Supplementary Information

Probing the penetration of antimicrobial polymyxin lipopeptides into Gram-negative bacteria

Zakuan Z. Deris[1,2], James Swarbrick[3], Kade D. Roberts[3], Mohammad A. K. Azad [1], Jesmin Akter[1], Andrew. S. Horne[3], Roger L. Nation[1], Kelly L. Rogers[4], Phillip E. Thompson[3], Tony Velkov[1*], Jian Li[1*]

[1]Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Victoria, Australia; [2]Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia;[3]Department of Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Victoria, Australia; and [4]Centre for Dynamic Imaging, The Walter & Eliza Hall Institute, Parkville, VIC 3052, Australia.

*Corresponding authors: Jian Li, Phone: +61-3-9903-9702; Fax: +61-3-9903-9583; Email: colistin.polymyxin@gmail.com or Tony Velkov, Phone: +61 3 9903 9539. Fax: +61 3 9903 9583. E-mail: Tony.Velkov@monash.edu

Supplementary Figures

Figure S1.

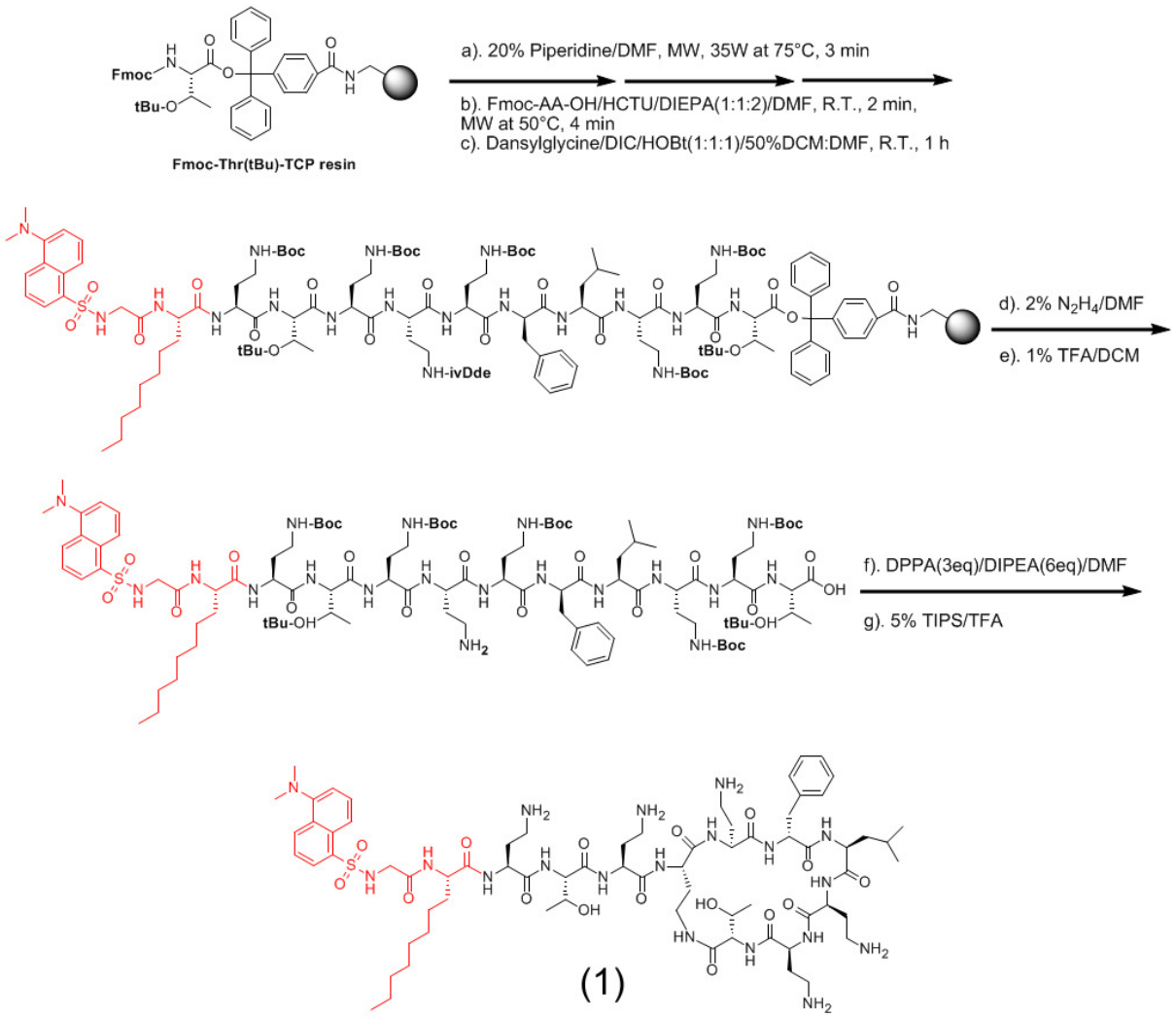
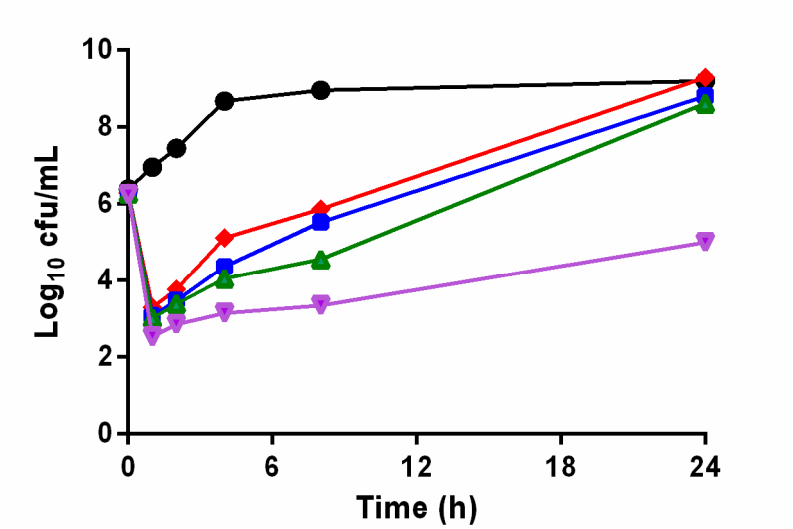


