Layer-by-layer Nanoparticles for Antibiotic Delivery and Biofilm Eradication

ACS Spring Conference

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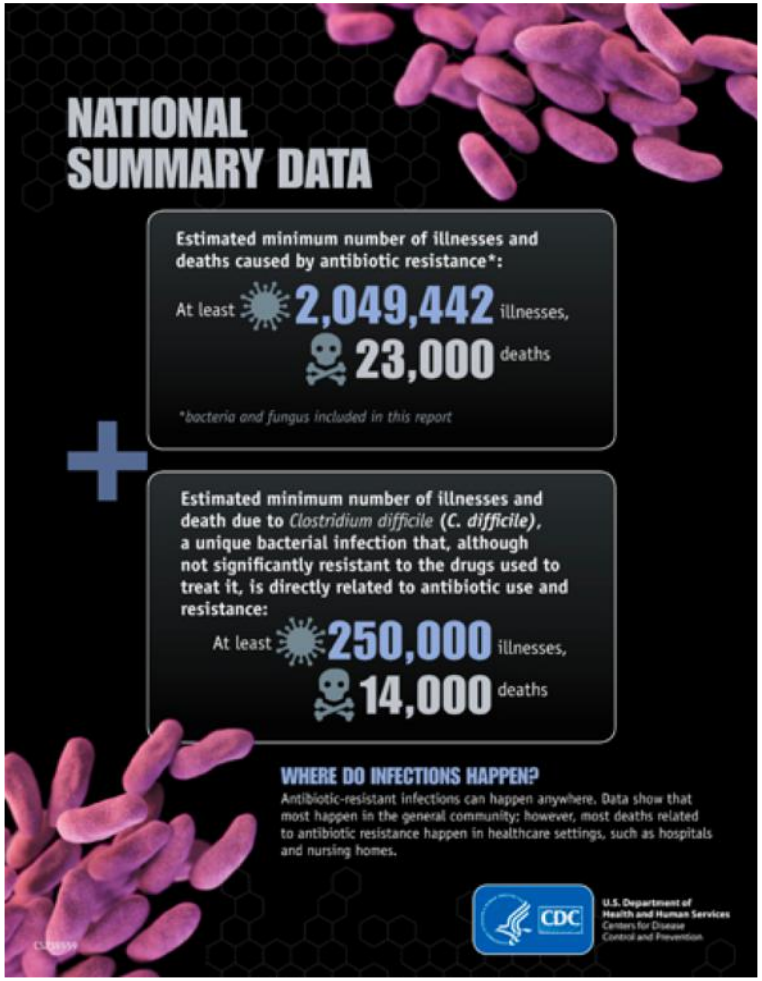
April 2020

Massachusetts Institute of Technology

Infectious Diseases Pose Serious Threat



Antibiotic Resistance Threats, **CDC**, 2013.
Antibiotic Resistance Threats, **CDC**, 2019.

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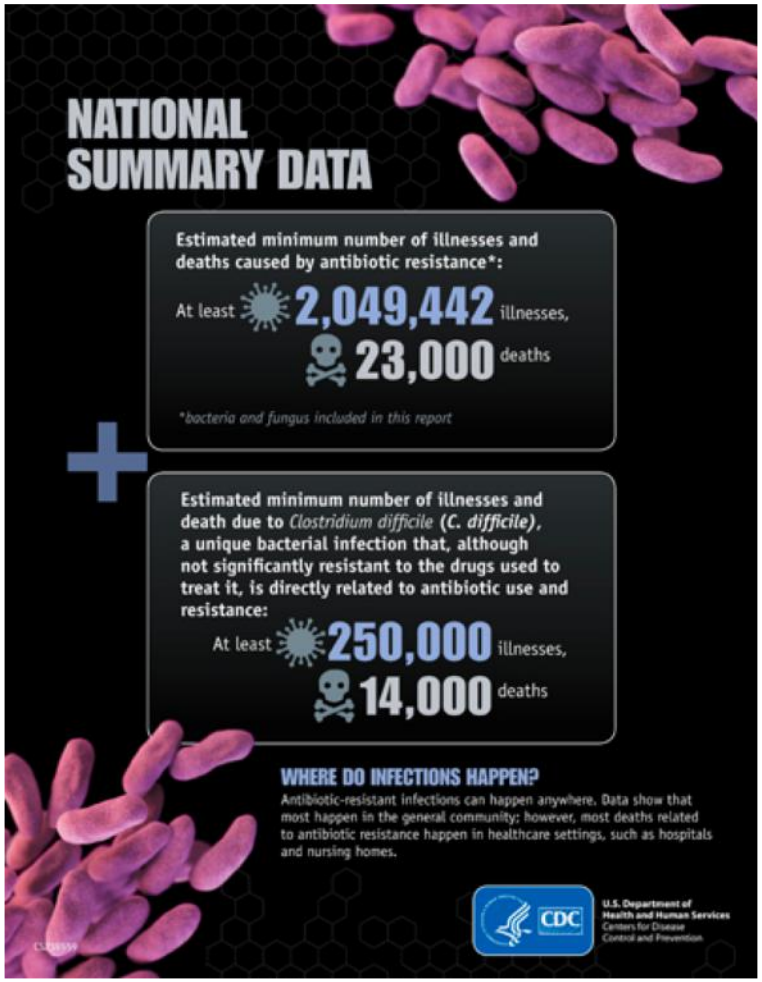

