THE UNIVERSITY OF
MELBOURNE

Bile-acid derived monomer and polymers as a broad-spectrum antimicrobial agent

Yijun Xiong

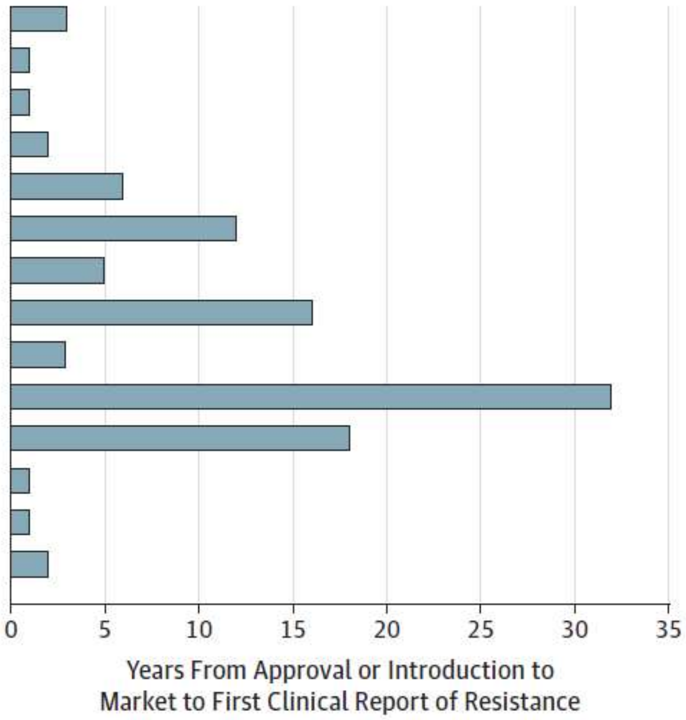
Antimicrobial resistance (AMR)

- AMR results in both health and economic cost

- Slow development of new antibiotic while fast AMR emergence

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

Class	Antibiotic	Year of Approval or Introduction to Market
β-Lactams	Penicillin	1942
	Methicillin	1960
	Cephalothin	1964
	Amoxicillin-clavulanic acid	1984
Carbapenems	Imipenem-cilastatin	1985
Amphenicols	Chloramphenicol	1950
Tetracyclines	Tetracycline	1953
Aminoglycosides	Streptomycin	1946
Macrolides	Erythromycin	1952
Glycopeptides	Vancomycin	1958
Quinolones	Nalidixic acid	1964
Streptogramins	Quinupristin-dalfopristin	1999
Oxazolidinones	Linezolid	2000
Lipopeptides	Daptomycin	2003



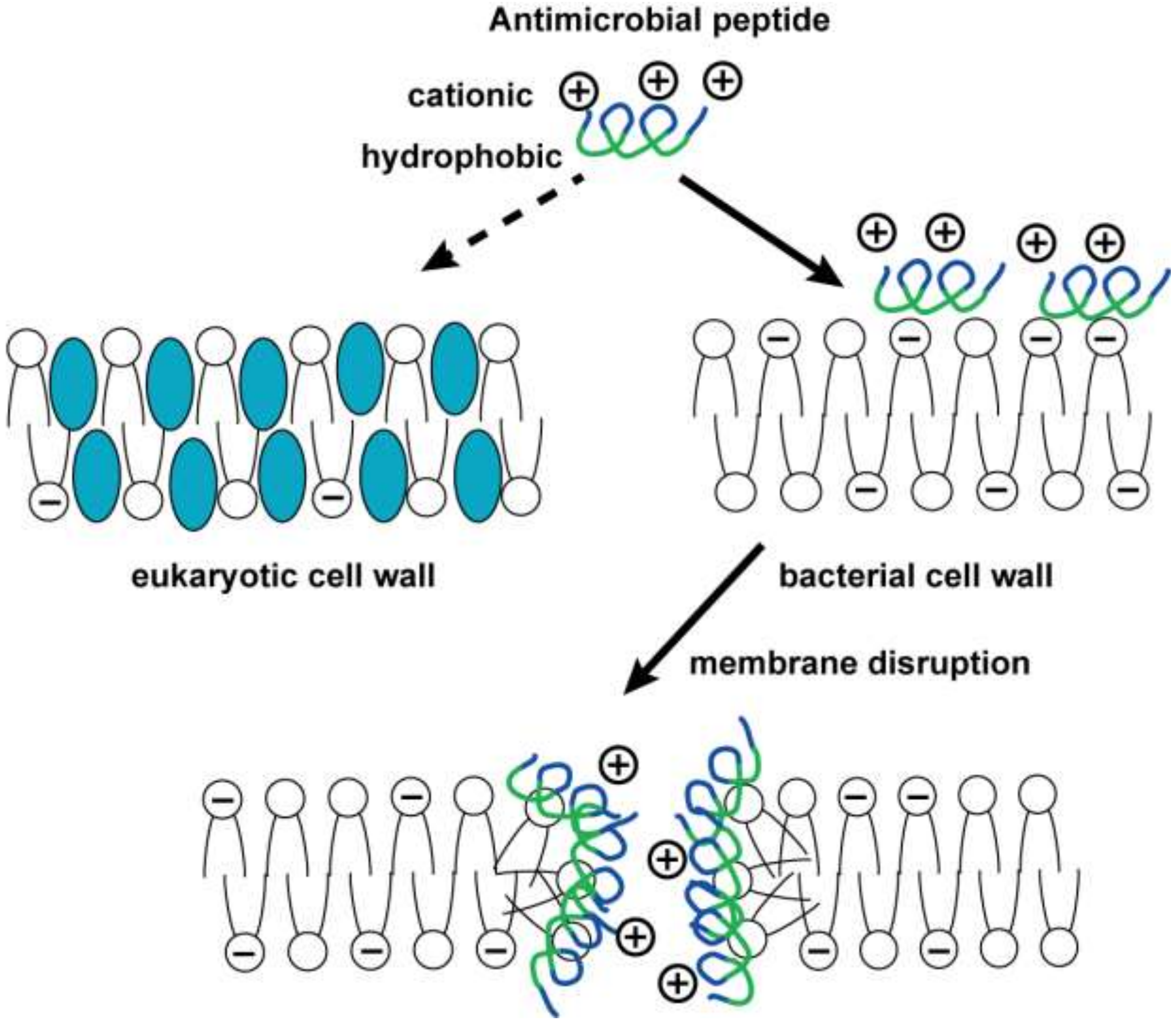
Marston, Hilary D., et al. "Antimicrobial resistance." *Jama* 316.11 (2016): 1193-1204.

