Design of Catheter Coatings for Localized Release of Antimicrobial Peptide Mimetics

Sean Palecek and David Lynn

DEPARTMENT OF
Chemical and Biological Engineering
College of Engineering *University of Wisconsin-Madison*

THE UNIVERSITY of WISCONSIN MADISON

β-peptide mimetics of antimicrobial peptides

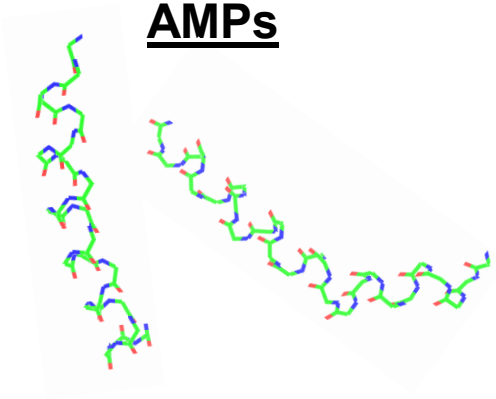
Antimicrobial α-peptides

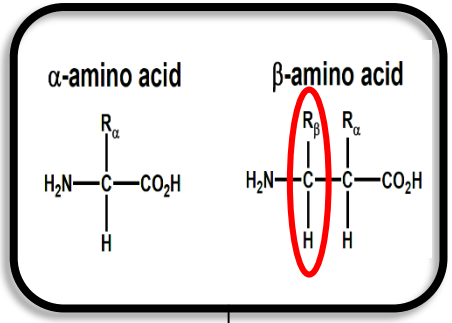
- Components of the innate immune system
- Kill bacterial and fungal pathogens
- Membrane disruption based mechanisms: more difficult for pathogens to develop resistance

Antimicrobial β-peptides

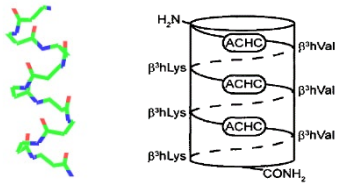
Oligomers of β-amino acids
Peptidomimetics of AMPs
Can be designed to fold into specific secondary structures

AMPs





β-Peptide



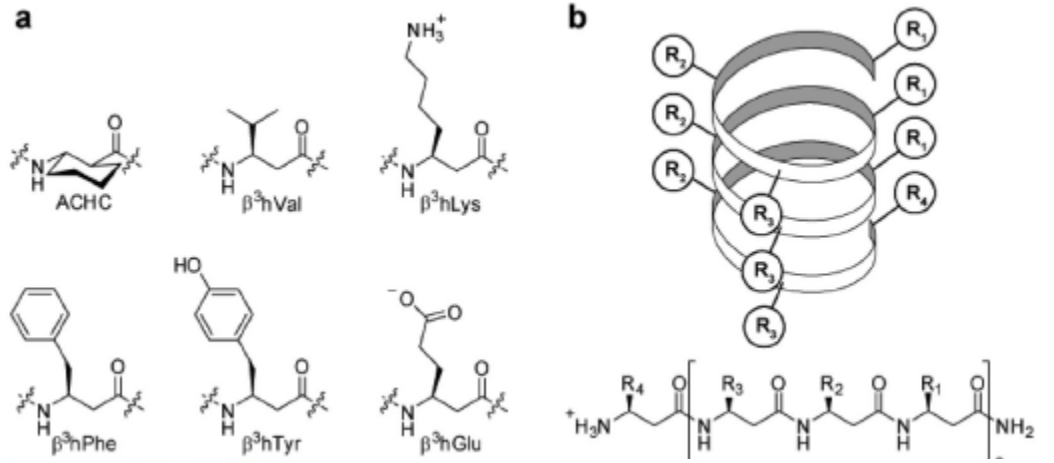
Limitations

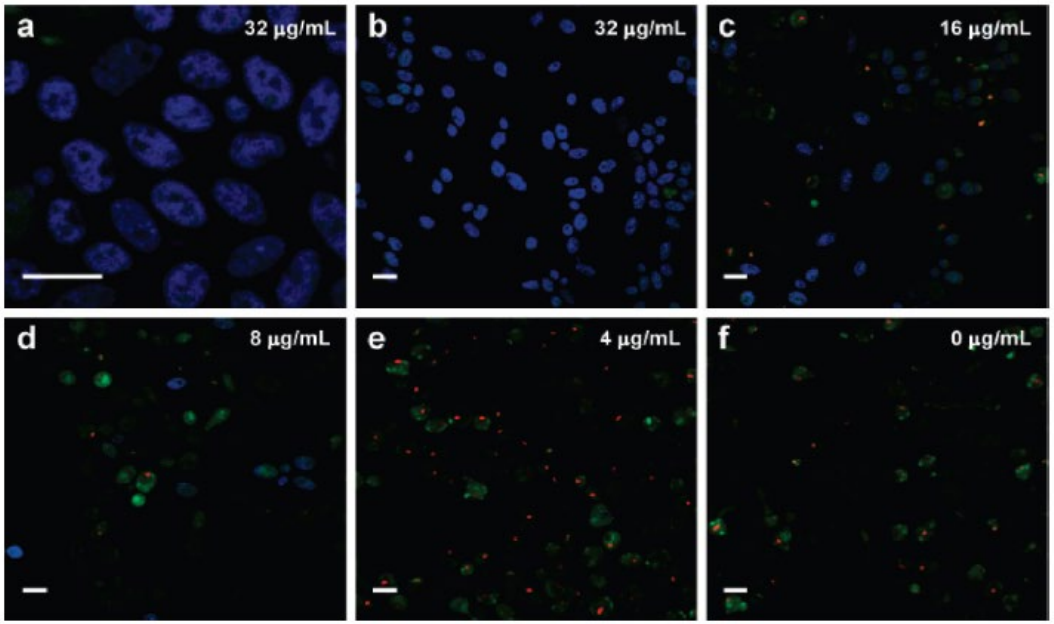
Degradation by cellular proteases
Low activity at physiologic ionic strength

Advantages

Stable in physiologic conditions, high ionic strength and against proteolytic enzymes

Antifungal β-peptides





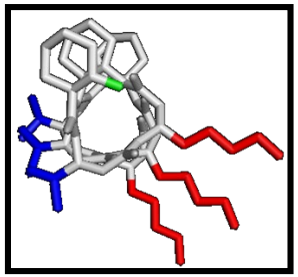
- Antifungal β-peptide
  - Globally amphiphillic
  - Length → 9-mer, 10-mer
  - Charge → +3, +4
- Identified β-peptides with antifungal activity against *C. albicans* and selective (non-toxic towards hRBCs)
- Fluorescently labeled peptide for tracking

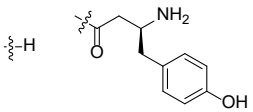
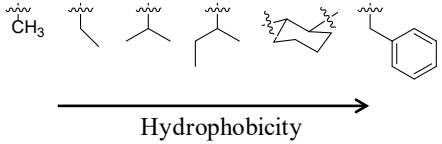
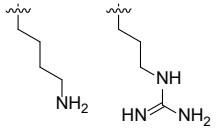
Blue – Labeled peptide
Green – Cytoplasm stain
Red – Intra-vacuolar structures
(only in metabolic active cells)

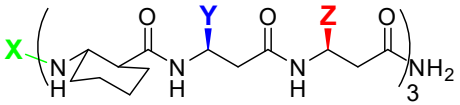
THE UNIVERSITY of WISCONSIN MADISON

Karlsson, A. J. et al., *ACS Chemical Biology* (2009)

Effect of hydrophobicity and helicity on β-peptide activity and selectivity

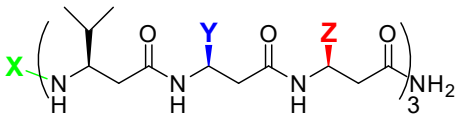


N-terminal (X)	Side Chain (Y)	Side Chain (Z)
		

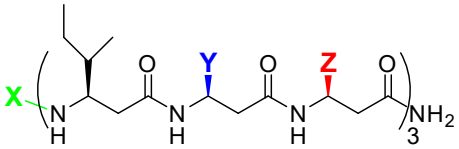


1 : X = H, Y = β^3-hAla, Z = β^3-hLys
2 : X = H, Y = β^3-Et, Z = β^3-hLys
3 : X = H, Y = β^3-Et, Z = β^3-hArg
4 : X = H, Y = β^3-hVal, Z = β^3-hLys
5 : X = H, Y = β^3-hVal, Z = β^3-hArg
6 : X = H, Y = ACHC, Z = β^3-hLys
7 : X = H, Y = ACHC, Z = β^3-hArg
8 : X = H, Y = β^3-hPhe, Z = β^3-hLys

9 : X = β^3-hTyr, Y = β^3-hAla, Z = β^3-hLys
10 : X = β^3-hTyr, Y = β^3-Et, Z = β^3-hLys
11 : X = β^3-hTyr, Y = β^3-Et, Z = β^3-hArg
12 : X = β^3-hTyr, Y = β^3-hVal, Z = β^3-hLys
13 : X = β^3-hTyr, Y = β^3-hVal, Z = β^3-hArg
14 : X = β^3-hTyr, Y = ACHC, Z = β^3-hLys
15 : X = β^3-hTyr, Y = ACHC, Z = β^3-hArg
16 : X = β^3-hTyr, Y = β^3-hPhe, Z = β^3-hLys