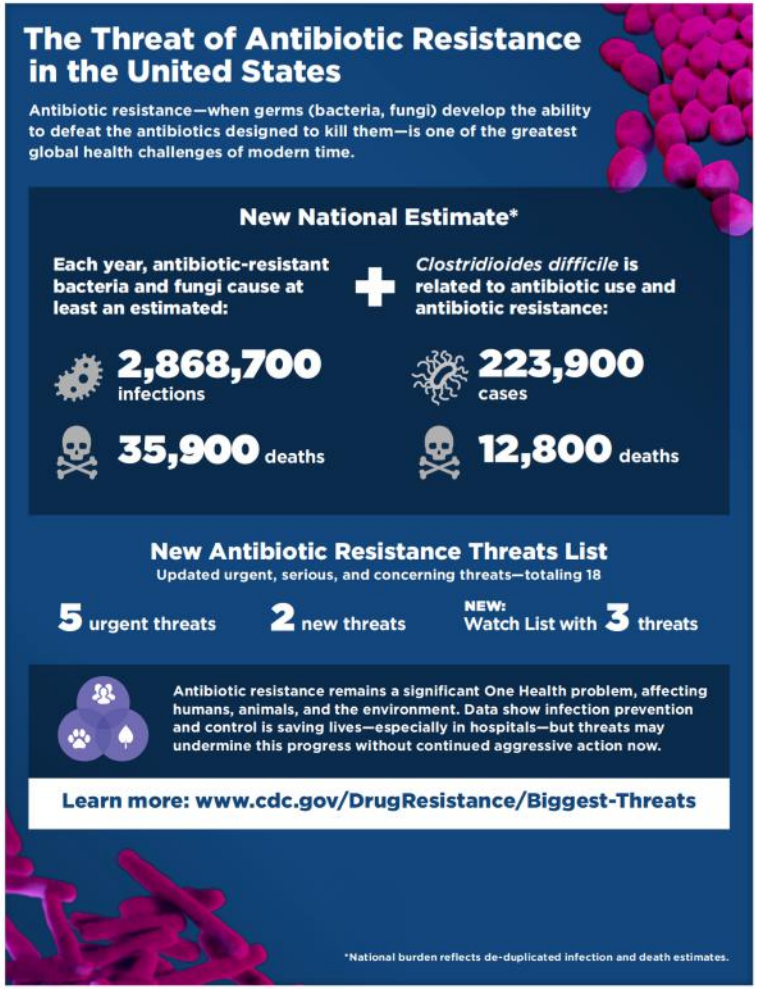

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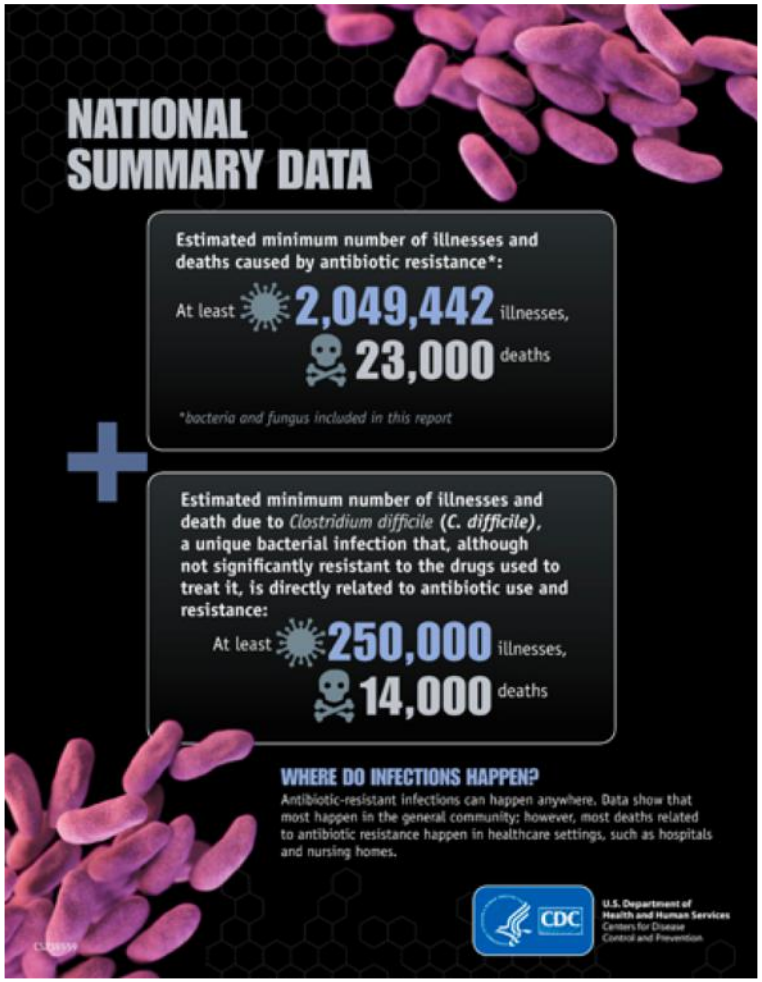
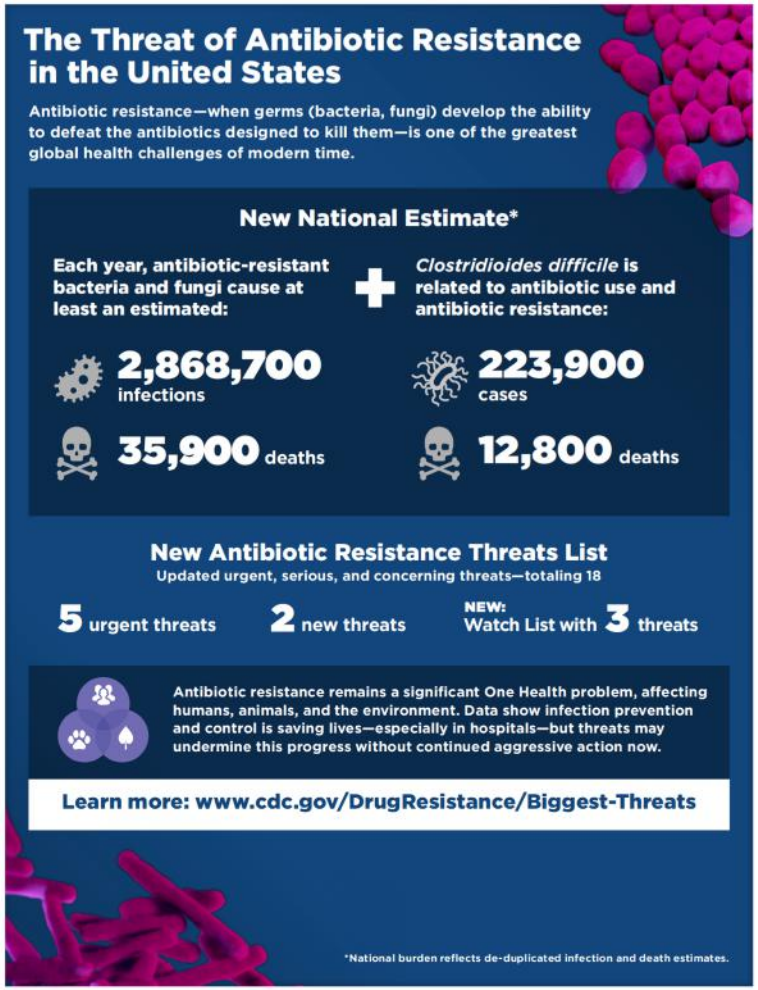


6 years apart

56% death increase



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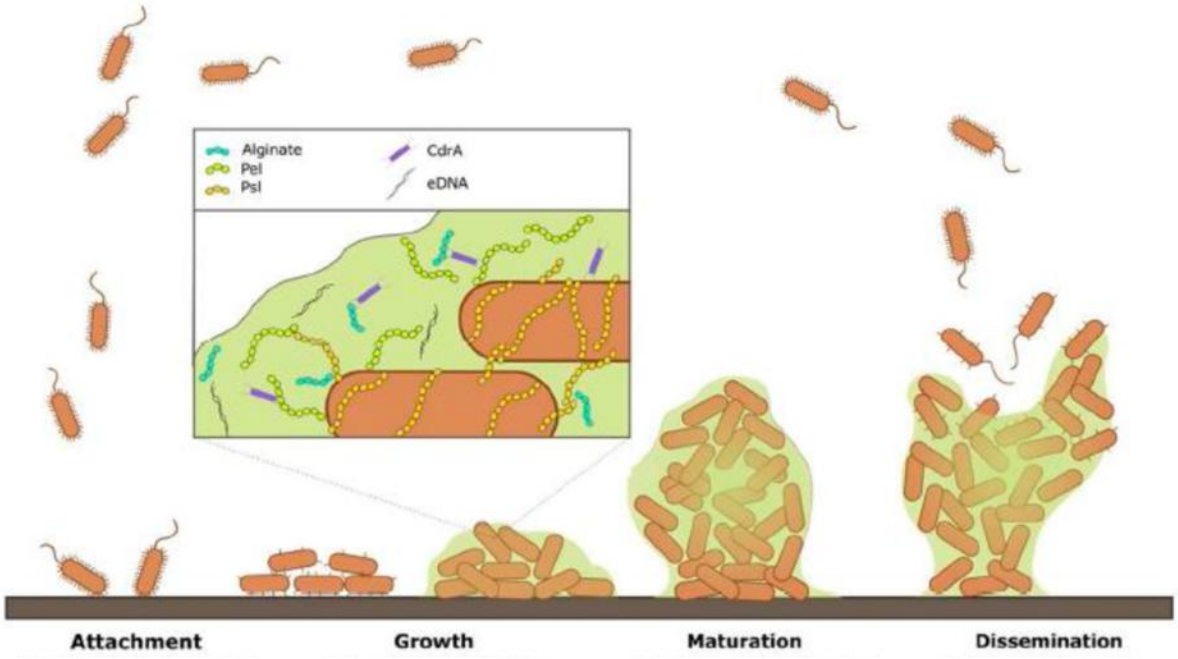
Key Stages in Biofilm Formation



Component	Composition %
Microbial Cells	2-5%
DNA and RNA	1-2%
Polysaccharides	1-2%
Proteins	<1-2%
Water	95%

Maunders, E.; Welch, M. *FEMS Micro. Let.* **364**, 2017.
Fleming, H. *Nature Rev. Micro.* **8**, 2010.

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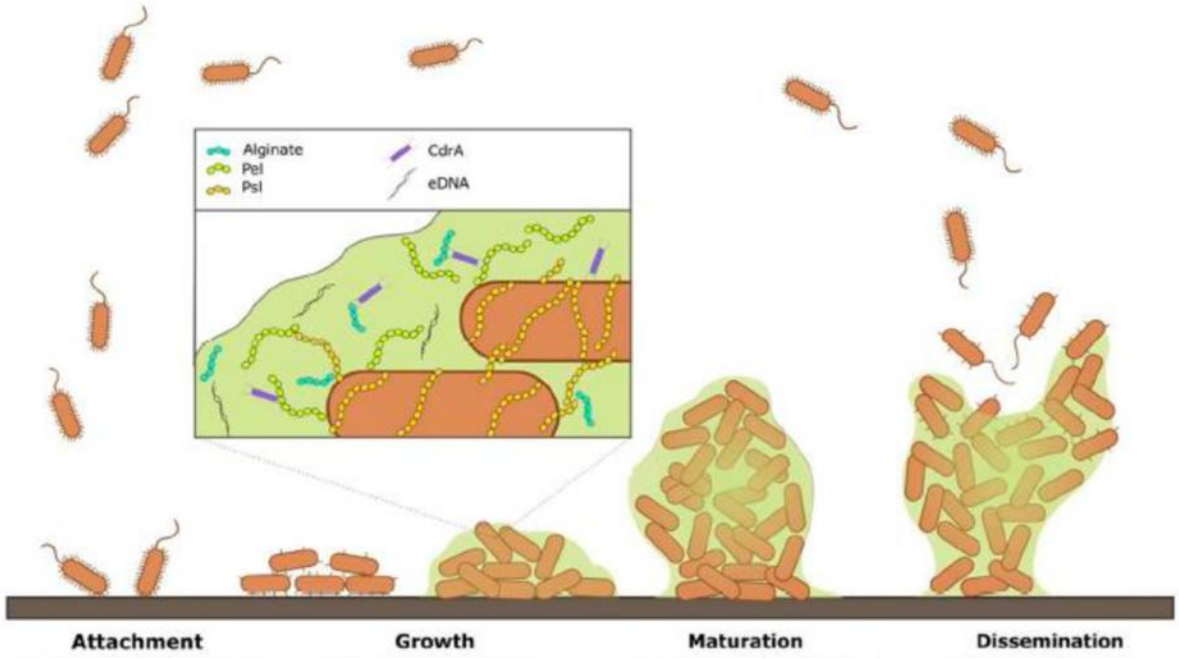

