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Supporting Information

The hydrophobic nature of amphiphilic polymethacrylates in the antimicrobial and hemolytic activities

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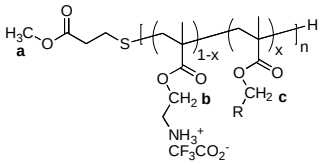
Instrumentation. ^{1}H spectra were obtained on Varian Unity 500 NMR spectrometer. Fluorescence spectra and intensity measurements were recorded using an Aviv Automated Titrating Differential/Ratio Spectrofluorometer (Aviv Biomedical, Lakewood, NJ).

Characterization of copolymers by ^{1}H NMR

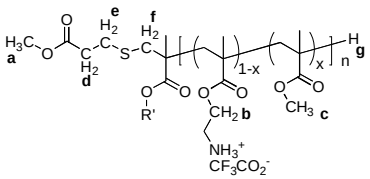
Copolymers with ethyl, butyl or hexyl side chains.



^{1}H NMR analysis provided determination of the degree of polymerization (DP) and the mole percentage of alkyl methacrylate (f_{HB}) for the polymers except methyl methacrylate through integration of the signals from the methylene protons (**b** and **c**) in the monomer side chains and the methyl protons of MMP in the polymer terminus (**a**).

^{1}H NMR (500MHz, Methanol-d_4) for the representative polymer with ethyl side chains. (f_{HB} = 0.35 and DP= 15): d4.4-4.2 (**b**, bs, 19.64H), 4.2-4.0 (**c**, bs, 10.66H), 3.723 (**a**, s, 3H), 2.8-2.5 (m, 8.62H), 2.3-1.8 (m, 24.34H), 1.7-0.8 (m, 61.12H).

Copolymers with methyl side chains.



For polymers having methyl side chains, there is considerable overlap of the chemical shifts of methyl groups in side chains (**c**) and polymer terminus (**a**), which prevents using the same method for the polymers with longer alkyl chains. Therefore, the integration of the signals from the following groups was used: Methyl groups in side chains (**c**, 3H) and at the polymer terminus (**a**, 3H), the methylnene (**c**, **d** and **e**, 6H), and in cationic side chains (**b**, 2H), and the proton in polymer terminus (**g**, 1H).

^{1}H NMR (500MHz, Methanol-d_4) for the representative polymer with methyl side chains. (f_{HB} = 0.38 and DP= 8.6: d7.7-7.2 (**b**, bs, 10.71H), 3.716, 3.666 (**a**, s, and **c**, s, 12.85H), 2.8-2.5 (**d, e, f, g**, m, 7H), 2.3-1.8 (m, 13.99H), 1.7-0.8 (m, 29.68H).

Copolymers with benzyl side chains. The f_{HB} and DP were determined through integration of the signals from the methylene protons (**b**) in the cationic monomer side chains, the methyl protons of MMP in the polymer terminus (**a**) and protons in phenyl groups.

The representative polymer (f_{HB} = 0.17 and DP= 16): d7.7-7.2 (bm, 13,98H), 4.4-4.2 (bs 26.42H), 3.7 (m, 3H), 2.8-2.5 (m, 7.02H), 2.3-1.8 (m, 29.40H), 1.7-0.8 (m, 57.67H).