17 : X = H, Y = β^3-hVal, Z = β^3-hLys
18 : X = H, Y = β^3-hVal, Z = β^3-hArg
19 : X = β^3-hTyr, Y = β^3-Et, Z = β^3-hArg
20 : X = β^3-hTyr, Y = β^3-hVal, Z = β^3-hLys
21 : X = β^3-hTyr, Y = β^3-hVal, Z = β^3-hArg

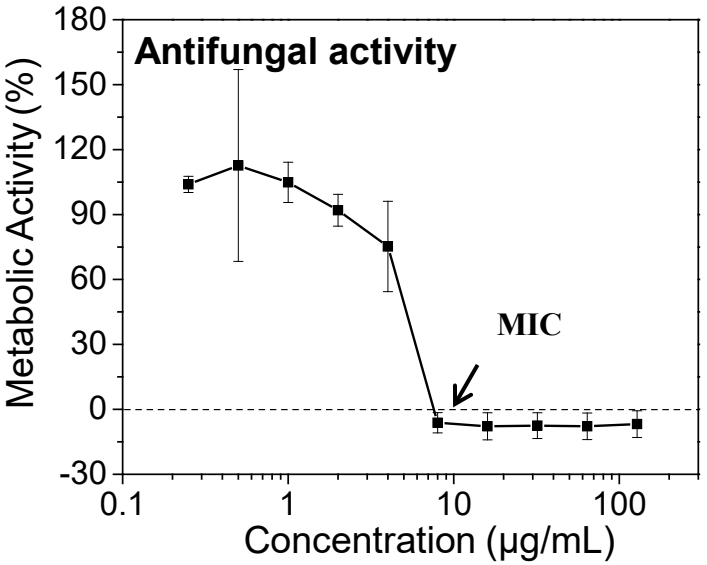


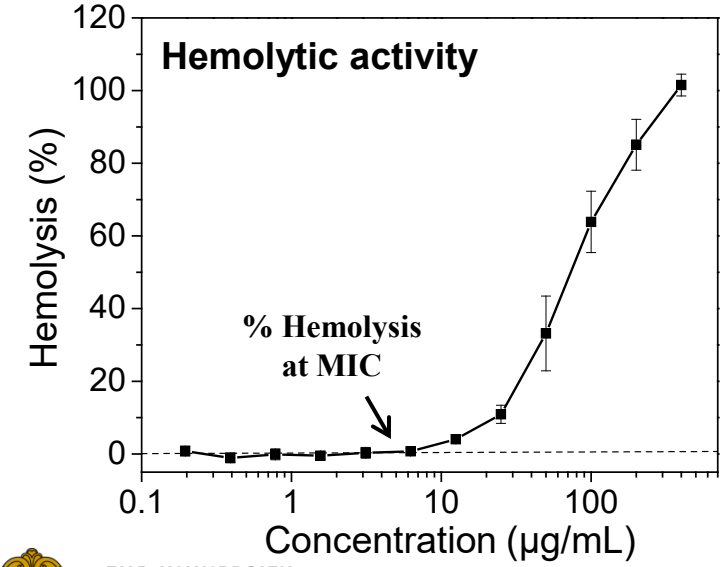
22 : X = H, Y = β^3-Et, Z = β^3-hLys
23 : X = H, Y = β^3-Et, Z = β^3-hArg
24 : X = β^3-hTyr, Y = β^3-Et, Z = β^3-hLys
25 : X = β^3-hTyr, Y = β^3-Et, Z = β^3-hArg

THE UNIVERSITY of WISCONSIN MADISON

Lee, M.-R.*, Raman, N*., et al. *ACS Chemical Biology*, (2014) 9, 1613

β-peptide antifungal and hemolytic activity



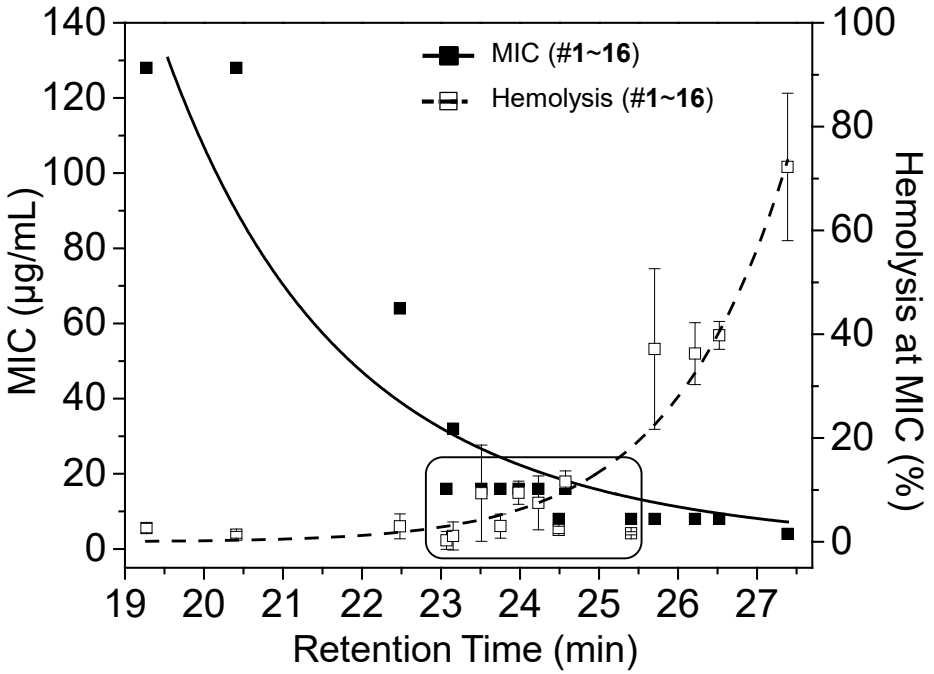


β-Peptide	RT (min ± SD)	MIC (μg/mL)	% Hemolysis at MIC ± SD
1	19.3 ± 0.1	> 128	2.6 ± 0.9*
2	22.5 ± 0.2	64	3.0 ± 2.4
3	23.2 ± 0.1	32	1.1 ± 2.7
4	**24.5 ± 0.2**	**8**	**2.3 ± 0.7**
5	**25.4 ± 0.1**	**8**	**1.6 ± 0.3**
6	**23.1 ± 0.2**	**16**	**0.3 ± 1.7**
7	**23.8 ± 0.1**	**16**	**3.0 ± 2.3**
8	26.2 ± 0.2	8	36.4 ± 6.0
9	20.4 ± 0.2	128	1.4 ± 0.6
10	**23.5 ± 0.1**	**16**	**9.4 ± 9.3**
11	**24.2 ± 0.1**	**16**	**7.5 ± 5.2**
12	25.7 ± 0.1	8	37 ± 15
13	26.5 ± 0.2	8	39.8 ± 2.7
14	**24.0 ± 0.2**	**16**	**9.5 ± 2.2**
15	**24.6 ± 0.2**	**16**	**11.6 ± 2.1**
16	27.4 ± 0.2	4	72 ± 14
17	22.5 ± 0.1	128	2.8 ± 0.1
18	23.5 ± 0.1	64	0.9 ± 1.9
19	22.7 ± 0.2	128	3.2 ± 2.9
20	24.3 ± 0.2	32	8.8 ± 3.6
21	**25.2 ± 0.2**	**16**	**4.2 ± 2.0**
22	22.8 ± 0.2	> 128	3.1 ± 4.4*
23	23.8 ± 0.1	128	4.5 ± 3.2
24	24.6 ± 0.1	32	7.2 ± 5.0
25	**25.7 ± 0.2**	**16**	**7.2 ± 3.4**