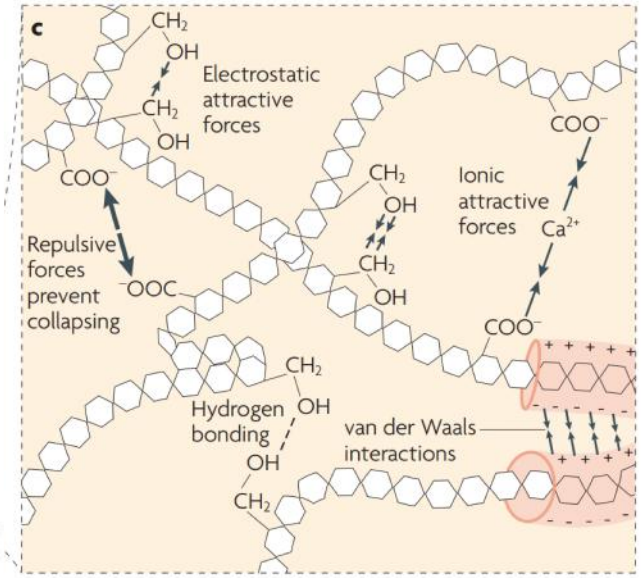

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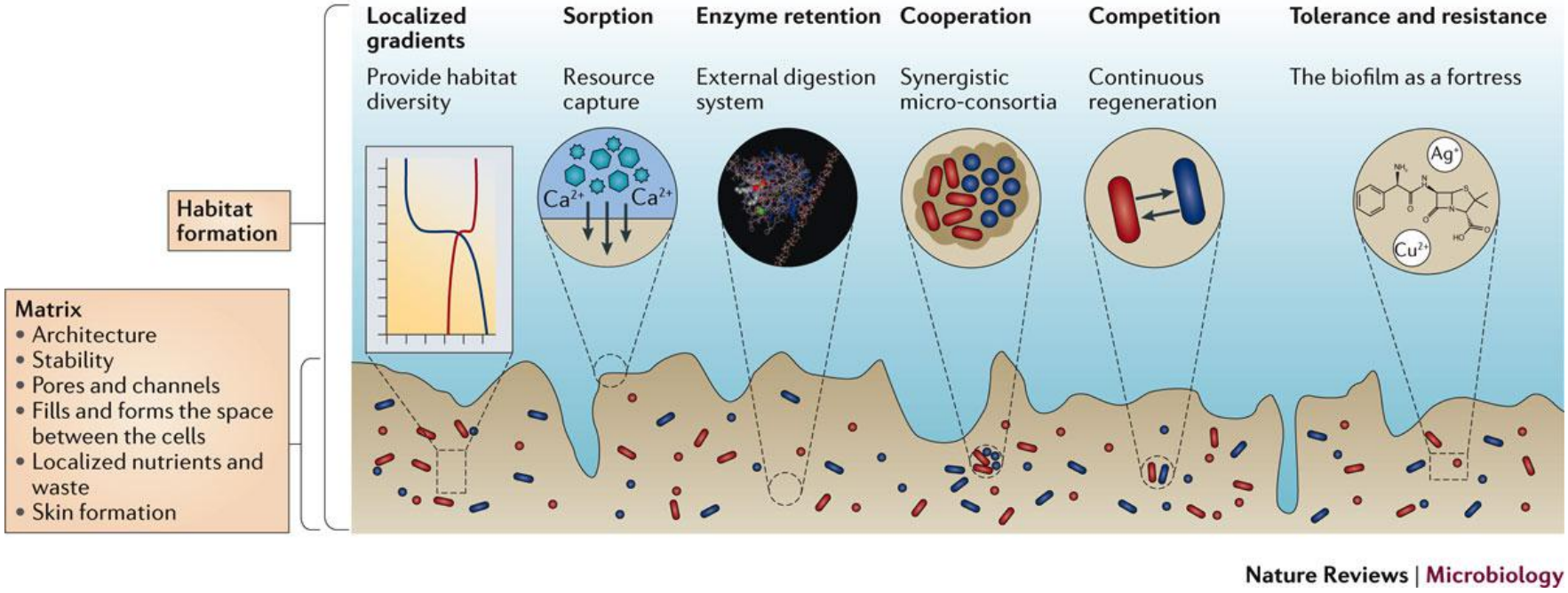
Biofilms Confer Competitive Advantages



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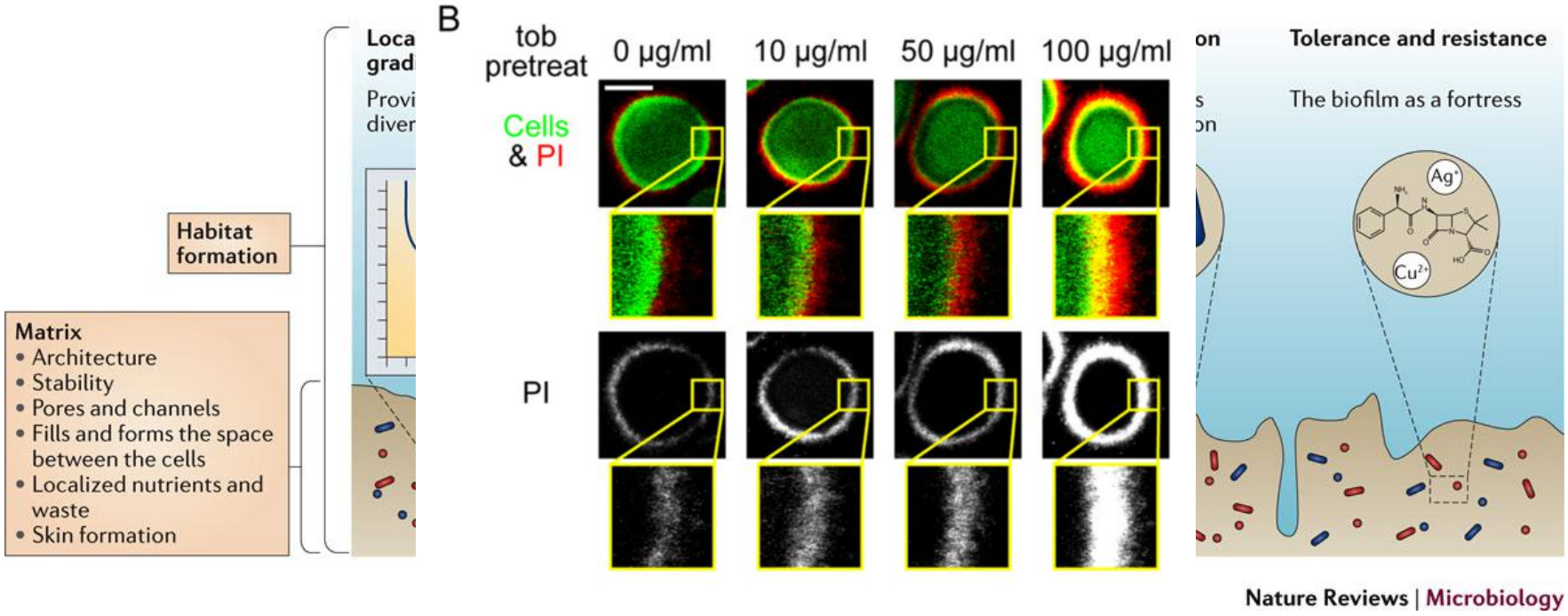

