity** and **biofilm matrix–cell membrane fusogenicity**, significantly improve the effectiveness of antimicrobial agents and reduce the recurrence of infections.

- It has been observed in treating **periodontitis** (gum disease), that due to **deeper penetration of the vesicles into the biofilm**, the antibacterial polypeptide chains on the vesicles and the drug ciprofloxacin worked together to kill bacteria and thus were more effective than a single mechanism.

Wang Y. J. Appl. Microbiol. 2021;131:2626–2639.

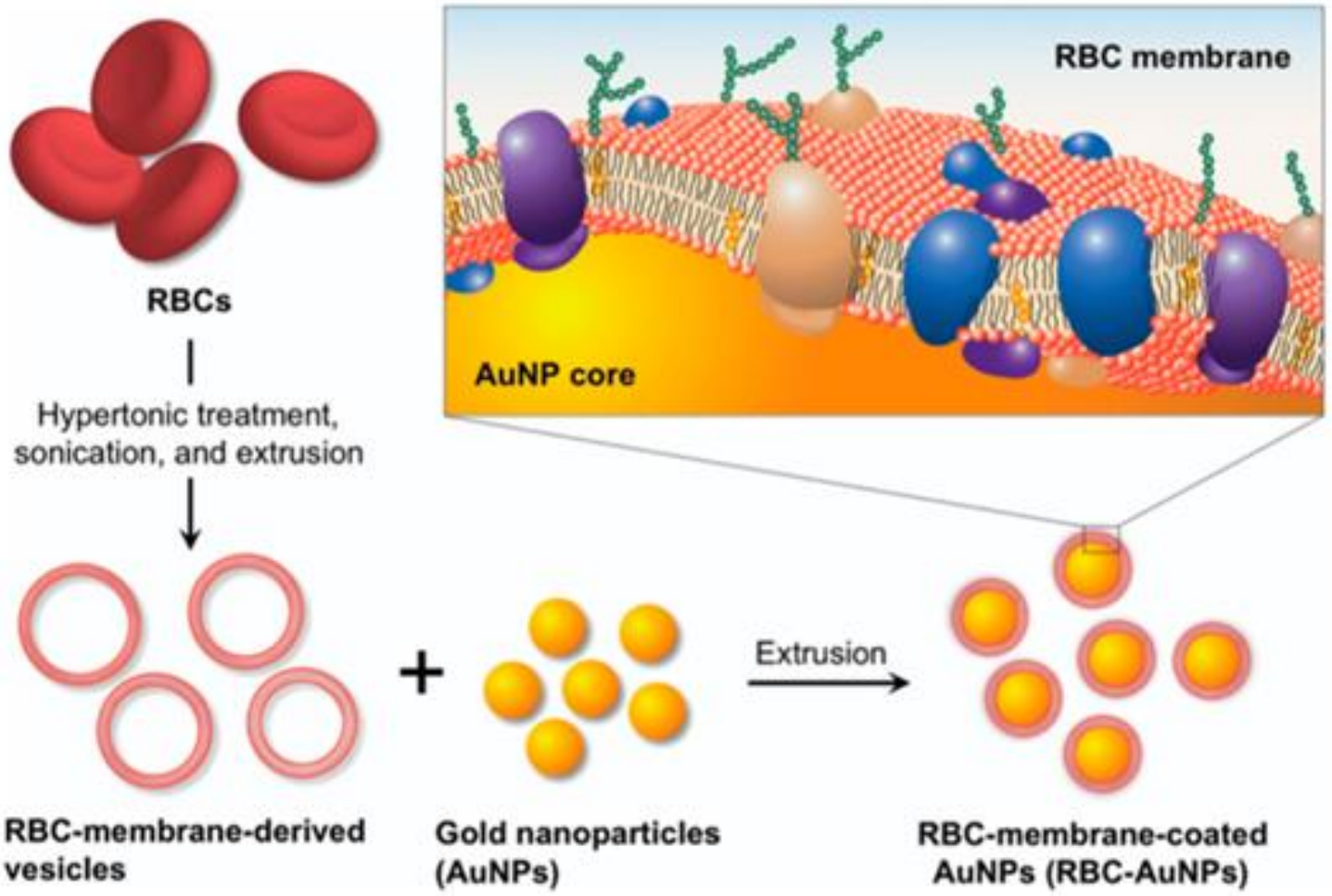
C. Biomimetic Nano-Delivery Systems

- **Biomimetic nano-delivery systems** are a relatively novel biological delivery strategy with properties such as **long circulation**, **focal site targeting** and **immune escape**.