Figure S1. Scheme for the synthesis of dasnyl-polymyxin probe (**1**).

Figure S2.

A.



B.

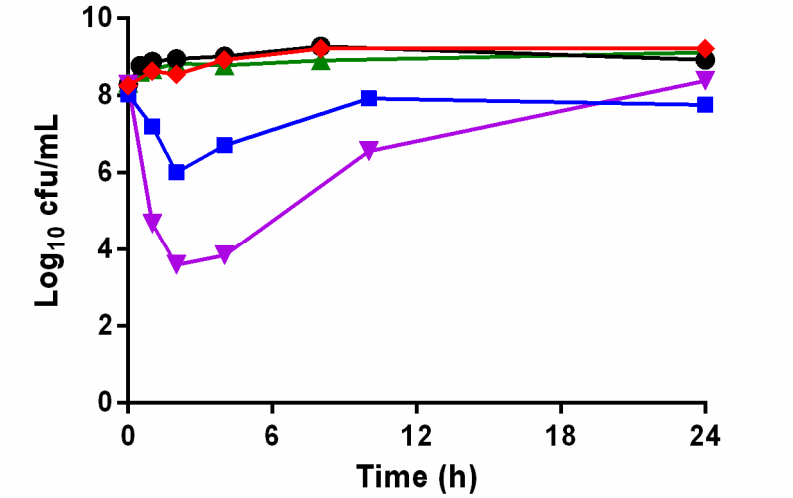


Figure S2. Static time-kill kinetics of (**1**) and colistin against *K. pneumoniae* ATCC 13883. At the indicated times, cultures were plated and the cfu per mL were enumerated. (**A**) Initial inoculum 10^6cfu/mL. (**B**) Initial inoculum 10^8cfu/mL.Control (●); colistin 0.5× MIC (♦); colistin 5× MIC (■); probe (**1**) 0.5× MIC (▲) and probe (**1**) 5× MIC (▼).