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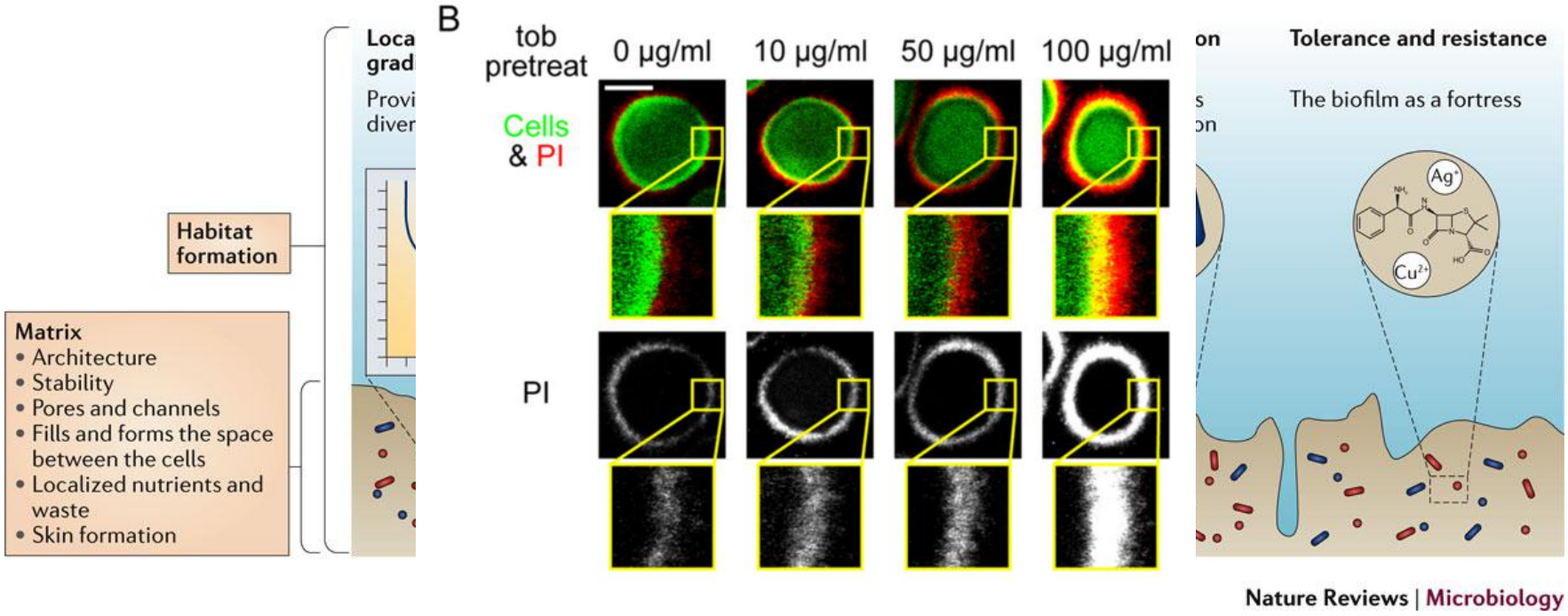
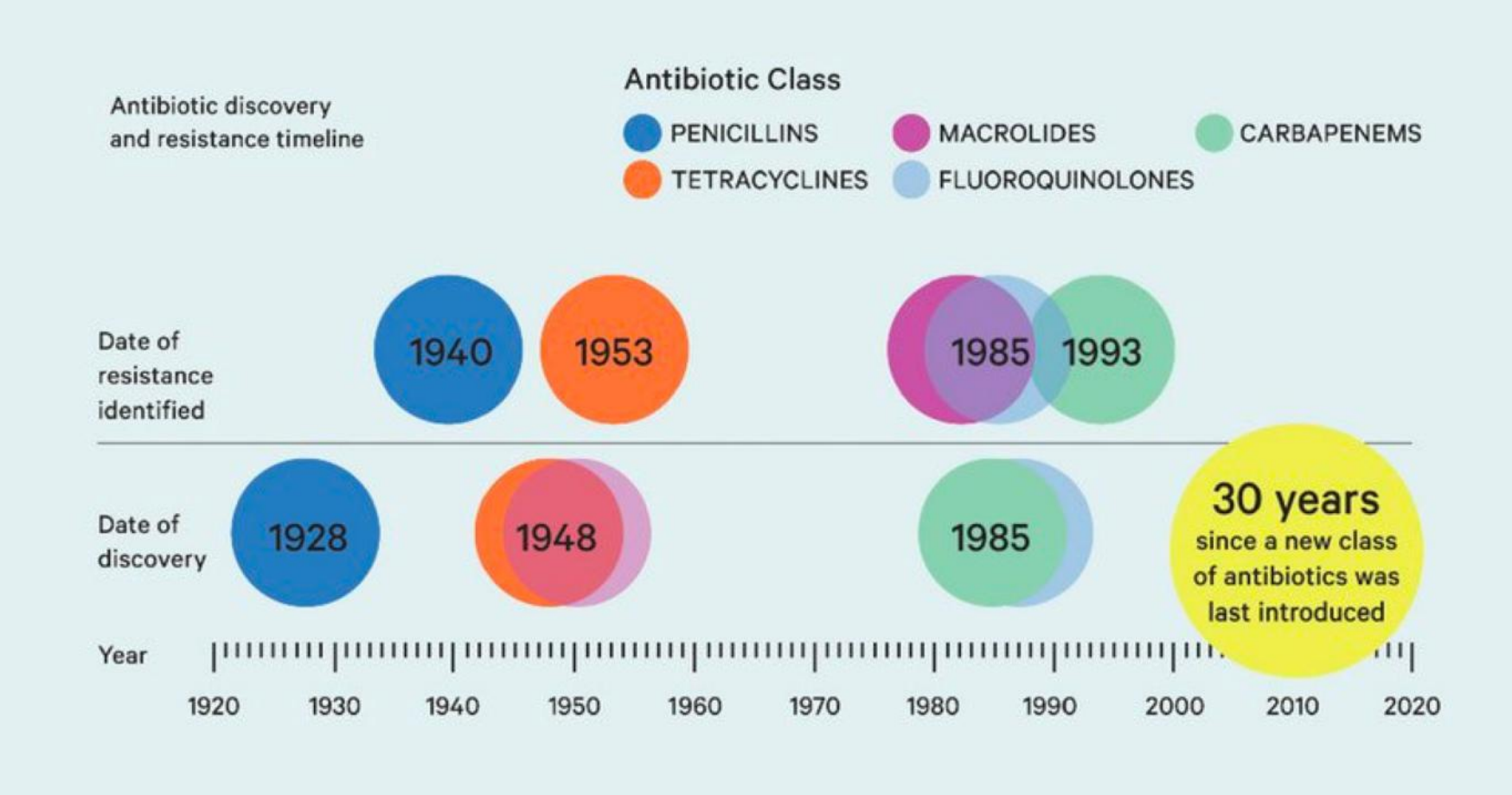


Heterogeneous matrix structure requires alternate strategies for enhanced penetration and prolonged exposure with lethal antibiotic doses

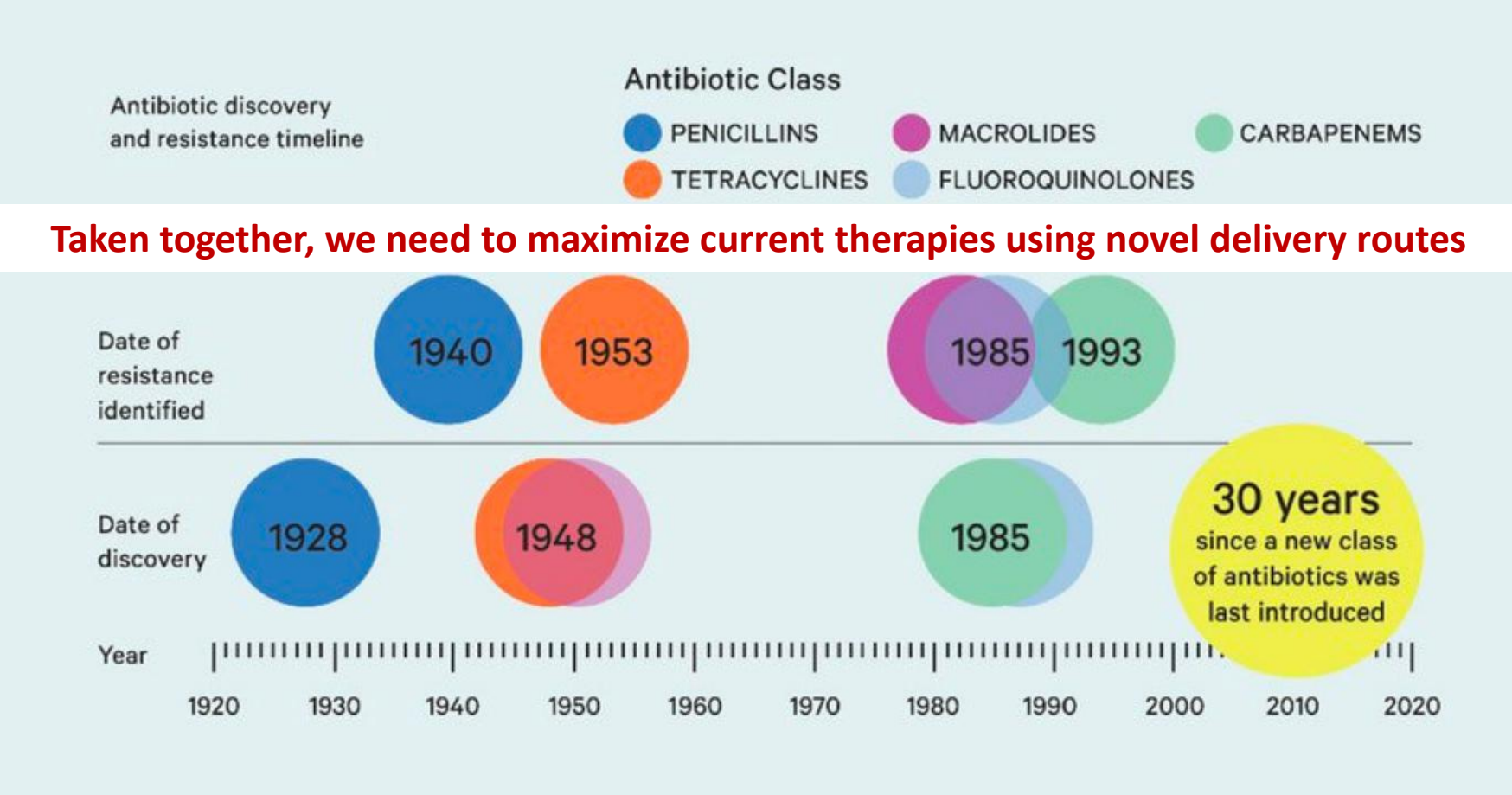
Fleming, H. *Nature Rev. Micro.* **8**, 2016.
Tseng, B. S., *et al. Env. Microbiol.* **15**, 2013.