- In recent years, **cell membrane-encapsulated carriers** have been increasingly used in the biomedical field.

a. Erythrocytes

- **Erythrocytes (RBCs)** can **camouflage** nanoparticles.
- **RBCs membrane nanovesicles** can be used as a **carrier system** for delivering drugs, enzymes, peptides and antigens in vivo.
- RBCs have the advantages of **good biocompatibility**, a **long circulation cycle**, and **high targeting**, due to the **intact retention of the structure** and **surface proteins** of the erythrocyte membrane.
- Accordingly, **erythrocyte membrane-encapsulated nanoparticles** have become a potential nano-drug delivery platform in recent years.


RBC membrane
AuNP core
RBCs
Hypertonic treatment,
sonication, and extrusion
Extrusion
RBC-membrane-derived
vesicles
Gold nanoparticles
(AuNPs)
RBC-membrane-coated
AuNPs (RBC-AuNPs)

- An **erythrocyte membrane-coated nanogel (RBC-nanogel)** system with combined antiviral and reactive antibiotic delivery for the **treatment of MRSA infection** has been reported.

- The RBC-nanogels were shown to **effectively neutralize MRSA-associated toxins** in the extracellular environment, and the neutralization of toxins in turn **promoted bacterial uptake by macrophages.**

- Once inside the cell, the RBC-nanogels showed **accelerated drug release**, which resulted in more effective bacterial inhibition.

Zhang Y., et. al. J. Control. Release. 2017;263:185–191.

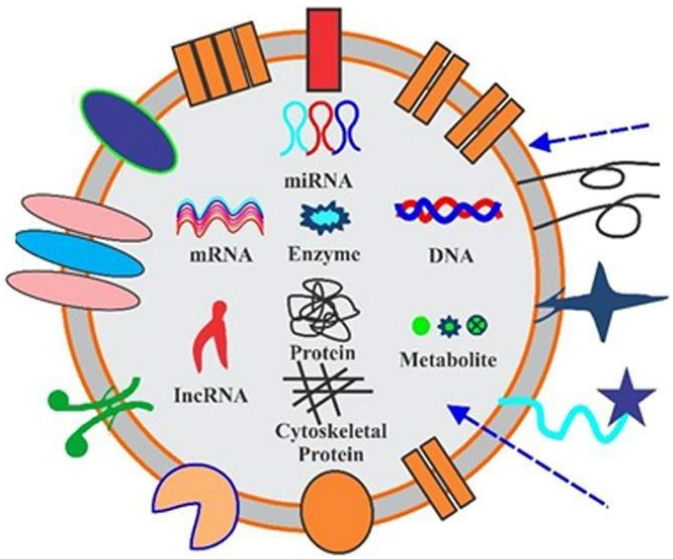
b. Exosomes

- **Exosomes** are **intracellular membrane-based vesicles** secreted by almost all types of cells.

- Exosomes play a **crucial role in intercellular communication** and are **effective in delivering drugs to receptor cells**.

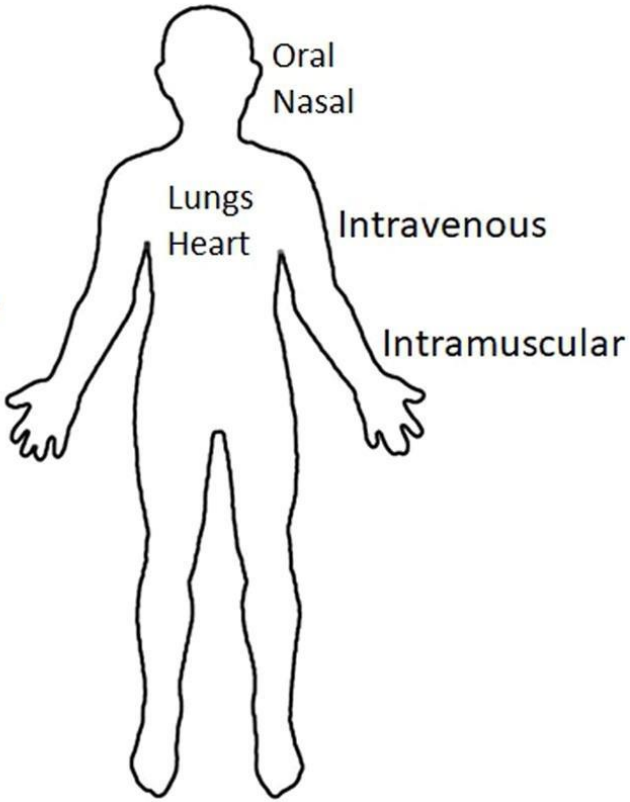
- In a study**, Mannosylated exosomes (MEXos)** have been used as drug carriers that were **preferentially engulfed by macrophages** and subsequently delivered **lysozyme (MEXoL)** and **vancomycin (MEXoV)** to **intracellular pathogens**.