Antimicrobial peptides (AMPs)

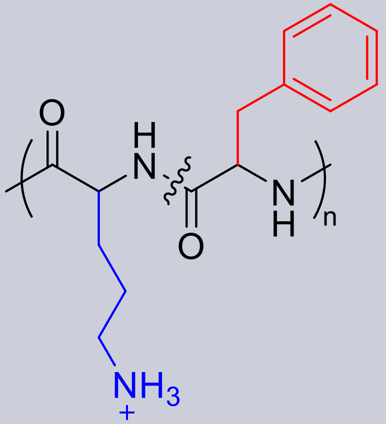
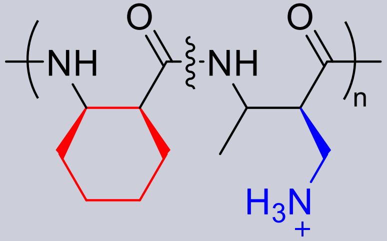
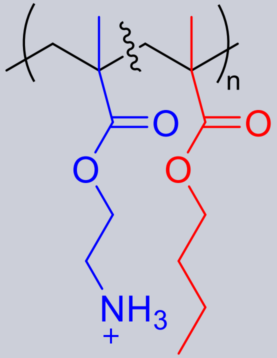
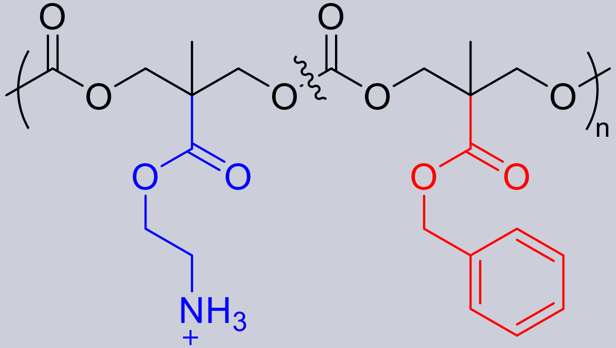
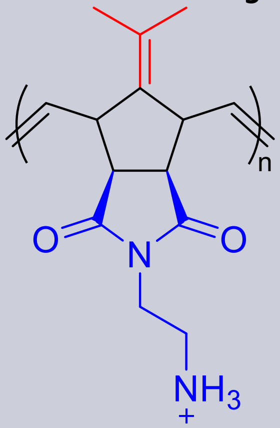
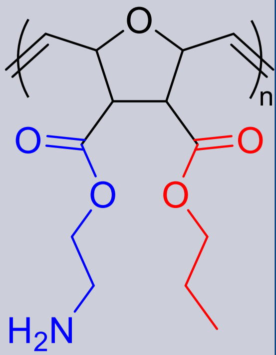
• AMPs feature a cationic charge and a substantial hydrophobic residues

• Cationic charge renders selectivity towards negatively charged microbial membrane instead of zwitterionic mammalian membrane

• Hydrophobicity facilitates membrane interaction

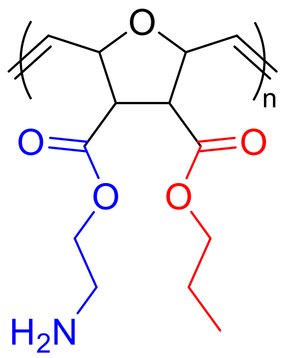


Antimicrobial polymers

Synthetic peptide		polymethacrylate	polycarbonate	polynorbornene	
1 	2 	3 	4 	5 	6 

[1] Zhou, Chuncai, et al. "High potency and broad-spectrum antimicrobial peptides synthesized via ring-opening polymerization of α-aminoacid-N-carboxyanhydrides." *Biomacromolecules* 11.1 (2010): 60-67.
[2] Mowery, Brendan P., et al. "Mimicry of antimicrobial host-defense peptides by random copolymers." *Journal of the American Chemical Society* 129.50 (2007): 15474-15476.
[3] Kuroda, Kenichi, and William F. DeGrado. "Amphiphilic polymethacrylate derivatives as antimicrobial agents." *Journal of the American Chemical Society* 127.12 (2005): 4128-4129.
[4] Nimmagadda, Alekhya, et al. "Polycarbonates with potent and selective antimicrobial activity toward gram-positive bacteria." *Biomacromolecules* 18.1 (2017): 87-95.
[5] Ilker, M. Firat, et al. "Tuning the hemolytic and antibacterial activities of amphiphilic polynorbornene derivatives." *Journal of the American Chemical Society* 126.48 (2004): 15870-15875.
[6] Lienkamp, Karen, et al. "Antimicrobial polymers prepared by ROMP with unprecedented selectivity: a molecular construction kit approach." *Journal of the American Chemical Society* 130.30 (2008): 9836-9843.

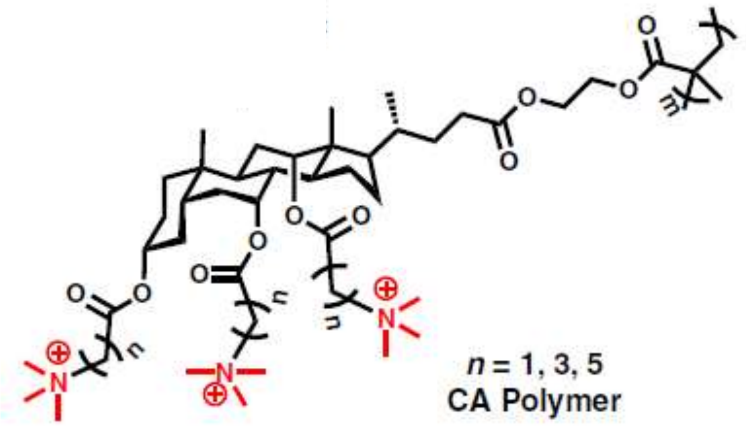
Antimicrobial polymers



	MIC$_{90}$ (µg/mL)		
Sample	*E. coli*	*S. aureus*	HC$_{50}$ (µg/mL)
monomer (n=1)	>200	>200	n.d.
oligomer (n=2)	200	<3.75	1050
oligomer (n=3)	100	6.25	800
oligomer (n=4)	200	25	1250
oligomer (n=5)	>200	50	>2000
oligomer (n=6)	100	50	1000
oligomer (n=7)	50	25	150
polymer (Mn~3k)	6.25	15	50
polymer (Mn~10k)	3.75	200	<50

Lienkamp, Karen, et al. "Antimicrobial polymers prepared by ROMP with unprecedented selectivity: a molecular construction kit approach." *Journal of the American Chemical Society* 130.30 (2008): 9836-9843.

