The Impact of Polymer Architecture on the Membrane Interaction of Antimicrobial Polymers

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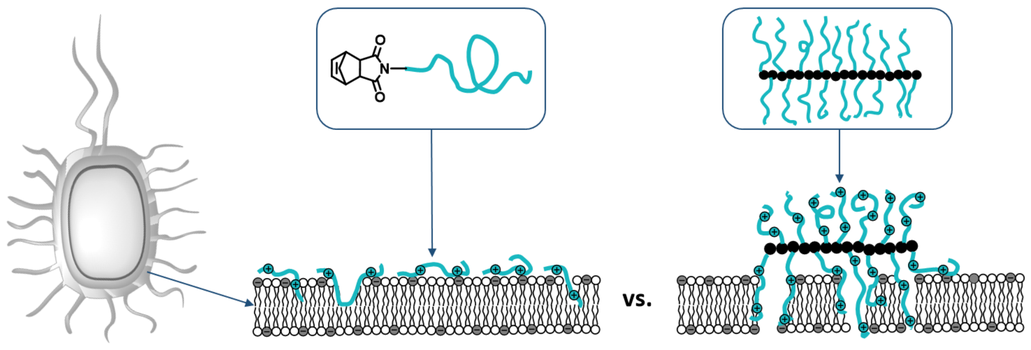
Motivation

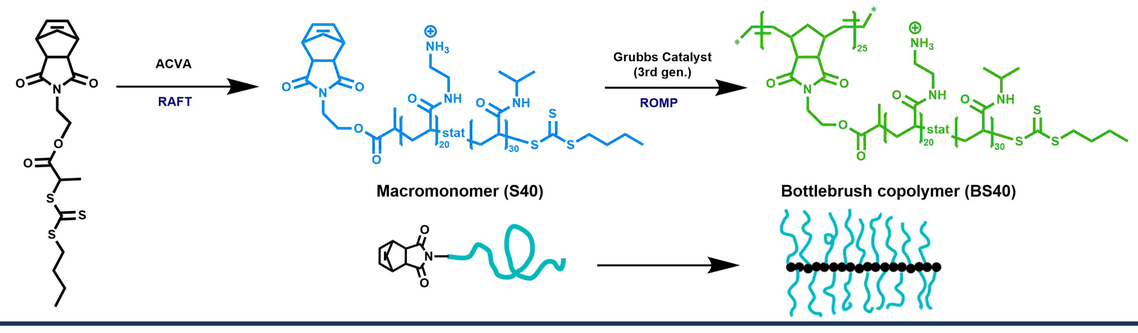
Antimicrobial resistance (AMR) is an increasing threat to global public health as the number of effective antibiotics decreases. The development of antimicrobial polymers (APs) is a promising potential solution to this problem as APs target the bacterial membrane and are therefore less susceptible to development of AMR. In addition to the amphiphilic balance, the polymer architecture plays an important role in the structure-activity relationship.

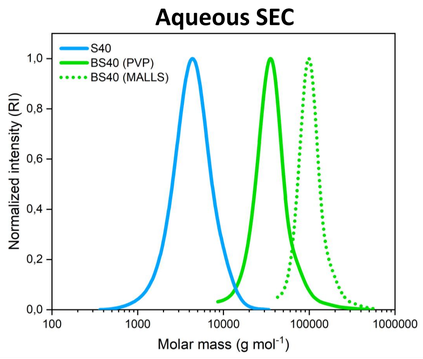
We previously developed multivalent APs of bottlebrush architecture (**BS40**) which showed to possess improved antibacterial activity and hemocompatibility compared to their linear counterpart (**S40**), despite their identical amphiphilic composition ratio (40% cationic monomer).

The herein presented work elucidates the differences between the intrinsic physicochemical characteristics of the two polymer architectures and their mechanisms of interaction with bacterial membranes using liposomes as cell models.