Antimicrobial and hemolyitc activities of polymers

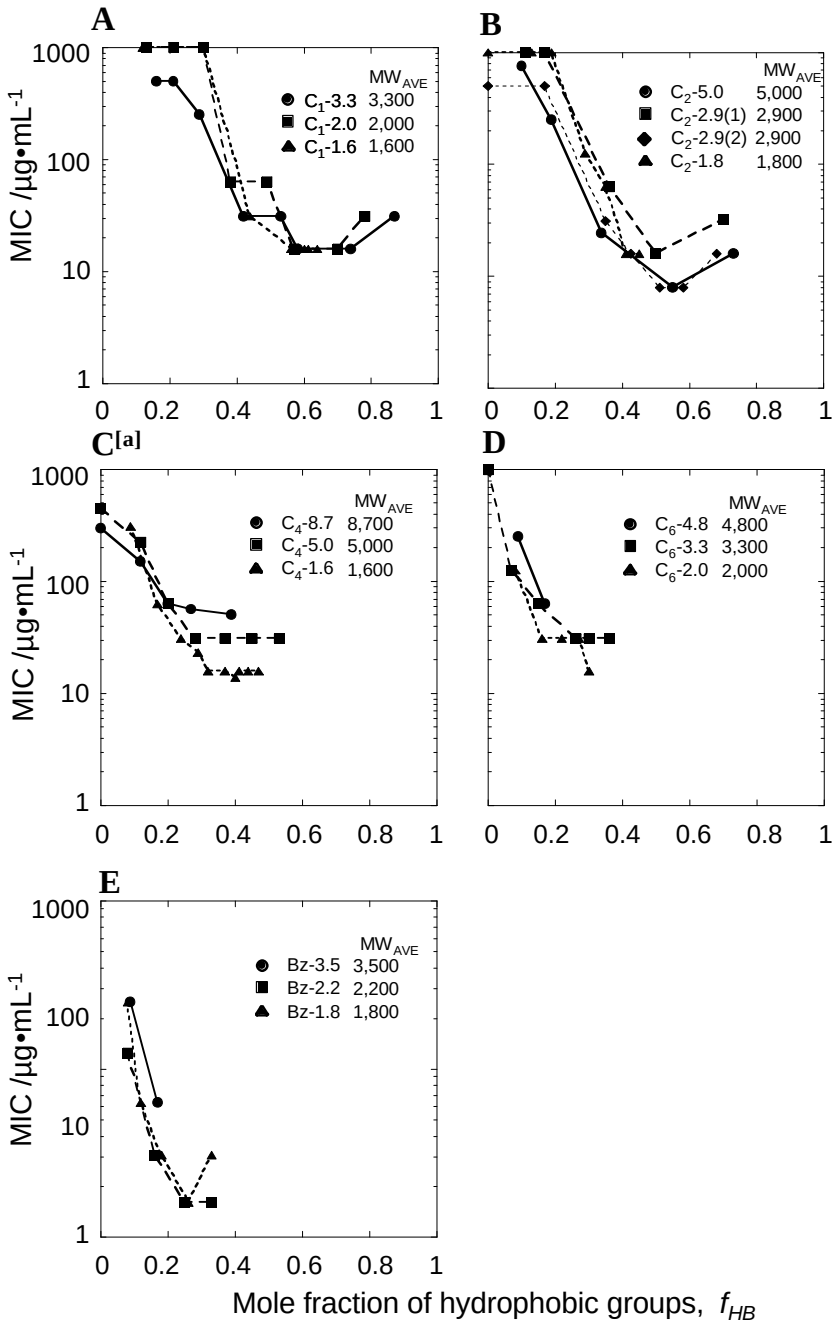


Figure S-1. Antimicrobial activity of cationic random copolymers of various molecular weights with (A) methyl, (B) ethyl, (C) butyl, (D) hexyls, and (E) benzyl groups. The MIC values are given in μg/mL. The average molecular weights for a given polymer series are indicated in the legend. [a] The data were previously reported.[1]

