Non-amphiphilic Polymers as Antimicrobial Materials

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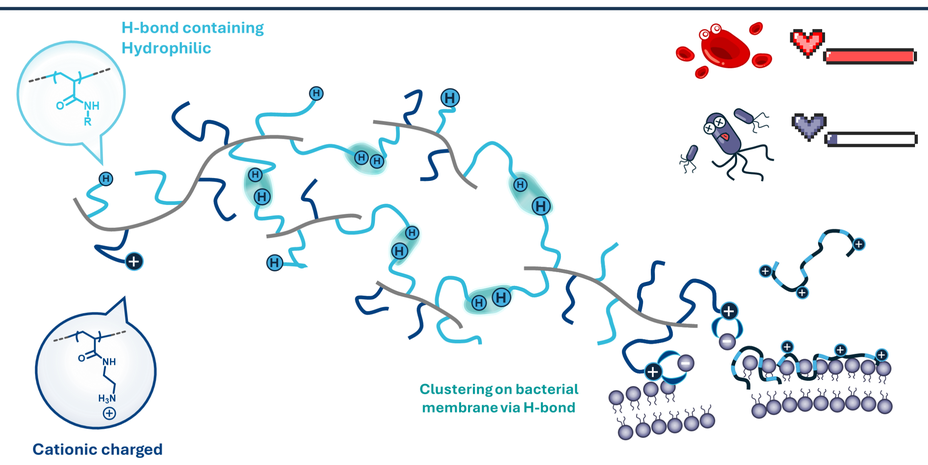
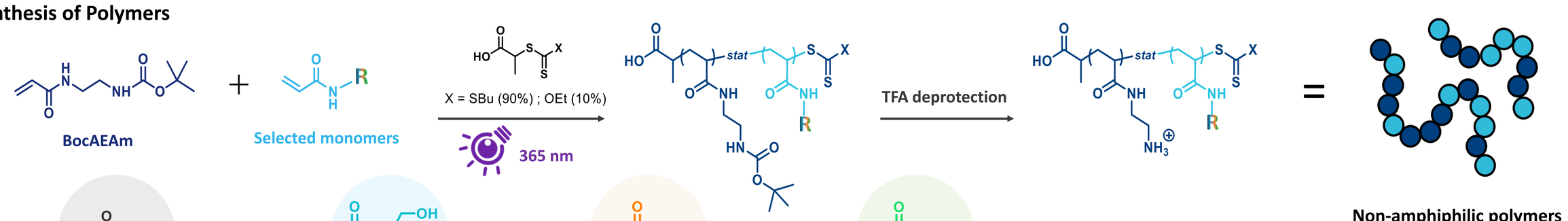
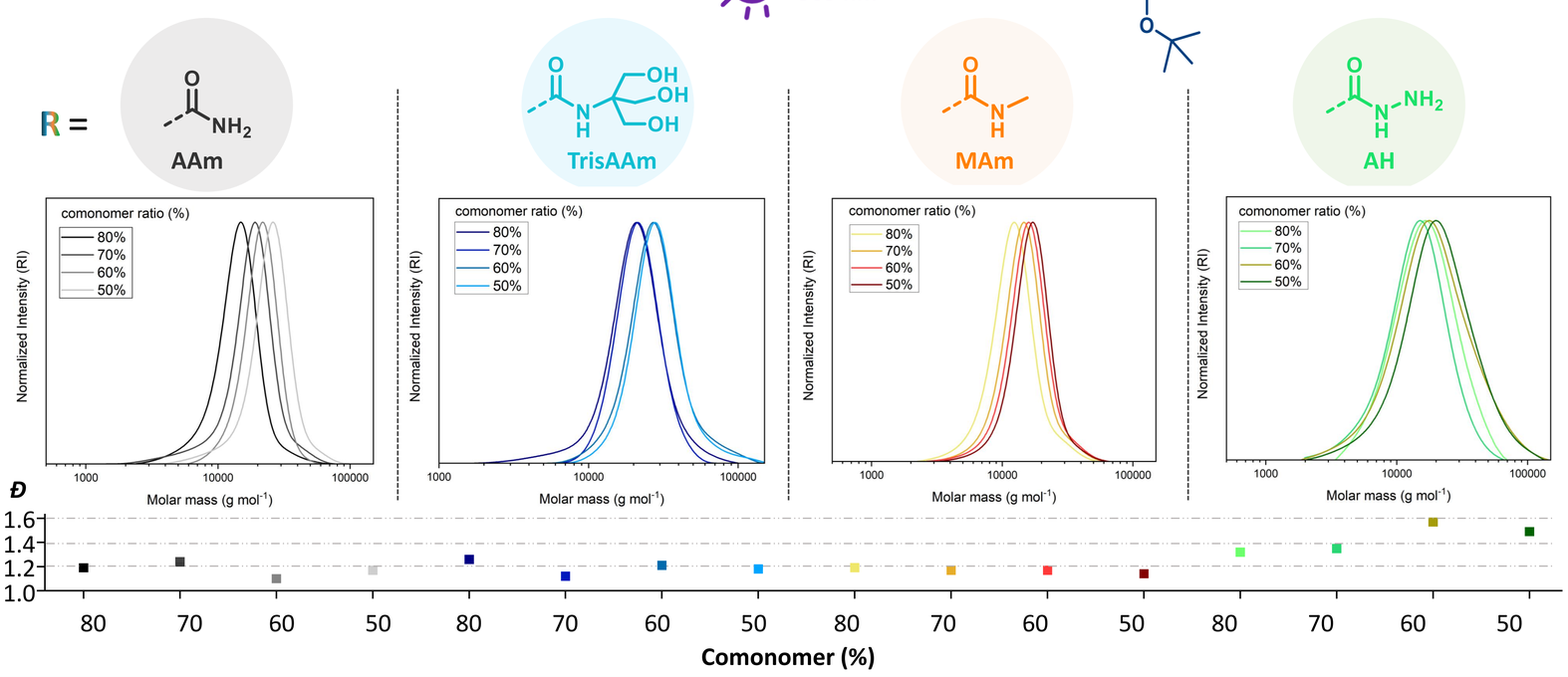


