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Singapore Centre for Environmental Life Sciences Engineering

Conjugated Oligoelectrolytes: A Versatile Platform for Membrane Modifications

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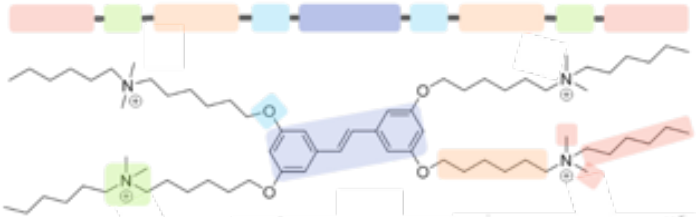
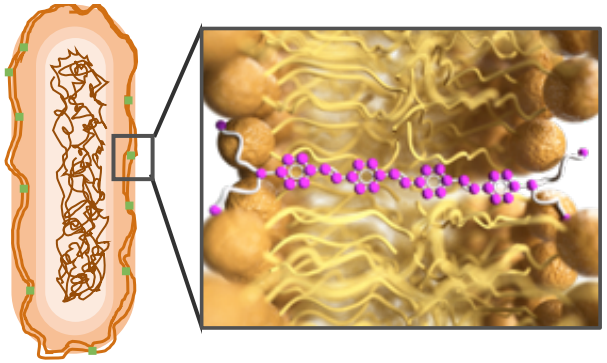
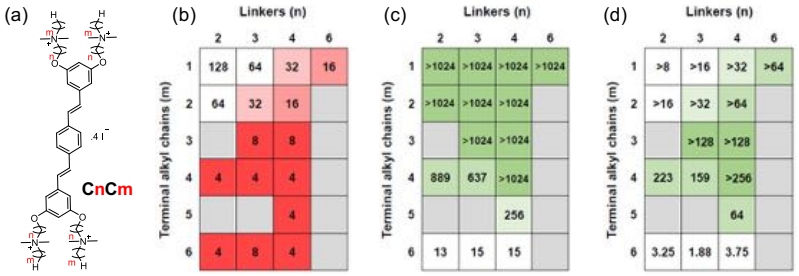
Outline

Conjugated *Oligo*electrolytes

- -structural considerations
- -impact on membrane properties
- -modification of biophysical characteristics
- -impact of molecular structure on antibiotic activity
- -translational efforts

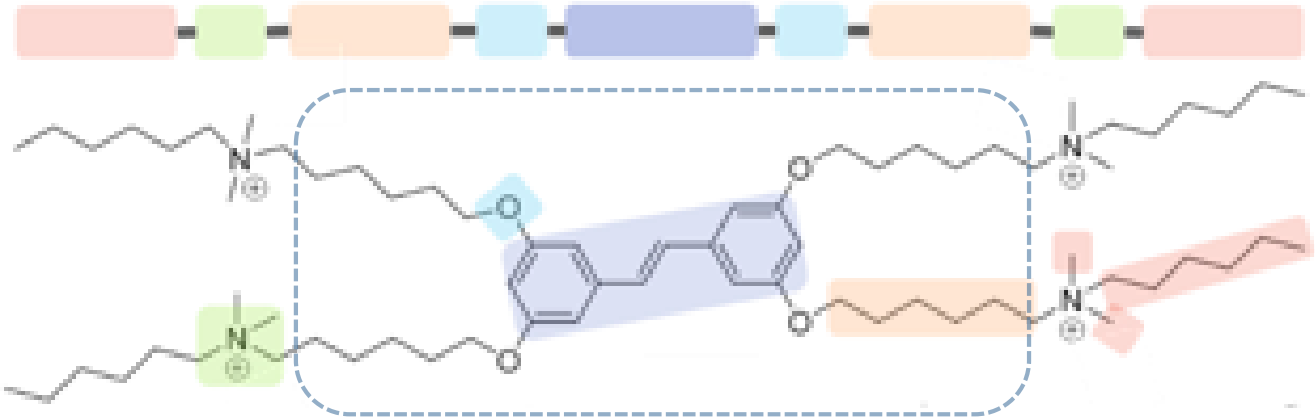


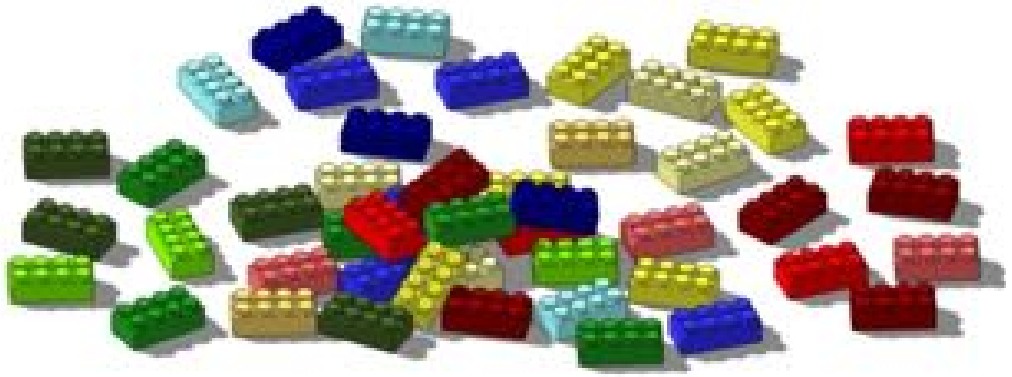




Conjugated Oligoelectrolytes

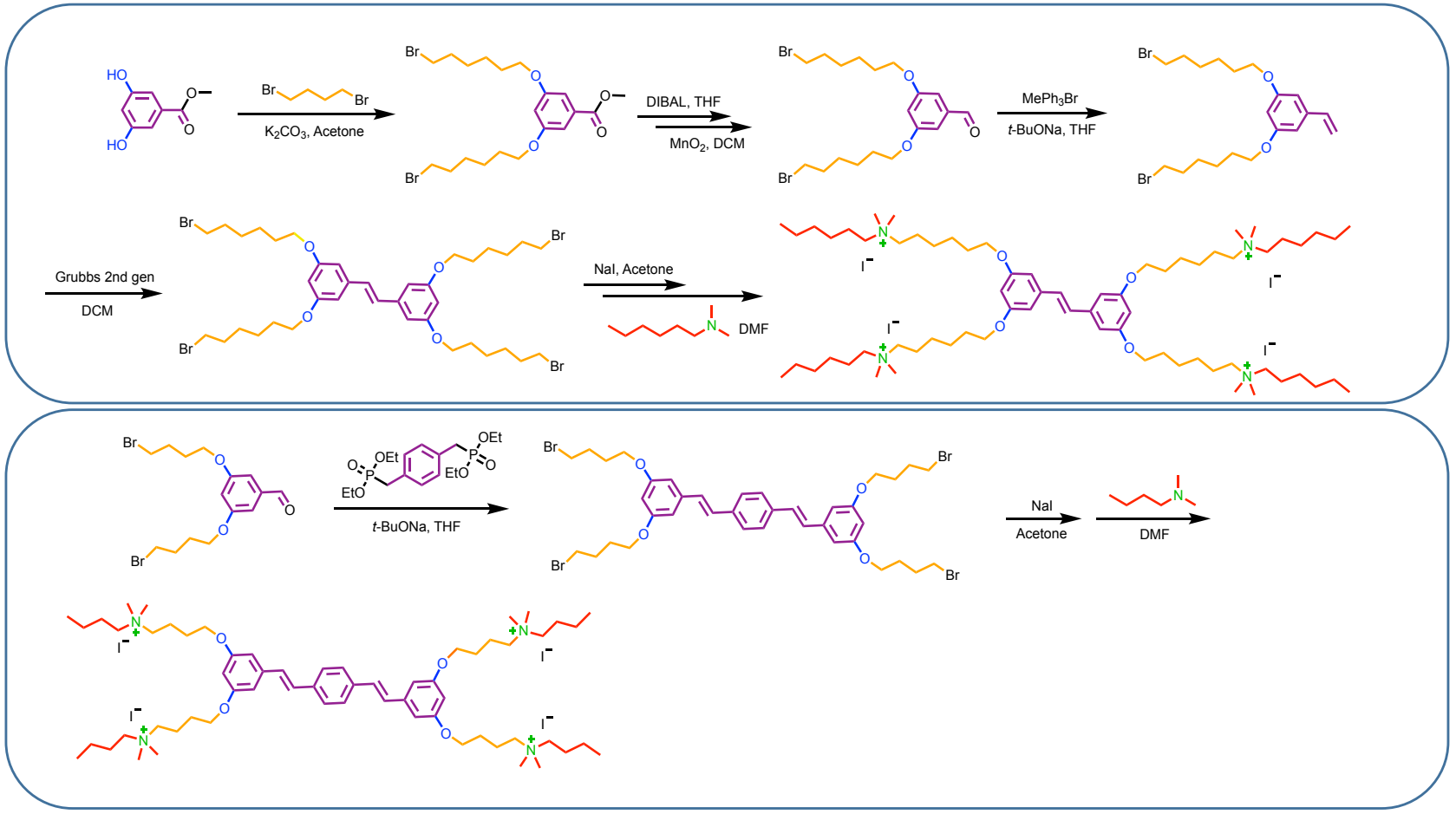
Structural considerations:





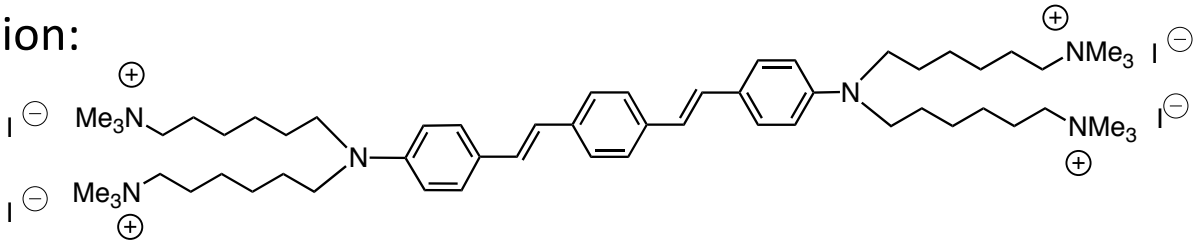
Conjugated Oligoelectrolytes

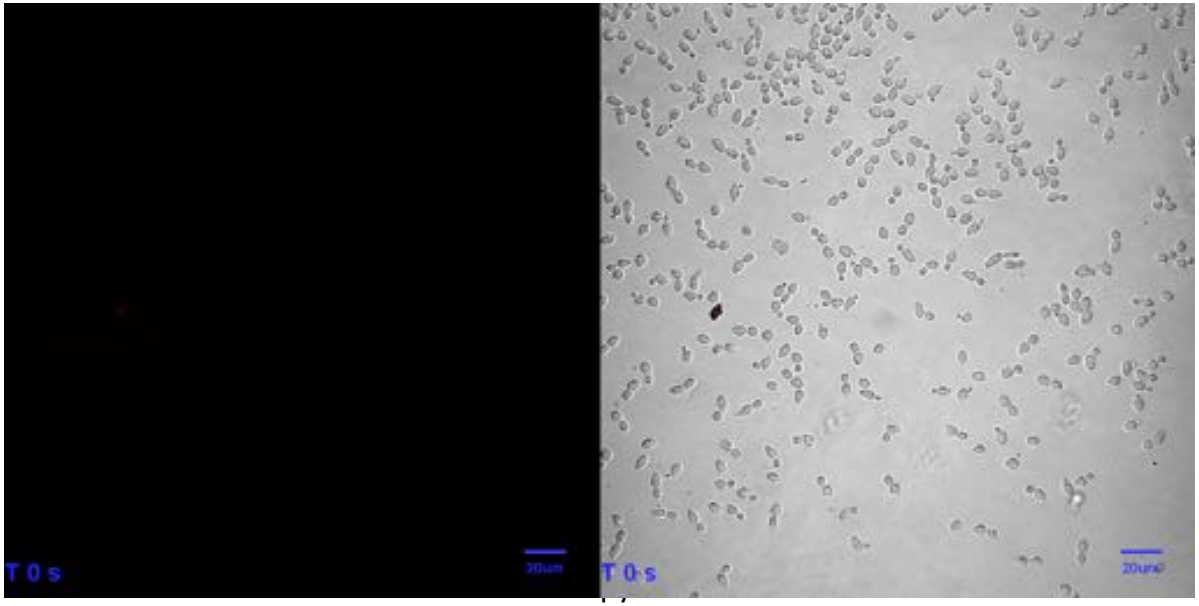
Structural Diversity:

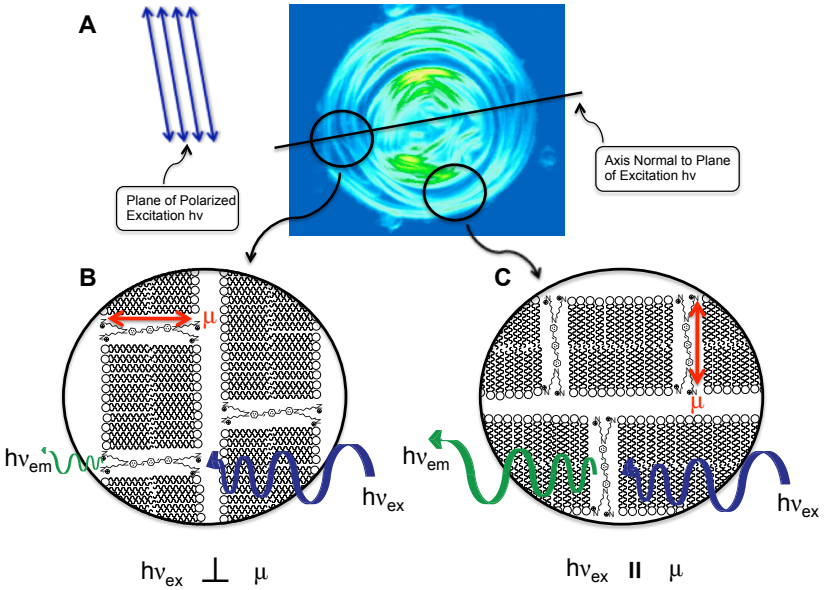


Conjugated Oligoelectrolytes

Membrane intercalation:

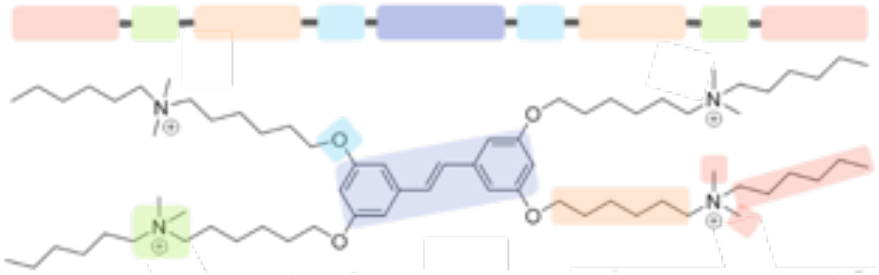


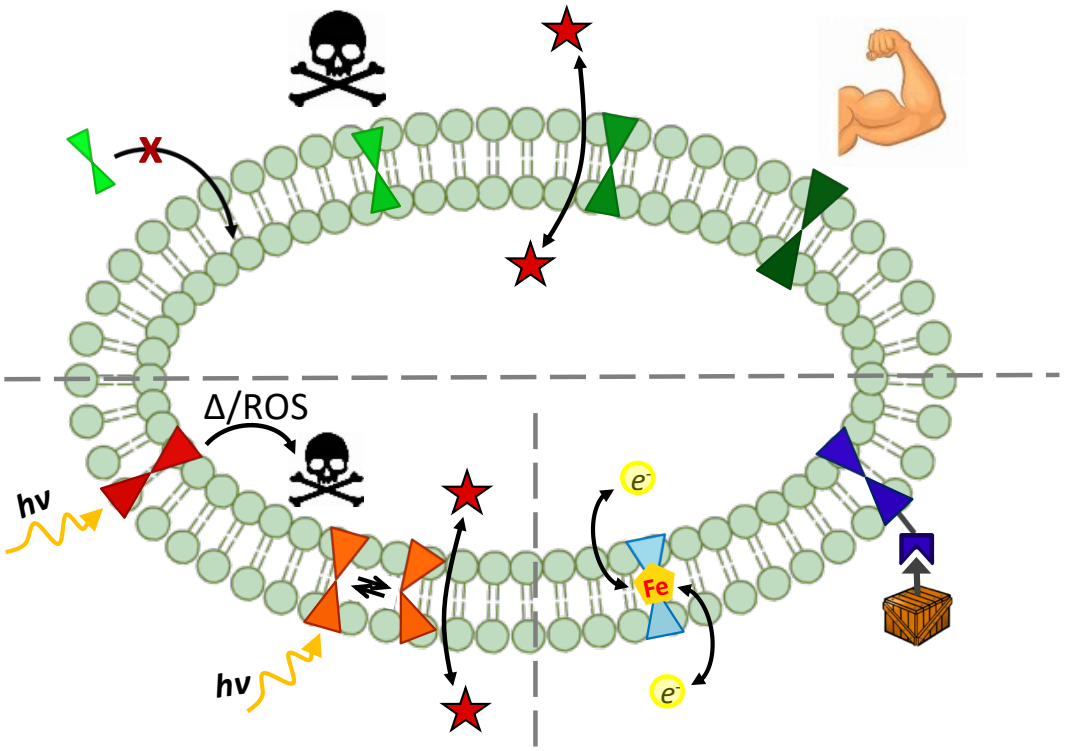




Different Effects

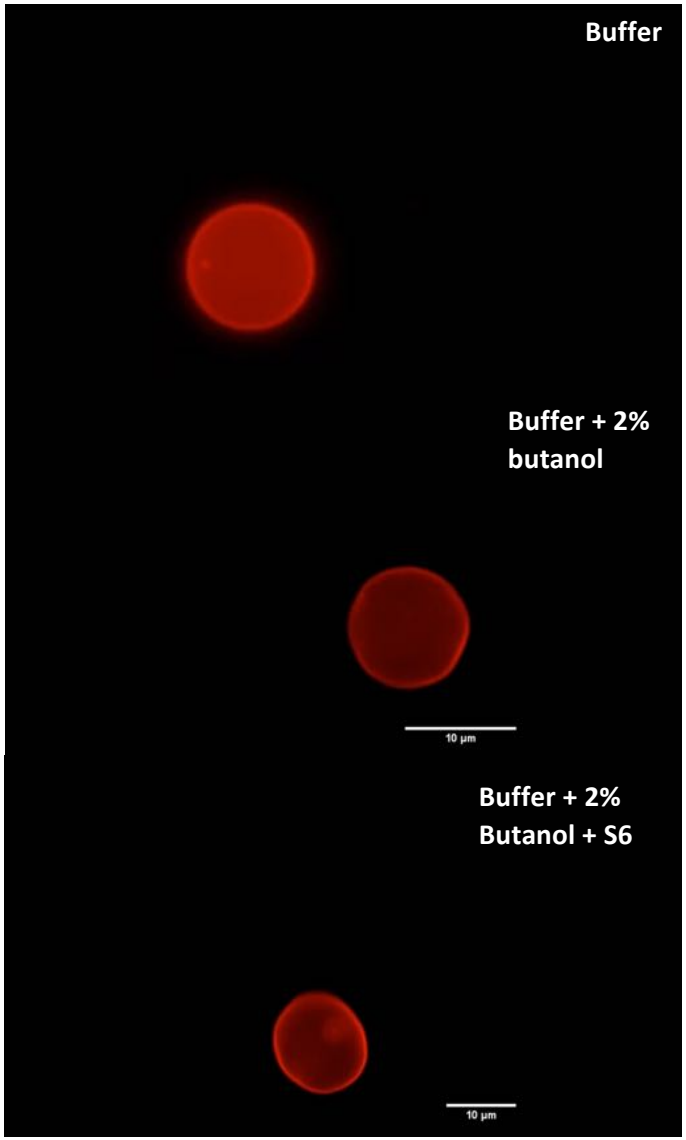
A diversity of COE structures enables a range of modifications:

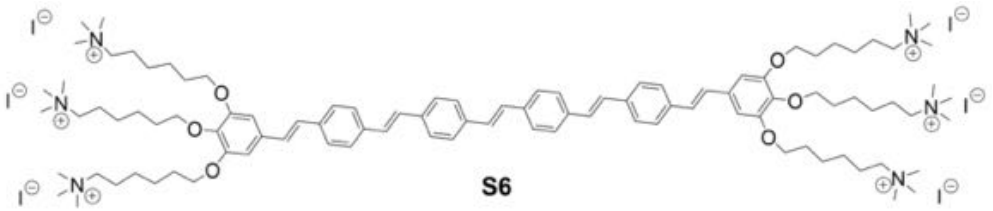


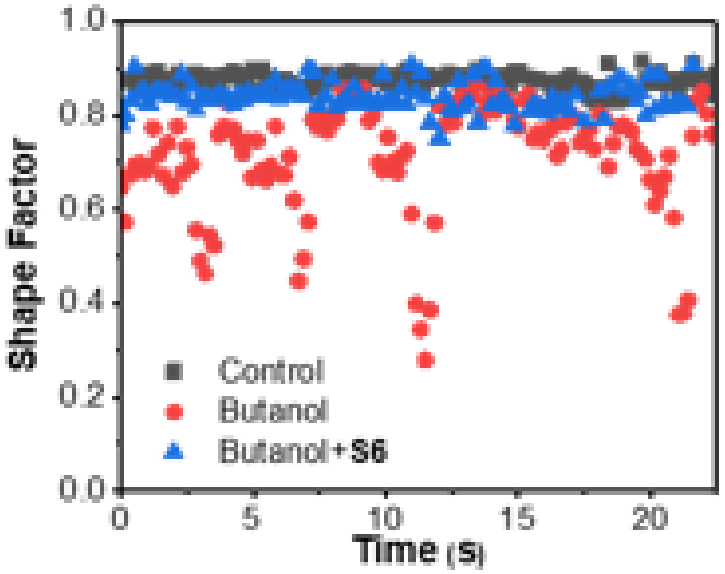


Dr. Logan Garner, Dr. Cheng Zhou, Geraldine Chia, Dr. Alex Thomas, Prof. Scott Rice, Dr. Jenny Du, Dr. James Sumner (ARL), Prof. Joachim Loo (NTU, SCELSE), Prof. Bin Liu (NUS), Dr. Jamie Hinks (SCELSE), Prof Derek Lovley (UMass), Prof. K. Kline (SCELSE), Dr. Thomas Seviour (SCELSE)

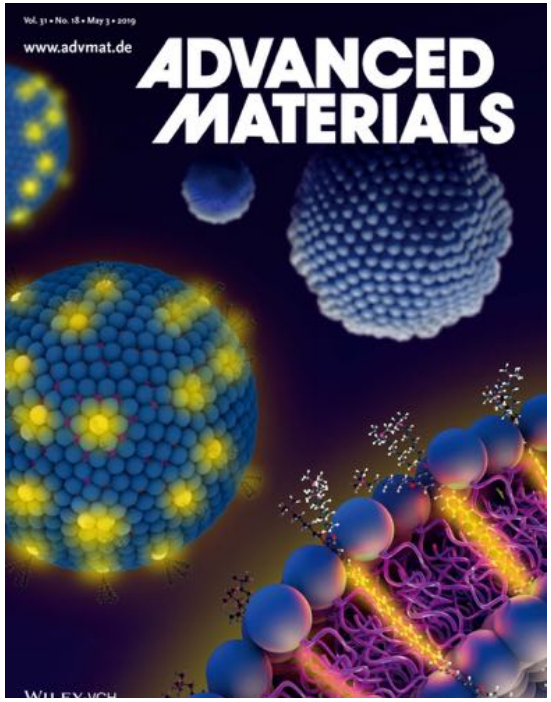
Unilamellar Vesicles





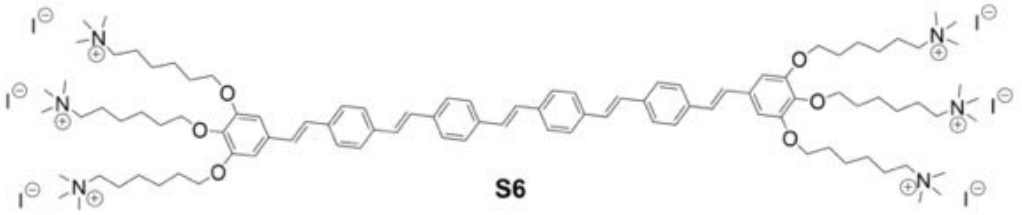


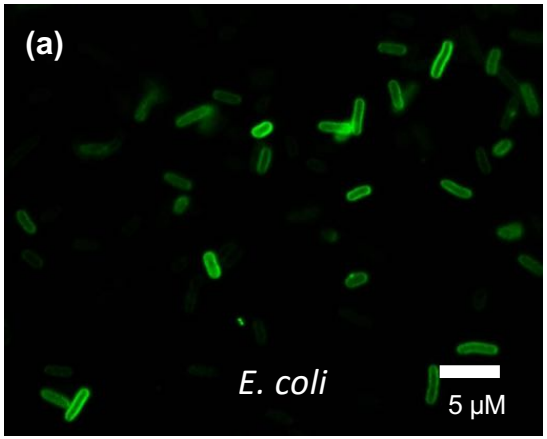
1-Palmitoyl-2-oleoylphosphatidylethanolamine (POPE), 1-palmitoyl-2-oleoylphosphatidylglycerol (POPG) and fluorescent 1,2-dipalmitoyl-*sn*-glycero-3- phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl)

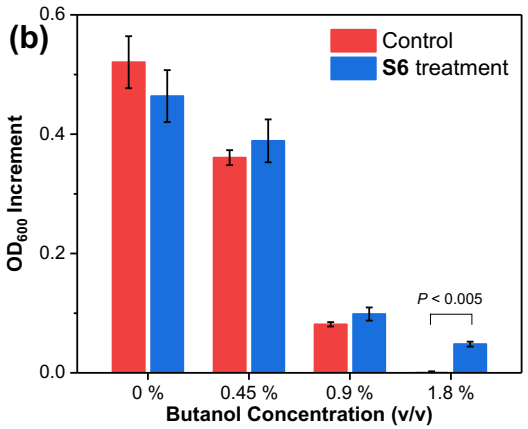


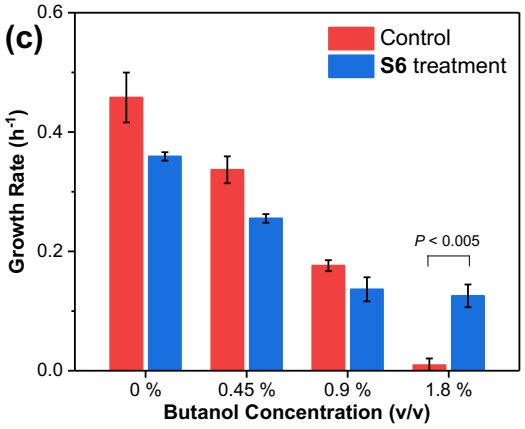
E. coli/Butanol

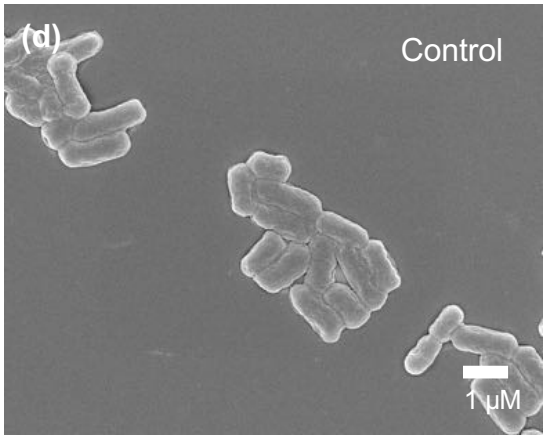
Increased tolerance of environmental perturbations:

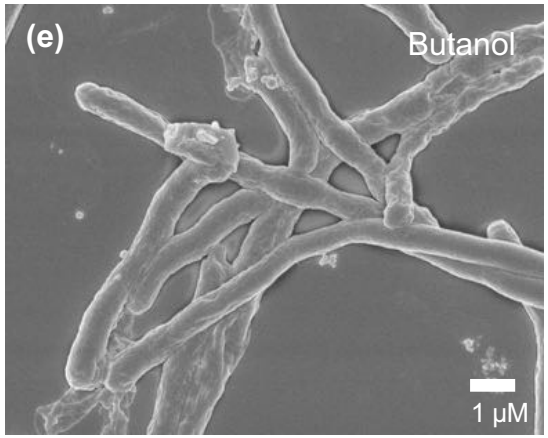


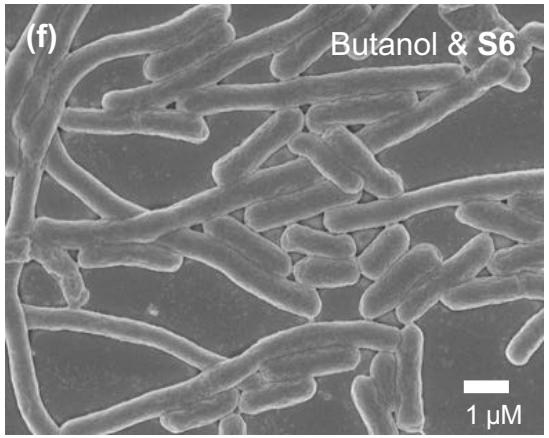






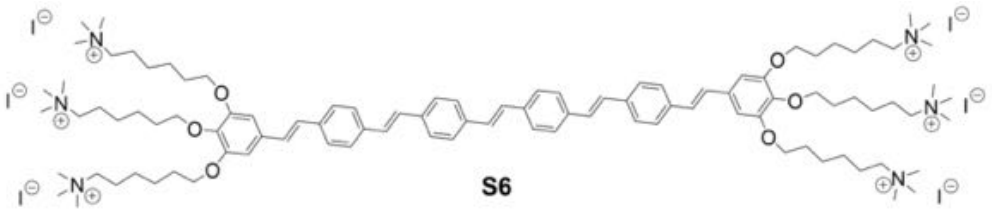


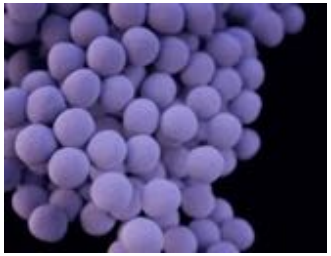




Gram Selective Labeling

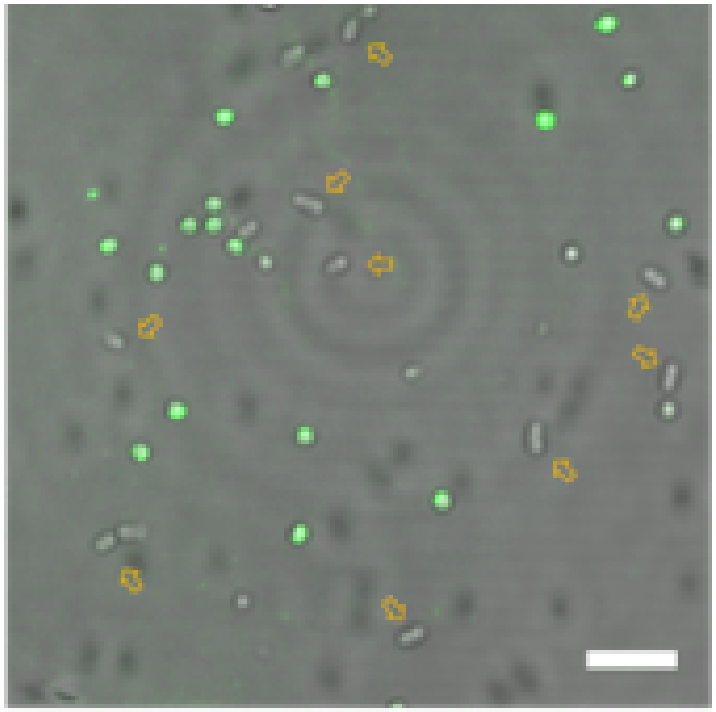
Selectivity toward cell wall type:



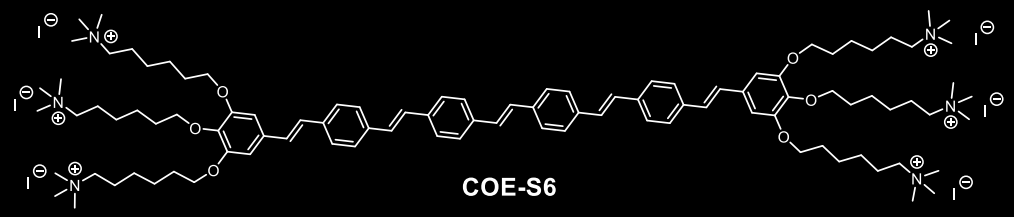


S. aureus
Gram-positive
round-shaped

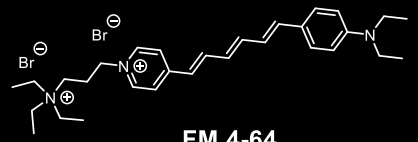
P. aeruginosa
Gram-negative
rod-shaped



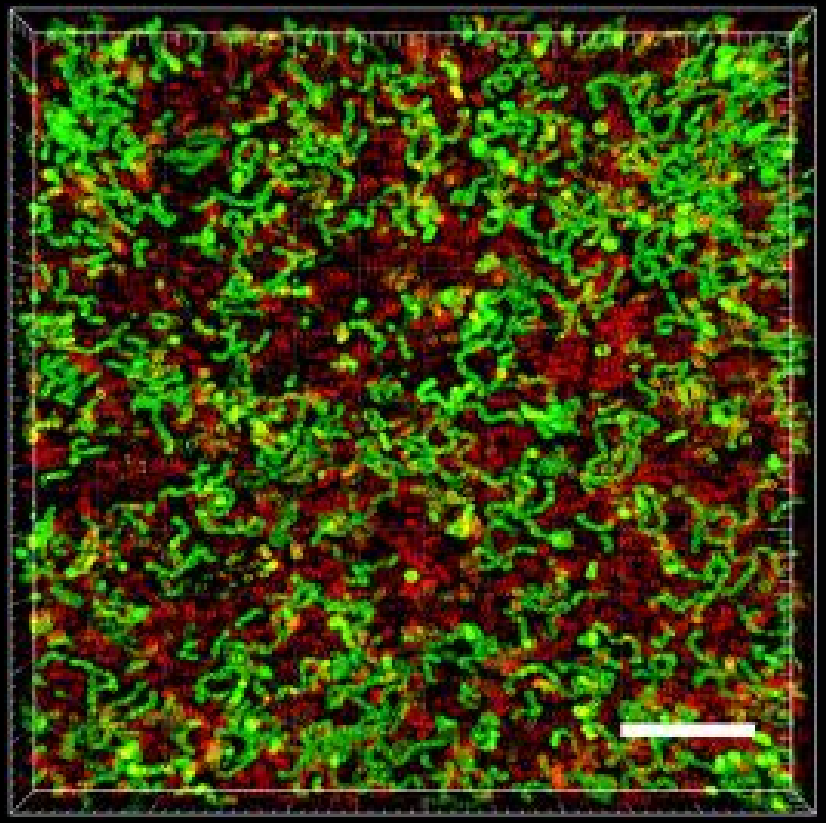
Zhou C., Ho J., Chia G., Kjelleberg S., Hinks J.*,
Bazan G.C.*, et al. *Adv. Funct. Mater.* **2020**, 2004068