Dry Antibiotic Pipeline



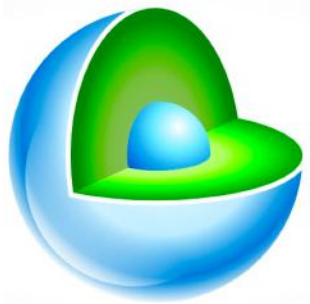
Public Health England, 2015.

Dry Antibiotic Pipeline



Public Health England, 2015.

NP Toolbox

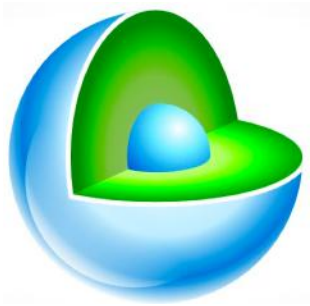


1. High SA/vol
2. Controlled release
3. High valency

NP Toolbox

Passive

- Leaky vasculature (EPR)
- Increase $t_{1/2}$ within body
- Ex: hydration layer avoids opsonization and RES clearance

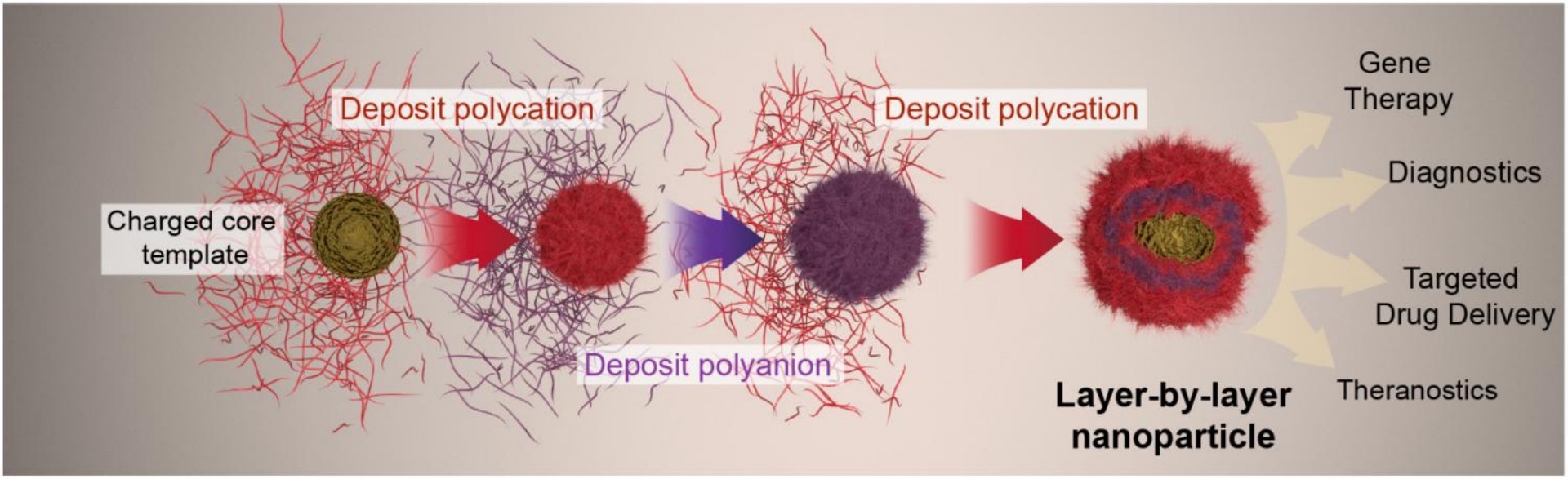


1. **High SA/vol**
2. **Controlled release**
3. **High valency**

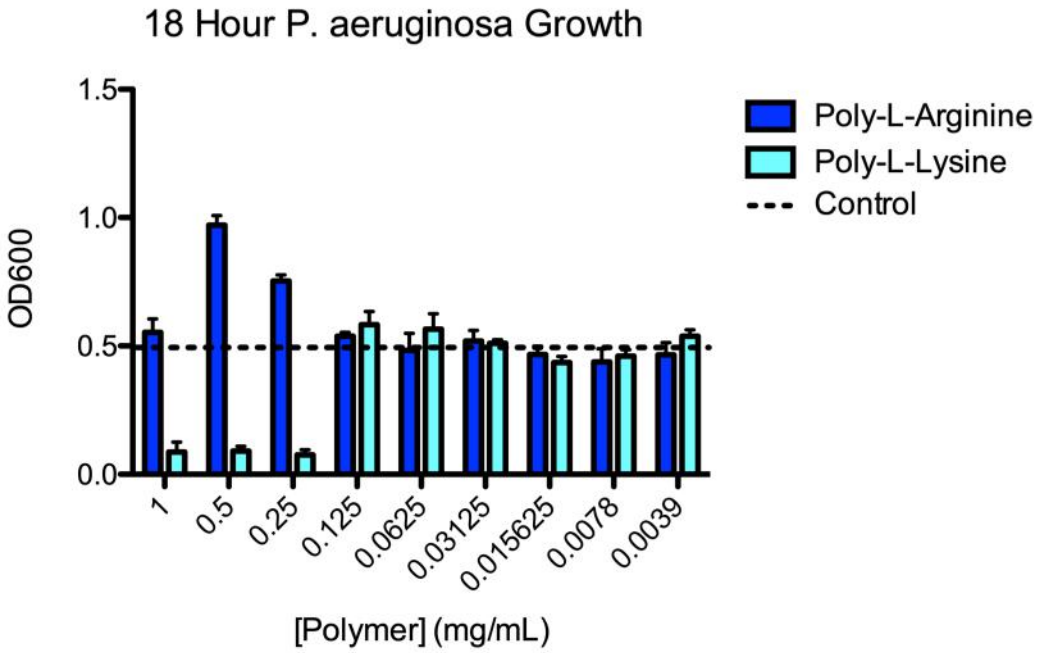
Active

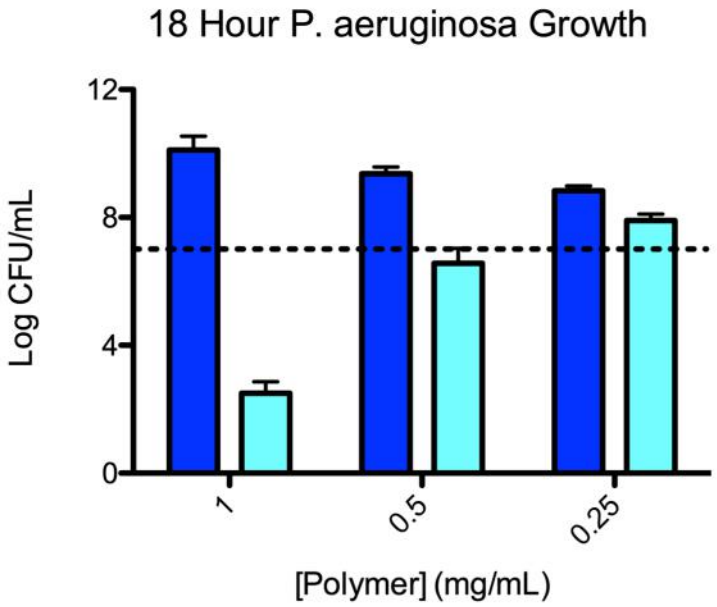
- Specific binding moieties
- Increase local accumulation
- Ex: NPs functionalized with carbohydrates for lectin attachment

Layer-by-Layer Nanoparticles



Positive Poly-Amino Acid Antimicrobial Effects





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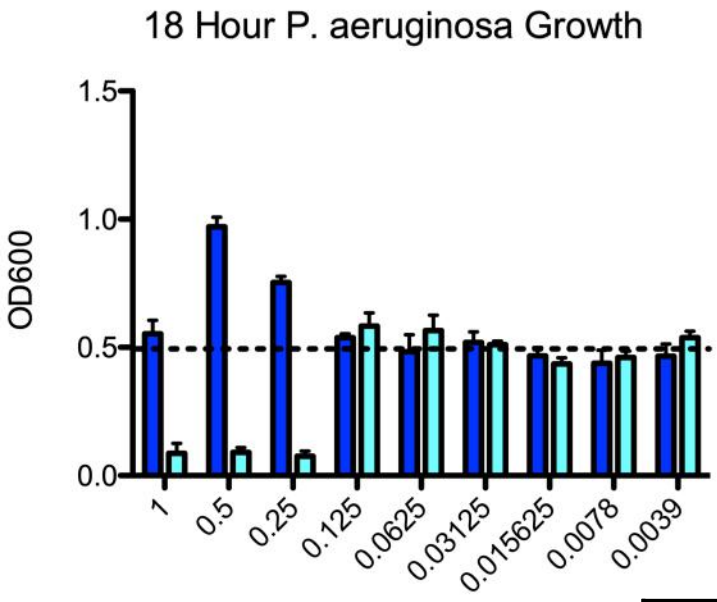