THE UNIVERSITY of WISCONSIN MADISON

Lee, M.-R.*, Raman, N*., et al. *ACS Chemical Biology*, (2014) 9, 1613

β-peptide hydrophobicity correlates with antifungal and hemolytic activity



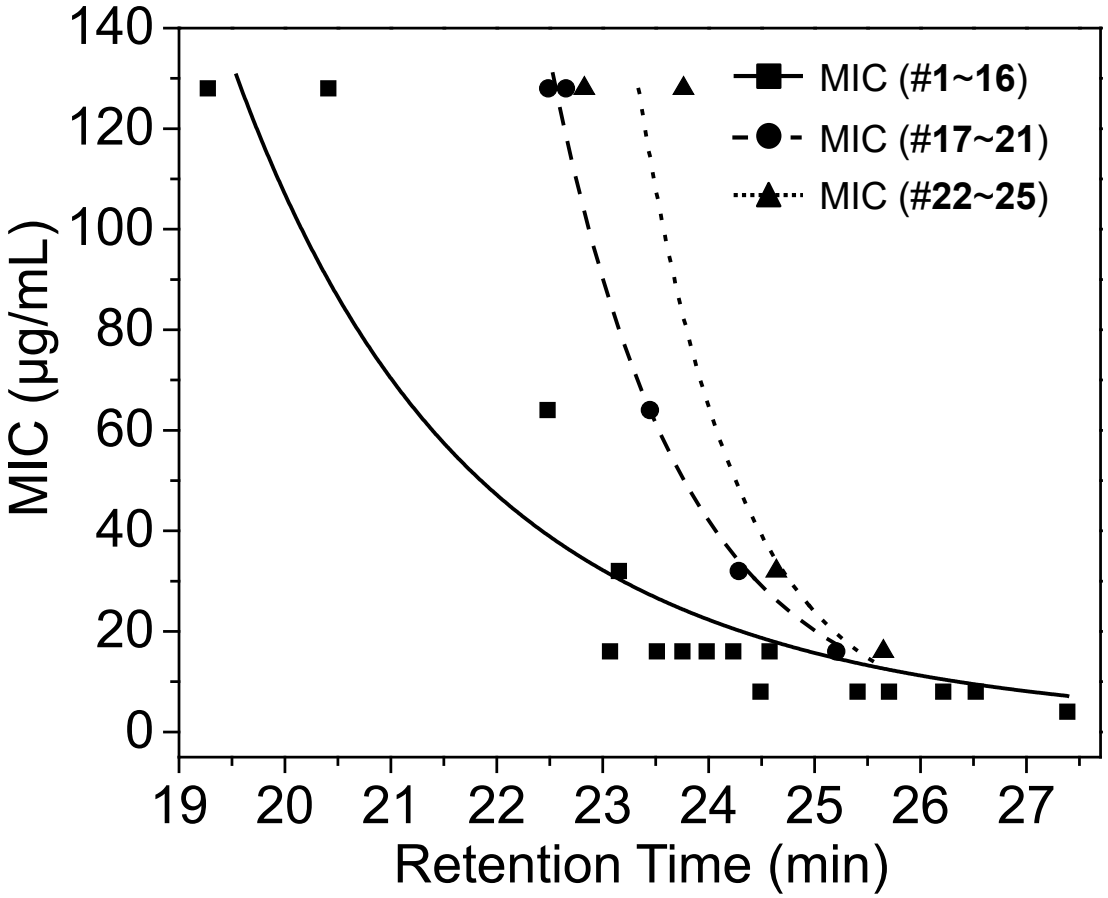
Peptide #	RT [a] (min)	MIC [b] (μg/mL) ATCC90028	K1	SC5314
1	19.3	>128	>128	>128
2	22.5	64	64	64
6	23.1	32	32	16
3	23.2	32	32	32
7	23.8	32	16	16
4	24.5	16	16	8
5	25.4	16	8	8
8	26.2	8	4	8

- Window of hydrophobicity where β-peptides have selective antifungal activity
- β-Peptide planktonic MICs were similar across multiple *C. albicans* strains

THE UNIVERSITY of WISCONSIN MADISON

Lee, M.-R.*, Raman, N*., et al. *ACS Chemical Biology*, (2014) 9, 1613

β-peptide helicity affects antifungal activity



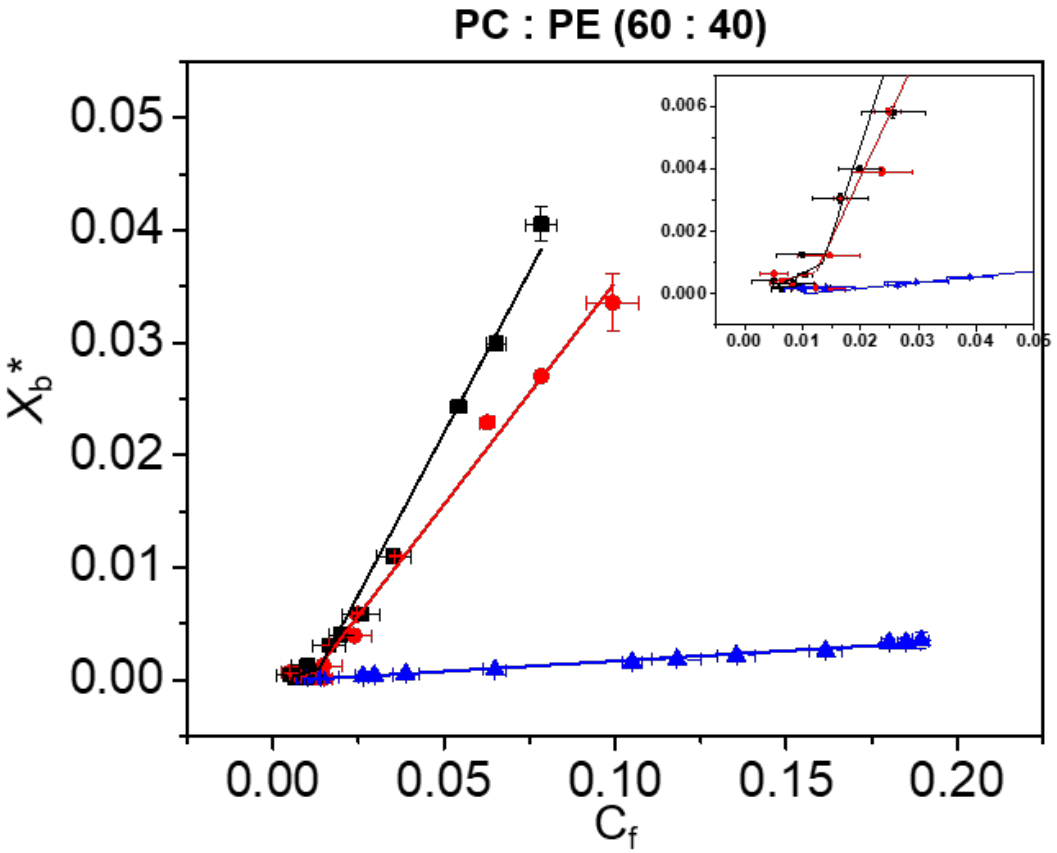
1-16: 3 ACHC side chains
17-25: no ACHC

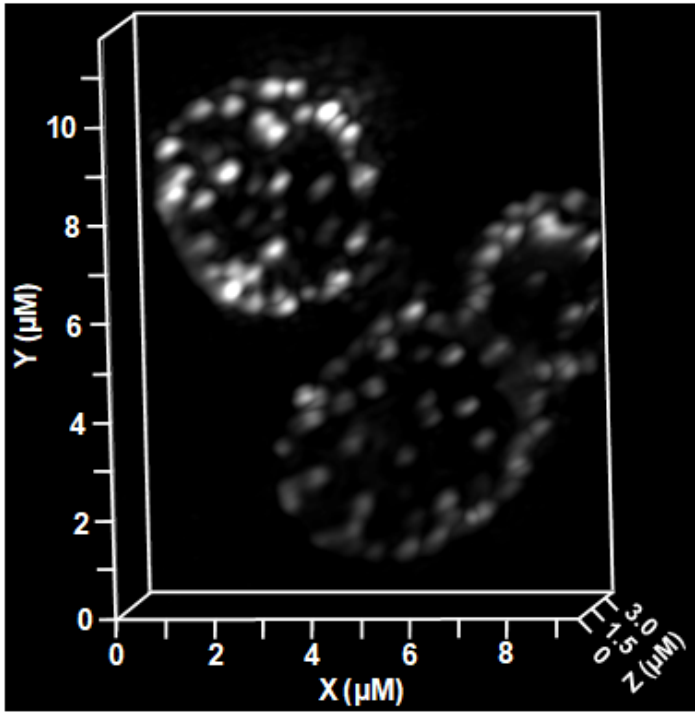
➢ ACHC incorporation increases antifungal activity in more hydrophobic β-peptides

THE UNIVERSITY of WISCONSIN MADISON

Lee, M.-R.*, Raman, N*., et al. *ACS Chemical Biology*, (2014) 9, 1613

β-peptides form pore-like structures in the plasma membrane





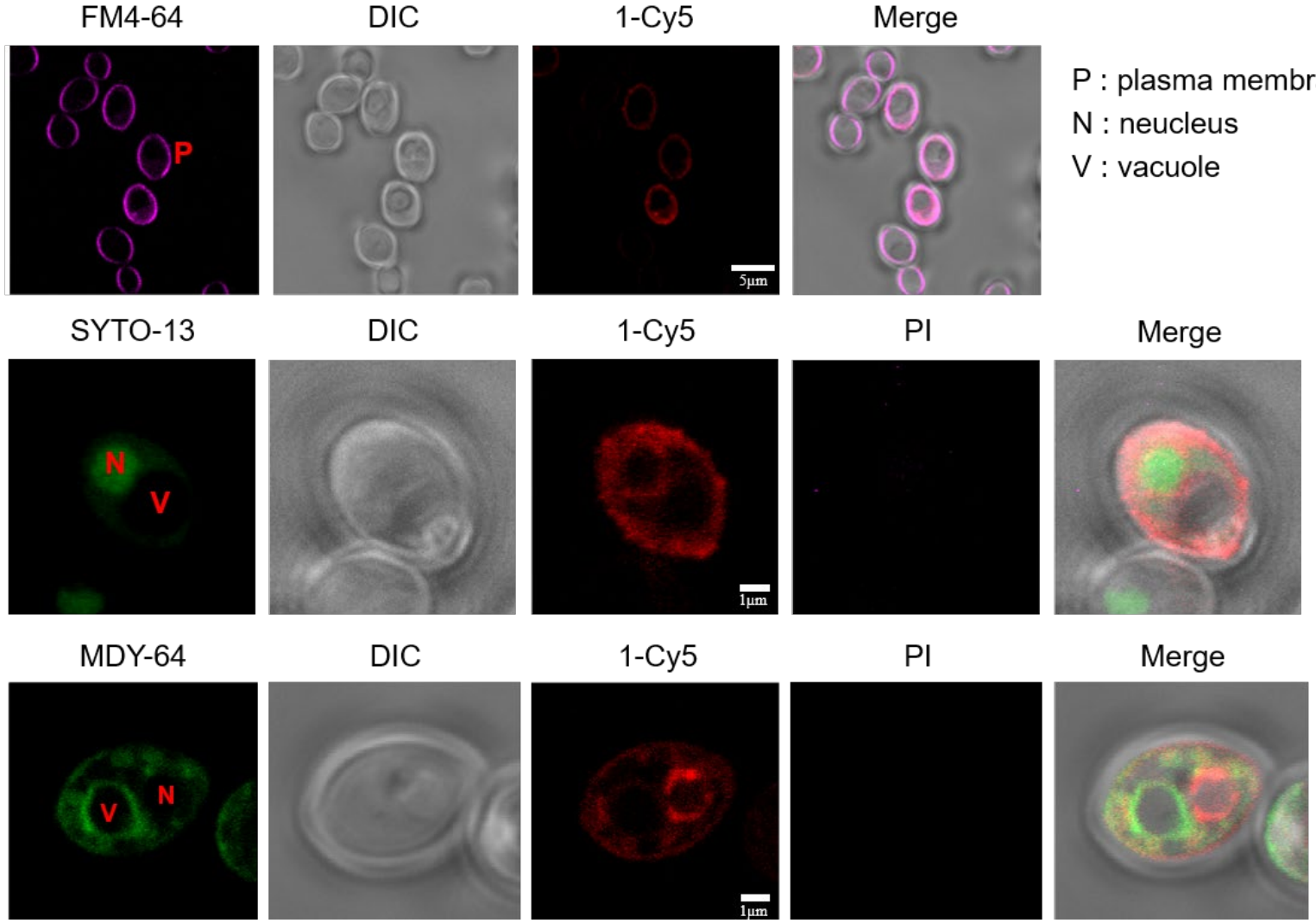
Cooperative binding of β-peptides to synthetic vesicles