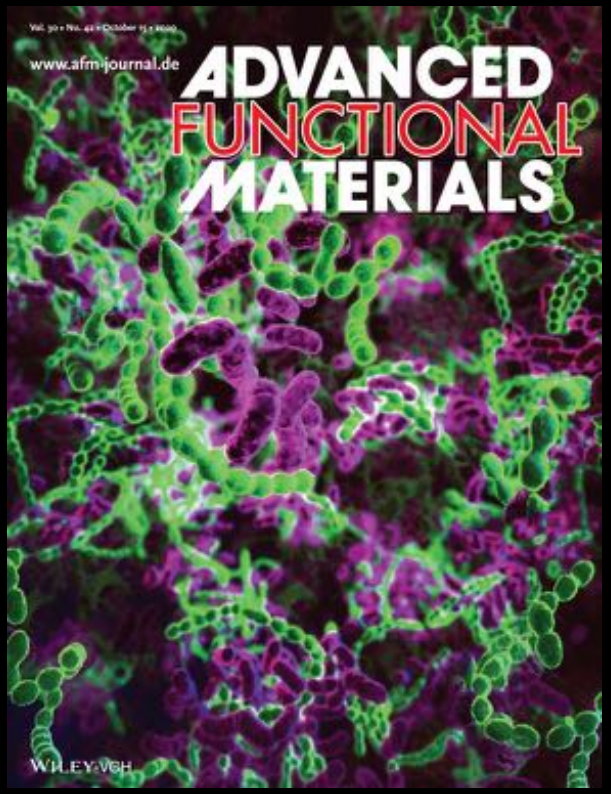


COE-S6



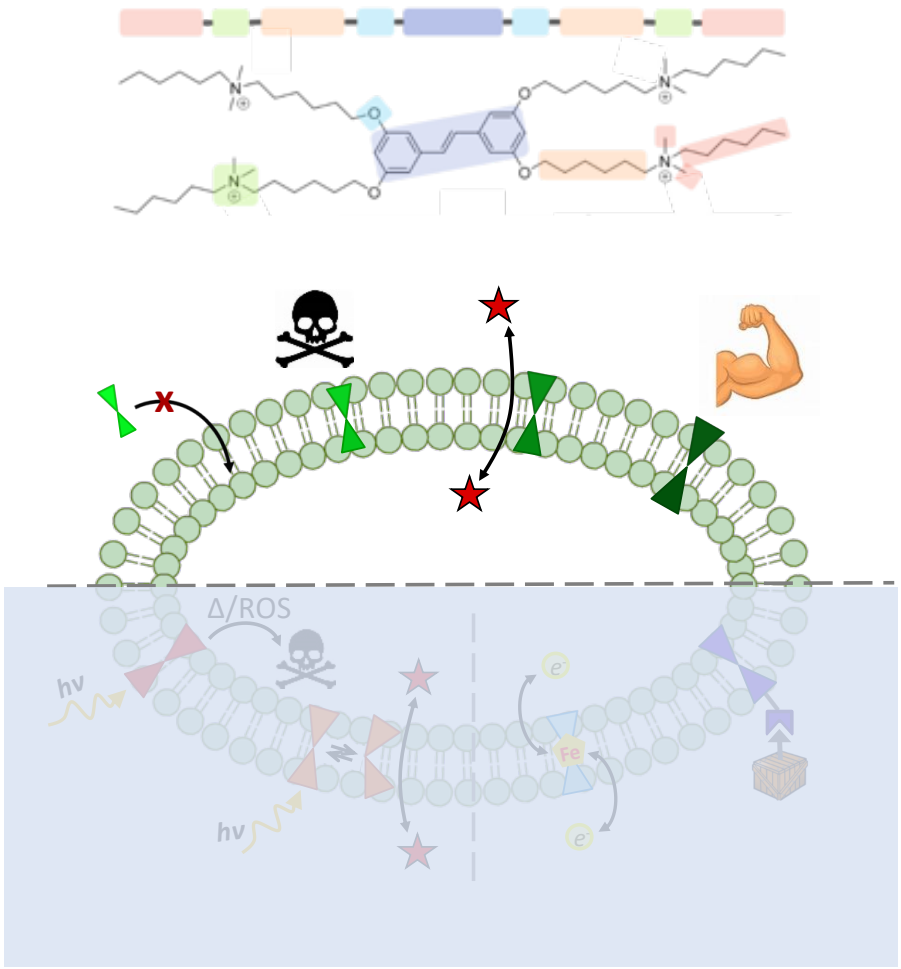
FM 4-64

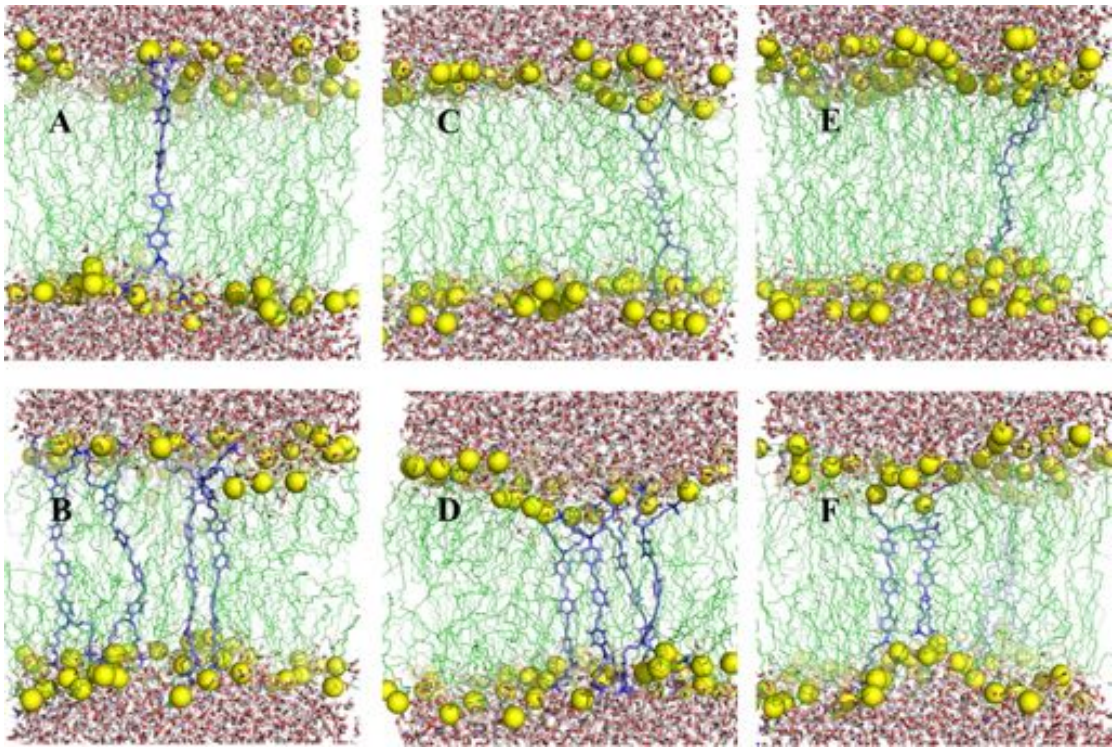




E. faecalis (**COE-S6**, green) and *E. coli* (**FM 4-64**, red) dual-species biofilm. Scale bar is 20 µm.

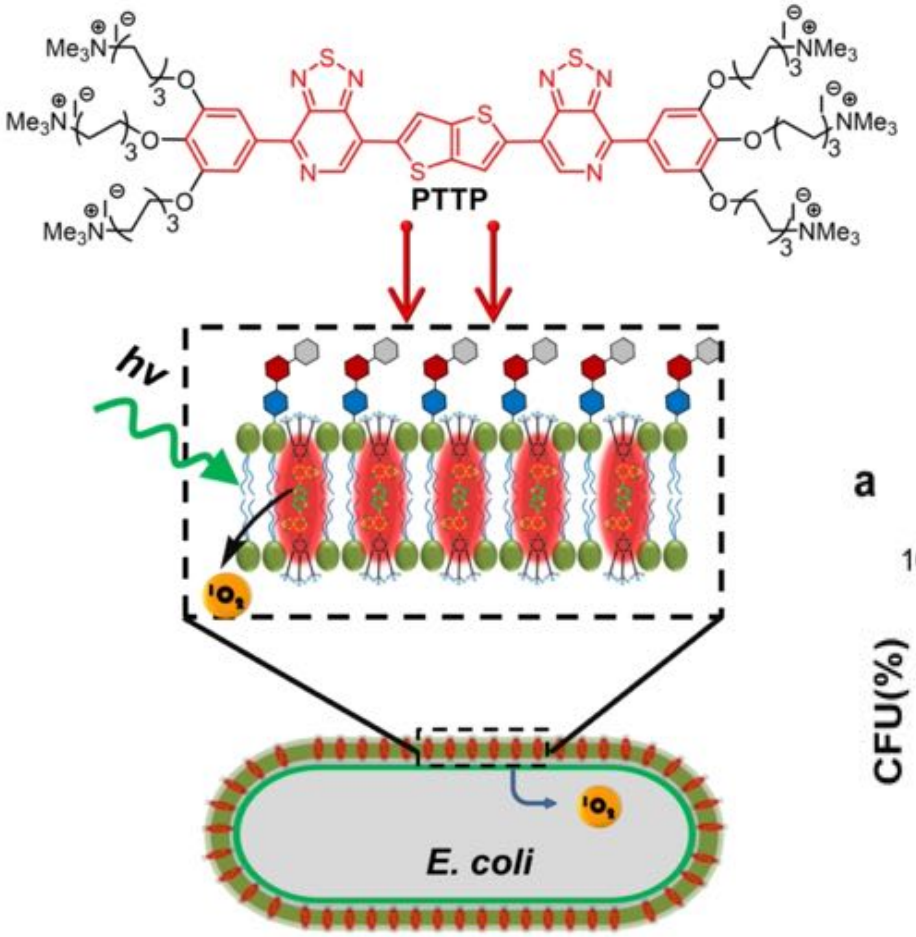
Modeling of Intercalation Biophysics

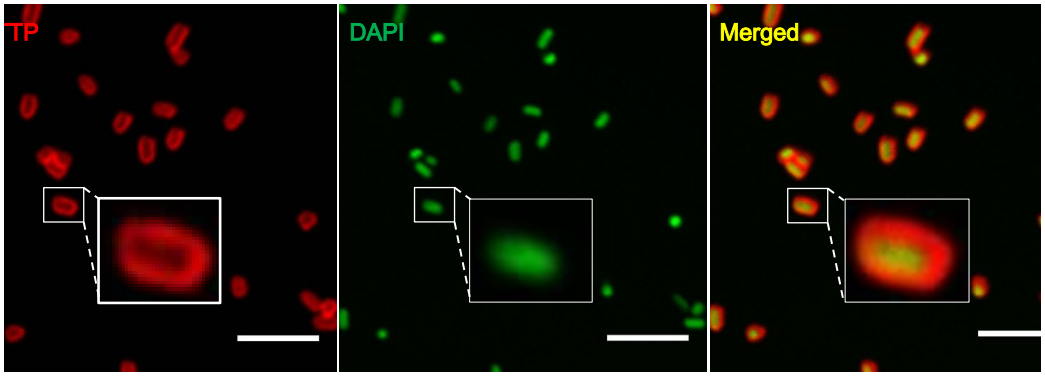


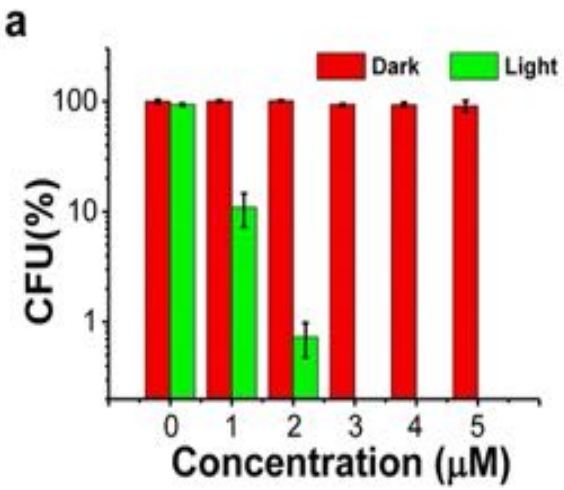


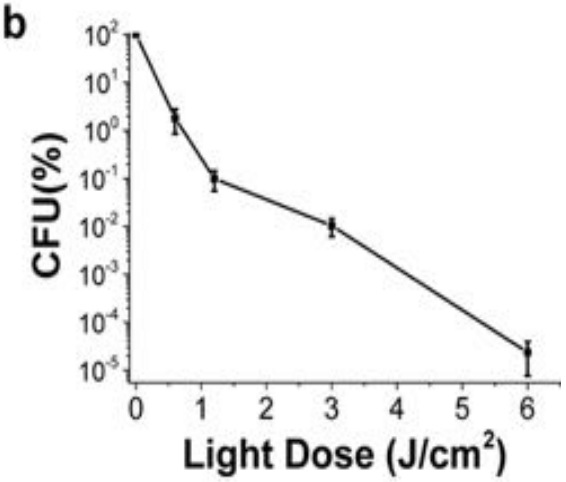
Jamie Hinks; Yaofeng Wang; Wee Han Poh; Bogdan C. Donose; Alexander W. Thomas; Stefan Wuertz; Say Chye Joachim Loo; Guillermo C. Bazan; Staffan Kjelleberg; Yuguang Mu; Thomas Seviour; *Langmuir* **2014,** 30, 2429-2440.