Figure S3.

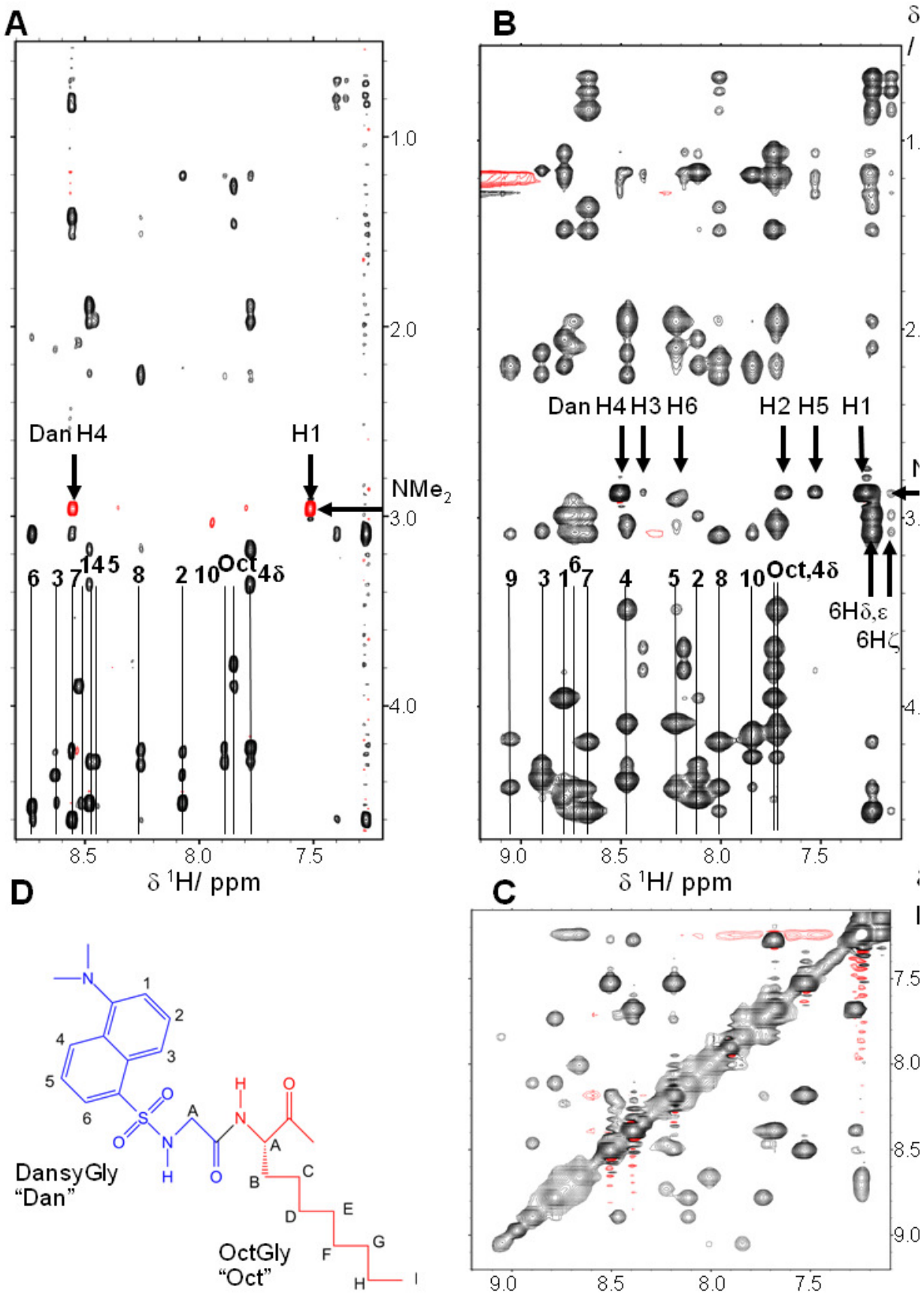

A
B
C
D
Dan H4
H1
NMe2
Dan H4 H3 H6
H2 H5 H1
6Hδ,ε
6Hζ
δ 1H/ ppm
δ 1H/ ppm
DansyGly
"Dan"
OctGly
"Oct"