β-peptides form 300 nm aggregates on the surface of *C. albicans* cells

THE UNIVERSITY of WISCONSIN MADISON

Lee, M.R. et al. *Cell Chem Biol.* 2018.

β-peptides disrupt intracellular organelles



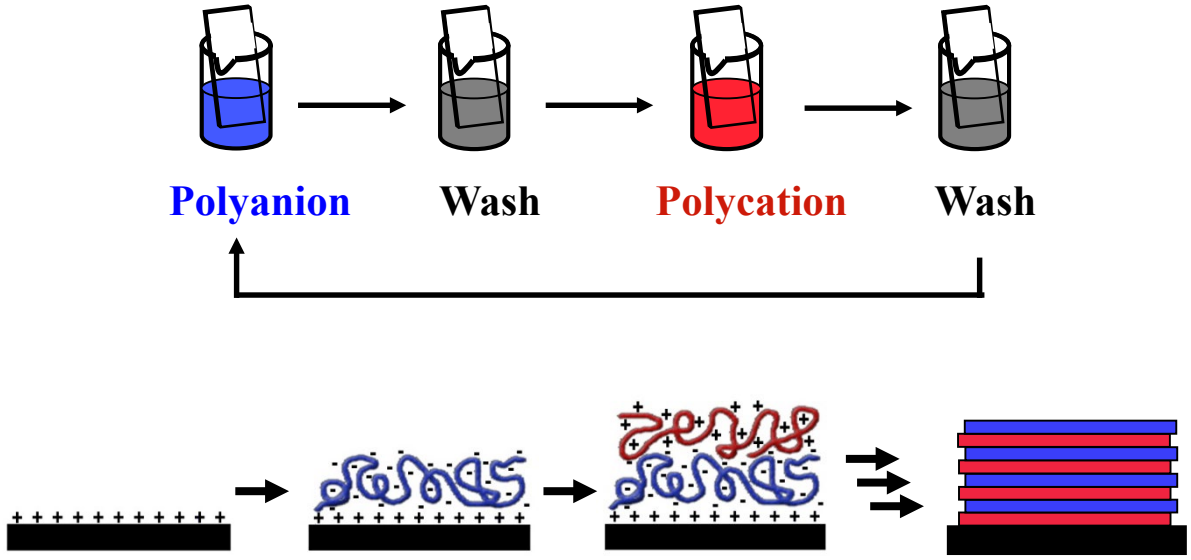
β-peptides disrupt the cell membrane, enter the cell and lyse the nucleus and vacuole

THE UNIVERSITY of WISCONSIN MADISON

Lee, M.R. et al. *Cell Chem Biol.* 2018.

Polyelectrolyte multilayer films

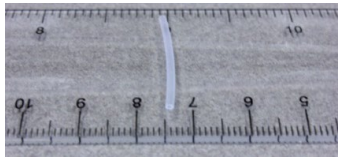
- Layer-by-layer adsorption of oppositely charged polyelectrolytes



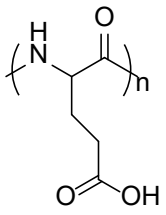
- Electrostatic self assembly process
- Compatible with biomolecules
- Versatile, inexpensive
- Coat topologically complex substrates

PEM fabrication in catheters

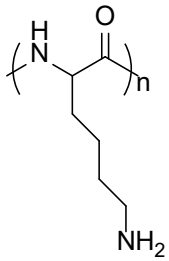
Catheter Segment



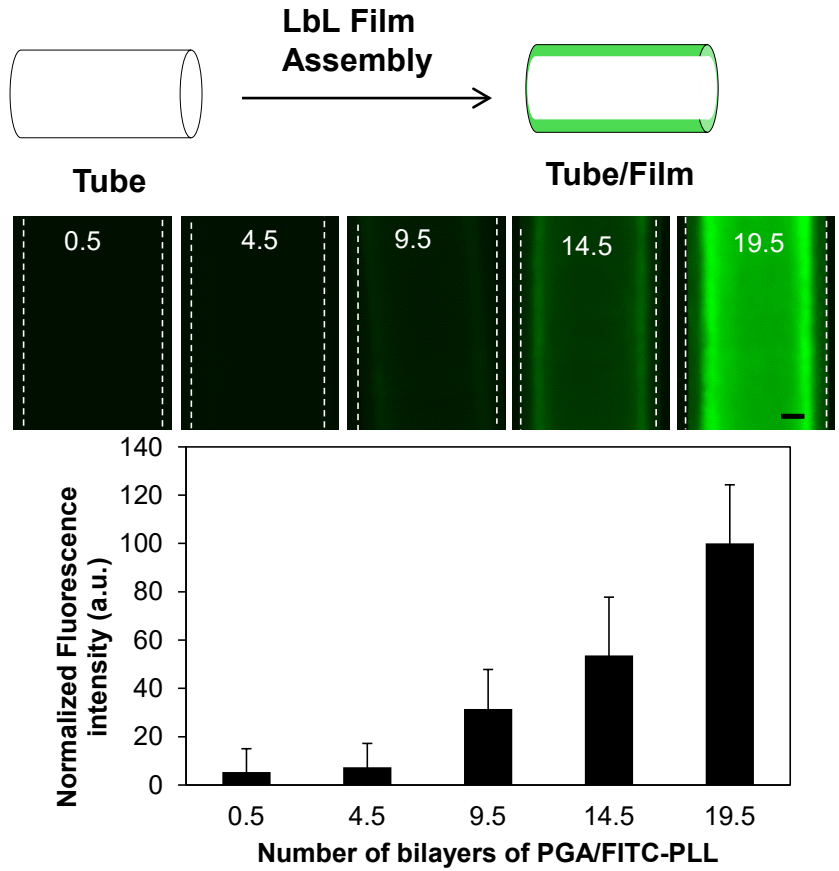
860 µm id polyethylene tube



Poly(L-glutamic acid)
(PGA)

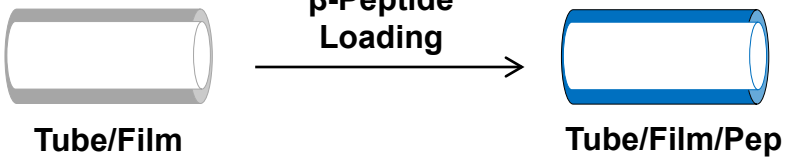


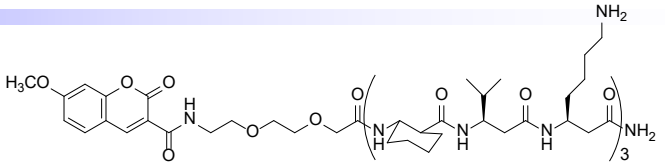
Poly(L-lysine)
(PLL)



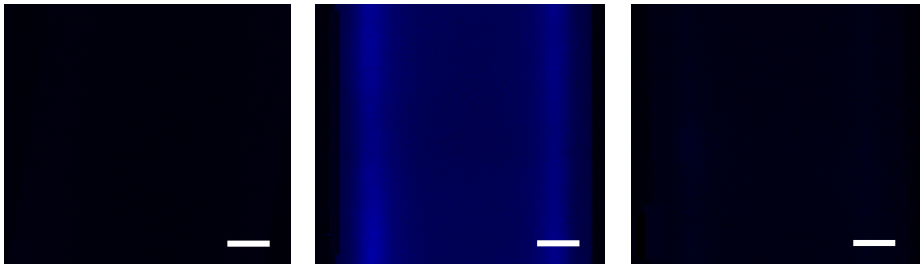
- Supra-linear PEM film growth of PGA/PLL multilayers fabricated on the inner surfaces of catheter tubes