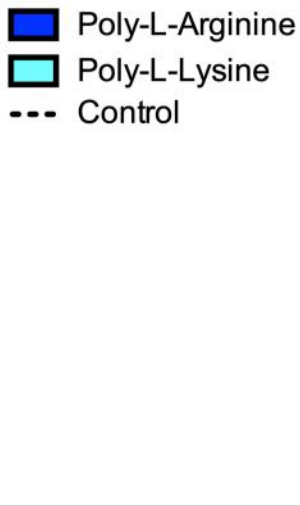

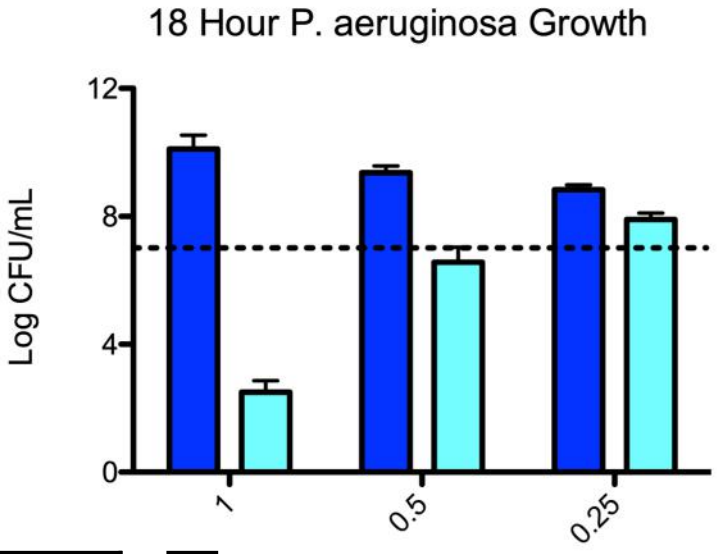

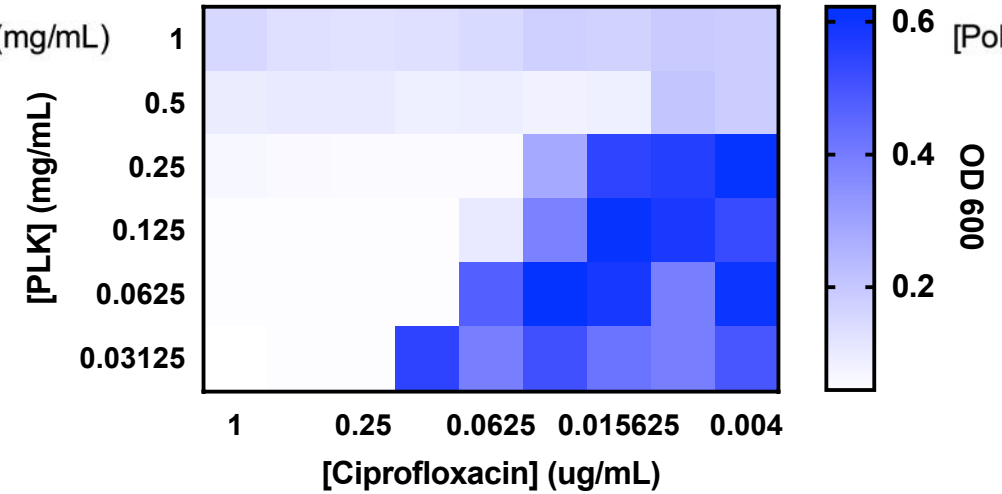

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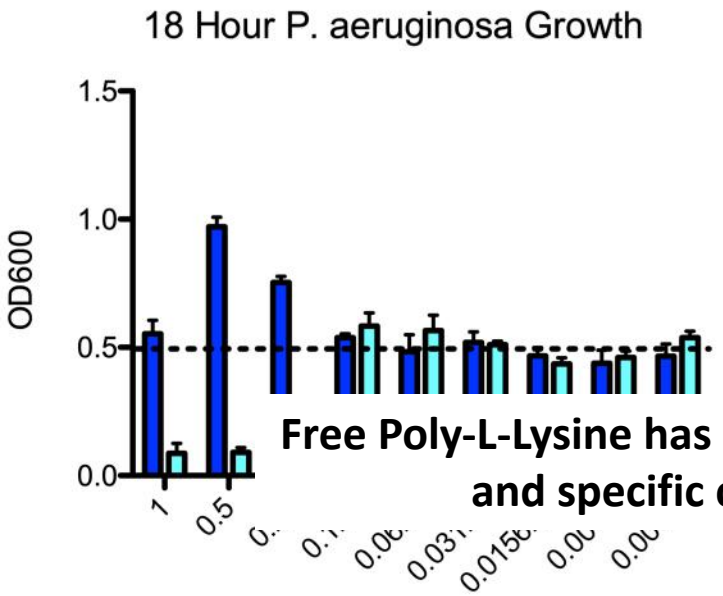
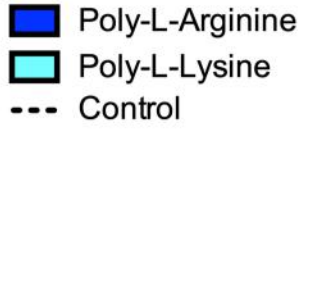
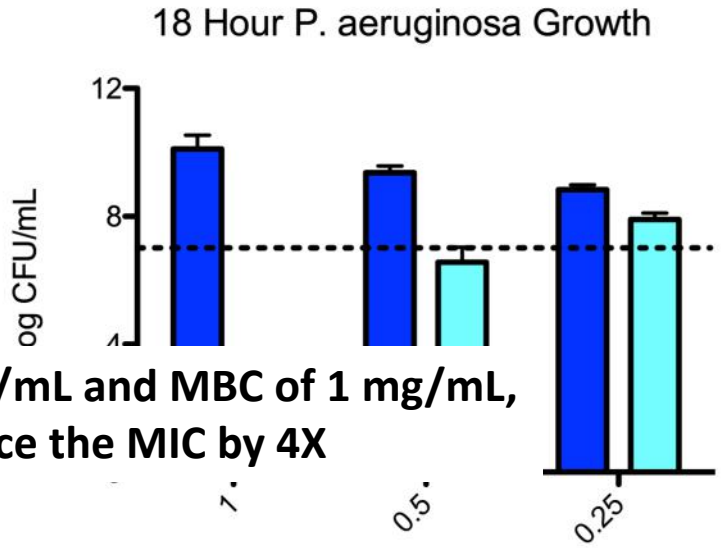
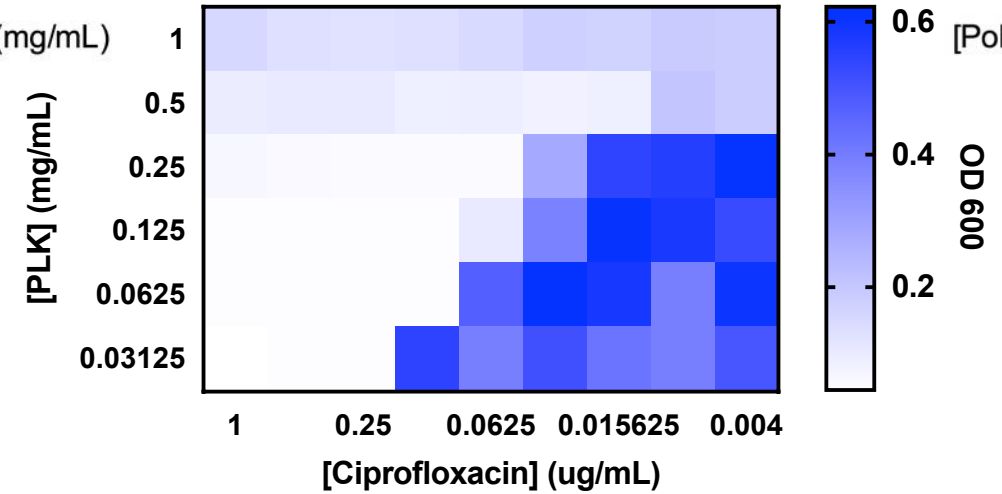


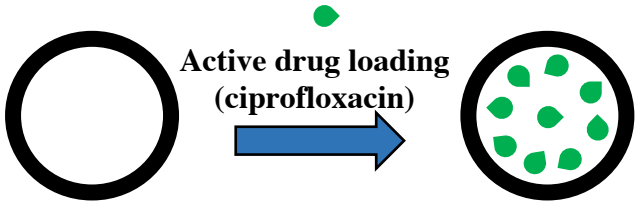


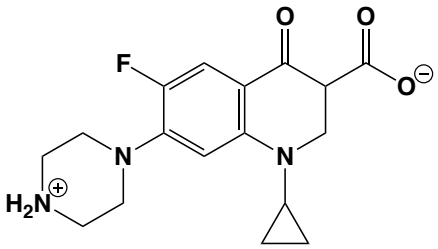


Free Poly-L-Lysine has an MIC of 0.25 mg/mL and MBC of 1 mg/mL, and specific combinations reduce the MIC by 4X