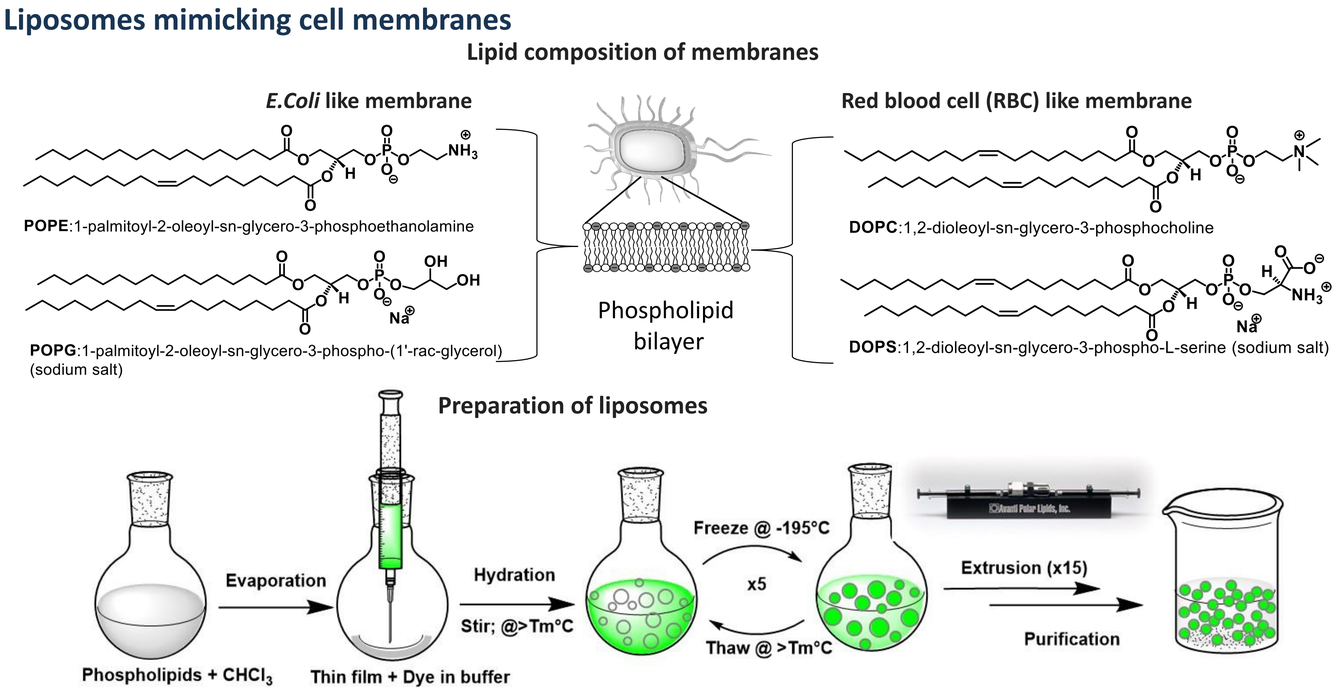
Synthesis and characterization of polymers





Polymer	SEC (PVP Calibration) M_n (g mol^{-1})	Đ	SEC (LS calibration) M_n (g mol^{-1})	Đ	dn/dc	DP	pH titration pK_a
S40	3,600	1.35	-	-	-	-	8.0
BS40	32,000	1.32	99,000	1.16	0.135	28	7.5