Photodynamic Antimicrobial Activity

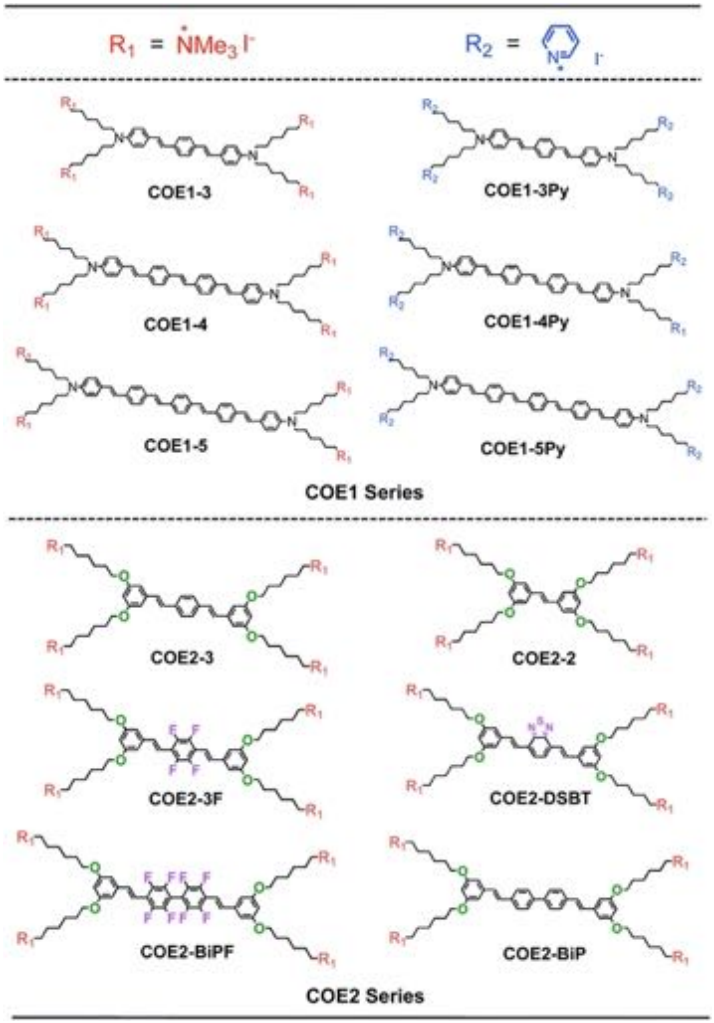






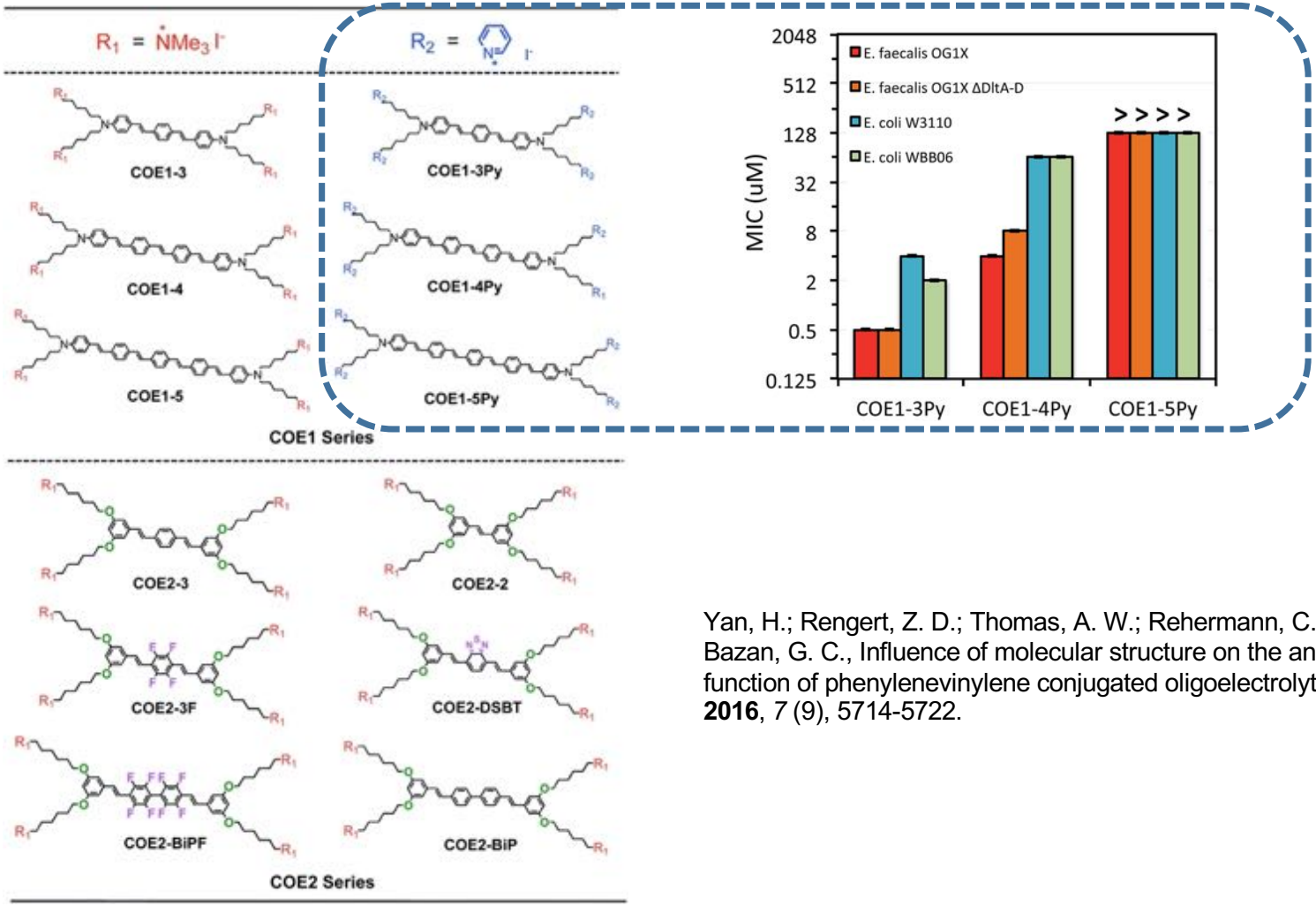


Effect of Chain Length on Antibiotic Activity



Yan, H.; Rengert, Z. D.; Thomas, A. W.; Rehermann, C.; Hinks, J.; Bazan, G. C., Influence of molecular structure on the antimicrobial function of phenylenevinylene conjugated oligoelectrolytes. *Chem. Sci*. **2016**, *7* (9), 5714-5722.

Effect of Chain Length on Antibiotic Activity



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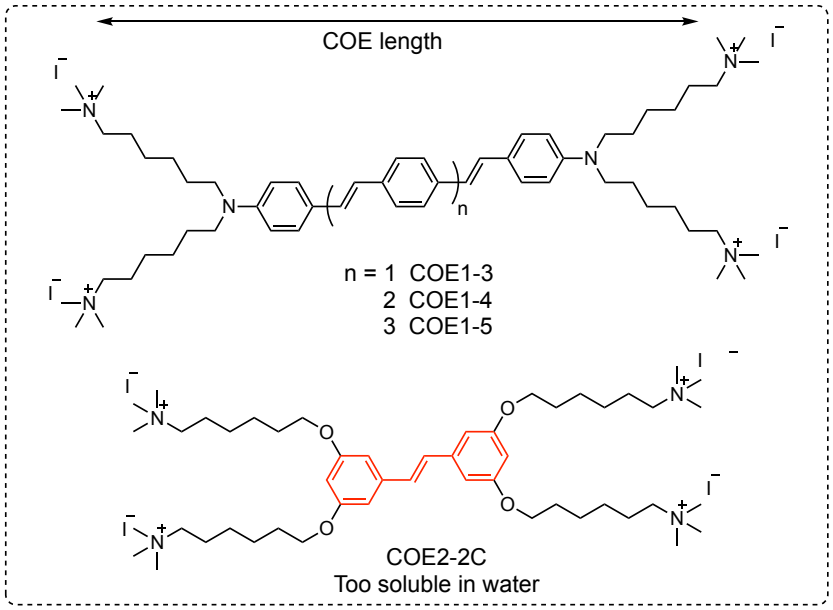
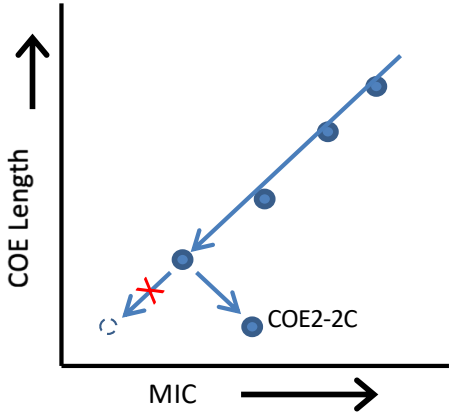
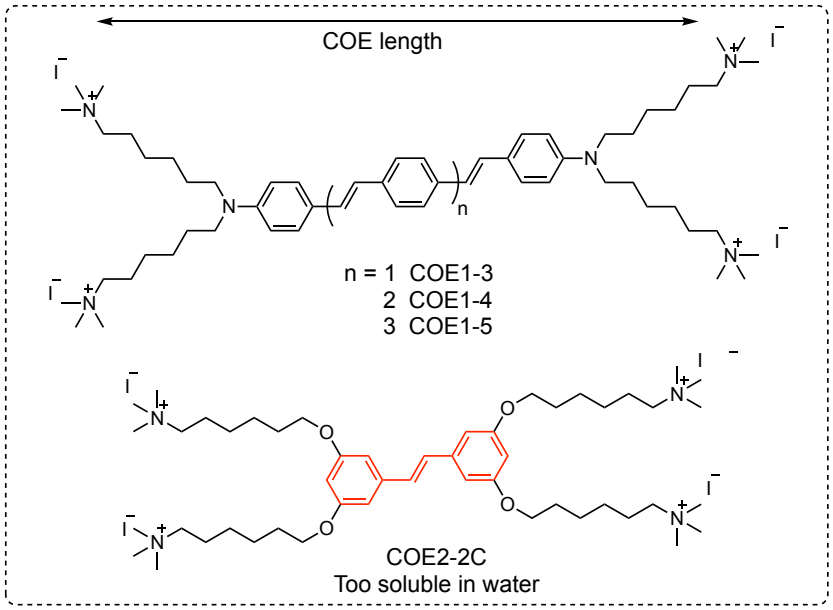
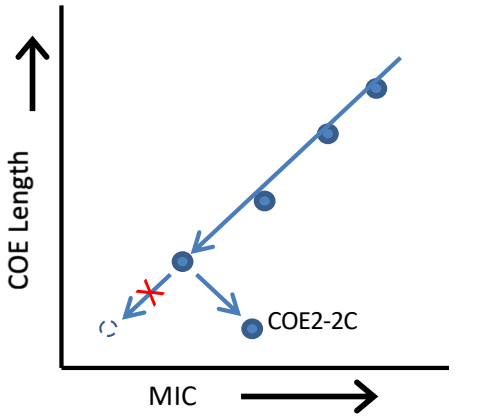
Relevance of Molecular Design