Loading and release of β-peptide from PEM-coated catheters

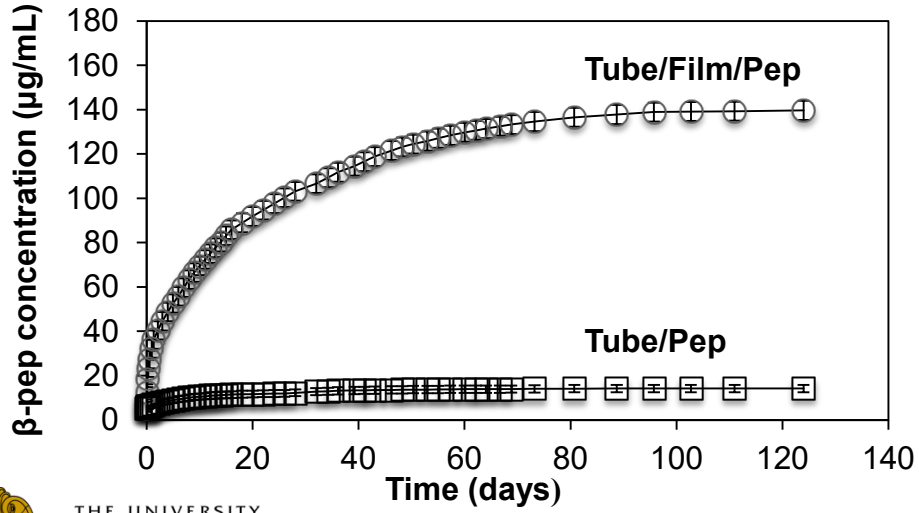




Coumarin-linker-(ACHC-β³Val-β³Lys)$_3$



Tube/Pep Day 0 | **Tube/Film/Pep Day 0** | **Tube/Film/Pep Day 120**

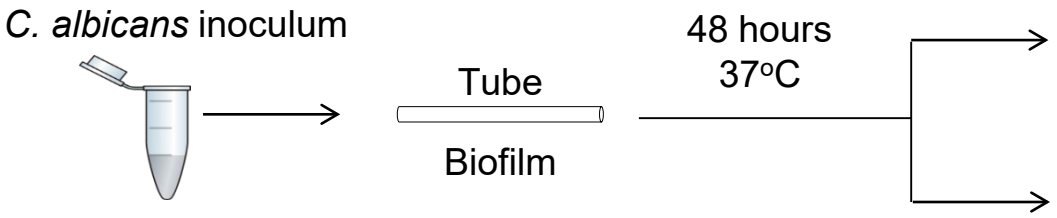


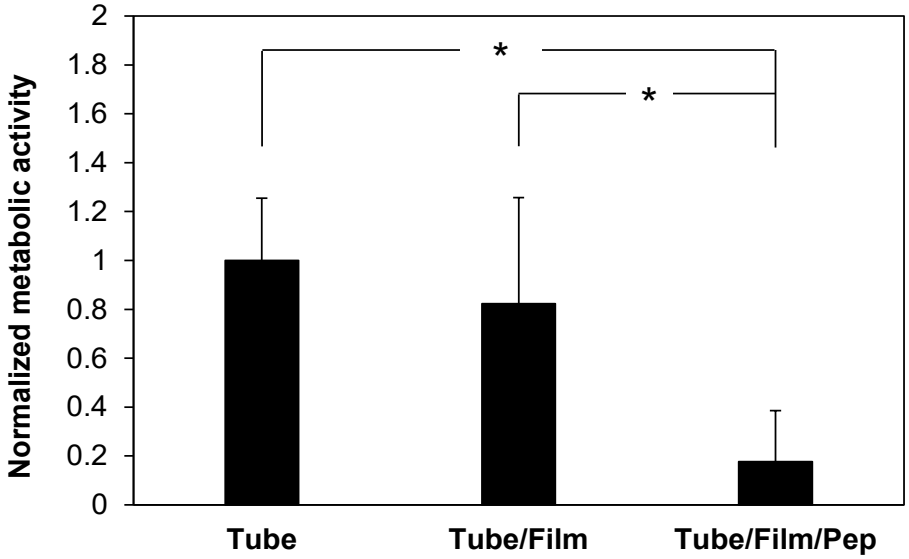
- β-Peptide can be loaded post PEM fabrication
- Multilayer film enhances the localization of the peptide on the inner surface of the catheter
- β-Peptide is gradually released from film coated, peptide loaded catheters over the course of 60-80 days.

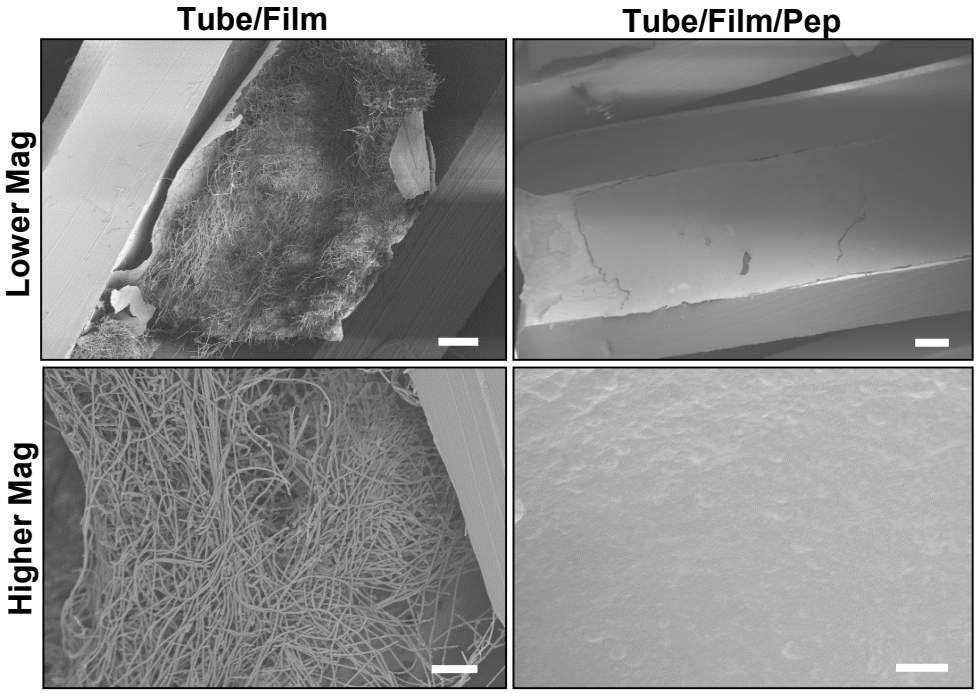
THE UNIVERSITY of WISCONSIN MADISON

Raman, N., et al., *Journal of controlled release* (2014) 191, 54

Prevention of *C. albicans* biofilms in β-peptide-loaded catheters







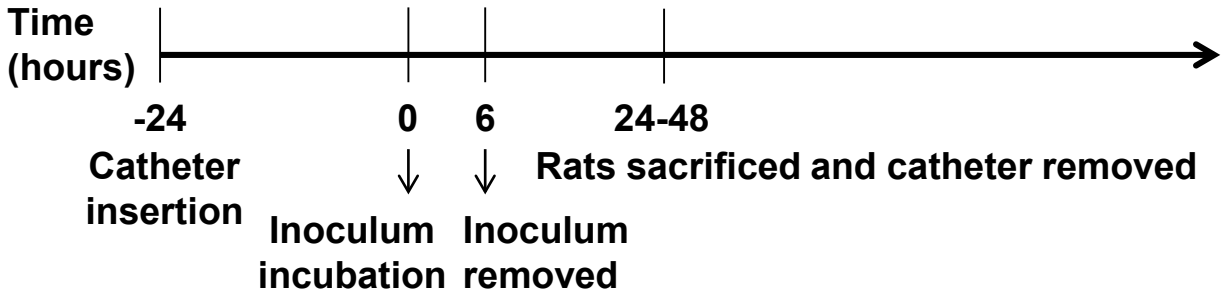
- Catheters coated with β-peptide-loaded films inhibit *C. albicans* biofilm formation