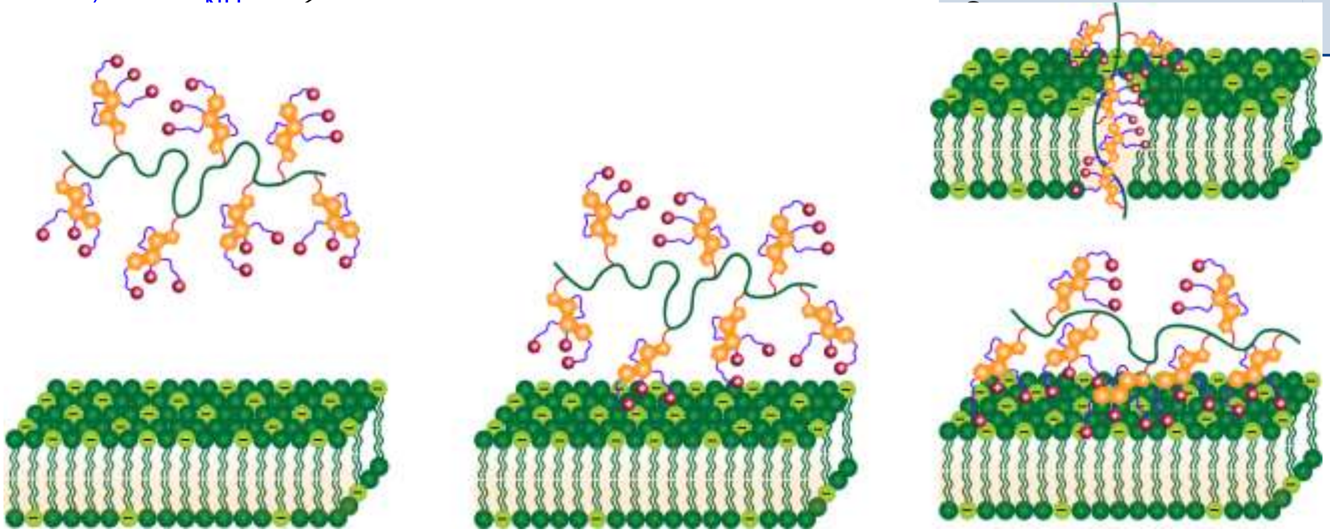
Ceragenins



n = 1, 3, 5
CA Polymer

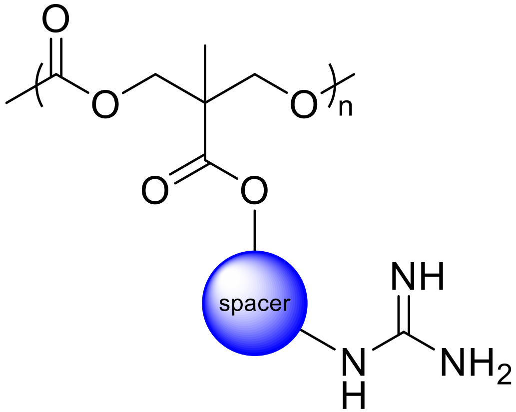
	MIC (µg/mL)			
sample	E. coli	P. aeruginosa	S. aureus	HC_{50} (µg/mL)
monomer	22.3	12.8	25.6	n.d.
polymer (Mn~10k)	6.4	3.0	19.1	>110
polymer (Mn~19k)	11.2	3.1	15.1	>306
polymer (Mn~25k)	11.4	10.5	11.9	>315
polymer (Mn~32k)	12.2	19.4	27.4	>1886

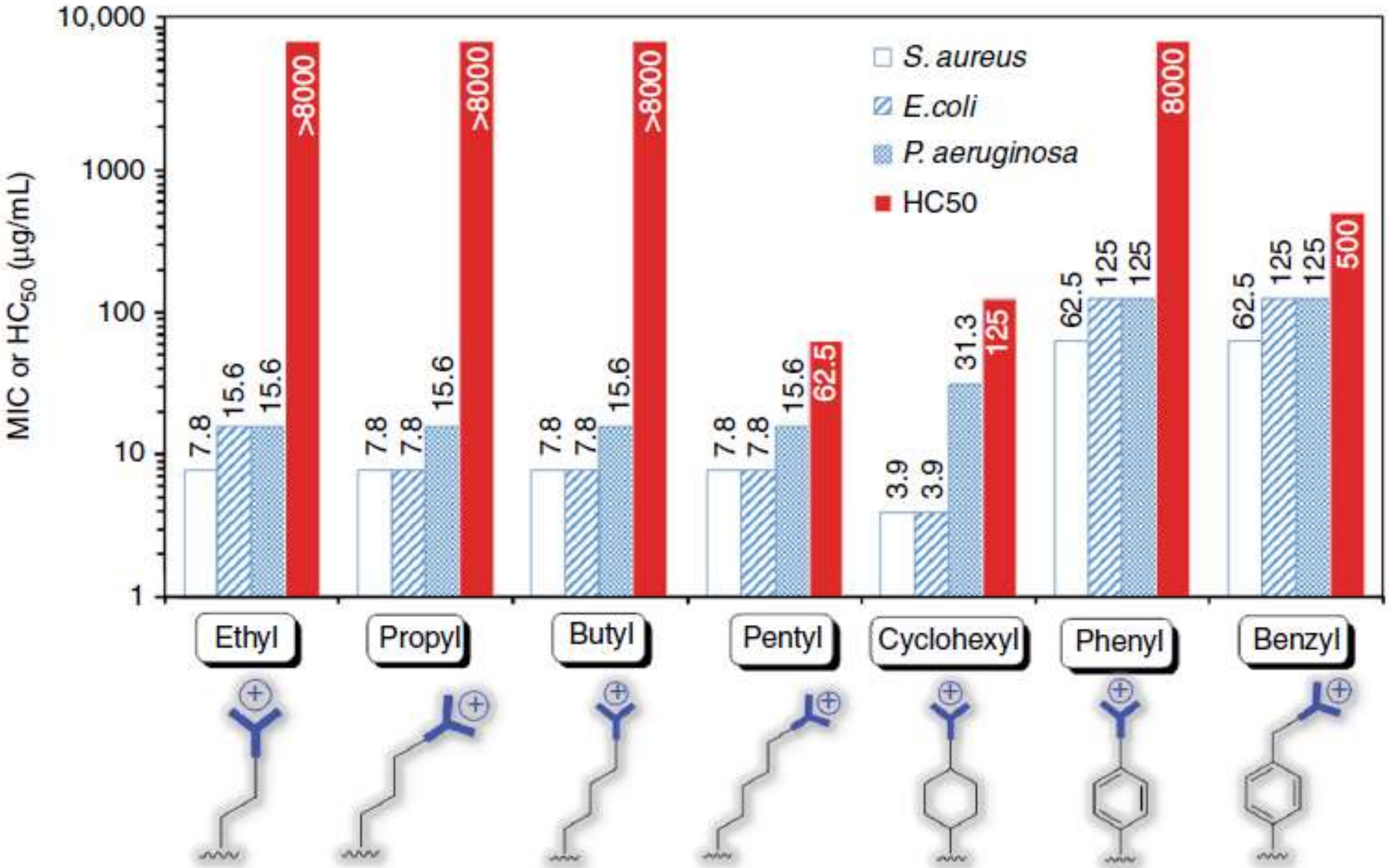
	MIC (µg/mL)
E. coli	3.0
P. aeruginosa	2.0
	0.4



Rahman, Md Anisur, et al. "Macromolecular-clustered facial amphiphilic antimicrobials." *Nature Communications* 9.1 (2018): 5231.

