School of Chemical Engineering

Shape matters: Effect of Amphiphilic Polymer topology on Antibacterial activity and Hemocompatibility

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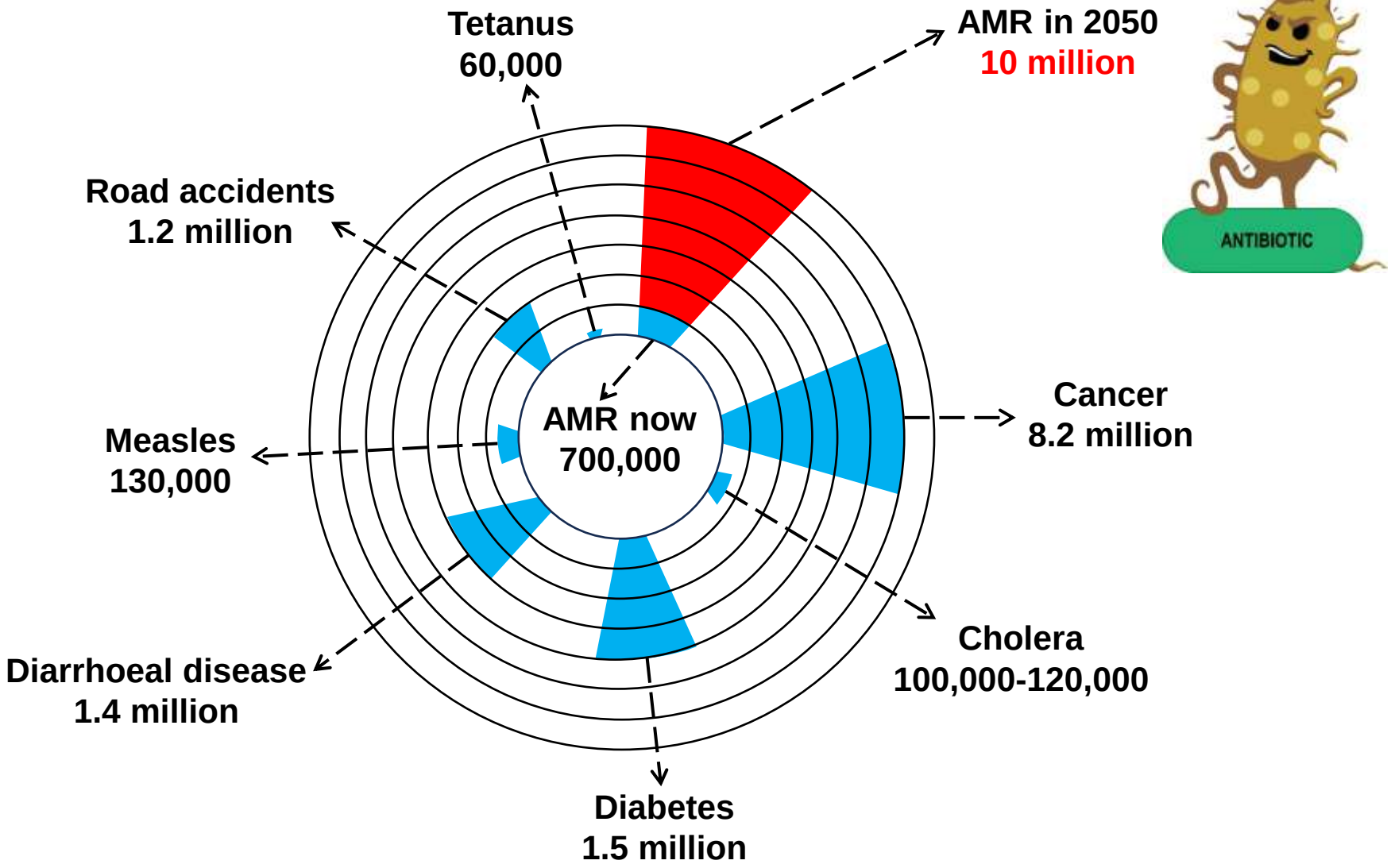
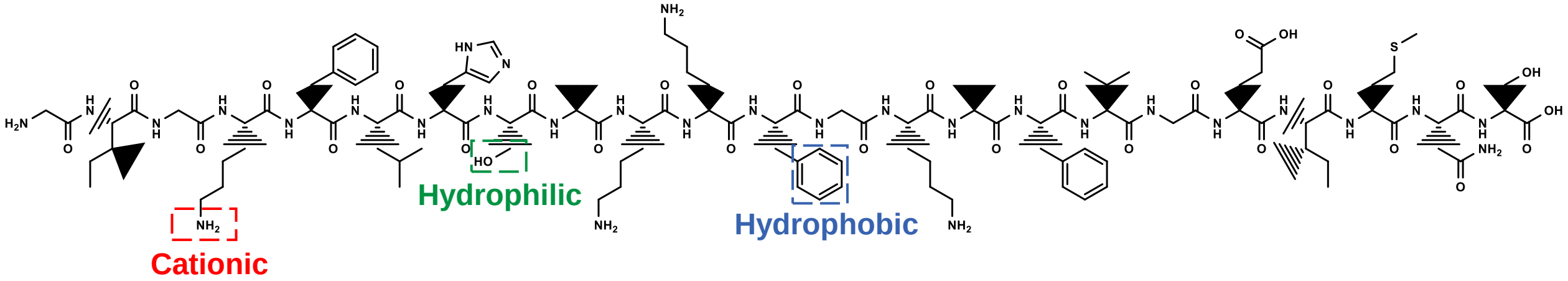
Why is Antimicrobials (Antibiotic) Research Important ?