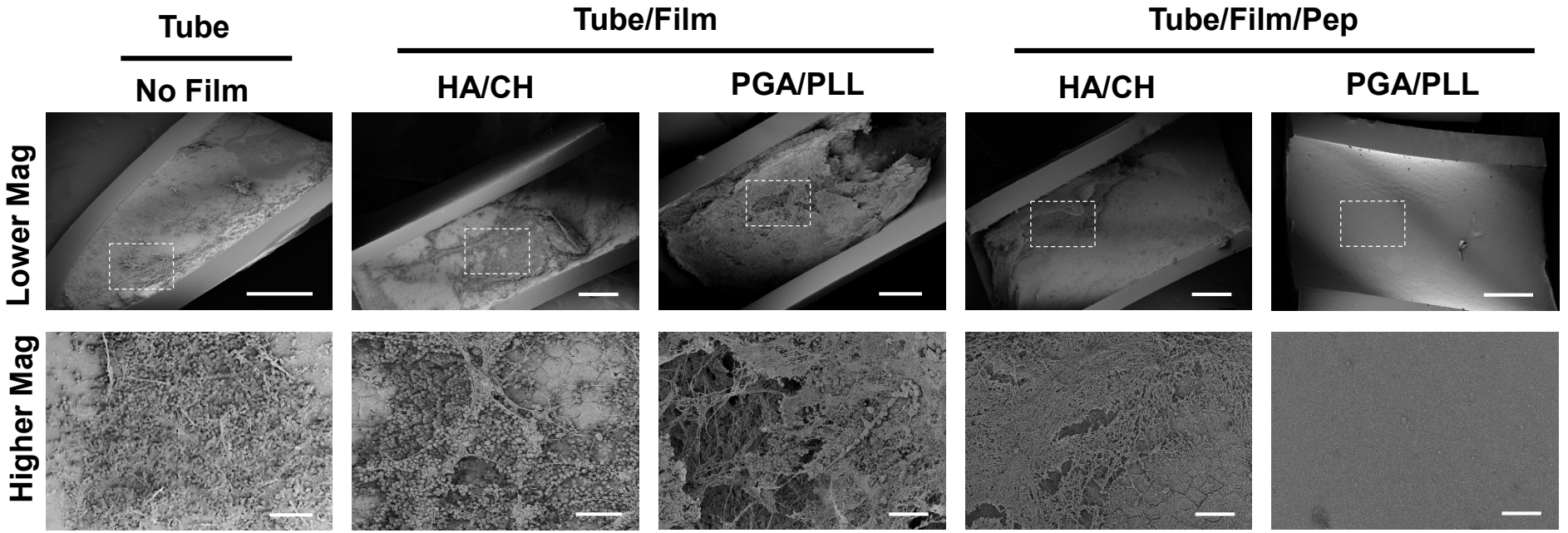
THE UNIVERSITY of WISCONSIN MADISON

Raman, N., et al., *Journal of controlled release* (2014) 191, 54

Reduction of biofilm formation *in vivo* on β-peptide loaded catheters

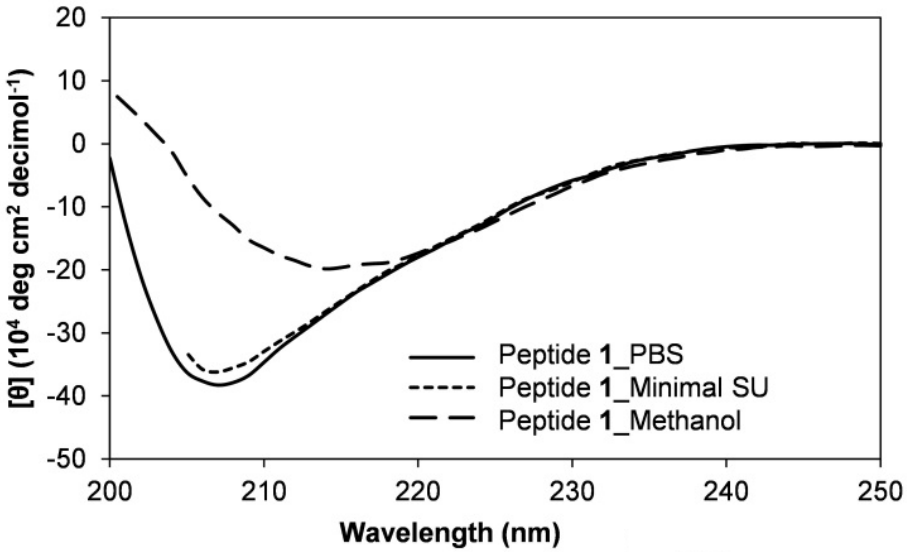
- **Rat central venous catheter model**





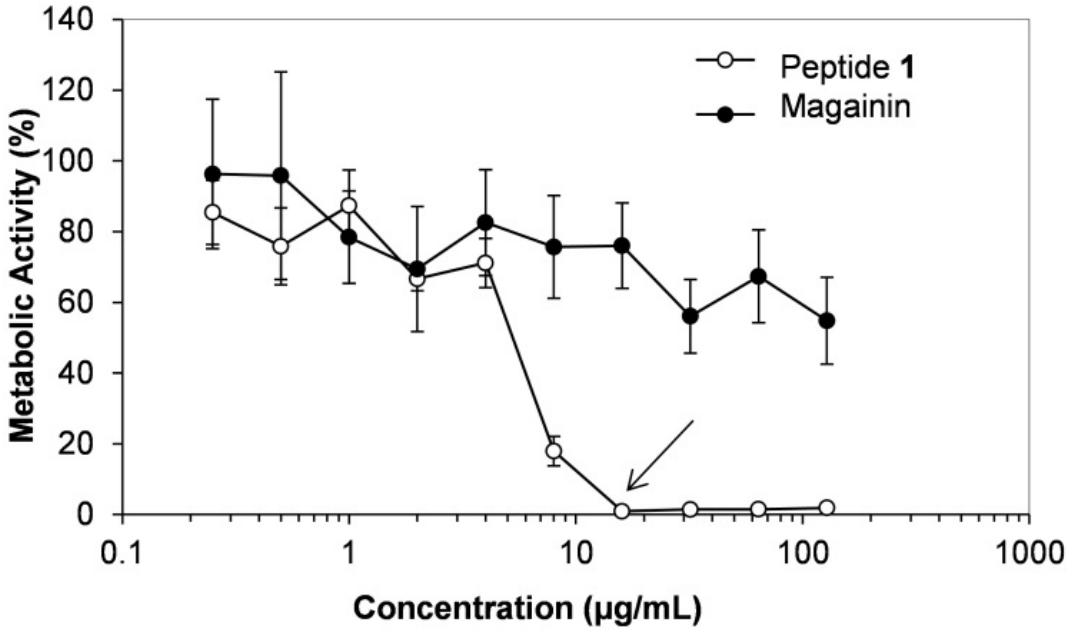
Raman, N. et al., *ACS Biomaterials Science & Engineering* (2016) 2, 112

β-peptide activity in synthetic urine



β-peptide remains helical in synthetic urine

MIC=16 μg/ml (8 in RPMI)
MBPC=32 μg/ml (32 in RPMI)