Design of Experiment for Ciprofloxacin Loading





Ciprofloxacin

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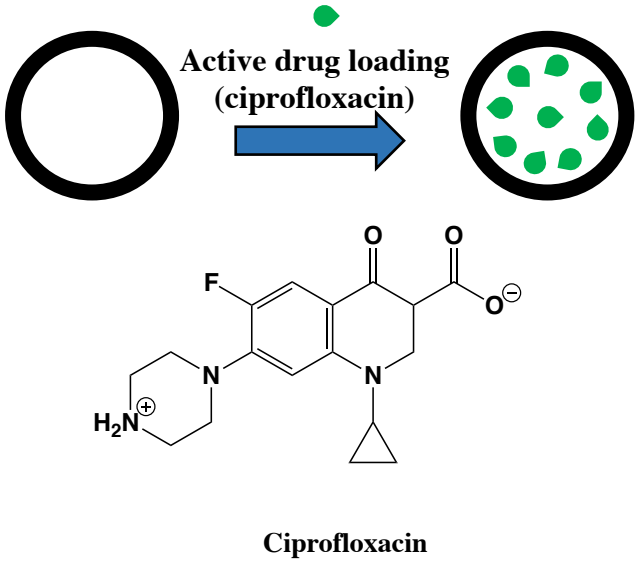

Ciprofloxacin

Note: 1.38 mg cipro added

Temperature	Exterior Solution	[NP]	Encapsulation Efficiency	Loading Efficiency
50C	pH 6	1 mg/mL	53.2%	7.34%
65C	pH 6	1 mg/mL	29.2%	4.03%
50C	pH 7.4	1 mg/mL	10.7%	1.48%
65C	pH 7.4	1 mg/mL	0.94%	0.13%
50C	pH 6	5 mg/mL	84.8±8.9%*	11.7±1.2%*
65C	pH 6	5 mg/mL	33.7%	4.65%
50C	pH 7.4	5 mg/mL	N/A	N/A
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*n=3 syntheses

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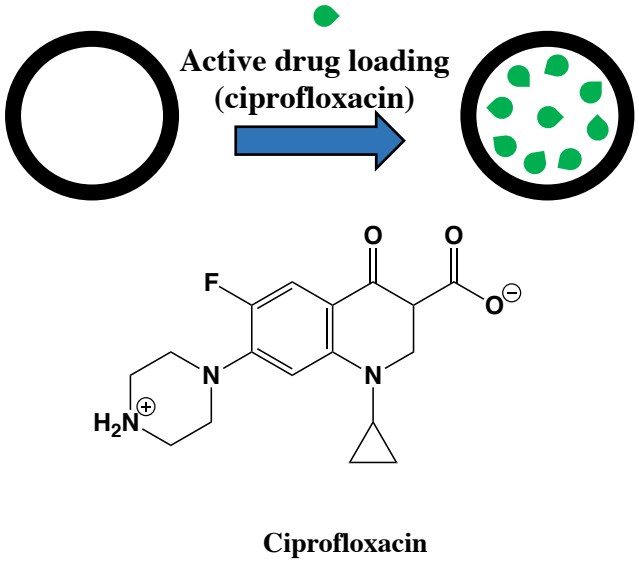

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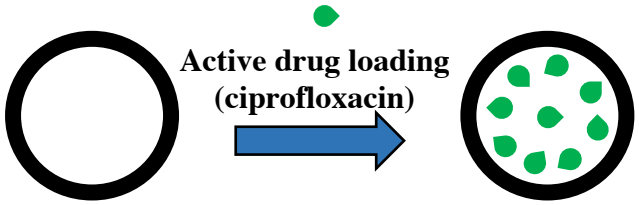

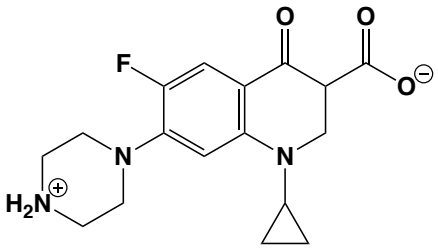

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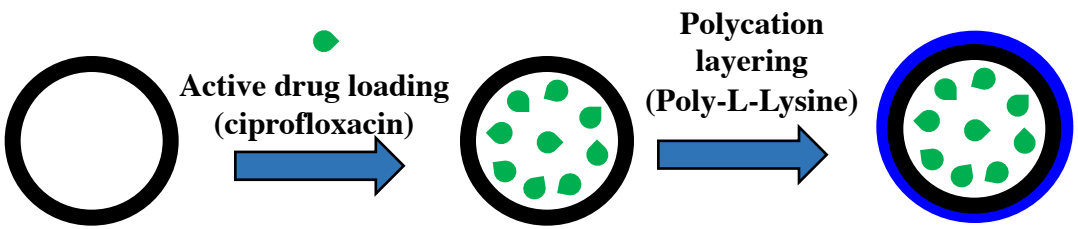
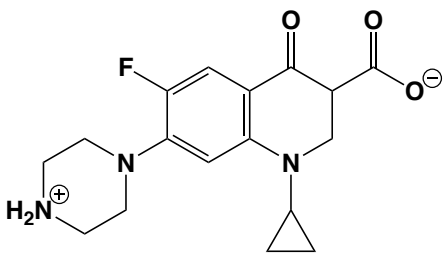
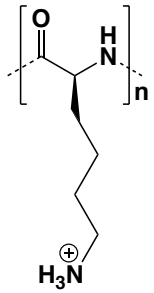
Mg Cipro Added	Temperature	Exterior Solution	[NP]	Encapsulation Efficiency	Loading Efficiency
4	50C	pH 6	5 mg/mL	42.0%	16.8%
7	50C	pH 6	5 mg/mL	15.3%	10.7%
10	50C	pH 6	5 mg/mL	N/A	N/A

Drug Loaded LbL Scheme





Ciprofloxacin



Poly-L-Lysine

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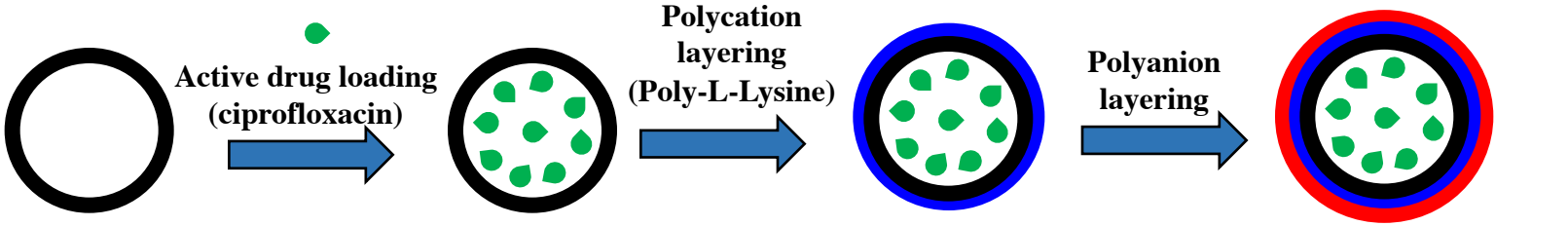

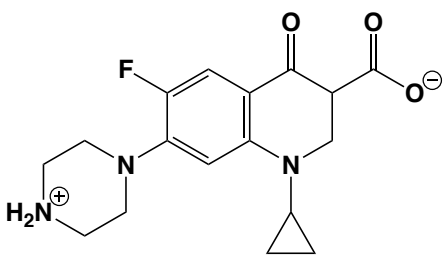

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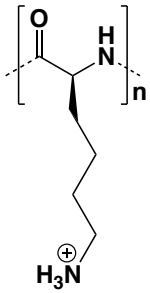

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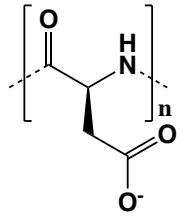
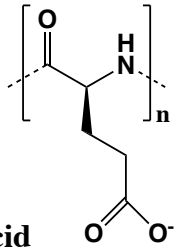
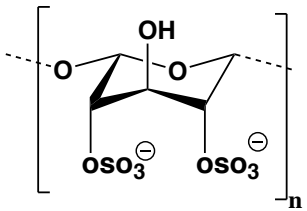