O'neill JI. Review Antimicrobial. Resistance. 2014.
O'Neill J. Review Antimicrobial. Resistance. 2016.
Ikuta KS, Swetschinski LR, Aguilar GR, Sharara F, Mestrovic T, Gray AP, Weaver ND, Wool EE, Han C, Hayoon AG, Aali A. The Lancet. 2022 Dec 17;400(10369):2221-48.
https://www.istockphoto.com/

Host-Defence Peptides (HDPs) as an Alternative to Antibiotics

HDPs are part of the **innate immune system** produced by multicellular organisms for their **self-defence** against invasive **pathogens**.



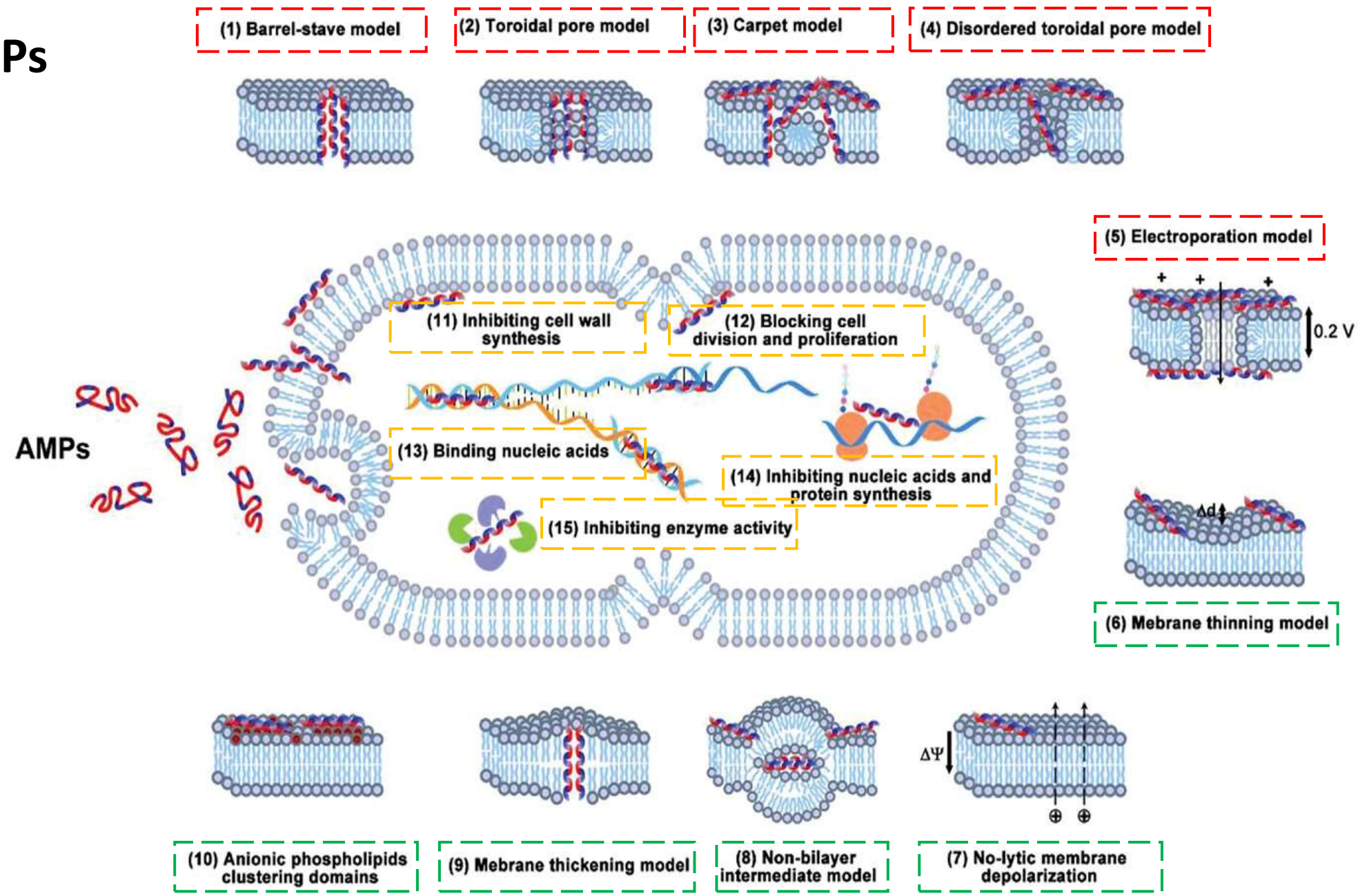
Chemical structure of **Magainin II**, a natural **HDP**.