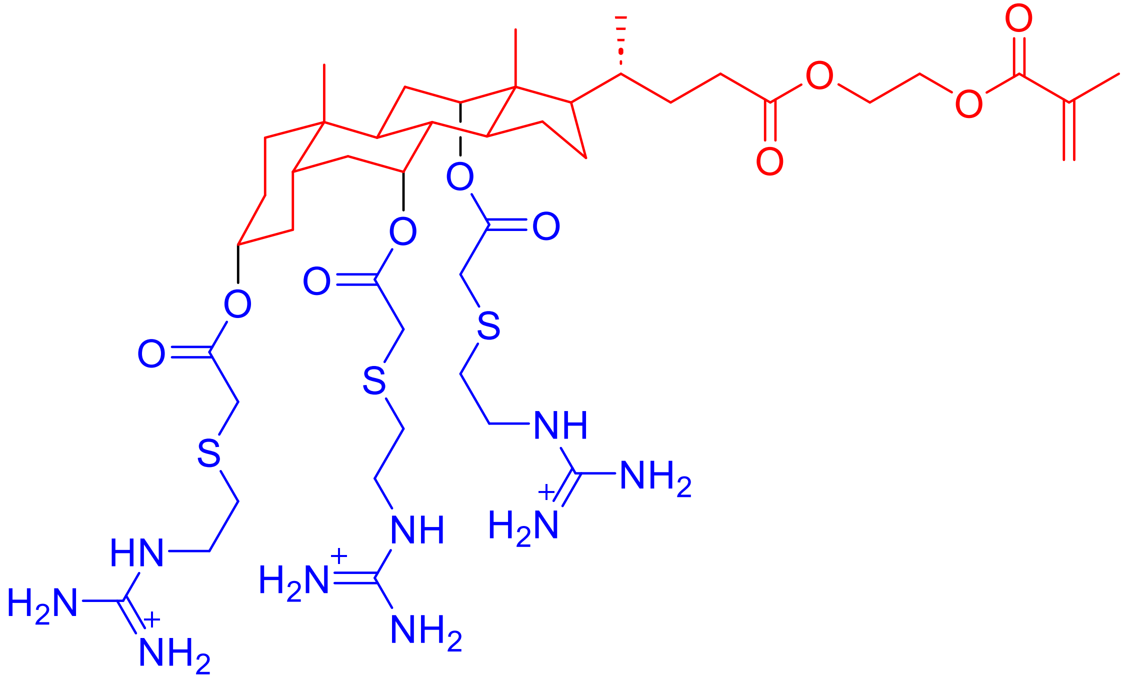
Guanidine





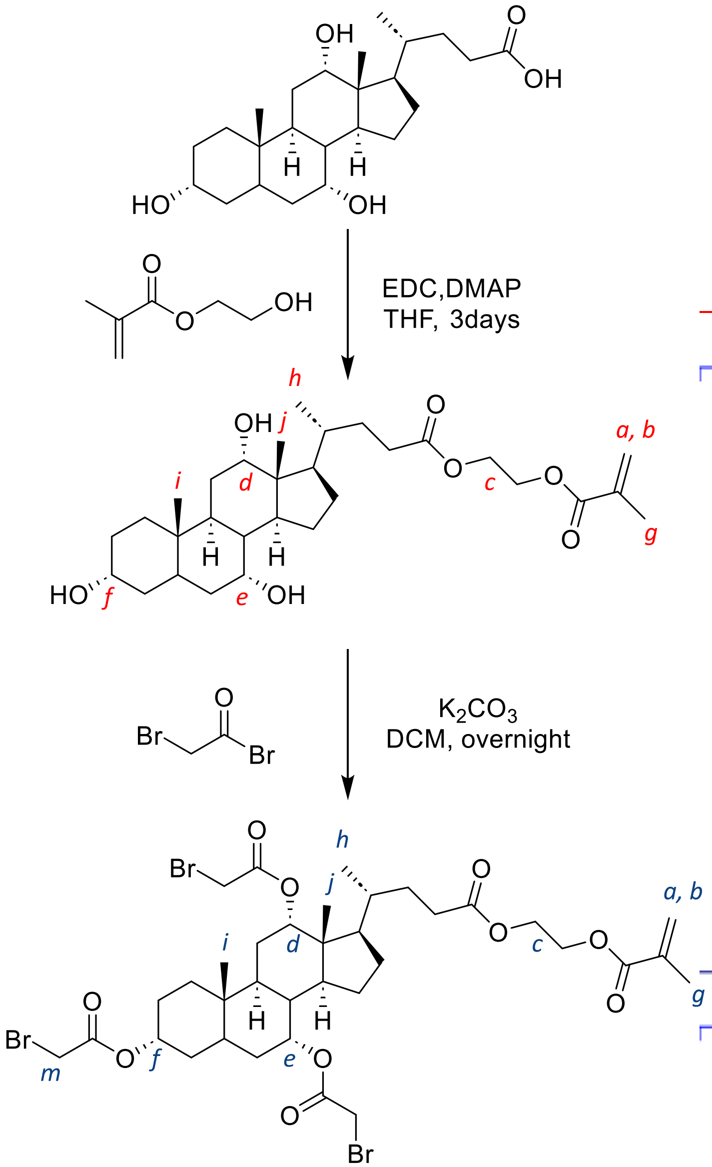
Chin, Willy, et al. "A macromolecular approach to eradicate multidrug resistant bacterial infections while mitigating drug resistance onset." *Nature communications* 9.1 (2018): 917.