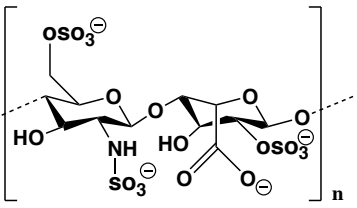
Poly-L-Aspartic Acid



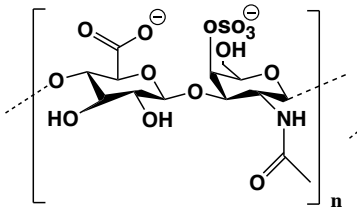
Poly-L Glutamic Acid



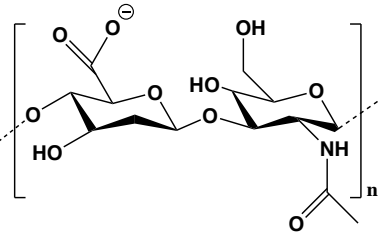
Dextran Sulfate



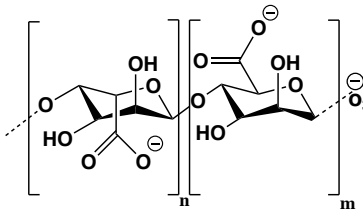
Heparin Sulfate



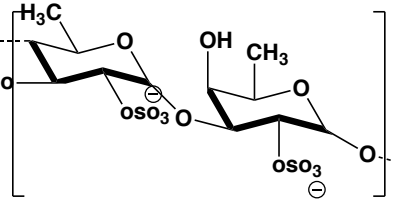
Chondroitin Sulfate A



Hyaluronic Acid

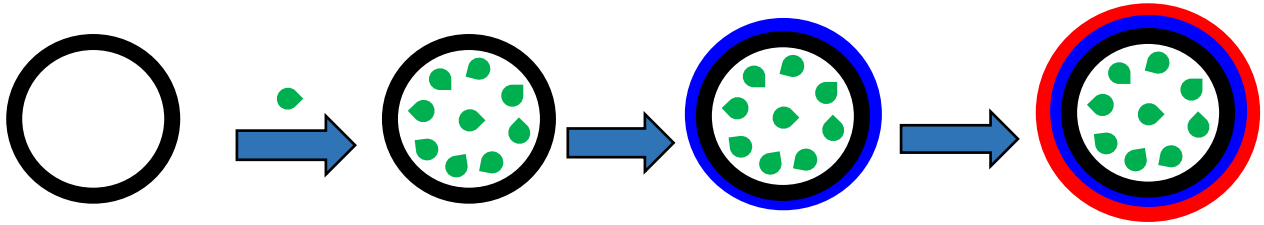


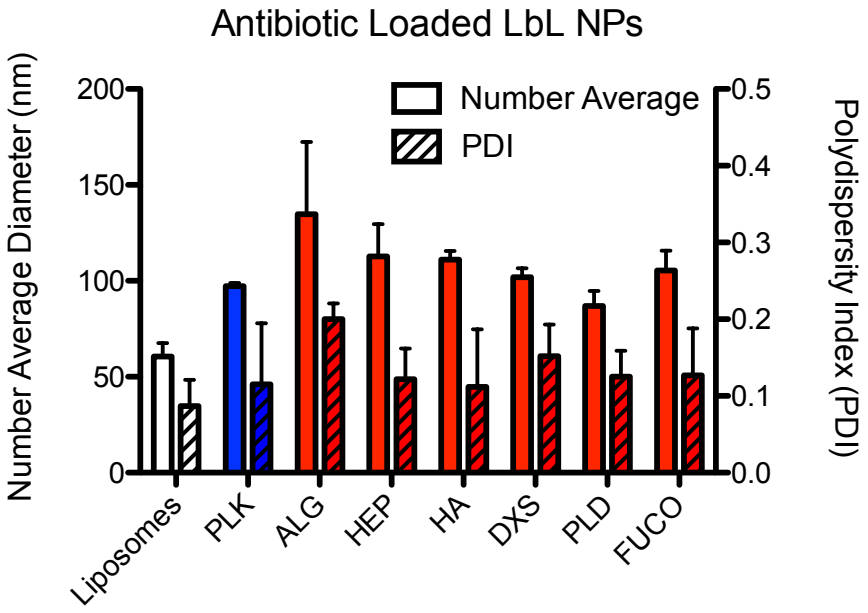
Alginic Acid

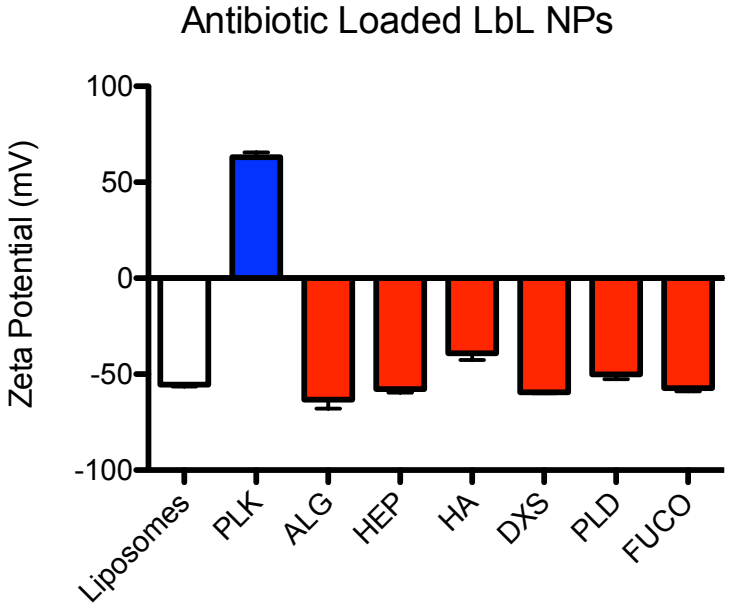


Fucoidan

Polyelectrolyte Layering







Particle Efficacy

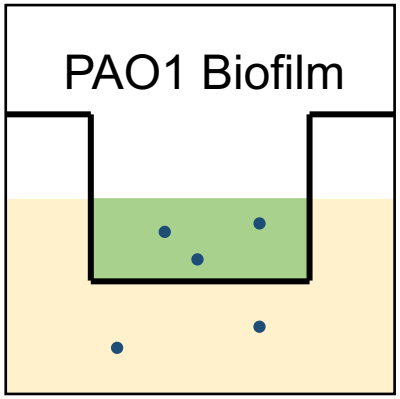
Nanoparticle Formulation	MIC (μgram/mL)	MBC (μgram/mL)	MBEC (μgram/mL)
Cipro (free)	0.2	0.8	12.5
LipoC	0.1	0.2	25
LipoC/PLK/PLD	0.4	0.8	>25
LipoC/PLK/PLE	0.1	0.2	25
LipoC/PLK/DXS	0.2	0.2, 0.2, 0.4	>25
LipoC/PLK/HEP	0.4	0.4	12.5
LipoC/PLK/HA	0.4	0.8	25
LipoC/PLK/CSA	0.2	0.4, 0.4, 0.8	25
LipoC/PLK/ALG	0.05	0.2	6.25
LipoC/PLK/FUCO	0.1	0.2	12.5

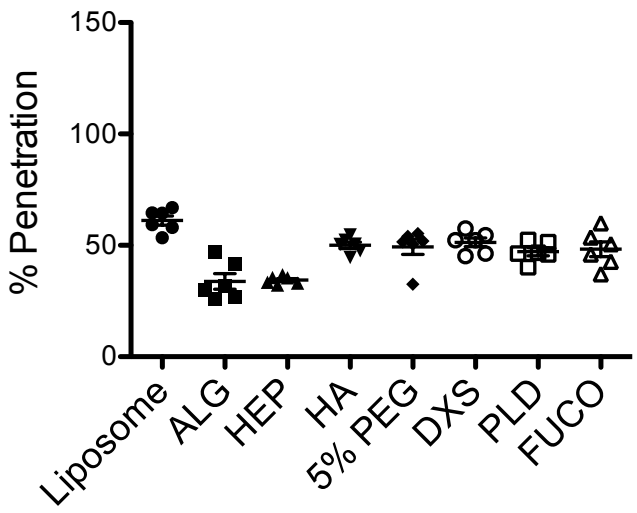
Transwell Penetration of NP Library

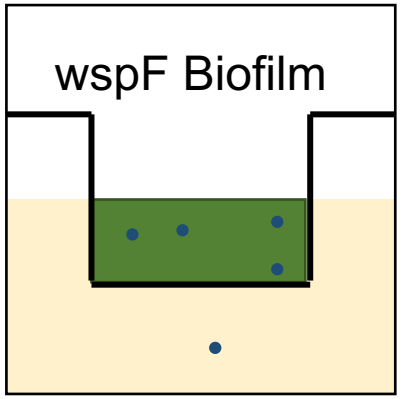
Wild-type microbe

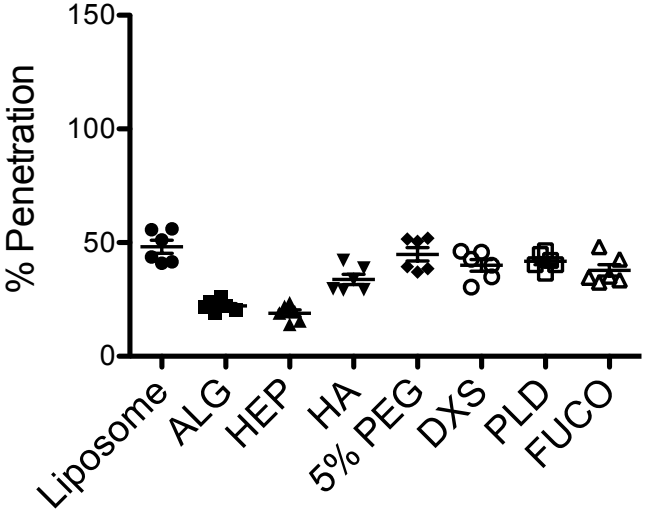
4 hour incubation

Polysaccharide overproducer









Thank you!

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