Mechanism of HDPs

I. Membrane destructive

II. Non-destructive membrane

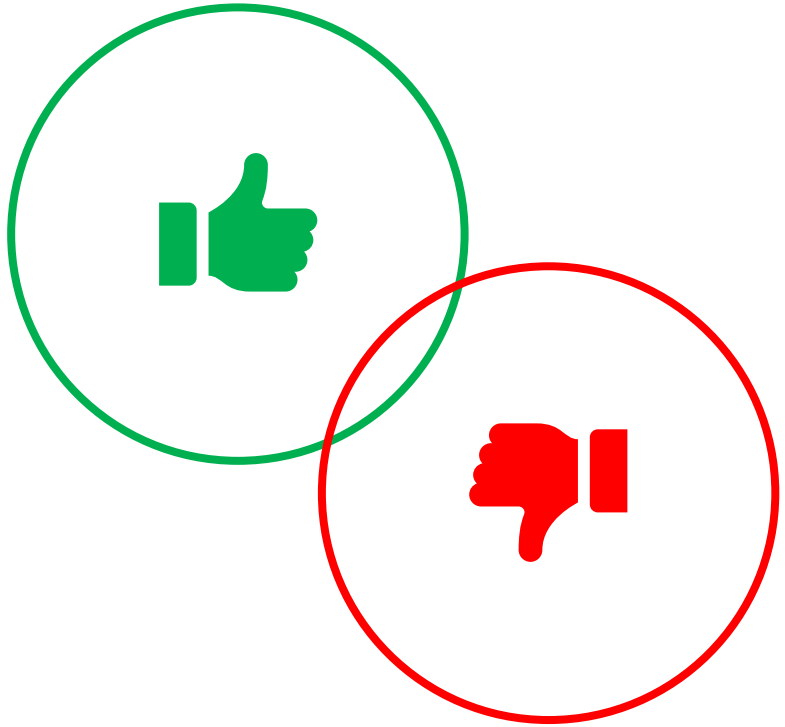
III. Intracellular targeting



Yu L, Li K, Zhang J, Jin H, Saleem A, Song Q, Jia Q, Li P. Antimicrobial peptides and macromolecules for combating microbial infections: from agents to interfaces. ACS Applied Bio Materials. 2022 Jan 24;5(2):366-93

Clinical Advantages and Disadvantages of HDPs

- ❑ Good safety
- ❑ Good efficacy
- ❑ Direct killing
- ❑ High specificity
- ❑ Immune modulation
- ❑ Slower/No resistance generation



- ❑ Low stability
- ❑ Short half-life
- ❑ Rapid clearance
- ❑ Poor bioavailability
- ❑ High production costs
- ❑ Proteolytic degradation

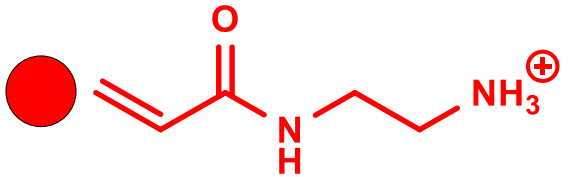
Synthetic Polymers can mimic HDPs/AMPs and Could Offer a Potential Alternative for Targeting Bacteria

Synthetic Polymers: As a Promising New Generation of Antibacterials

Previous research mainly focused on composition of linear polymers to optimize antibacterial activity and biocompatibility

❑ Cationic Group:

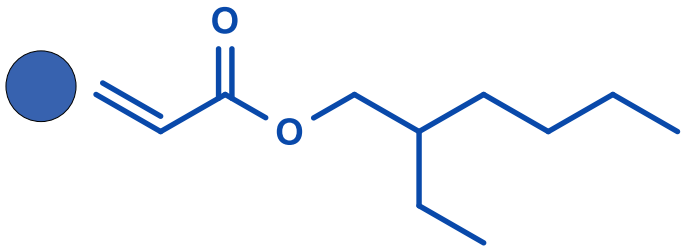
- **Primary amines** displayed **superior bioactivity**



Primary ammonium

❑ Hydrophobic Group:

- **Branched** hydrophobic groups were **more antibacterial**

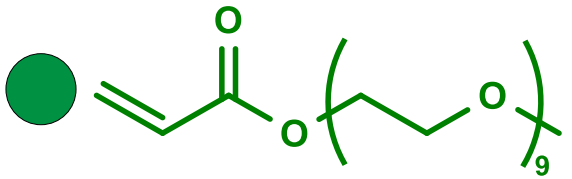


2-ethylhexyl acrylate

❑ Hydrophilic Group:

- **PEG**-containing hydrophilic groups offer the **highest selectivity**

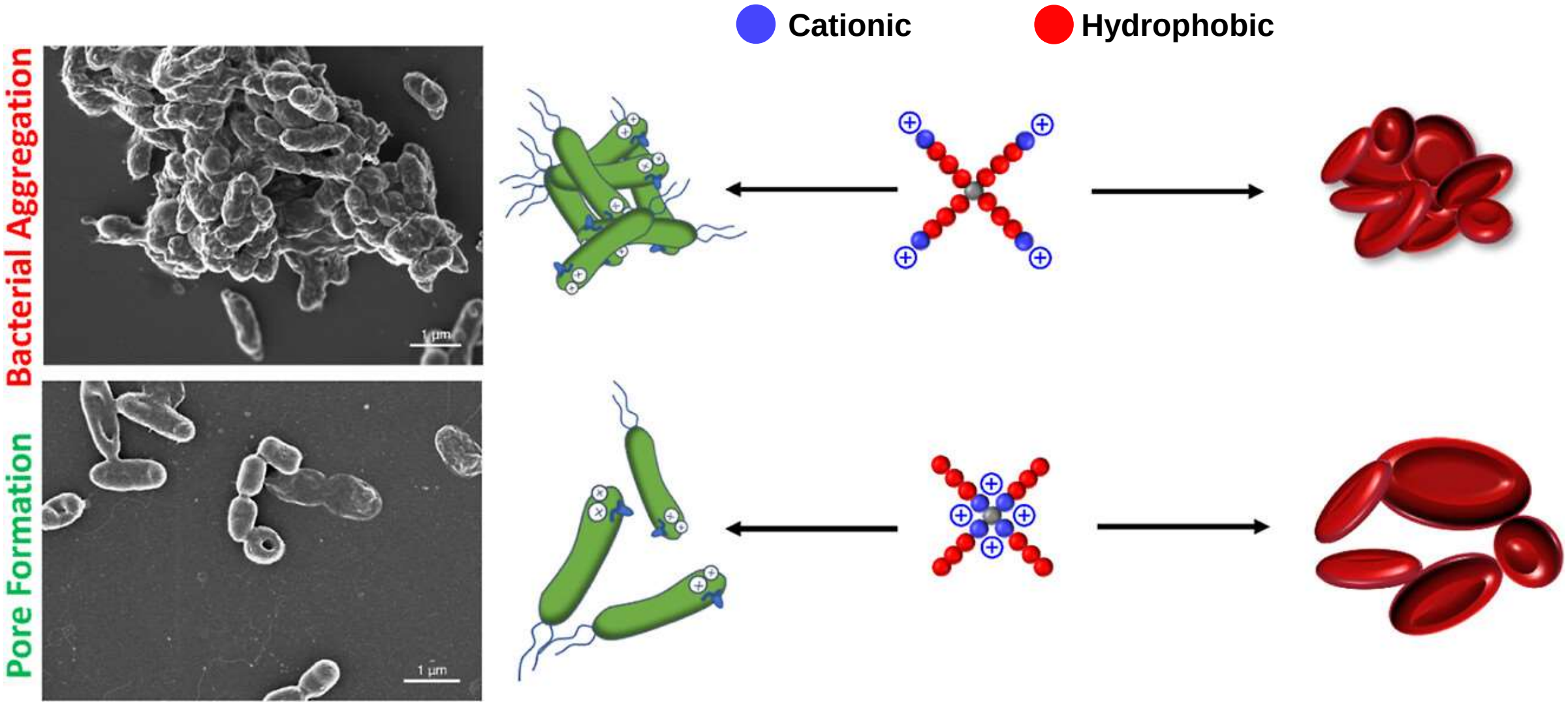
But What About Topology



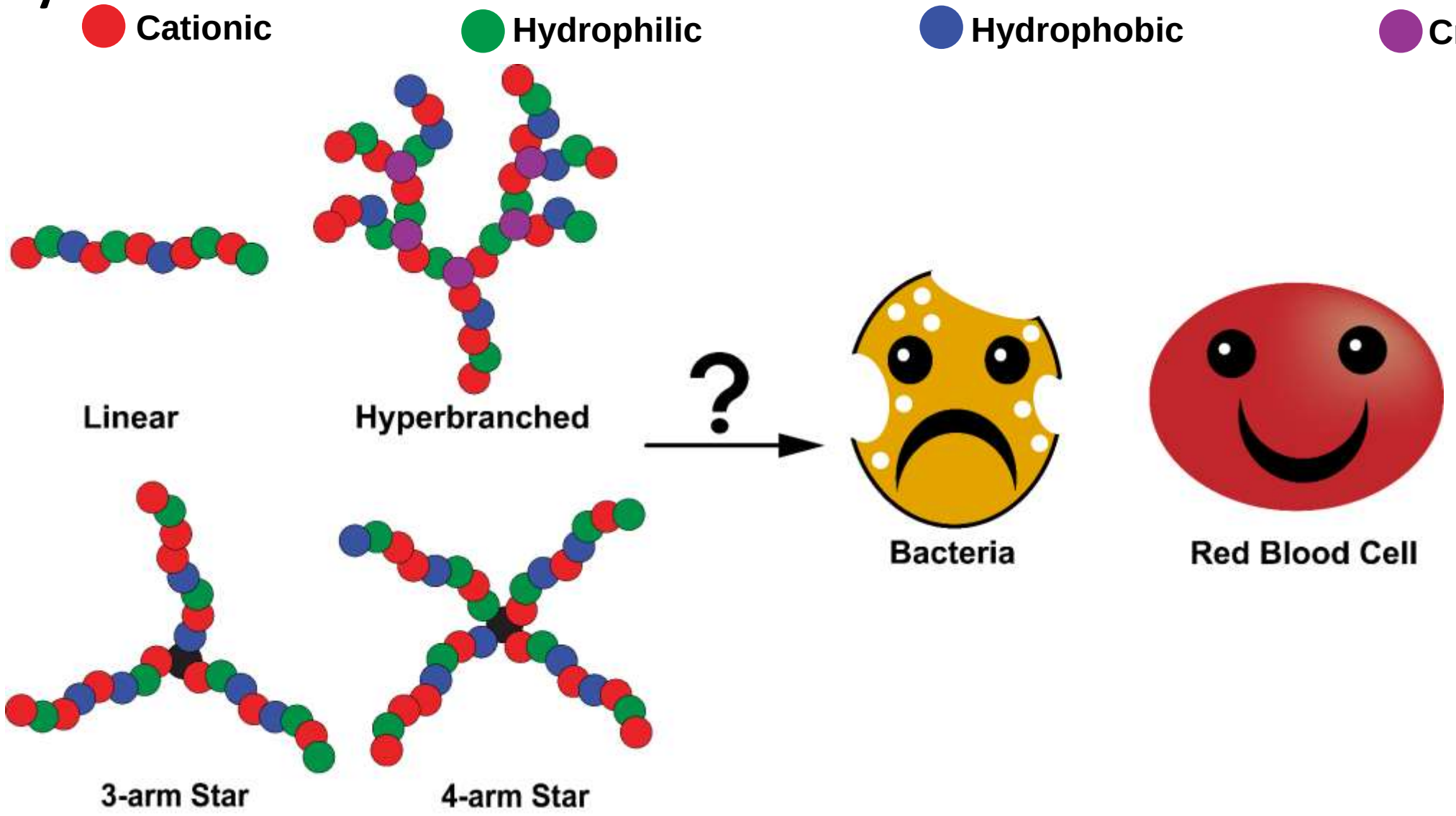
Poly(ethylene glycol) methyl ether acrylate

Pham P, Oliver S, Nguyen DT, Boyer C. Macromolecular Rapid Communications. 2022 Nov;43(21):2200377.
Nguyen TK, Lam SJ, Ho KK, Kumar N, Qiao GG, Egan S, Boyer C, Wong EH. ACS Infectious Diseases. 2017 Mar 10;3(3):237-48.
Pham P, Oliver S, Wong EH, Boyer C. Polymer Chemistry. 2021;12(39):5689-703.