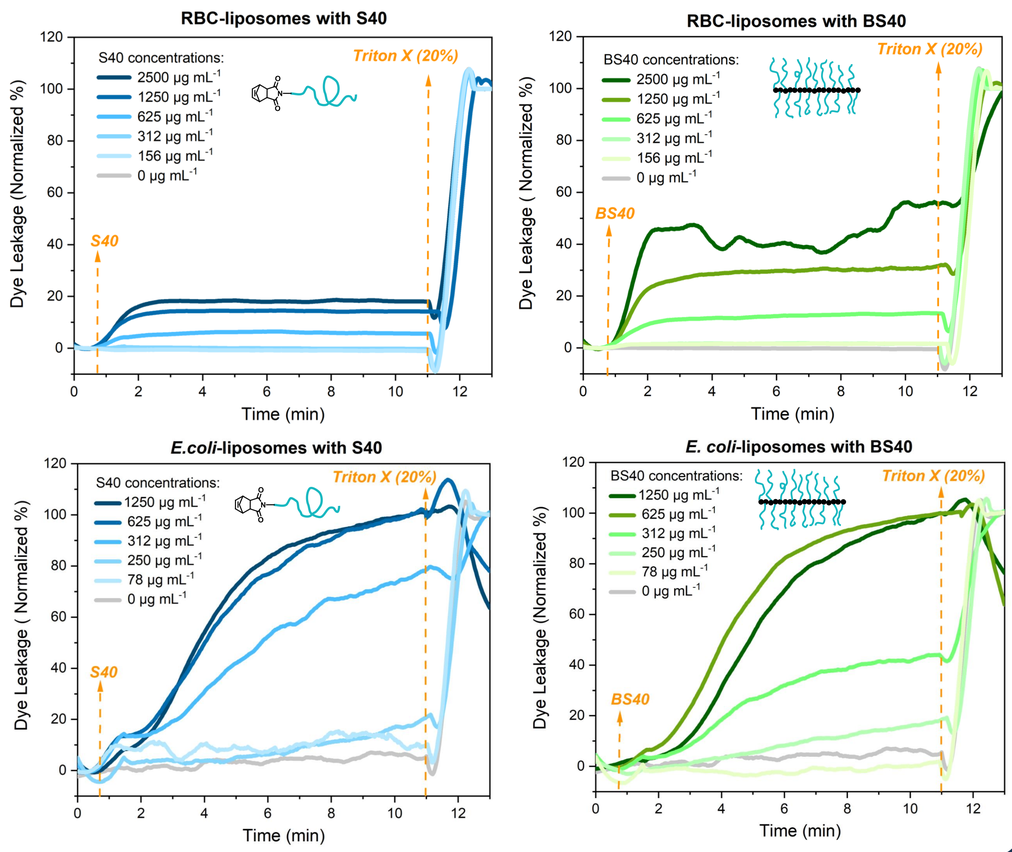
Liposomes mimicking cell membranes



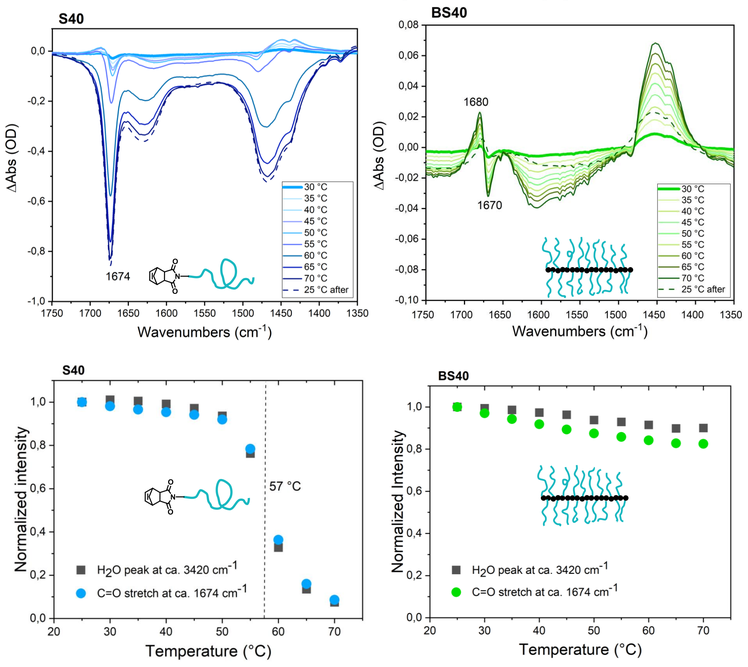
- Both linear S40 and bottlebrush BS40 copolymers induced a concentration-dependent dye leakage from both liposomes' types
- Both APs are selective toward *E.coli*-liposomes, confirming their hemocompatibility

Polymer	Dye leakage fluorimetry assays	
	E. coli-liposomes EC_{50} (µg mL^{-1})	RBC-liposomes EC_{50} (µg mL^{-1})
S40	279	> 2,500
BS40	344	> 2,500