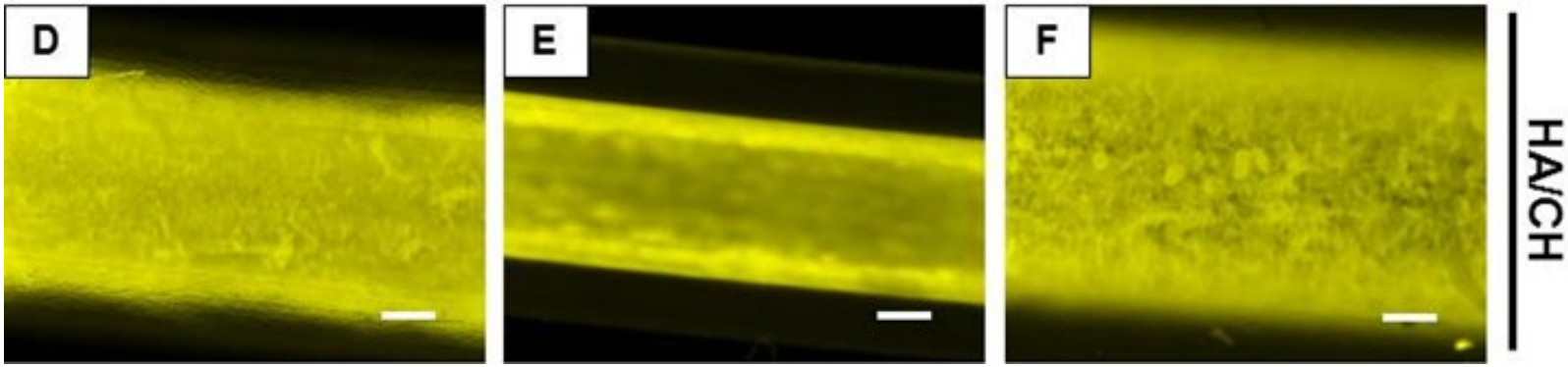


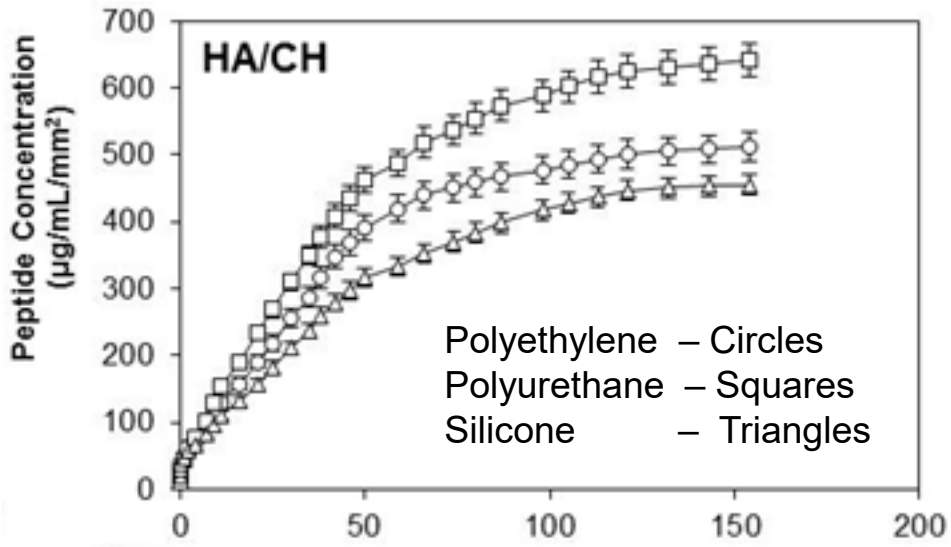
THE UNIVERSITY of WISCONSIN MADISON

β-peptide release from films on urinary catheters

polyethylene polyurethane silicone

Fluorescent β-peptide



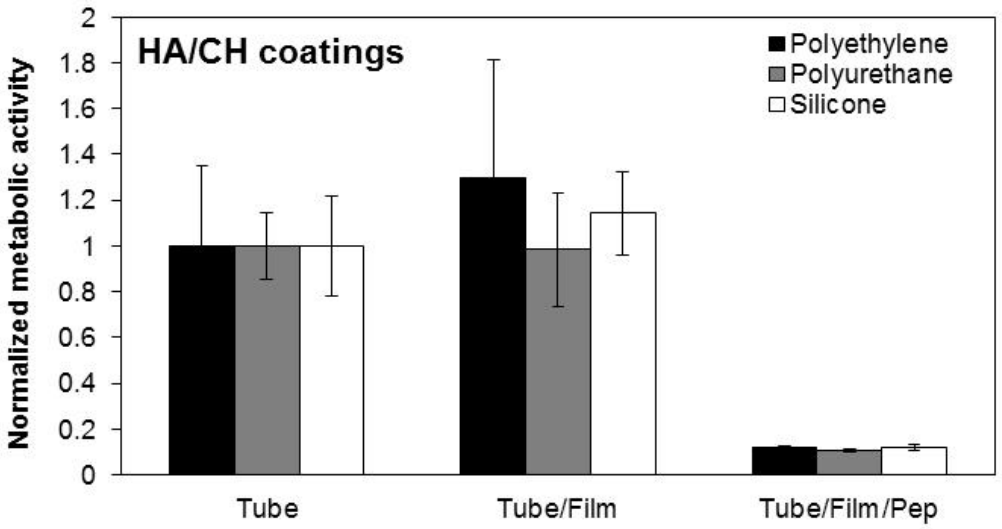


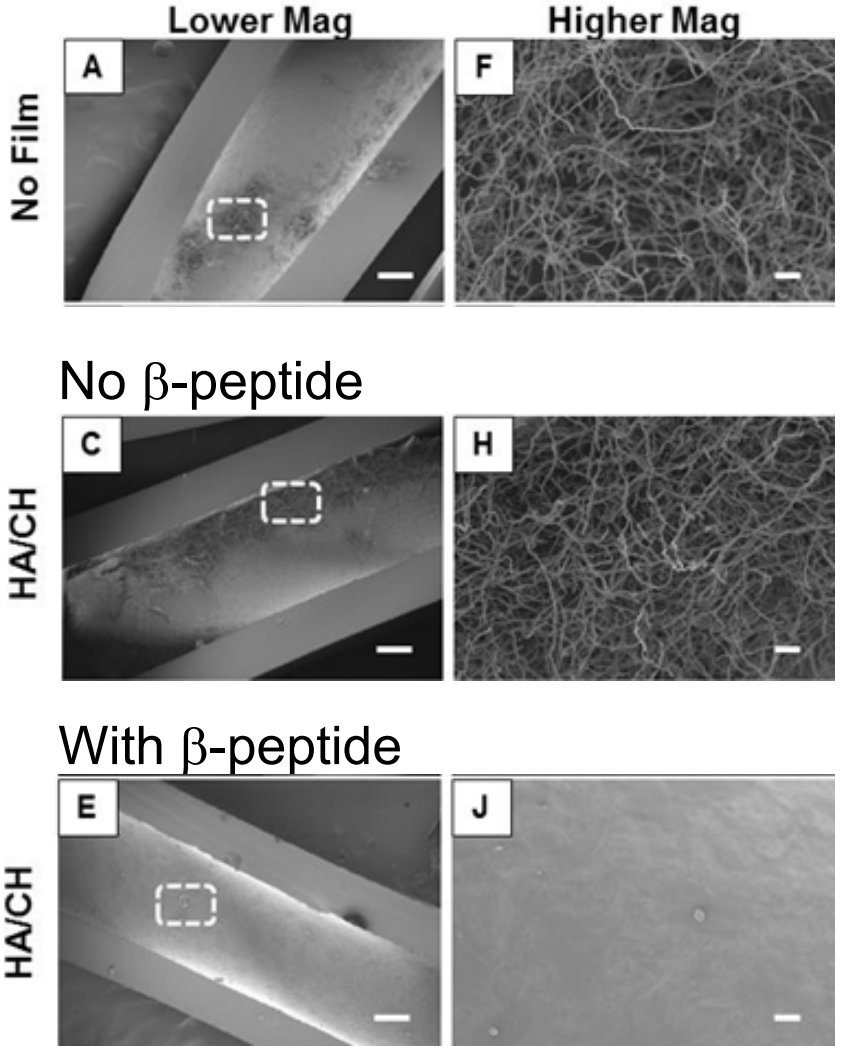
β-peptide release over 2-4 months

THE UNIVERSITY of WISCONSIN MADISON

Raman, N. et al. *Acta Biomater.*. 2016. 43:240-250.

β-peptide-loaded catheters resist *C. albicans* biofilm formation in SU





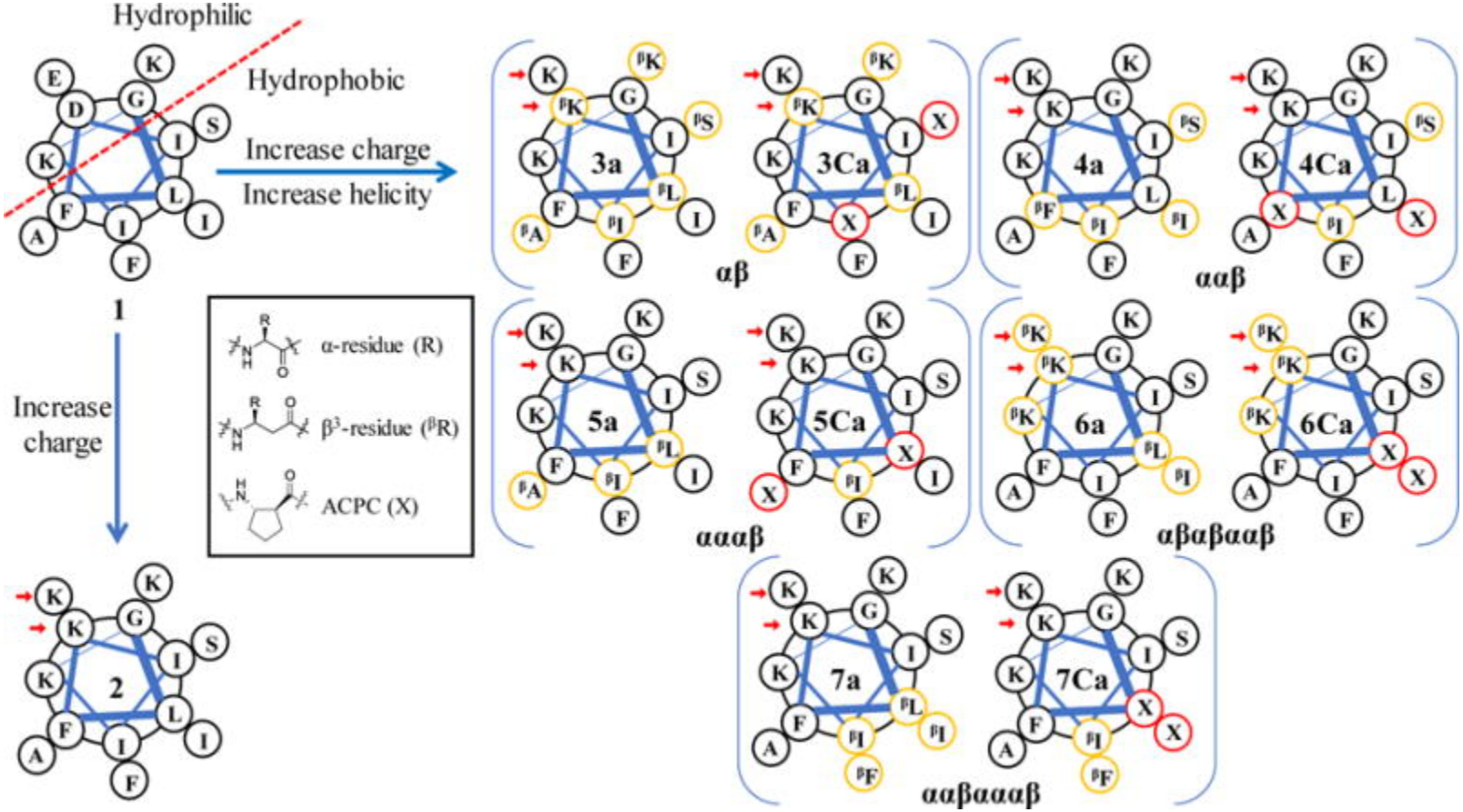
HA/CH films loaded with β-peptide prevent *C. albicans* biofilm formation on catheters *in vitro*

THE UNIVERSITY of WISCONSIN MADISON

Raman, N. et al. *Acta Biomater.*. 2016. 43:240-250.