Previous Study

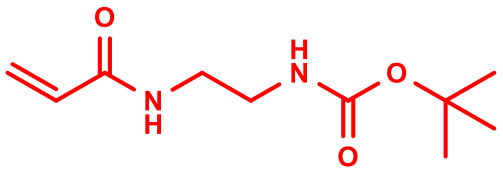


Our Study



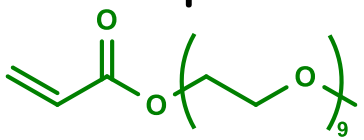
Aquib M, Schaefer S, Gmedhin H, Corrigan N, Bobrin VA, Boyer C. European Polymer Journal. 2024;205:112698.

Polymer Synthesis by PET-RAFT



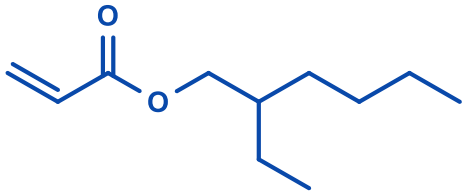
tert-butyl (2-acrylamidoethyl carbamate (Boc-protected)

+



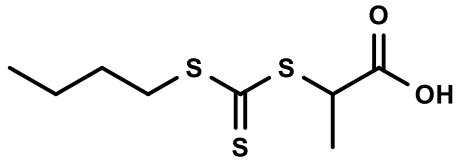
Poly(ethylene glycol) methyl ether acrylate

+



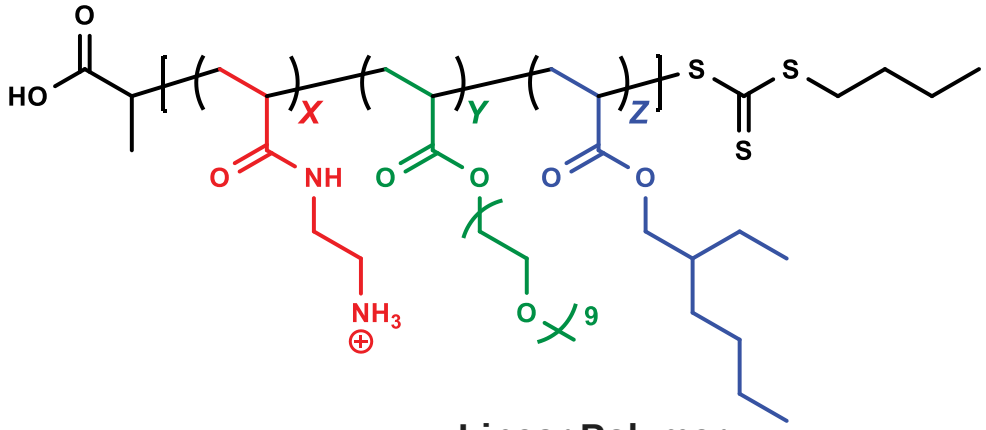
2-ethylhexyl acrylate

+



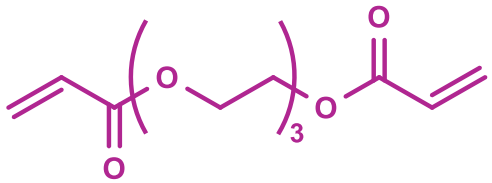
BTPA*

After Deprotection

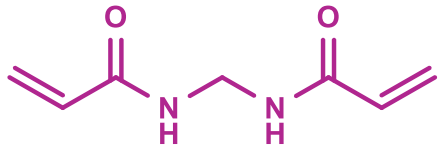


Linear Polymer

Crosslinking Monomers for Hyperbranched Polymers

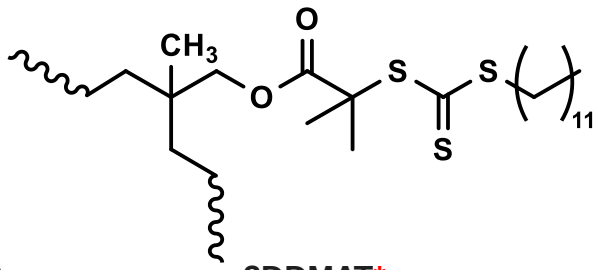


Poly(ethylene glycol) diacrylate (P)



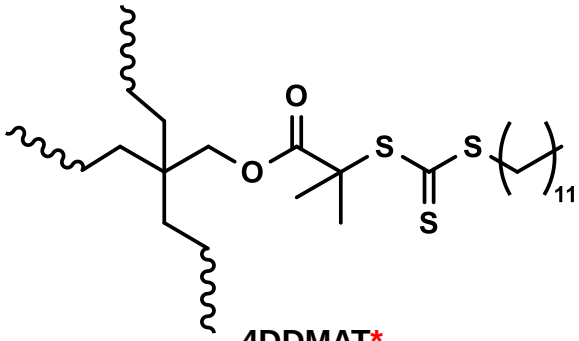
N,N'-methylenebis(acrylamide) (M)

RAFT-agent for 3-Arm Star Polymers (SP)