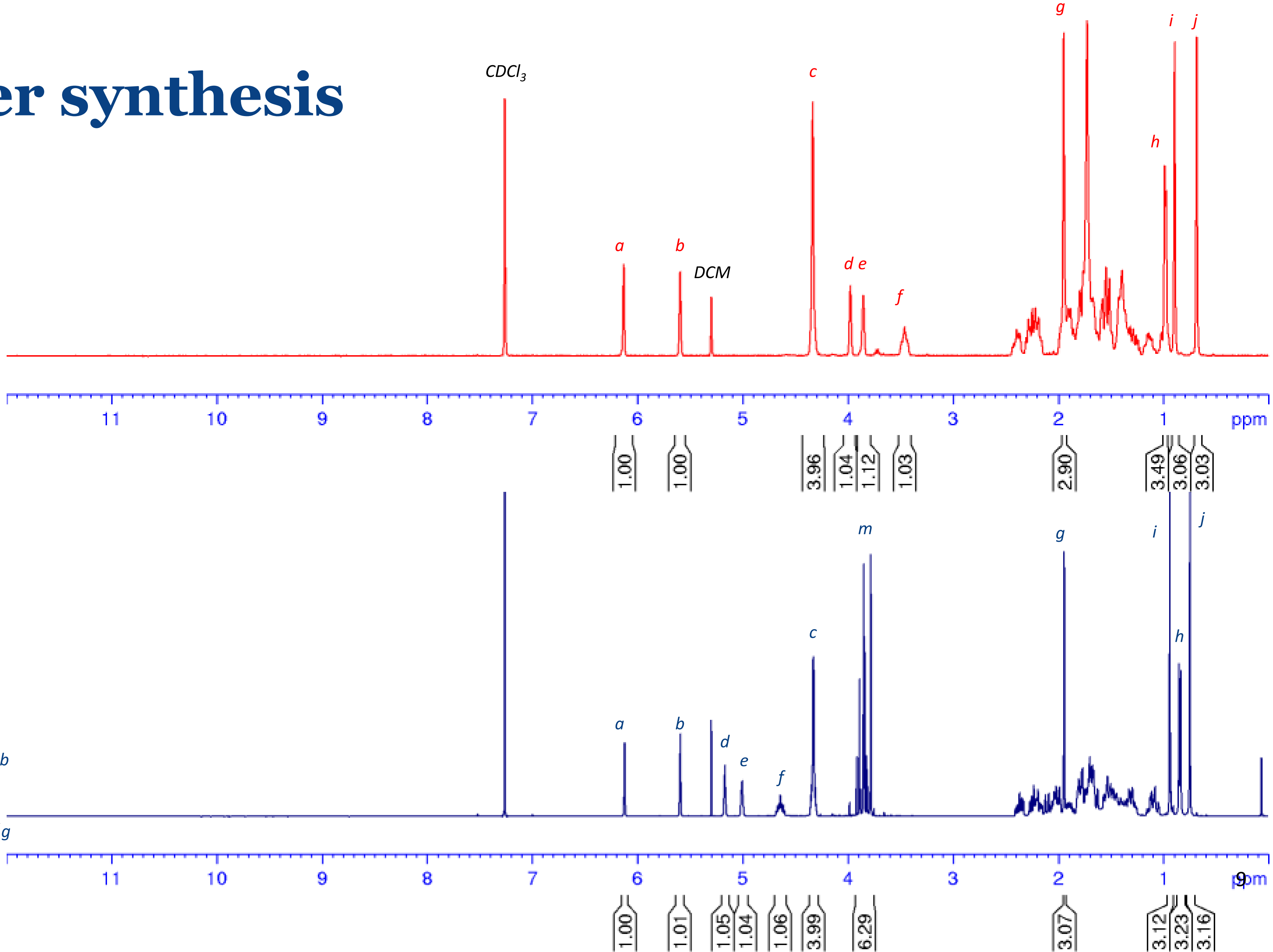
Design



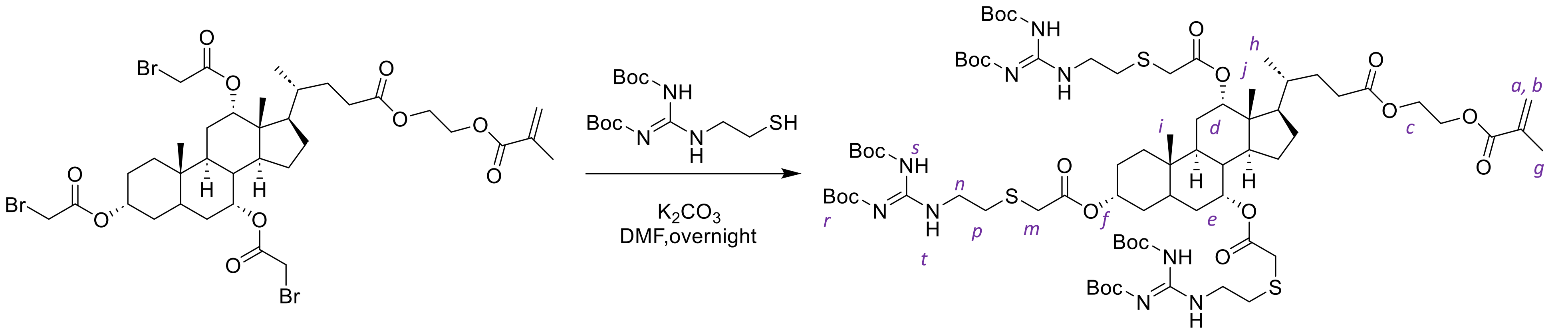
- Facial amphiphilicity
- Guanidine featured