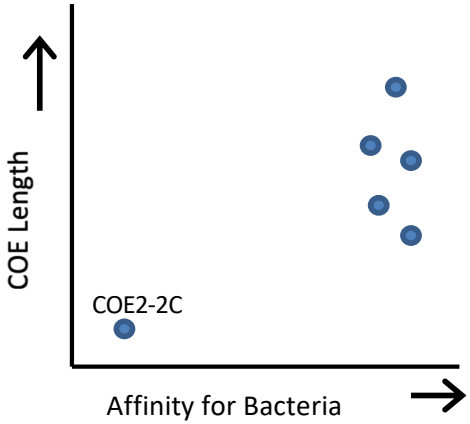




Relevance of Molecular Design







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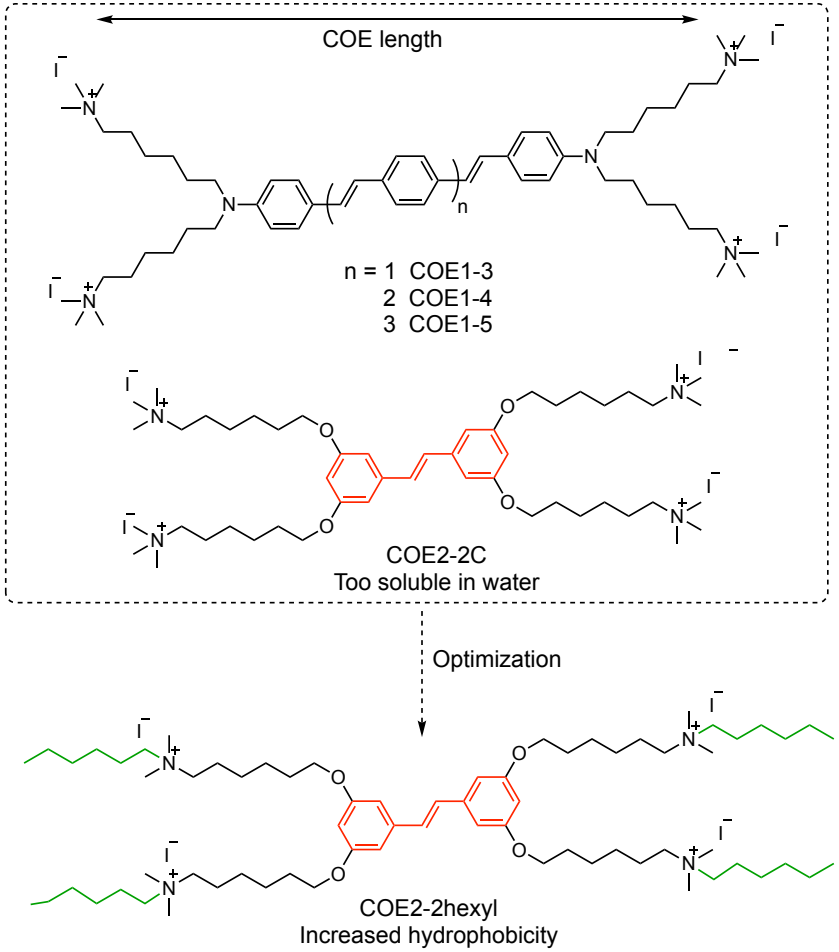
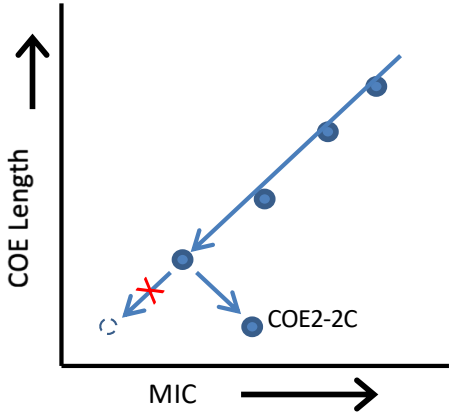
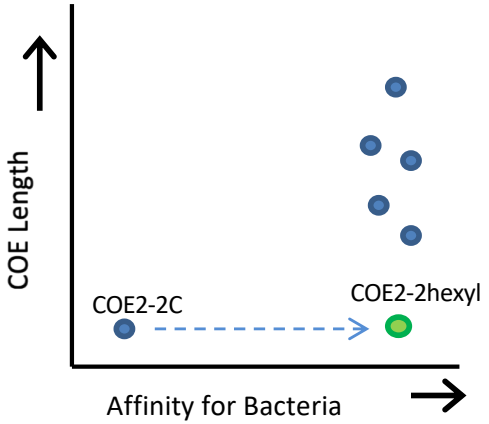
Relevance of Molecular Design







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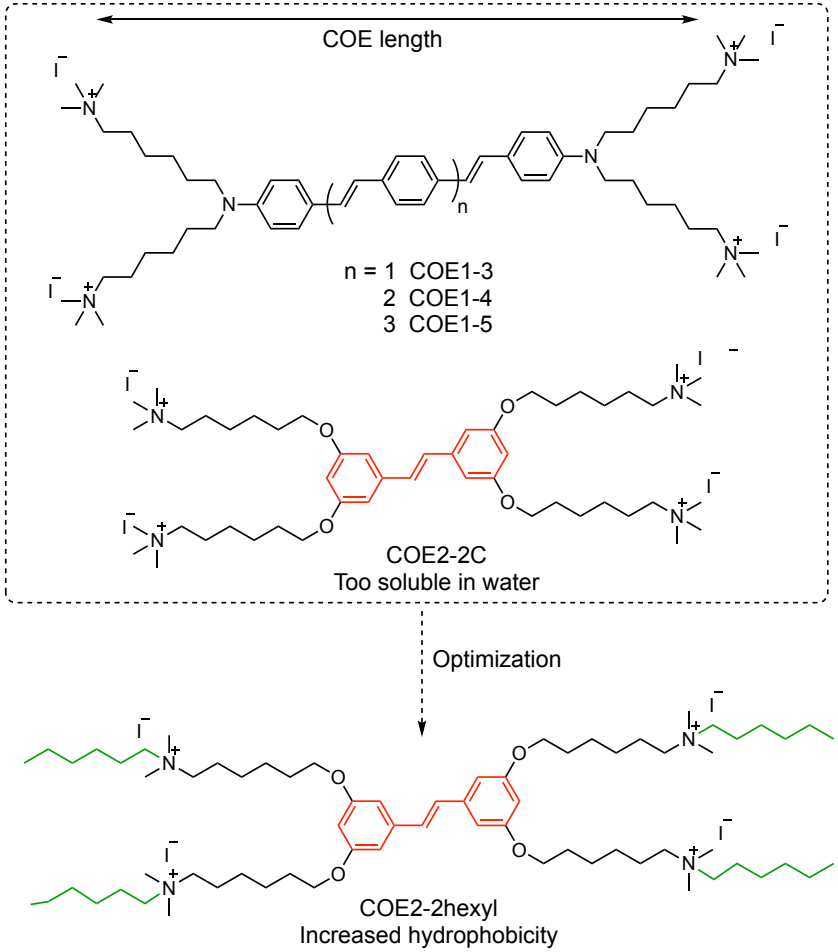
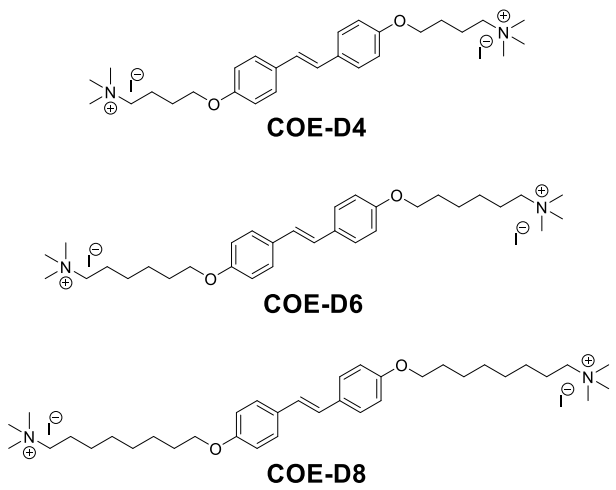
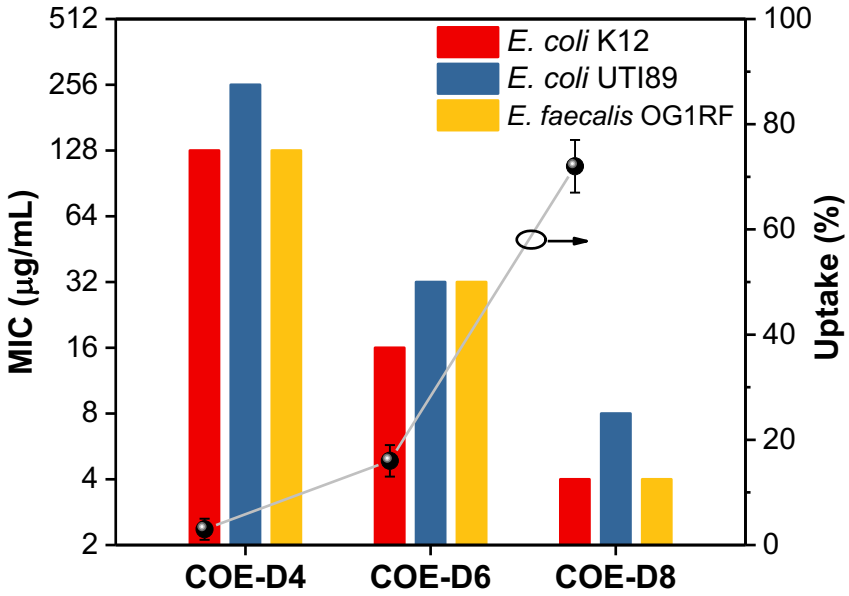
Broad Spectrum Antibiotic Function

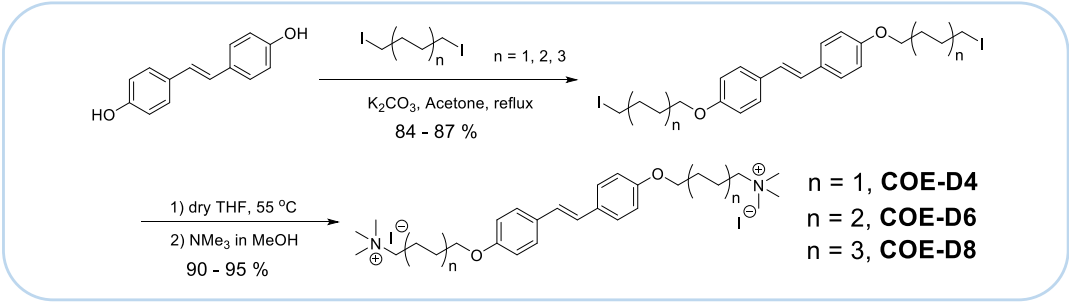


	MIC values (μg/mL)	
Pathogen	AZM	COE2-2hexyl
S. Typhimurium	4	2
E. coli	4	2
P. aeruginosa	128	8
K. pneumoniae (CRE)*	256	4
S. flexneri	2	2
Y. pseudotuberculosis	8	1
A. baumannii	64	4
N. gonorrhoeae	0.03	0.5
S. pneumoniae	8	8
S. aureus (MRSA)*	128	1

Relevance of Molecular Design





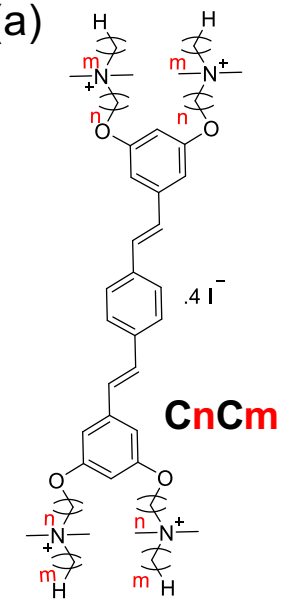


Zhou C., Chia G., Ho J., Kjelleberg S., Hinks J., Bazan G.C., et al. *Angew. Chem. Int. Ed.* **2018**, *57,* 8069.