3DDMAT*

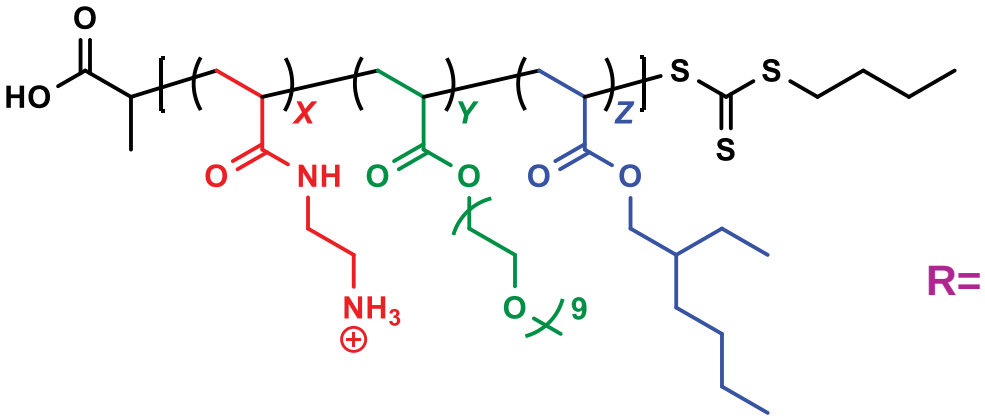
RAFT-agent for 4-Arm SP



4DDMAT*

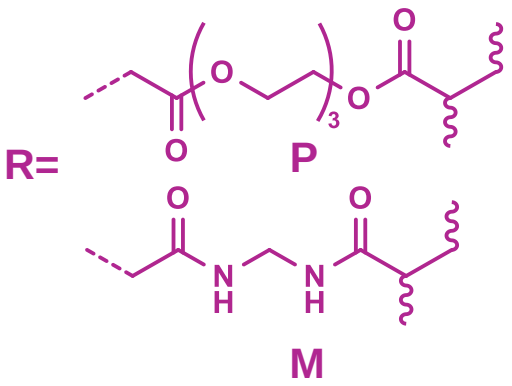
Aquib M, Schaefer S, Gmedhin H, Corrigan N, Bobrin VA, Boyer C. European Polymer Journal. 2024;205:112698.

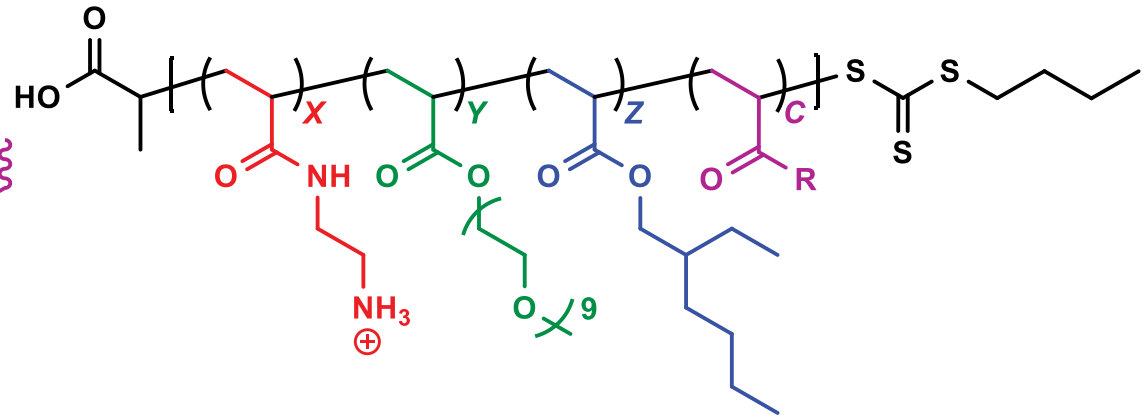
The Compositional Structures and Architectures of the Amphiphilic Copolymers



Linear Polymer (LP)

Polymer	X/ Y/ Z
LP-20	50/30/20
LP-25	50/25/25
LP-30	50/20/30





Hyperbranched Polymer (HP)

Polymer	X/ Y/ Z/C
HPP1-20	50/30/20/1
HPP2-20	50/30/20/2
HPP3-20	50/30/20/3
HPP4-20	50/30/20/4
HPP5-20	50/30/20/5
HPP2-25	50/25/25/2
HPP5-25	50/25/25/5
HPP2-30	50/20/30/2
HPP5-30	50/20/30/5

Polymer	X/ Y/ Z/C
HPM1-20	50/30/20/1
HPM2-20	50/30/20/2
HPM3-20	50/30/20/3
HPM4-20	50/30/20/4
HPM5-20	50/30/20/5
HPM2-25	50/25/25/2
HPM5-25	50/25/25/5
HPM2-30	50/20/30/2
HPM5-30	50/20/30/5

X= Cationic
Y= Hydrophilic
Z= Hydrophobic
P= Poly(ethylene glycol) diacrylate
M= *N,N'*-methylenebis(acrylamide)
} **C=** Number of crosslinker groups