α/β-peptide analogues of aurein 1.2

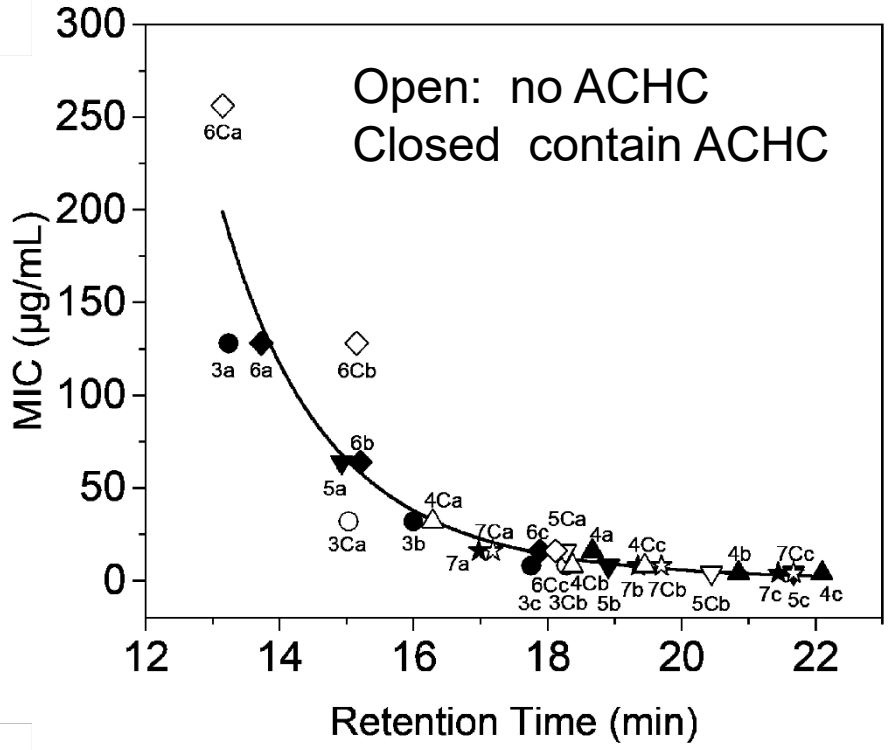
α/β peptides mimic sequence and structure of aurein 1.2



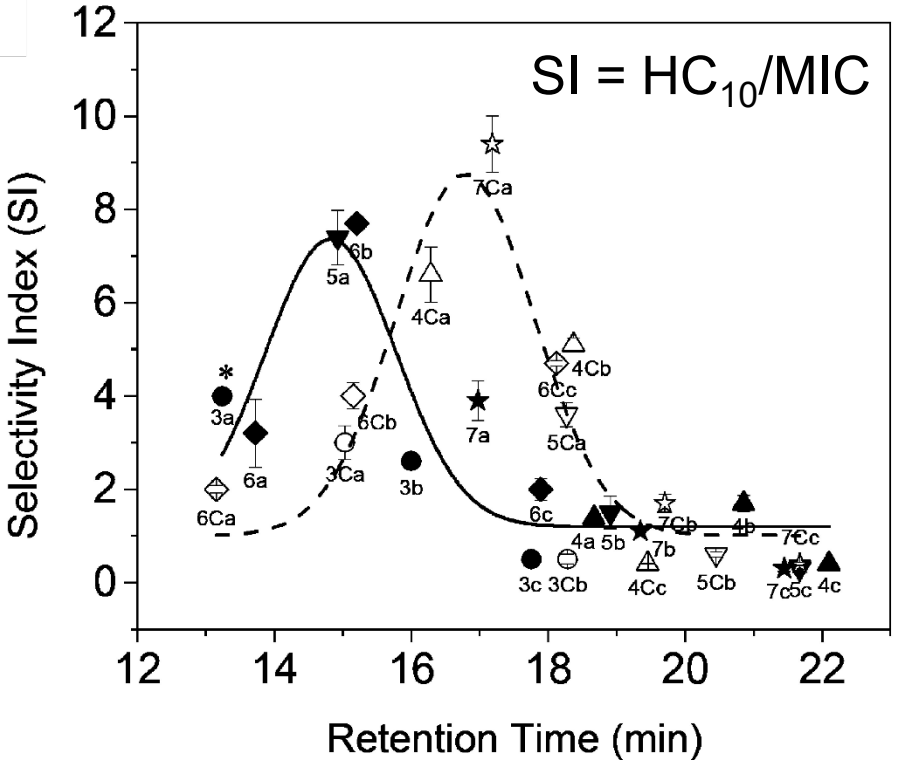
Lee, M.R. et al. *ACS Chem Biol.* 2017. 12:2975-2980

Active and selective α/β-aurein analogues

Peptide activity



Peptide selectivity



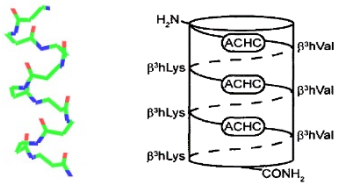
Active and selective α/β-aurein analogues at intermediate hydrophobicity

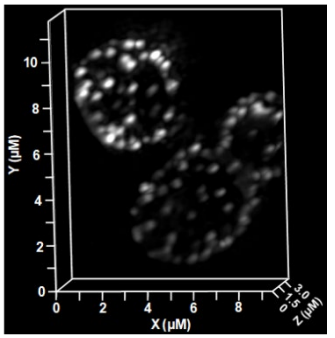
THE UNIVERSITY of WISCONSIN MADISON

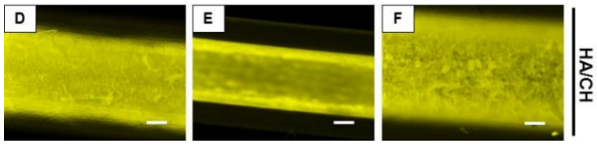
Lee, M.R. et al. *ACS Chem Biol.* 2017. 12:2975-2980

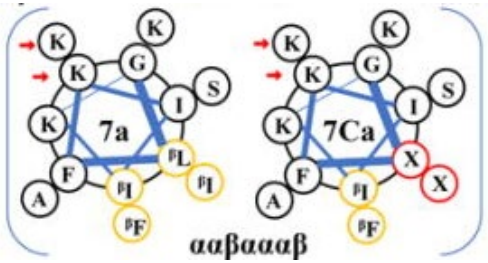
Summary

- Amphiphilic, helical β-peptides exhibit selective antifungal activity
- β-peptides kill *C. albicans* by plasma and intracellular membrane lysis
- Delivery of antifungal β-peptides from polyelectrolyte multilayers prevents *C. albicans* biofilms on catheters *in vitro* and *in vivo*
- α/β-analogues of aurein 1.2 have been engineered for selective antifungal activity

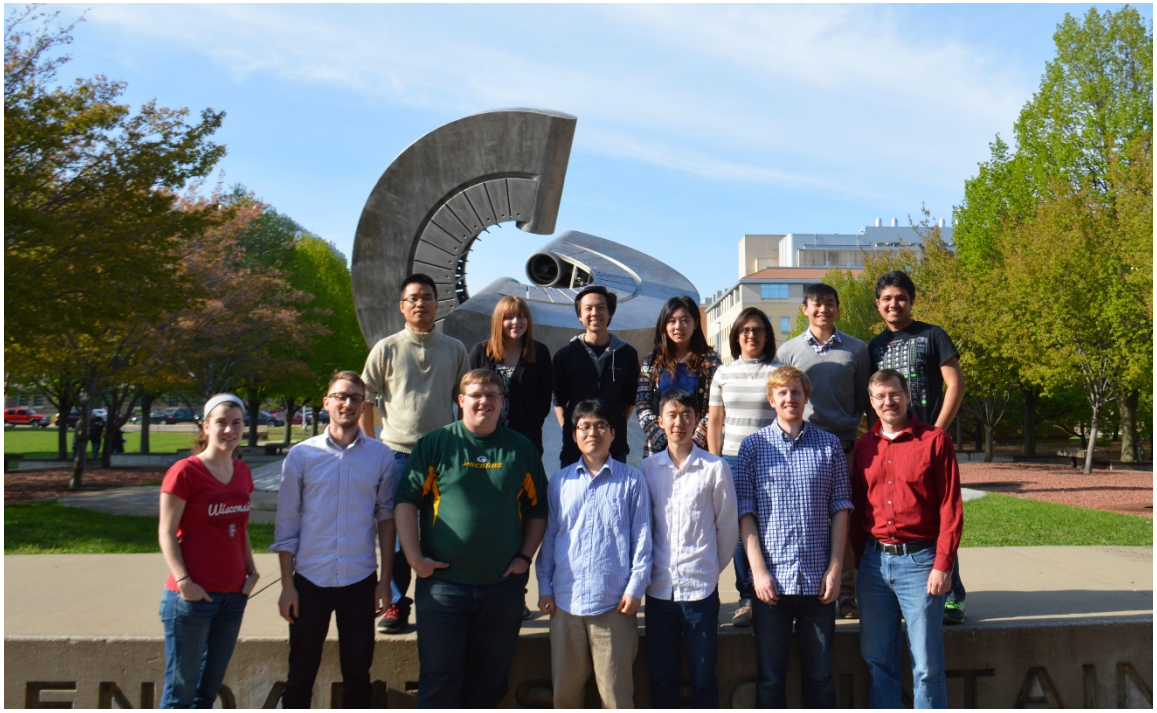








Acknowledgements



David Lynn

David Andes

Funding
NIH/NIAID
NSF NSEC

Namrata Raman

Sam Gellman

Recruiting postdocs: peptide engineering, delivery, applications

THE UNIVERSITY of WISCONSIN MADISON