Fluorescent dye leakage from liposomes

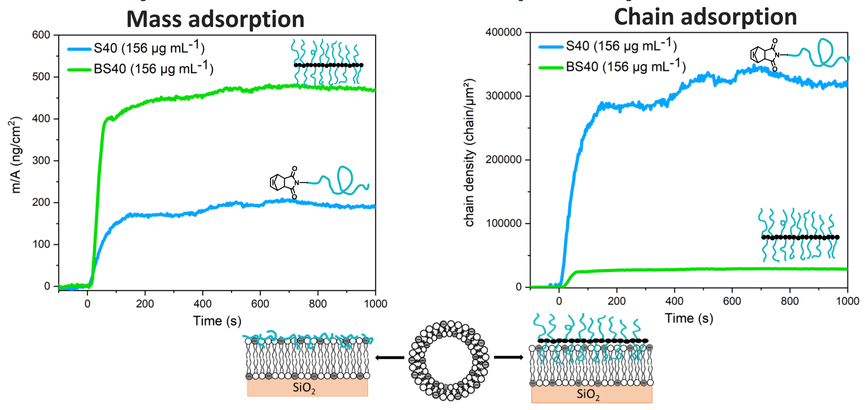


Fourier-transform infrared spectroscopy

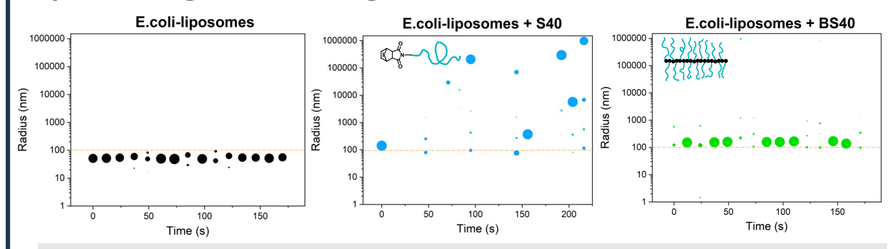


- The linear S40 is more solvated and characterized by facial amphiphilicity and thermo-sensitivity, with irreversible changes in vibrational transitions
- The bottlebrush BS40 has a more stable secondary conformation with the presence of more H-bonding between C=O and ammonium

Quartz Crystal Microbalance: solid lipid bilayer

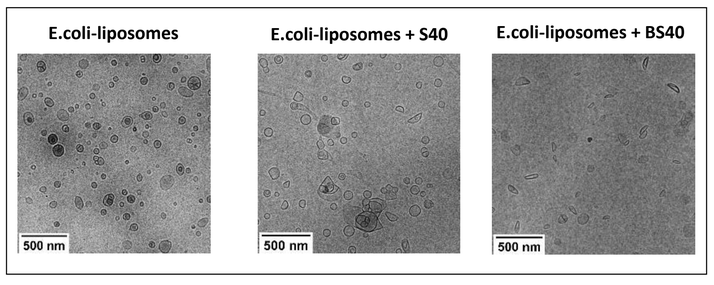


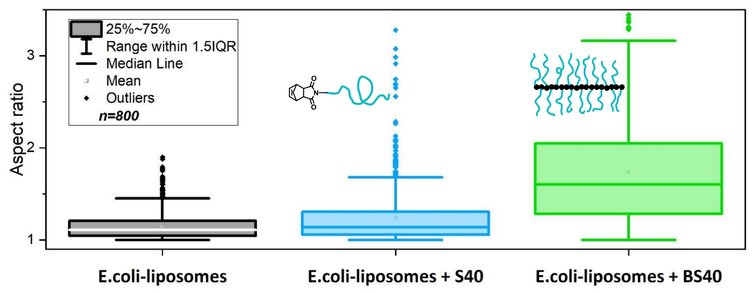
Dynamic Light Scattering: radius distribution

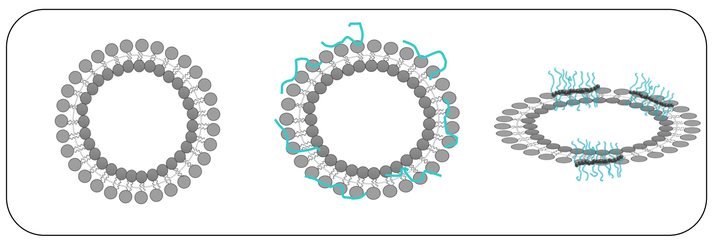


- The linear chains S40 are adsorbed in abundance on liposomes surface and with higher propensity to random aggregation
- The bottlebrushes BS40 are adsorbed faster on membranes' surface thanks to their multivalence and at higher mass per area due to their density and, therefore, inducing significant changes in aspect ratio of liposomes

Cryo-Electron Microscopy







Conclusion and Outlook

The linear and bottlebrush polymers proved to be more selective to *E.coli* like liposomes than to red blood cell like liposomes. The densely packed architecture of bottlebrush copolymers with stable secondary conformation and their multivalence could explain the advantageous and well-defined mechanism of interaction with bacterial membranes. Future work will focus on designing bottlebrush copolymers of different grafting densities to potentialize the activity of these promising APs.

References

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