***HP** [Crosslinker]:[RAFT agent] =1:1 to 5:1
Hydrophobic content = 20–30 %

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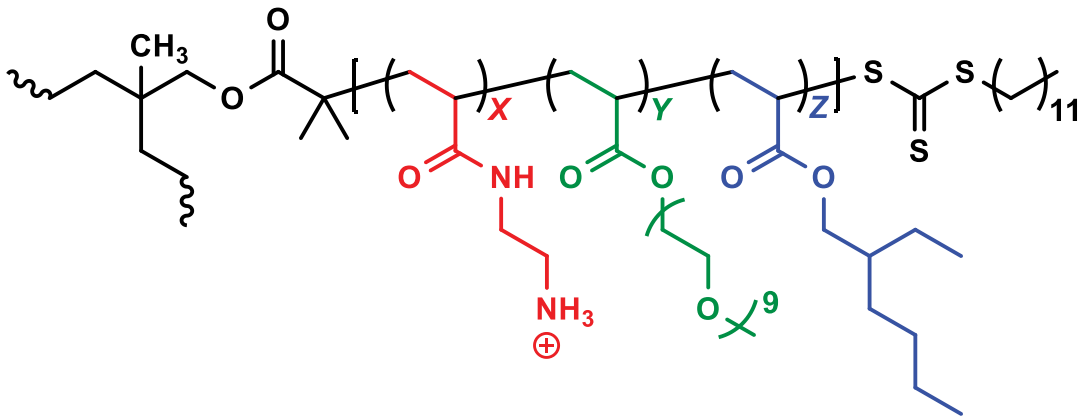
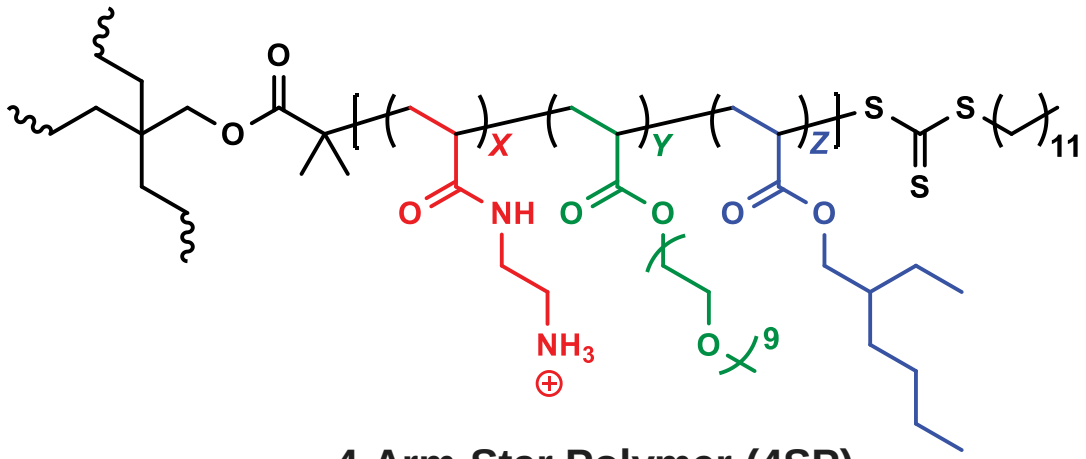
The Compositional Structures and Architectures of the Amphiphilic Copolymers



3-Arm Star Polymer (3SP)

Polymer	X/ Y/ Z
3SP-20	50/30/20
3SP-25	50/25/25
3SP-30	50/20/30

X= Cationic
Y= Hydrophilic
Z= Hydrophobic



4-Arm Star Polymer (4SP)

Polymer	X/ Y/ Z
4SP-20	50/30/20
4SP-25	50/25/25
4SP-30	50/20/30

We synthesized 27 polymers with different topologies (LPs HPs, 3SPs, and 4SPs)

Aquib M, Schaefer S, Gmedhin H, Corrigan N, Bobrin VA, Boyer C. European Polymer Journal. 2024;205:112698.

Overview of the Polymer Properties

Polymers	Average Hydrodynamic Diameter (nm)
Linear and Star Polymers	**34.5–93.5 nm**
Hyperbranched Polymers	**10.1–113.8 nm**

Higher crosslinker ratio can **reduce** the **average hydrodynamic diameter** in the **HPs**

All the **copolymers** exhibited a **positive zeta potential** indicating the presence of **cationic ammonium moieties**

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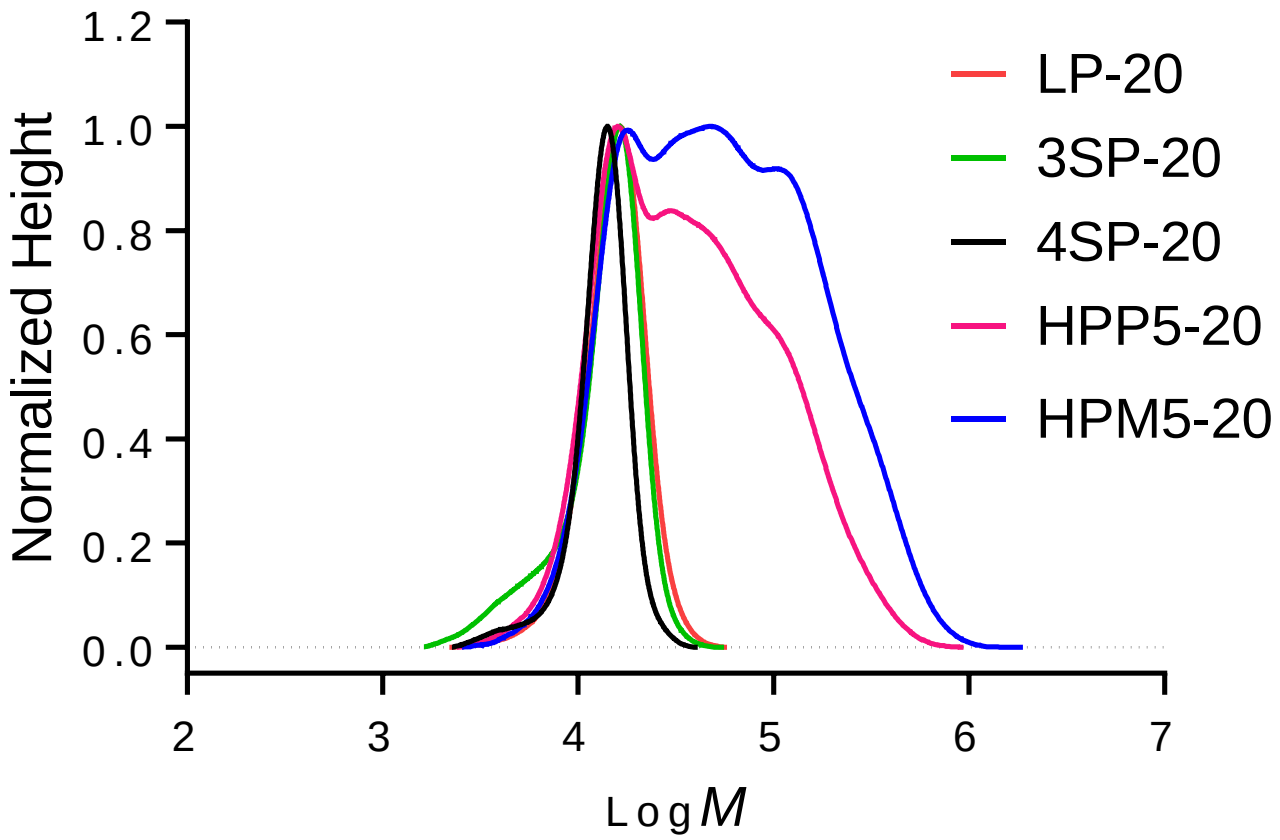
Size Exclusion Chromatography