Motivation

- Antimicrobial resistance is a critical global issue that leads to significant casualties and places a heavy burden on healthcare systems.
- Traditional antimicrobial polymers (APs), composed of cationic and hydrophobic units, are inherently toxic due to their amphiphilic nature.
- **Non-amphiphilic APs** are thus developed and studied as safer alternatives.
- Non-amphiphilic APs stay isolated in solution but cluster via hydrogen bonds upon contact with negatively charged bacterial membranes.
- This study aims to show that hydrophobicity is not essential for APs, highlighting non-amphiphilic polymers as a new design motif.

Synthesis of Polymers

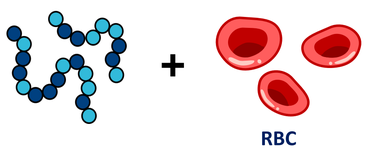




Polymer Synthesis and Characterization

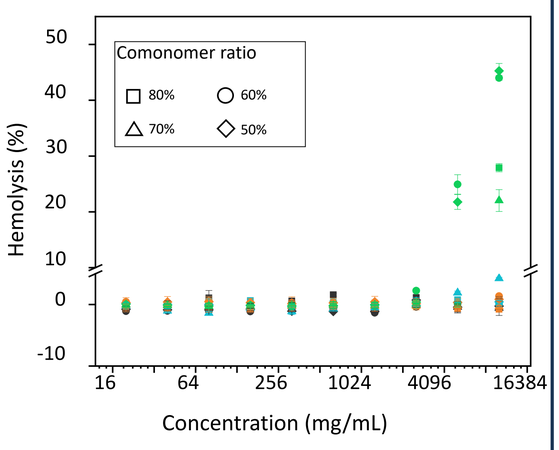
- This platform combines AEAm as the cationic unit with selected hydrophilic monomers (AAm, TrisAAm, MAm, AH) at varying comonomer ratios.
- Xanthate-supported photo-iniferter (XPI)-RAFT polymerization is employed to produce copolymers to ensure controlled and efficient polymerization process.
- Followed by deprotection and purification, targeted copolymers are produced.
- SEC traces show that the obtained polymers have desirable dispersity, with no shoulders or bimodal distributions.