Figure S3. (**A**) Fingerprint region of the 400 ms 2D NOESY spectrum of 2 mM probe (**1**) in 90% H_2O/10% D_2O at pH 4.5 and 25ºC. Positive and negative peaks (with respect to the positive diagonal) are shown in black and red respectively. (**B**) Fingerprint and (**C**) Amide region of the 150 ms 2D NOESY spectrum of 1.4 mM FADDI-043 in 90% H_2O/10% D_2O / 150 mM DPC. The amide proton assignments are given as vertical lines in F2. Assignments of the napthalene protons of the dansyl group are also shown. (**D**) Structure of the dansylGly and OctGly residues.

Figure S4.

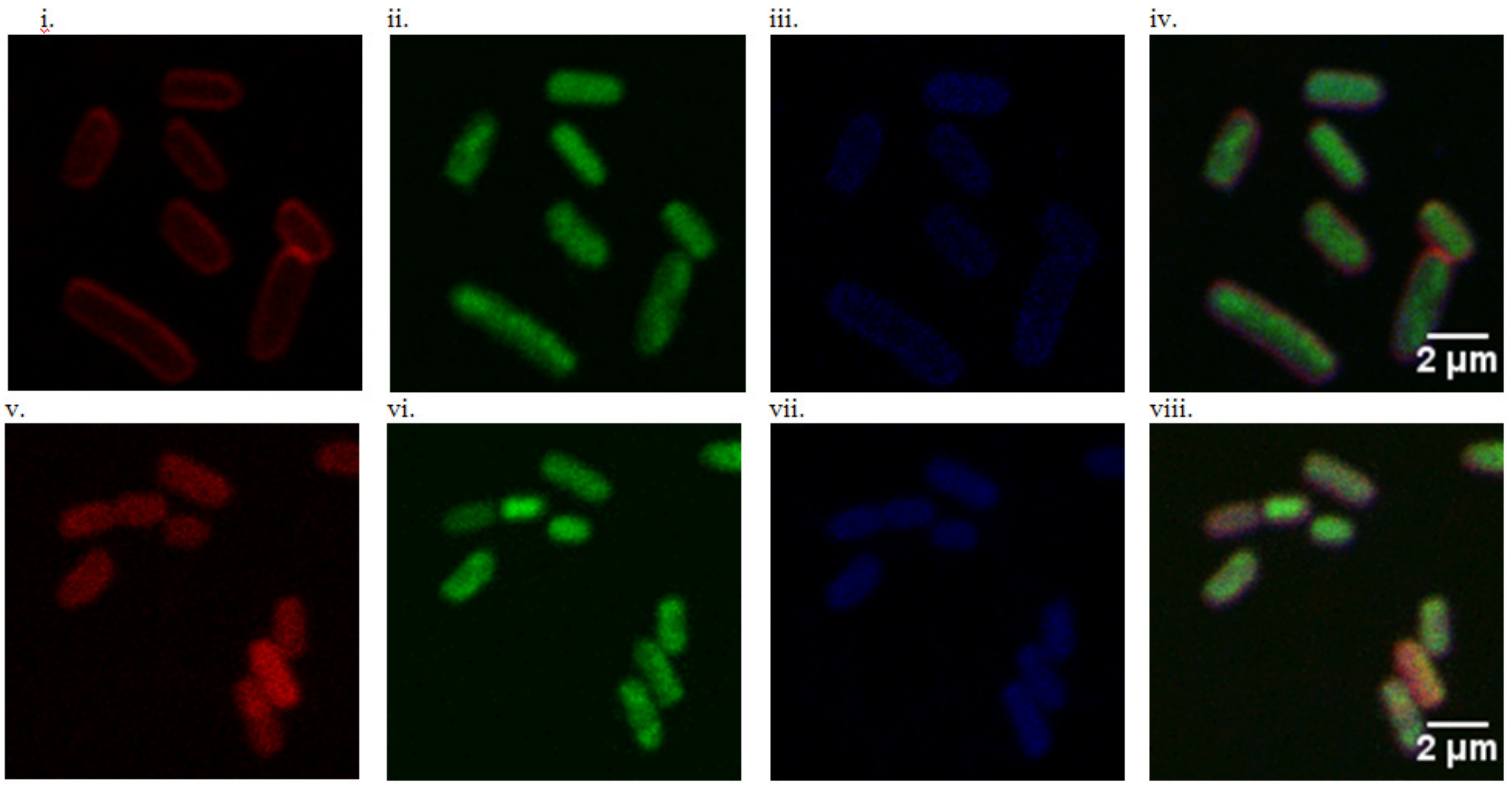
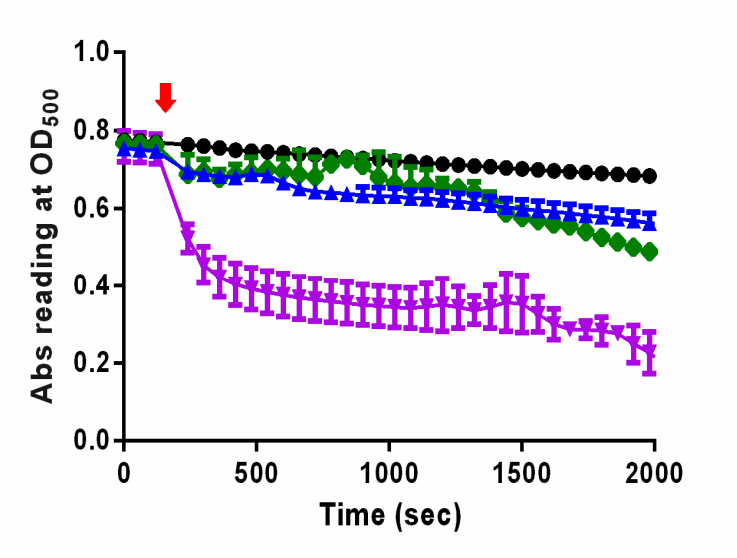