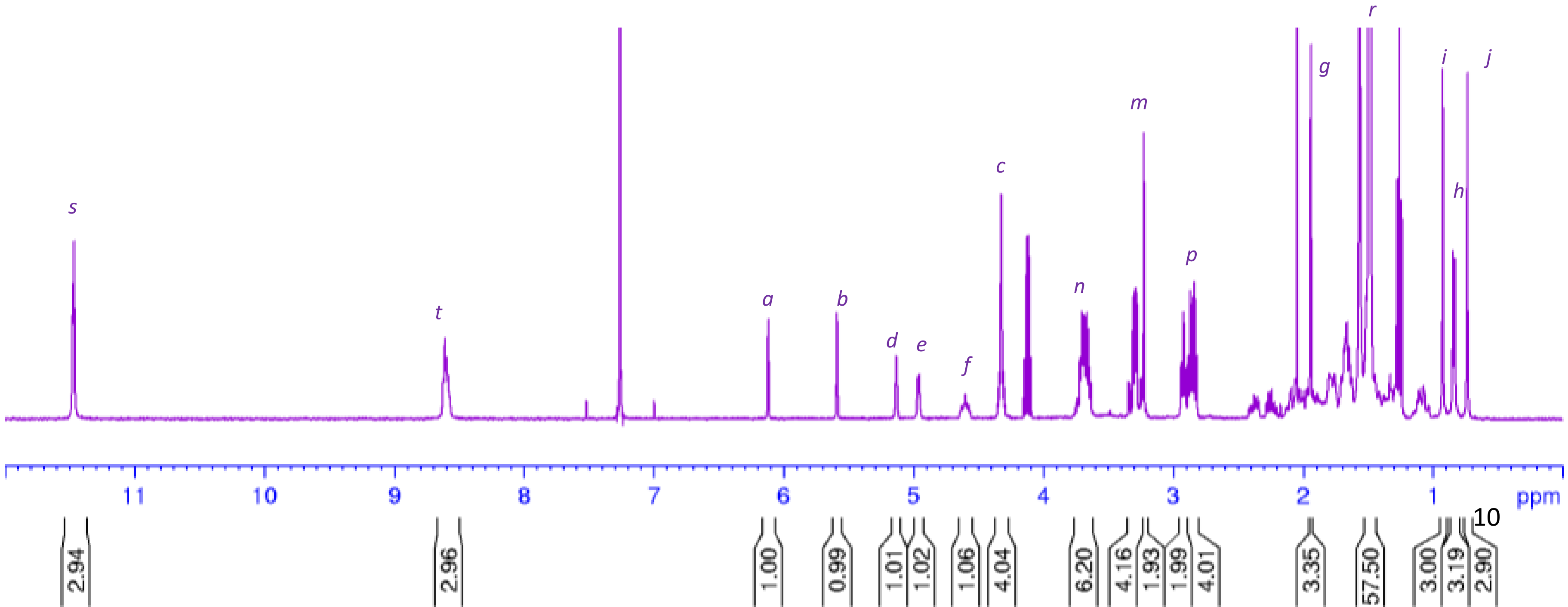
Monomer synthesis



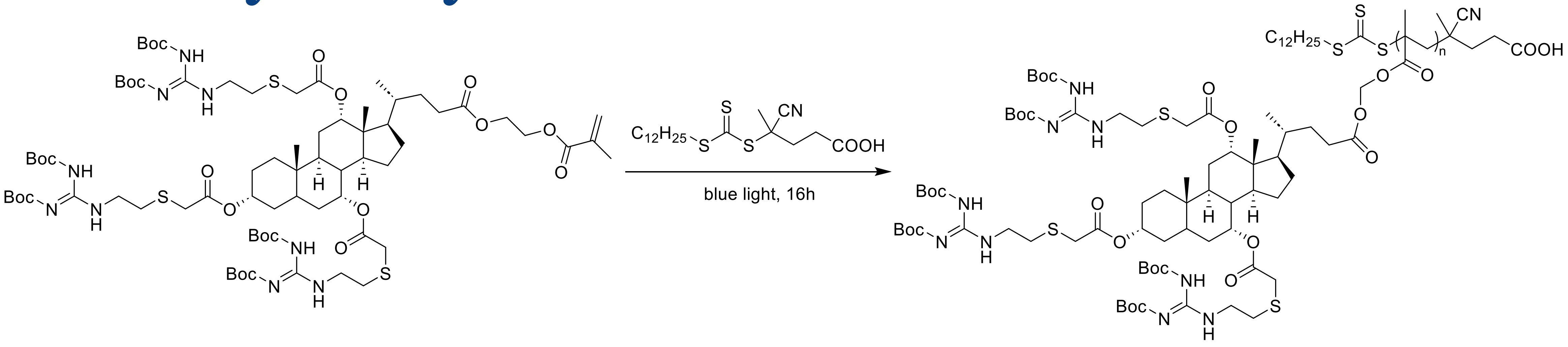


Monomer synthesis





Polymer synthesis

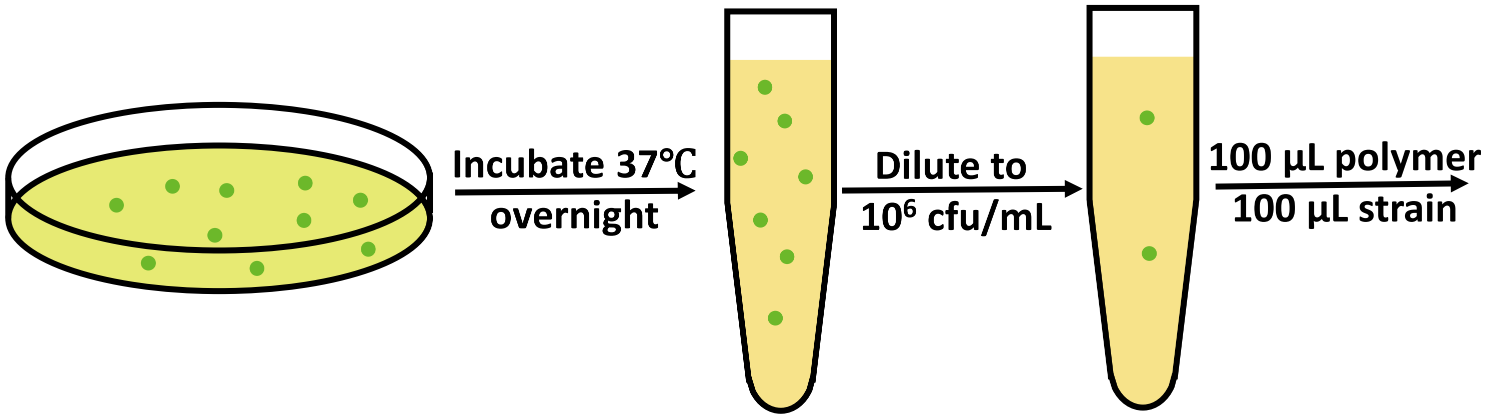


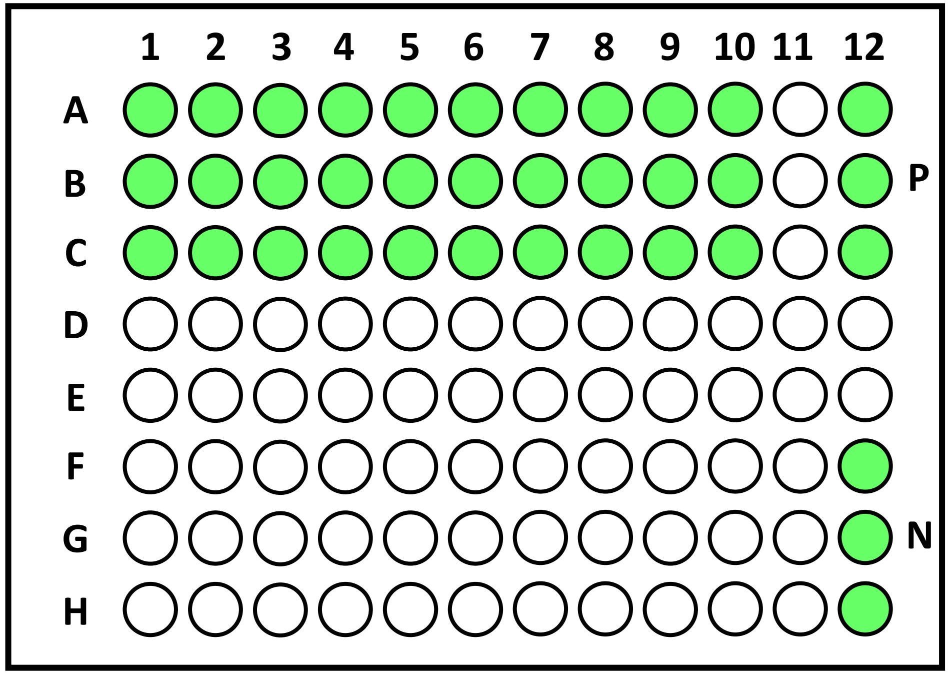
PG₃₋₁₄

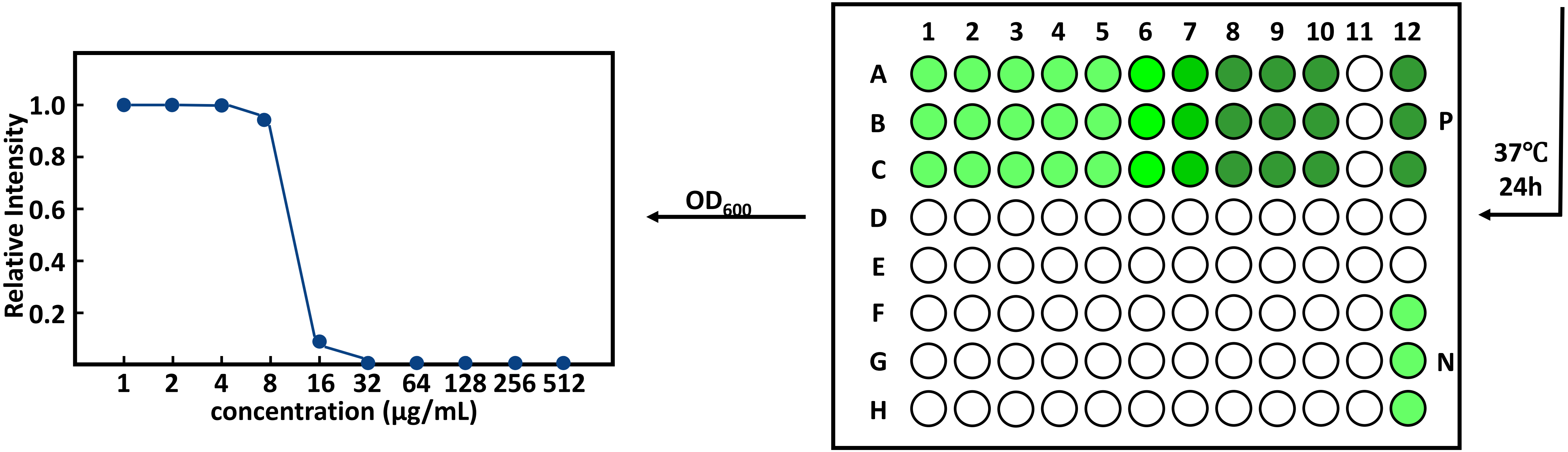
Entry	Mn (SEC)[a] (kDa)	DP	Mn (theor.)[b] (kDa)	Đ
PG_3	8.8	3	4.8	1.16
PG_6	10.7	6	10.6	1.20
PG_{14}	11.5	14	22.8	1.35
PA_9	10.4	9	10.8	1.21