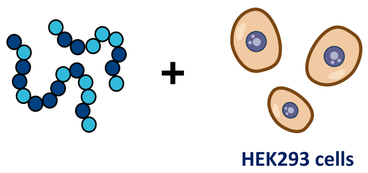
Hemotoxicity & Biocompatibility



Hemotoxicity

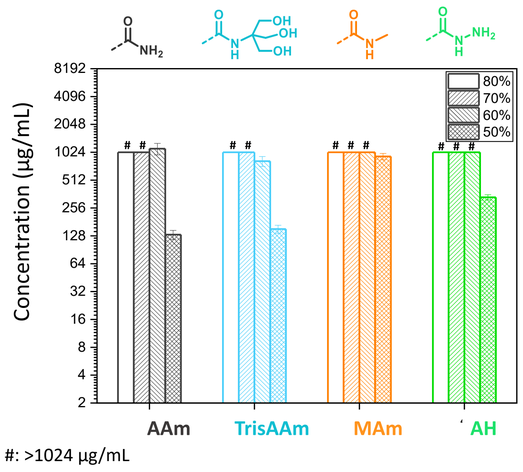
- Hemotoxicity tests reveal that most polymers are non-toxic to blood cells, with only a subset of acrylohydrazide (AH) copolymers exhibiting toxicity



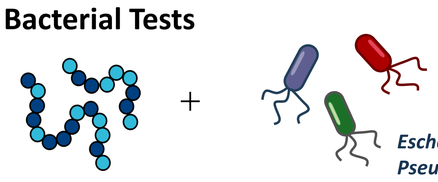


Cytotoxicity Test – CC50

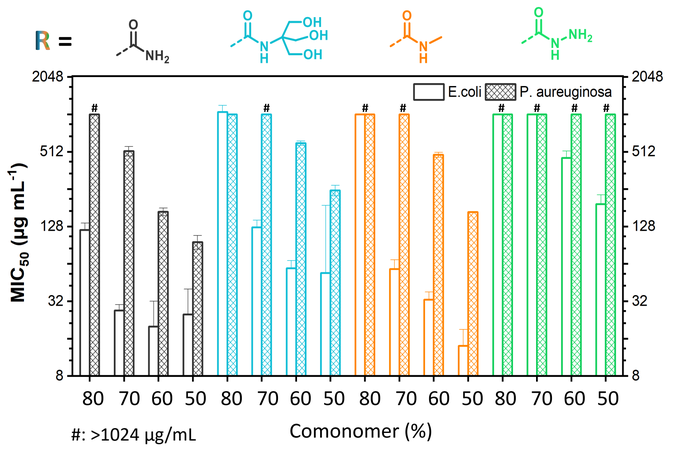
- Cytotoxicity on HEK cells is assessed using the resazurin assay.
- Increased cationic subunit content correlated with higher toxicity toward mammalian cells is observed.



Bacterial Tests



$$\text{Therapeutic index (TI)} = \frac{CC_{50,\ HEK\ cells}}{MIC_{50,\ bacteria}} \qquad \text{Selectivity index (SI)} = \frac{HC_{10,\ RBC}}{MIC_{50,\ bacteria}}$$



Comonmer	Ratio (%)	TI		SI	
		EC	*PA*	*EC*	*PA*
AAm	80	>9	-	>85	-
	70	**38**	>8.5	**>380**	>20
	60	**56**	7	**>512**	>61
	50	5	2	**>410**	>107
TrisAAm	80	1	-	>10	-
	70	>8	-	>81	-
	60	14	1.36	>174	>17
	50	3	1	>190	>41
MAm	80	-	-	-	-
	70	**18**	-	**>177**	-
	60	**31**	>2	**>310**	>21
	50	**59**	5.5	**>731**	>61
AH	80	-	-	<6	<6
	70	-	-	<6	<6
	60	>2	-	8	<4
	50	2	>0.3	22	<4

"-": No antibacterial activity or cytotoxicity was observed.