Figure S4. Laser scanning confocal microscopy imaging of *K. pneumoniae* ATCC 13883 cells following treatment with (**1**) at 0.5× MIC (top panels) and 5× MIC (bottom panels). The different channels for (**1**) (**i&v**), SYTOX green (**ii&vi**), FM4-64 (**iii&vii**) or all channels overlaid (**iv&viii**) are shown.

Figure S5

A.


1.0
0.8
0.6
0.4
0.2
0.0
Abs reading at OD500
0
500
1000
1500
2000
Time (sec)

B.

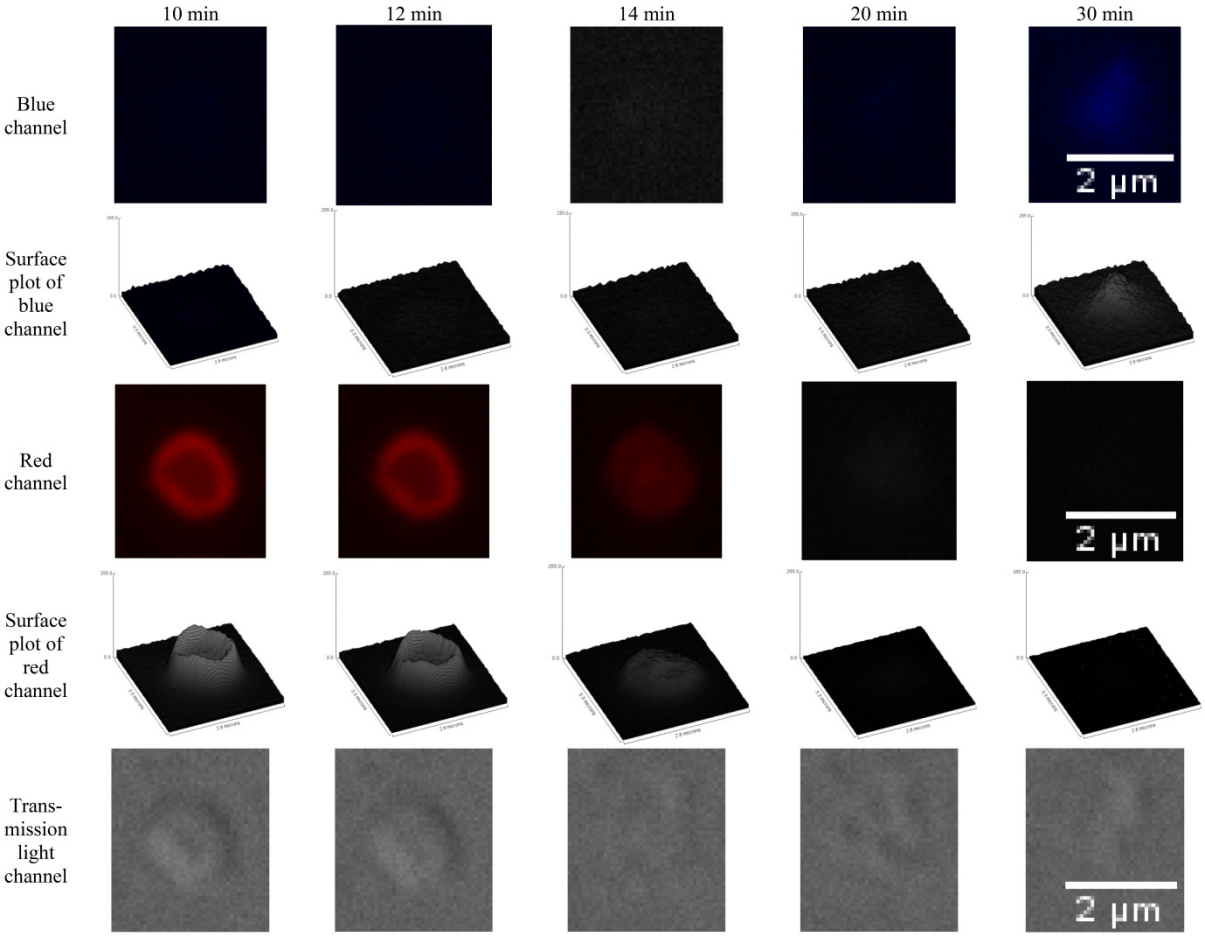

10 min
12 min
14 min
20 min
30 min
Blue channel
Surface plot of blue channel
Red channel
Surface plot of red channel
Trans-mission light channel
2 µm
2 µm
2 µm

Figure S5. (**A**) Lysis of a *K. pneumoniae* ATCC 13883 spheroplast suspension following treatment with probe (**1**). Spheroplast lysis was followed as the decrease in the absorbance at 500 nm; the red arrow designates the addition of (**1**). The experiment was performed in triplicate, the error bars indicate the standard deviation. Taurocholic acid treatment was used as a control lytic agent. Untreated spheroplast (●); taurocholic treated spheroplast (♦); peptide (**1**) treated spheroplast at 0.5× MIC (▲) and peptide (**1**) treated spheroplast at 5× MIC (▼). (**B**) Laser scanning confocal microscopy time-lapse imaging of *K. pneumoniae* ATCC 13883 spheroplasts following treatment with (**1**) at 5× MIC.

Table S1. ^{1}H and ^{13}C observed chemical shifts for probe (**1**) in 50 mM acetate, pH 4.5, 25°C.