Yang X., et al. J. Control Release. 2021;329:454–467.



Exosomes as drug delivery systems

Cargo – small molecules, nucleotides, proteins



Exosomes can be administered via various delivery routes

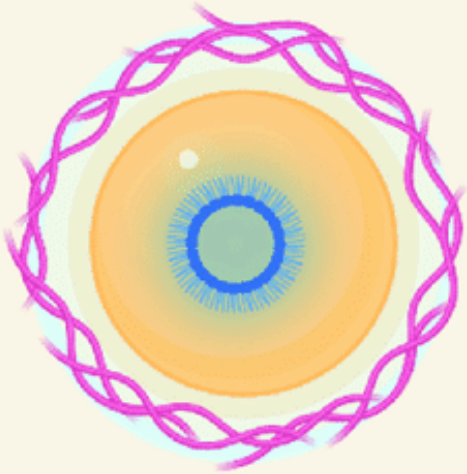
D. Polymer-Based Antibiotic Delivery Systems (PADSs)

- **PADSs** can protect antibiotics from **premature metabolism** and **optimize their pharmacokinetics**.

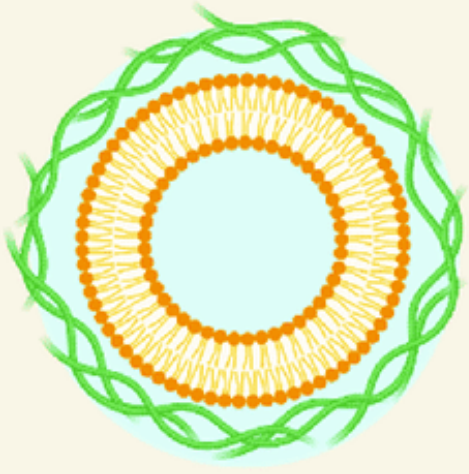
- Several PADSs have been reported, such as **polymeric liposomes**, **polymeric micelles**, **highly branched polymers and dendrimers**, and **polymeric nanogels**.

- Antimicrobial polymers are of great interest because of their **low cost, simple preparation, antimicrobial effect** and **easy modifications.**

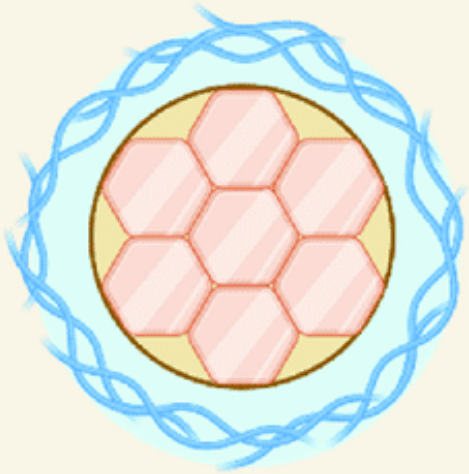
You K., et al. J. Mater. Chem. B. 2022;10:1005–1018.



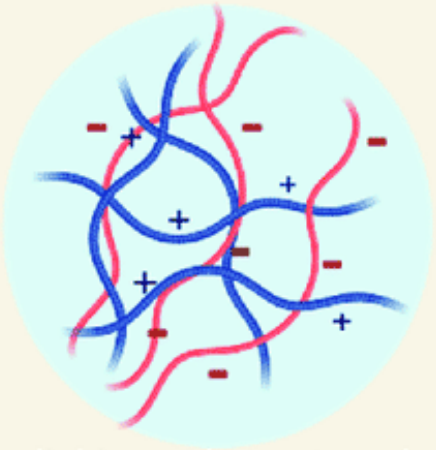
Polymer-emulsified emulsion



Polymer coated liposome



Polymer coated solid lipid nanoparticles (SLN)



Cross-linking polymer to polymer or protein to polymer

Polymer conjugation


Thank You!