Improvements in *in vitro* Toxicity Profiles

Molecular architecture design achieves selectivity:

(a)



(b)

Linkers (n)

Terminal alkyl chains (m)	2	3	4	6
1	128	64	32	16
2	64	32	16	
3		8	8	
4	4	4	4	
5			4	
6	4	8	4	

(c)

Linkers (n)

Terminal alkyl chains (m)	2	3	4	6
1	>1024	>1024	>1024	>1024
2	>1024	>1024	>1024	
3		>1024	>1024	
4	889	637	>1024	
5			256	
6	13	15	15	

(d)

Linkers (n)

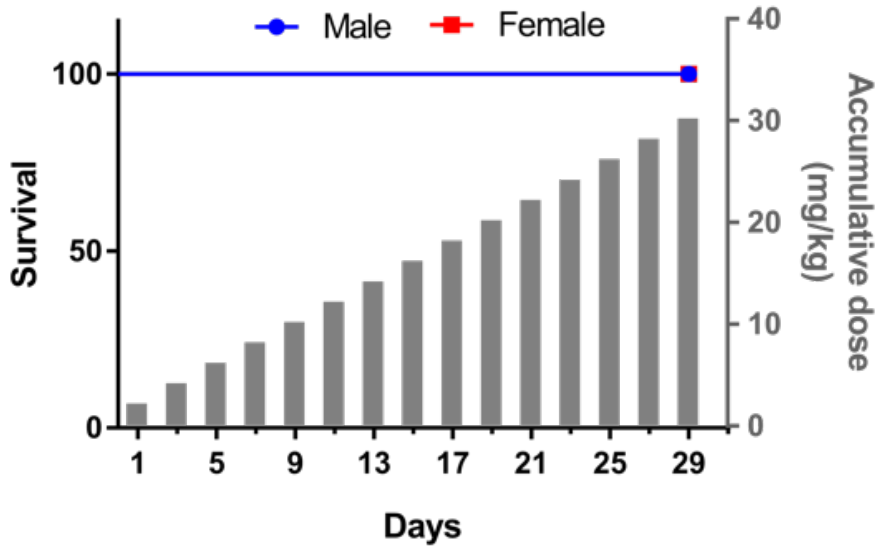
Terminal alkyl chains (m)	2	3	4	6
1	>8	>16	>32	>64
2	>16	>32	>64	
3		>128	>128	
4	223	159	>256	
5			64	
6	3.25	1.88	3.75	

A series of COEs **CnCm** generated by modulating two molecular features (modules): linker length (**n**) and terminal alkyl chains length (**m**), while keeping the core constant. **(b)** MICs of COEs against *E. coli* K12. **(c)** IC_{50} of COEs against HepG2 cell line. **(d)** Heatmap of IC_{50}/MIC values of each COE. The intensity of the green color indicates higher selectivity toward bacterial cells. All MIC and IC_{50} values are reported in μg mL^{-1}. COE **C4C4** therefore achieves the highest selectivity index with IC_{50}/MIC > 256 as a result of module optimization. Limwongyut, J.; Nie, C.; Moreland, A. S.; Bazan, G. C. *Chemical Science*, **2020**, *11*, 8138-8144.

In-vivo Tests: Murine Infection Models

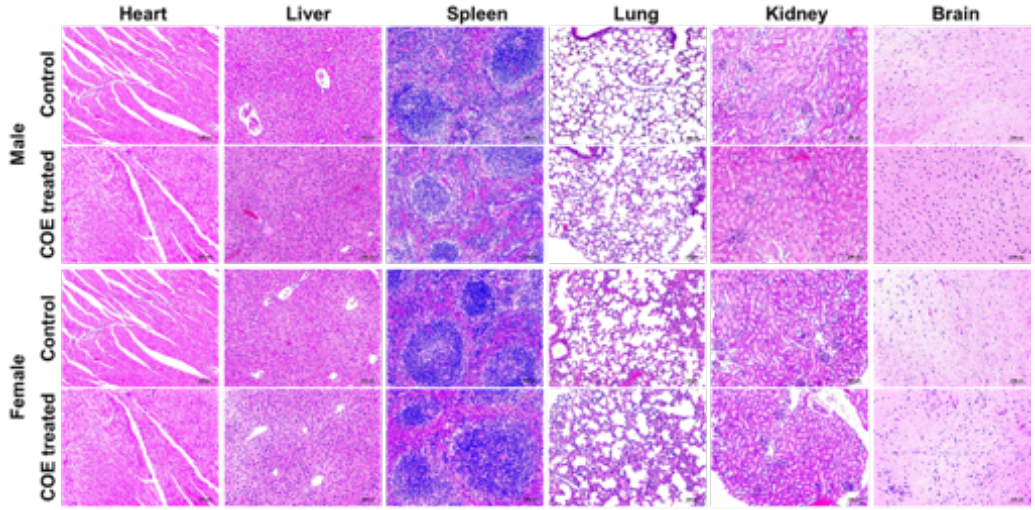
COEs clears MRSA and carbapenem-resistant K. pneumoniae in mice models		
	Survivors	
Pathogen	Treated	Untreated
MRSA*	10/10	0/10
*K. pneumoniae**	10/10	0/10

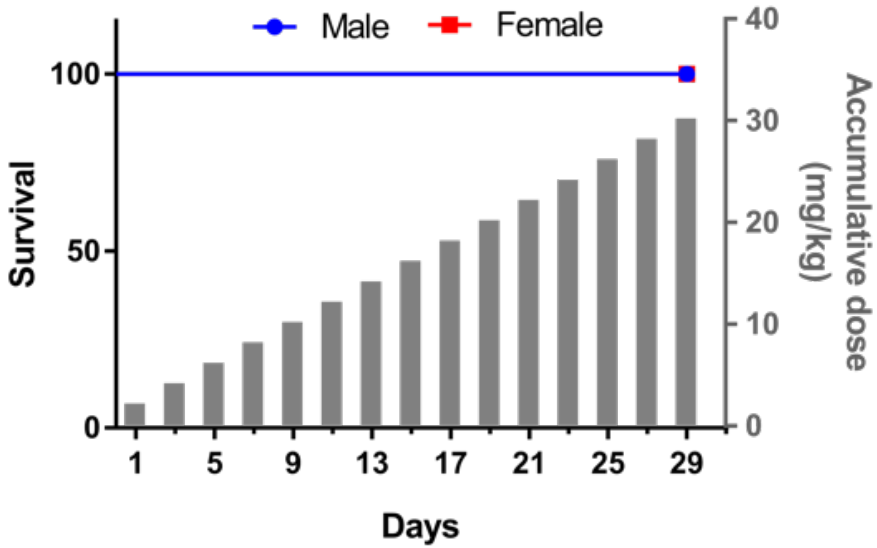
*Denotes patient expired from infection
Dose 2 mg/kg/day



Collaboration with Prof. Mike Mahan and Prof. David Low (UCSB), and Prof. Lin (Shenzhen University).

In-vivo Tests: Safety Profiles

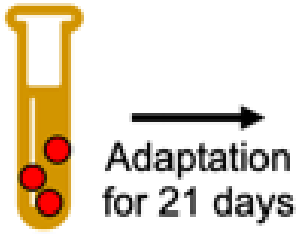


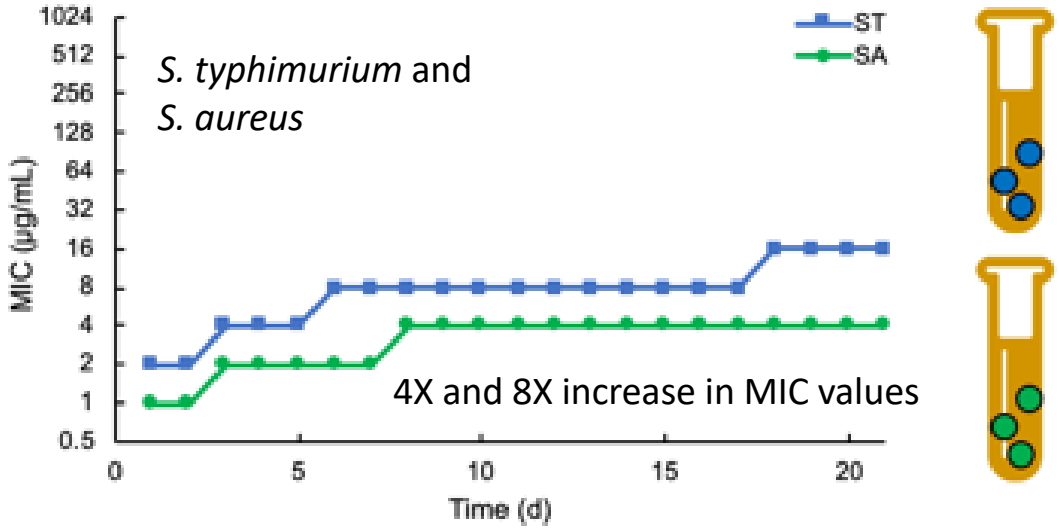


Collaboration with Prof. Mike Mahan and Prof. David Low (UCSB), and Prof. Lin (Shenzhen University).

Slow development of *in-vitro* resistance

Twenty-one days serial passage:





Fourteen days serial passage:

Strain	Description	Growth conditions	MIC		
			COE1-3C	COE1-3Py	Daptomycin
E. faecalis OG1RF	Wild type	BHI	2 µM	1 µM	1.23 µM
EFC3C[a]	COE1-3C resistant	BHI	8 µM	1 µM	9.87 µM
EFC3Py[b]	COE1-3Py resistant	BHI	8 µM	16 µM	9.87 µM
DAP 21[c]	Daptomycin resistant	BHI	2 µM	1 µM	78.96 µM
DAP 22[d]	Daptomycin resistant	BHI	2 µM	1 µM	78.96 µM

For comparison, **Daptomycin** exhibits a 256X and 512X increase in MIC against *E. faecalis*. See: Hinks et al, *RSC Adv.*, **2018**, *8*, 10284.

Translation into Startup Activity

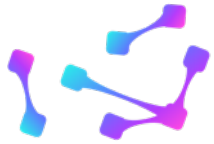
XIRETSA™

Building the Future of Antibiotics

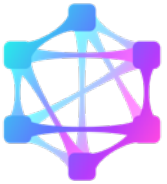
-Holds IP from UCSB and SCELSE (NTU and NUS)
-Winner of 2021 California Biotech Startup Competition
-https://www.xiretsapharm.com/
-https://www.youtube.com/watch?v=KxqOIoOBHGM

Revolutionary Patented Technology

Modular Design	Resistance	First-in-class	Broad Spectrum
Simple synthesis	Limited effect on MIC	Novel mode of action	G+, G-, and Fungi
Rapid derivatization	Low frequency	Paradigm-changing	Bactericidal
Flexible matrix of opportunities	No cross-resistance observed	Unique IP position to secure range of meds	Biofilms
Low-cost, scalable, and adaptable; Can be tailored to specific indications	Long marketable lifetime; Reduced risk for treatment failures	Unique IP position to secure a range of new anti-infective medicines	Capable of addressing various current/ emerging markets and health threats



Molecular Fragments



Life-saving Antibiotic

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Thank You!