Dansyl[1]	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**HA**	**NMe**	
	7.51	7.79	8.36	8.56	7.74	8.30	3.77	2.96	
	119.19	132.88	122.05	133.17	126.97	132.73	47.54	47.85	
OctyGly[1]	**HA**	**HB**	**HC**	**HD**	**HE**	**HF**	**HG**	**HH**	**HI**
	3.89	1.45 1.26	1.04	1.19	1.23	1.24	1.24	1.28	0.87
	56.72	33.35	27.46	30.85	31.09	33.42	33.91	24.82	16.31
	HN	**aCH**	**bCH**	**gCH**	**dCH**	**eCH**	**Other**		
1 LDab	8.53	4.51	2.20 2.07	3.06					
		53.95	31.21	39.331					
2 Thr	8.07	4.35	4.23	1.19					
		61.722	69.962	21.536					
3 LDab	8.63	4.50	2.24 2.12	3.12					
		54.05	31.53	39.40					
4 LDab	8.48	4.29	1.97 1.88	3.35 3.17			**dNH** 7.77		
		54.54	33.40	39.08					
5 LDab	8.45	4.52	2.05 1.95	2.91 2.82					
		53.32	32.65	38.90					
6 DPhe	8.73	4.60	3.10		7.27	7.40	**zCH** 7.36		
		58.53	39.37		131.76	131.75	130.18		
7 Leu	8.56	4.22	1.50 1.41	0.84			**d1CH** 0.77	**d2CH** 0.70	
		54.62	41.88	26.23			25.08	23.08	
8 LDab	8.25	4.30	2.25	3.16					
		54.78	30.77	39.40					

9 LDab	8.67 (10 °C)	4.28	2.19	3.10					
		54.67[2]	31.3[2]	39.64[2]					
10 Thr	7.89	4.21	4.27	1.19					
		62.27	69.12	21.91					

[1]HN of Dansy and OctylGly were not observed.

[2]The assignments for 9LDab are based on experiments recorded at 10 °C. At 25 °C the amide was not observed.

Table S2. ^{1}H and ^{13}C observed chemical shifts for probe (**1**) in 50 mM acetate, pH 4.5, 100 mM DPC (d38), 25°C.

Dansyl[1]	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**HA**	**NMe**	
	7.28	7.68	8.39	8.51	7.53	8.19	3.69 3.80	2.87	
	118.23	131.50	122.11	133.85	126.90	132.36	47.98	47.89	
OctGly[1]	**HA**	**HB**	**HC**	**HD**	**HE**	**HF**	**HG**	**HH**	**MeI**
	3.95	1.47	1.07	1.16	1.28	1.28	1.20	1.31	0.90
	56.29	34.18	28.11	32.01	32.15	34.63	34.17	25.28	16.68
	HN	**aCH**	**bCH**	**gCH**	**dCH**	**eCH**	**Others**		
1 LDab	8.78	4.49	2.19 2.05	2.99					
		53.71	30.99	39.25					
2 Thr	8.11	4.38	4.31	1.17					
		61.63	69.92	21.75					
3 LDab	8.90	4.39	2.24 2.13	3.08					
		54.65	30.99	39.33					
4 LDab	8.47	4.09	1.95	3.49 3.04			**dNH** 7.72		
		54.39	33.22	38.69					
5 LDab	8.23	4.43	2.10 1.96	2.90					
		53.49	31.92	39.06					
6 DPhe	8.74	4.55	3.08 2.98		7.24	7.24	**zCH** 7.15		
		58.33	40.35		131.18	132.04	129.28		
7 Leu	8.66	4.187	1.48 1.35	0.84			**d1CH** 0.74	**d2CH** 0.66	
		53.86	41.28	26.25			25.79	23.71	
8 LDab	8.01	4.43	2.24 2.156	3.09					

		53.84	31.88	39.40					
9 LDab	9.05	4.18	2.19	3.09					
		56.14	33.00[2]	39.21					
10 Thr	7.843	4.14	4.27	1.18					
		62.21	68.91	22.18					

[1]HN of Dansyl and OctGly were not observed.

[2]Tentative

Table S3. NMR statistics for the solution structure of probe (**1**) in DPC micelles.

NOE distance restraints	
Number	253
Intraresidual, $\|i - j\| = 0$	66
Sequential, $\|i - j\| = 1$	118
Medium range, $1 < \|i - j\| < 5$	33
Long range[a], $\|i - j\| \geq 5$	39
Maximal violation	0.30
Final CYANA target function value	0.55 +/- 0.038 $Å^2$
RMSD to mean coordinates	
Backbone atoms N, C[a], C'	0.34 +/- 0.08 Å
All heavy atoms	0.80 +/- 0.11 Å

[a]4 upper and 3 lower distance restraints were implemented to maintain an amide bond bond with trans geometry between Dab4 and Thr10 during the calculation.