[a]Mn and Đ were calculated from GPC characterization using THF as GPC mobile phase.
[b]Mn and DP were calculated from the crude ^1H NMR and the ratio of monomer/RAFT.

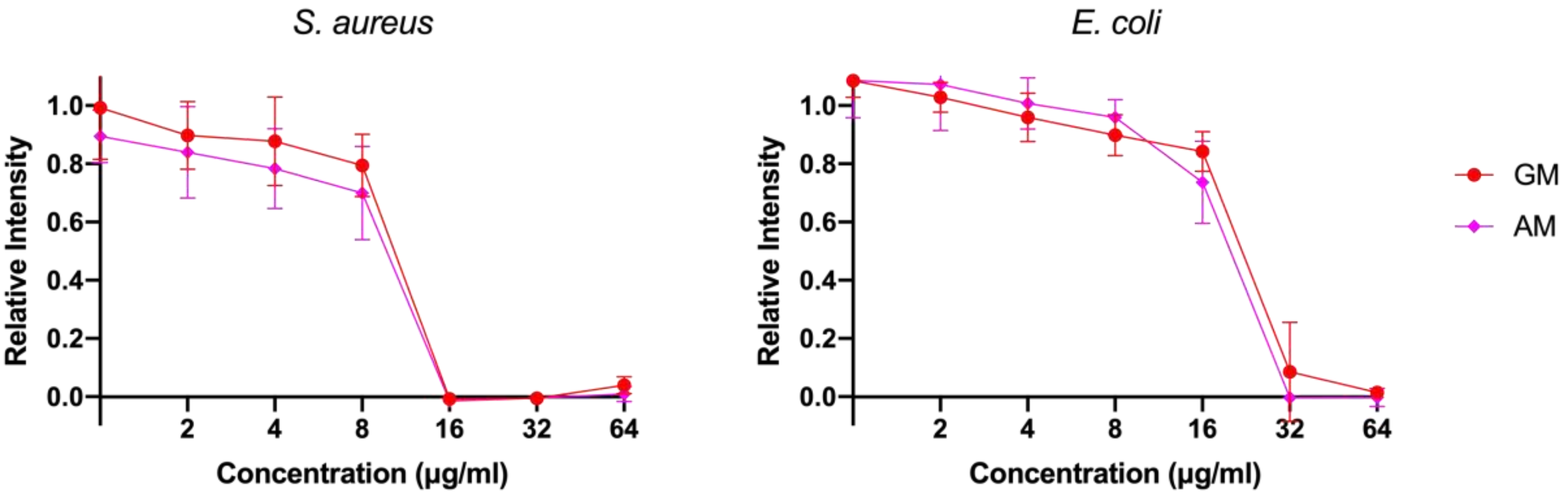
Antimicrobial tests





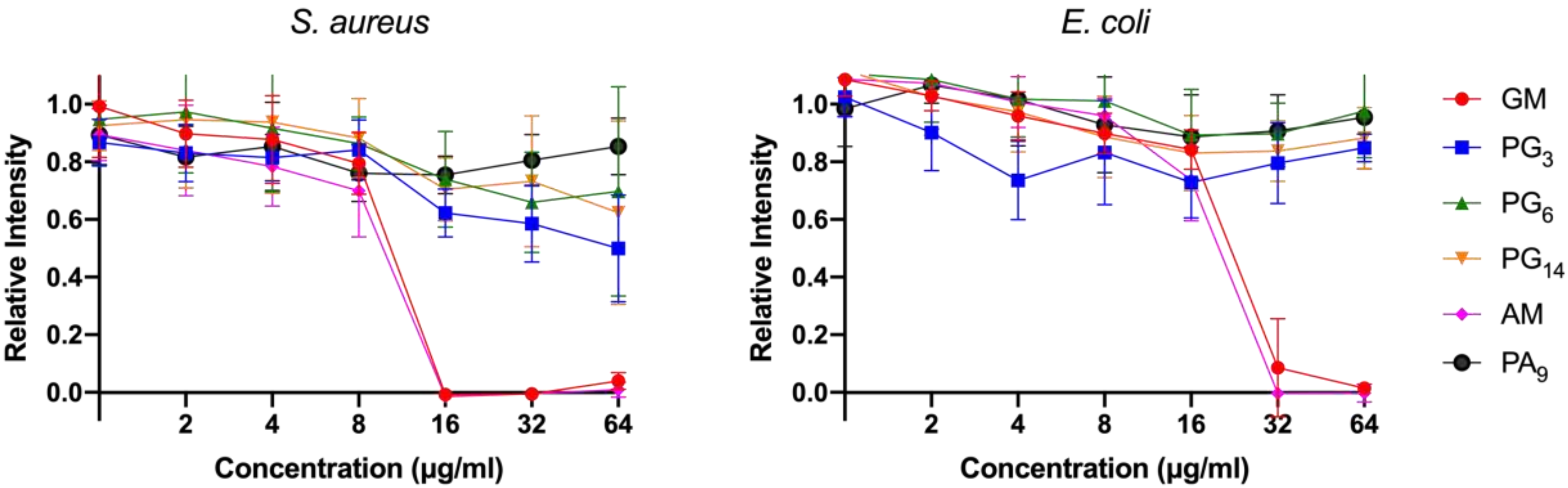


Antimicrobial tests



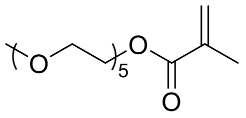
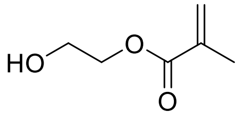
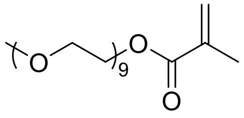
GM = guanidine monomer
AM = primary amine monomer