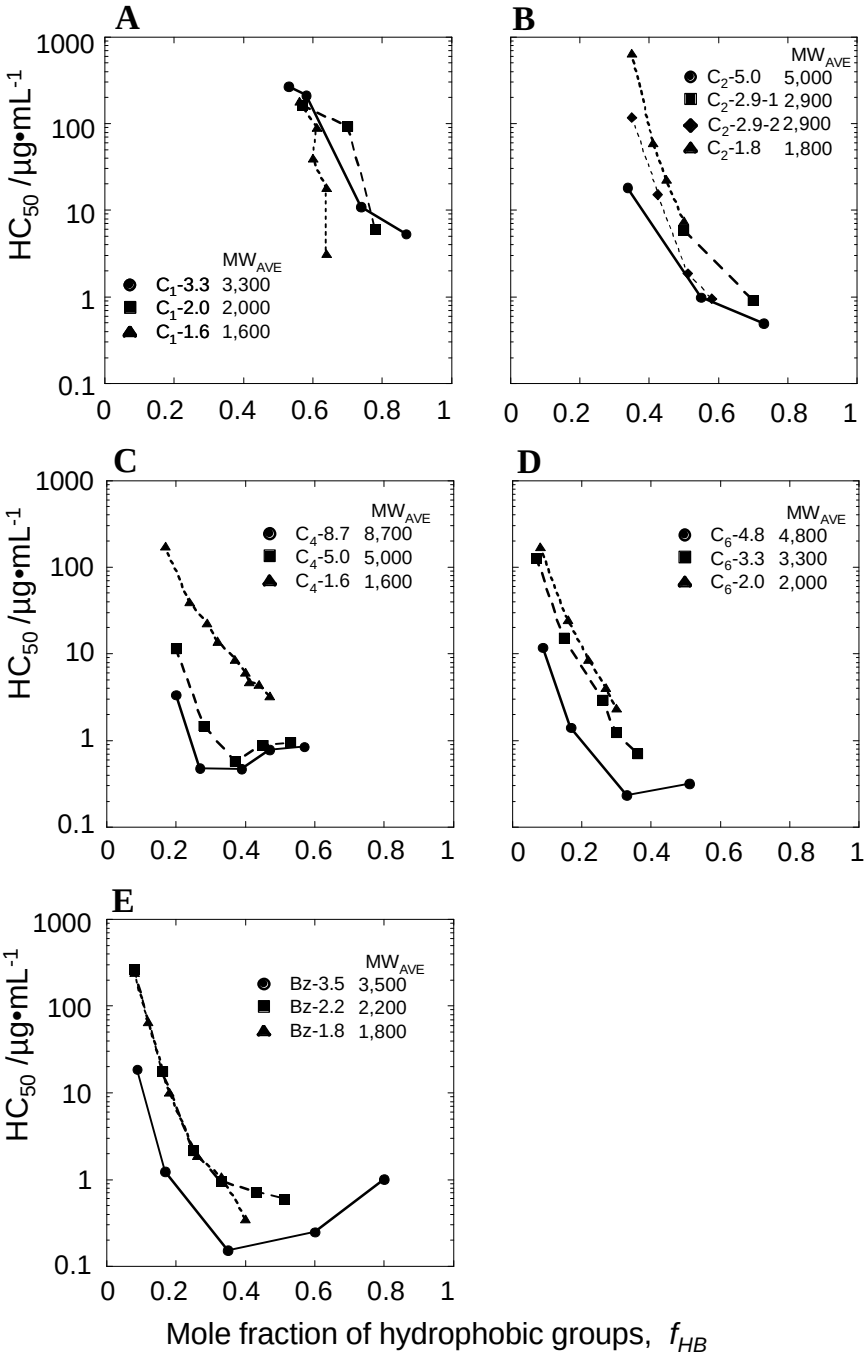


Figure S-2. Hemolytic activity of cationic random copolymers with (A) methyl, (B) ethyl, (C) butyl, (D) hexyls, and (E) benzyl groups. The HC_{50} values are given in µg/mL. The average molecular weights for a given polymer series are indicated in the legend. [a] The data were previously reported.[1]

Characterization and antimicrobial and hemolytic activities of polymers

The number-average molecular weight of polymers (M_n) referred as MW in the article was calculated by f_{HB}, the degree of polymerization (DP), and the molecular weights of monomers and methyl 3-mercaptopropionate.

Table S-1. Characterization and activities of polymers with methyl side chains

$f_{HB\ feed}$[a]	f_{HB}	DP	M_n	MIC/μM (μg/mL)	HC_{50}/μM (μg/mL)
C_1-3.3 ([MMP]/[total monomers] = 0.10)[b]					
0.20	0.16	16	3700	140 (500)	13%[c]
0.30	0.21	14	3100	160 (500)	12%[c]
0.40	0.29	16	3300	77 (250)	18%[c]
0.50	0.42	17	3200	9.6 (31)	20%[c]
0.60	0.53	19	3200	9.7 (31)	83 (270)
0.70	0.58	20	3400	4.7 (16)	63 (210)
0.80	0.74	21	3000	5.3 (16)	3.6 (11)
0.90	0.87	32	3900	8.0 (31)	1.4 (5.3)
C_1-2.0 ([MMP]/[total monomers] = 0.20)[b]					
0.20	0.13	7.6	1800	550 (1000)	11%[c]
0.30	0.21	8.3	1900	530 (1000)	10%[c]
0.40	0.30	9.9	2100	480 (1000)	Not determined.
0.50	0.38	8.6	1700	36 (63)	18%[c]
0.60	0.49	9.9	1800	34 (63)	30% [c]
0.70	0.57	10	1700	9.2 (16)	95 (160)
0.80	0.70	13	2000	7.9 (16)	46 (93)
0.90	0.78	20	2800	11 (31)	2.2 (6.0)

C_1-1.6 ([MMP]/[total monomers] = 0.30) [b]					
0.20	0.12	6.5	1600	630 (1000)	16% [c]
0.40	0.30	7.2	1600	640 (1000)	15% [c]
0.60	0.44	8.0	1600	20 (31)	270% [c]
0.70	0.56	8.9	1600	10 (16)	120 (180)
0.75	0.61	11	1900	8.5 (16)	48 (91)
0.80	0.60	9.6	1600	9.8 (16)	24 (40)
0.85	0.64	11	1700	9.3 (16)	10 (18)
0.90	0.64	7.9	1300	12 (16)	2.4 (3.2)

[a] The percentage of methyl methacrylate relative to total amount of monomers in polymerizations. [b] The ratio of methyl mercaptopropionate (MMP) to monomers in polymerizations. [c] The percentage of lysis at the highest polymer concentration (1mg/mL).