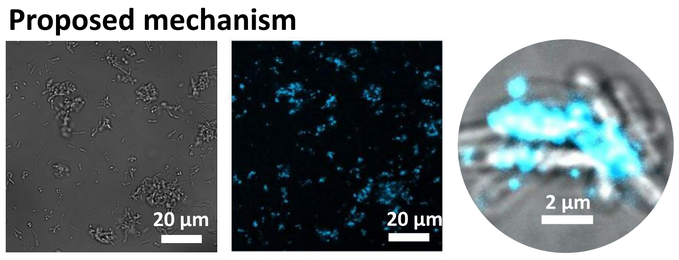
Antibacterial Tests - MIC_{50} value

- A subset of copolymers shows strong activity against *E. coli* and *P. aeruginosa*, except those containing AH.
- Polymer bioactivity is notably enhanced when the cationic charge and specific subunit ratio are balanced at 50:50.

TI & SI Assessment

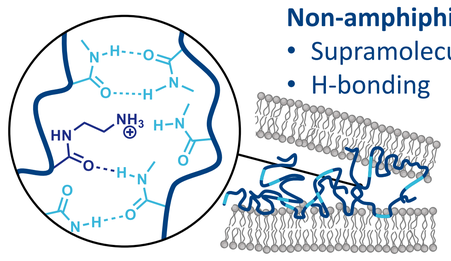
- A subset of AAm and MAm copolymers shows a high TI and SI, indicating strong activity against *E. coli and P. aeruginosa.*

Proposed mechanism



Confocal image

- Confocal microscopy images of bacteria treated with dye-labeled copolymers show that the non-amphiphilic polymers accumulate on the bacterial cell envelope and induce significant aggregation[4].



Non-amphiphilic APs

- Supramolecular multivalency & aggregation
- H-bonding

Polymer Recruiting Mechanism

- Non-amphiphilic polymers recruit each other via hydrogen bonding, leading to local enrichment on the bacterial membrane and enhancing disruption.

Conclusion and outlook

- A series of non-amphiphilic polymers was successfully synthesized, purified, and well-characterized.
- AAm and MAm copolymer series demonstrated significant selectivity against *E. coli*, acting as a promising material.
- Supported by prior research and current findings, this study confirms that hydrophobicity is not essential for effective AP design.

Reference

1. Pham, P.; Oliver, S.; Boyer, C., Design of Antimicrobial Polymers. *Macromolecular Chemistry and Physics* 2023, *224* (3), 2200226.
2. .Jain, A.; Duvvuri, L. S.; Farah, S.; Beyth, N.; Domb, A. J.; Khan, W., Antimicrobial Polymers. *Advanced Healthcare Materials* 2014, *3* (12), 1969-1985.
3. Ergene, C.; Yasuhara, K.; Palermo, E. F., Biomimetic antimicrobial polymers: recent advances in molecular design. *Polymer Chemistry* 2018, *9* (18), 2407-2427.
4. Bapolisi, A. M.; Lehnen, A.-C.; Wolff, M.; Kramer, J.; Kogikoski, S.; Steinbrecher, R.; Michler, N.; Kiesow, A.; Bald, I.; Obry, M.; Kersting, S.; Stensitzki, T.; Müller-Werkmeister, H. M.; Leiske, M. N.; Chiantia, S.; Hartlieb, M. Non-Amphiphilic Antimicrobial Polymers. Angewandte Chemie International Edition 2025, DOI: 10.1002/anie.202507564.

Acknowledgement

Emmy Noether-Programm

DFG Deutsche Forschungsgemeinschaft

Fraunhofer IAP

Fraunhofer IZI-BB