Antimicrobial tests



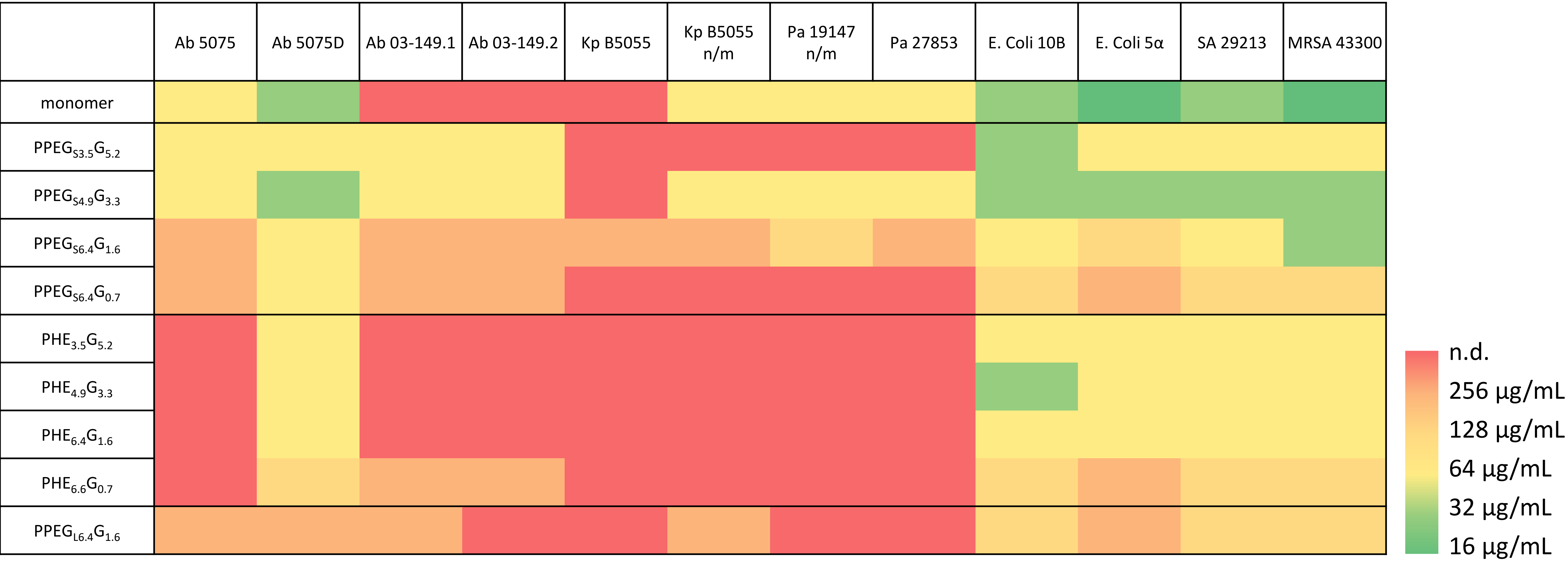
GM = guanidine monomer
AM = primary amine monomer

Copolymerization

Entry	Copolymer Ratio(%)	DP	Mn(theor.)[b] (kDa)	Đ[a]	Solubility in CaMHB (µg/mL)	Copolymer
$PPEG_{S3.5}G_{5.2}$	40	9	8.5	1.42	16	 Mw = 300 Da PEG_S
$PPEG_{S4.9}G_{3.3}$	60	8	6.3	1.62	32	
$PPEG_{S6.4}G_{1.6}$	80	8	4.5	1.39	512	
$PPEG_{S6.4}G_{0.7}$	90	7	3.3	1.33	512	
$PHE_{3.5}G_{5.2}$	40	9	7.8	1.42	16	 HE
$PHE_{4.9}G_{3.3}$	60	8	5.4	1.41	16	
$PHE_{6.4}G_{1.6}$	80	8	3.4	1.36	16	
$PHE_{6.6}G_{0.7}$	90	7	2.2	1.80	64	
$PPEG_{L6.4}G_{1.6}$	80	8	5.7	1.81	512	 Mw = 500 Da PEG_L

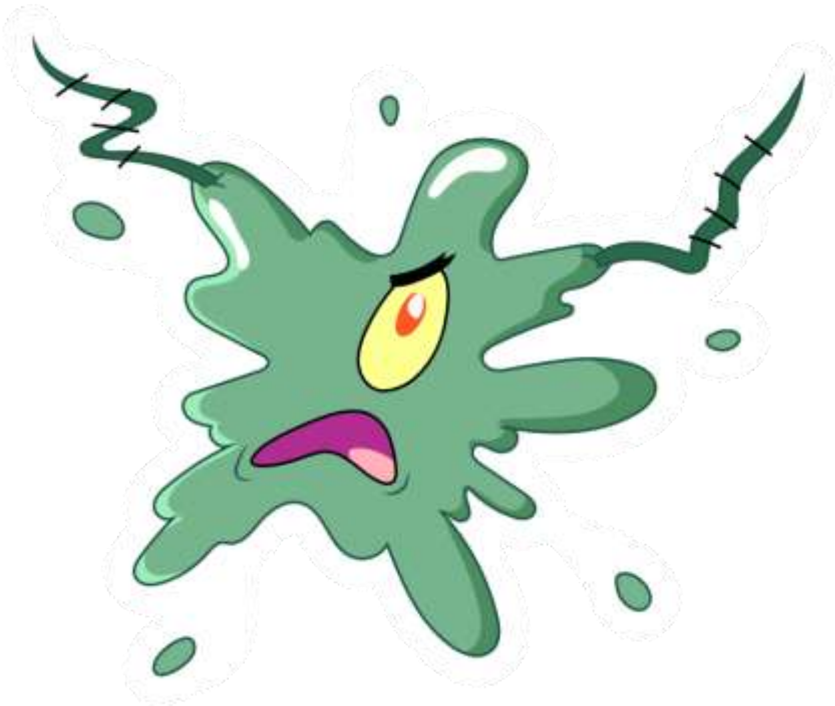
[a] Đ was calculated from GPC characterization using THF as GPC mobile phase.
[b] Mn and DP were calculated from the crude ^1H NMR and the ratio of monomer/RAFT.

Antimicrobial tests



Conclusion

- Synthesis of a new antimicrobial guanidine monomer and its polymers
- Homopolymer lost activity compared to its monomer
- Recovery of activity by copolymerization



Thanks

To my supervisors:
Dr. Georgina Such,
Dr Ben Muir
and everyone in my group

Design issue