- **Linear and Star Polymers**
  - **Monomodal** molecular weight distributions
  - **Dispersity** (*Đ*) values ranging from **1.12 to 1.25**
- **Hyperbranched Polymers**
  - HPP and HPM polymers **broad** and often **multimodal** distributions
  - **Dispersity** (*Đ*) values ranging from **1.21 to 3.31**

Aquib M, Schaefer S, Gmedhin H, Corrigan N, Bobrin VA, Boyer C. European Polymer Journal. 2024;205:112698.

Effect of Topology on Antibacterial and Hemolysis Activity

Polymer	20 % Hydrophobicity	$MIC_{90\%,\ 20\ h}$ (μg/mL) PAO1	$MIC_{90\%,\ 20\ h}$ (μg/mL) K12	$HC_{50\%\ 2\ h}$ (μg/mL)	Selectivity (PAO1)
LP-20		32	32	500–1000	16
HPP1-20		32	32	>2000	>62
HPP2-20		32–64	32–64	>2000	>31
HPP3-20		32–64	32–64	>2000	>31
HPP4-20		32–64	32–64	>2000	>31
HPP5-20		32–64	64	>2000	>31
HPM1-20		32	32	>2000	>62
HPM2-20		32–64	32	>2000	>31
HPM3-20		32–64	64	>2000	>31
HPM4-20		64	64	>2000	>31
HPM5-20		32–64	64	>2000	>31
3SP-20		32	16–32	No Hemolysis	>250
4SP-20		32–64	32	No Hemolysis	>250

$MIC_{90\%}$ scale (μg/mL): 8 — 64

$HC_{50\%}$ scale (μg/mL): >2000 — <125

Selectivity: >250 — <1

Antibacterial Activity
LP, HPs, and SPs have similar antibacterial activity

HPP and HPM family
No differences in the $MIC_{90\%}$

Hemocompatibility
SPs>HPs>LPs

Selectivity
SPs>HPs>LPs

PAO1 = *P. aeruginosa*
K12 = *Escherichia coli*

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Effect of Topology on Antibacterial and Hemolysis Activity

Polymer	25 % Hydrophobicity	$MIC_{90\%,\ 20\ h}$ (μg/mL) PAO1	$MIC_{90\%,\ 20\ h}$ (μg/mL) K12	$HC_{50\%\ 2\ h}$ (μg/mL)	Selectivity (PAO1)
LP-25		16	16	<125	n.d.
HPP2-25		16	32	<125	n.d.
HPP5-25		32	16–32	125–500	4–16
HPM2-25		16	16–32	<125	n.d.
HPM5-25		32	32–64	125–500	4–16
3SP-25		16–32	16–32	>2000	>62
4SP-25		32–64	32	>2000	>62

Polymer	30 % Hydrophobicity	$MIC_{90\%,\ 20\ h}$ (μg/mL) PAO1	$MIC_{90\%,\ 20\ h}$ (μg/mL) K12	$HC_{50\%\ 2\ h}$ (μg/mL)	Selectivity (PAO1)
LP-30		16	16	<125	n.d.
HPP2-30		16	16	<125	n.d.
HPP5-30		16–32	16	<125	n.d.
HPM2-30		16–32	16–32	<125	n.d.
HPM5-30		32–64	32–64	<125	n.d.
3SP-30		16–32	16–32	<125	n.d.
4SP-30		32	16–32	<125	n.d.
Colistin		8–16	8	No Hemolysis	>250

Antibacterial Activity

Increasing hydrophobic monomer proportion slightly enhanced the antibacterial property

Hemocompatibility

Enhancing the crosslinker ratio (2 to 5) in HPs) led to an increase in the $HC_{50\%}$ values

3SP-25 and 4SP-25 were greater (>2000 μg/mL) >LP-20 (500–1000 μg/mL) and = HPs with 20 % hydrophobicity group (>2000 μg/mL)

*** No hemagglutination***

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Conclusion/Outcomes

Linear Polymers

Antibacterial Activity

LP-20 < LP-25 and LP-30

Hemocompatibility

LP-20 (500–1000 μg/mL)> LP-25 and LP-30 (<125 μg/mL)

Hyperbranched Polymers

Antibacterial Activity

No differences in MIC90 %

Hemocompatibility

HPs HC50 % (>2000 μg/mL) > LPs (500–1000 μg/mL)

Star Polymers

Antibacterial Activity

3SP and 4SP potent and similar effects

Hemocompatibility

Increased hemocompatibility

Highest Selectivity

Aquib M, Schaefer S, Gmedhin H, Corrigan N, Bobrin VA, Boyer C. European Polymer Journal. 2024;205:112698.

Acknowledgements

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Camillo Taraborrelli, Lab Manager (UNSW)

Mark Wainwright Analytical Centre (MWAC), UNSW

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CAMD Centre for Advanced Macromolecular Design

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