Table S-2. Characterization and activities of polymers with ethyl side chains

$f_{HB\ feed}$[a]	f_{HB}	DP	M_n	MIC/μM (μg/mL)	HC_{50}/μM (μg/mL)
C_2-5.0 ([MMP]/[total monomers] = 0.10)[b], [e]					
0.10	0.10	26	6100	120 (750)	46%[c]
0.20	0.19	25	5600	45 (250)	36%[c]
0.40	0.34	23	4700	5.1 (24)	3.8 (18)
0.60	0.55	20	3600	2.2 (8)	0.27 (0.97)
0.80	0.73	32	4900	3.3 (16)	0.10 (0.49)
C_2-2.9-1 ([MMP]/[total monomers] = 0.20)[b], [e]					
0.10	0.11	12	2900	350 (1000)	59%[c]
0.20	0.17	13	3000	330 (1000)	68%[c]
0.40	0.36	11	2300	28 (63)	59%[c]
0.60	0.50	16	3000	5.4 (16)	20 (5.9)
0.80	0.70	22	3500	9.2 (32)	0.26 (0.90)
C_2-2.9-2 ([MMP]/[total monomers] = 0.10)[b]					
0	0	14	3600	140 (500)	100%[c], 8.6%[d]
0.10	0.9	14	3200	Not determined	100%[c], 6.4%[d]
0.20	0.17	11	2500	200 (500)	95%[c], 5.9%[d]
0.40	0.35	15	3100	10 (31)	38 (120)
0.50	0.425	16	3200	5.1 (16)	4.8 (15)
0.60	0.51	15	2700	3.0 (8)	0.69 (1.9)
0.70	0.58	16	2900	2.8 (8)	0.33 (1.0)
0.80	0.68	14	2300	6.9 (16)	Not determined

C_2-1.8 ([MMP]/[total monomers] = 0.30) [b]					
0	0	8.6	2200	450 (1000)	48% [c]
0.10	0.13	6.9	1700	590 (1000)	29% [c]
0.20	0.19	7.6	1800	560 (1000)	27% [c]
0.30	0.29	8	1800	71 (130)	28% [c]
0.40	0.35	8.3	1800	36 (63)	360 (640)
0.50	0.41	7.9	1600	9.9 (16)	37 (61)
0.60	0.45	8.2	1600	9.8 (16)	14 (23)
0.70	0.50	8.9	1700	Not determined	4.4 (7.6)

[a] The percentage of methyl methacrylate relative to total amount of monomers in polymerizations. [b] the ratio of methyl mercaptopropionate (MMP) to monomers in polymerizations. [c] The percentage of lysis at the highest polymer concentration (1mg/mL). [d] The percentage of lysis at [polymer] = 0.5mg/mL. [e] Prepared by the method reported previously.[1]

Table S-3. Characterization and activities of polymers with butyl side chains[e]

$f_{HB\ feed}$ [a]	f_{HB}	DP	M_n	MIC/μM (μg/mL)	HC_{50}/μM (μg/mL)
C_4-8.7 ([MMP]/[total monomers] = 0.05) [b], [c]					
0	0	32	7900	38 (300)	(>500)
0.10	0.12	36	8400	18 (150)	(>500)
0.20	0.20	36	8100	7.7 (63)	0.41 (3.3)
0.30	0.27	46	10000	5.6 (57)	0.048 (0.48)
0.40	0.39	41	8500	5.9 (50)	0.055 (0.47)
0.50	0.47	46	9100	Not determined[d]	0.085 (0.77)
0.60	0.57	46	8700	Not determined[d]	0.097 (0.84)

C_4-5.0 ([MMP]/[total monomers] = 0.10) [b], [c]					
0	0	20	5000	90 (450)	(>500)
0.10	12	19	4500	50 (230)	(>500)
0.20	20	21	4800	13 (63)	2.37 (11)
0.30	28	24	5300	5.9 (31)	0.28 (1.5)
0.40	37	23	4900	6.4 (31)	0.12 (0.57)
0.50	45	22	4500	6.9 (31)	0.20 (0.87)
0.60	53	31	6000	5.2 (31)	0.16 (0.96)
C_4-1.6 ([MMP]/[total monomers] = 0.50) [b], [c]					
0	0	4.8	1300	Not determined	(>500)
0.10	0.9	4.9	1300	247 (313)	(>500)
0.20	0.17	5.7	1400	45 (63)	130 (180)
0.30	0.24	6.5	1500	20 (31)	26 (40)
0.35	0.29	6.2	1400	16 (24)	16 (23)
0.40	0.32	7.7	1700	9.2 (16)	8.1 (14)
0.45	0.37	7.6	1700	9.5 (16)	5.1 (8.7)
0.50	0.40	8.6	1900	7.5 (14)	3.3 (6.1)
0.55	0.41	8.6	1900	8.6 (16)	2.6 (4.7)
0.60	0.44	8.7	1800	8.7 (16)	2.4 (4.4)
0.65	0.47	8.5	1784	9.0 (16)	1.8 (3.3)

[a] The percentage of methyl methacrylate relative to total amount of monomers in polymerizations. [b] The ratio of methyl mercaptopropionate (MMP) to monomers in polymerizations. [c] Prepared by the method reported previously.[1] [d] MIC was not available due to the low solubility of the polymers. [e] The data were converted from the previous results.[1]

Table S-4. Characterization and activities of polymers with hexyl side chains

$f_{HB\ feed}$[a]	f_{HB}	DP	M_n	MIC/μM (μg/mL)	HC_{50}/μM (μg/mL)
C_6-4.8 ([MMP]/[total monomers] = 0.10)[b], [d]					
0.10	0.9	20	4700	53 (250)	2.4 (12)
0.20	0.17	24	5600	11 (63)	0.25 (1.4)
0.40	0.33	20	4400	Not determined[f]	0.05 (0.24)
0.60	0.51	22	4600	Not determined[f]	0.07 (0.31)
C_6-3.3 ([MMP]/[total monomers] = 0.20)[b], [d]					
0	0	12.4	3100	320 (1000)	64% [c]
0.10	0.07	12	2900	43 (125)	43 (126)
0.20	0.15	11	2700	23 (63)	5.5 (15)
0.30	0.26	13	2900	11 (31)	1.0 (2.9)
0.40	0.30	18	4000	7.8 (31)	0.31 (1.2)
0.50	0.36	19	4100	7.6 (31)	0.17 (0.70)
C_6-2.0 ([MMP]/[total monomers] = 0.30)[b], [d]					
0	0	7.7	2000	(>1000)	>310 (>610)
0.10	0.8	7.5	1900	66 (130)	90 (170)
0.20	0.16	8	2000	16 (31)	13 (24)
0.30	0.22	7.8	1900	17 (31)	4.6 (8.6)
0.40	0.27	8.3	1900	16 (31)	2.1 (4.1)
0.50	0.30	9.9	2300	7.1 (16)	1.0 (2.4)

[a] The percentage of methyl methacrylate relative to total amount of monomers in polymerizations. [b] The ratio of methyl mercaptopropionate (MMP) to monomers in polymerizations. [c] The percentage of lysis at the highest polymer concentration (1mg/mL). [d] Prepared by the method reported previously.

Table S-5. Characterization and activities of polymers with benzyl side chains

$f_{HB\ feed}$[a]	f_{HB}	DP	M_n	MIC/μM (μg/mL)	HC_{50}/μM (μg/mL)
Bz-3.5 ([MMP]/[total monomers] = 0.10)[b], [c]					
0.10	0.09	17	4100	61 (250)	4.5 (19)
0.20	0.17	16	3800	17 (63)	0.32 (1.2)
0.40	0.35	17	3800	Not determined [f]	0.04 (0.15)
0.60	0.60	15	3200	Not determined [f]	0.08 (0.24)
0.80	0.80	13	2700	Not determined [f]	0.38 (1.0)
Bz-2.2 ([MMP]/[total monomers] = 0.20)[b], [c]					
0.10	0.08	9.5	2400	53 (130)	110 (270)
0.20	0.16	8	2000	16 (31)	8.8 (17)
0.30	0.25	9.5	2300	7.0 (16)	0.94 (2.2)
0.40	0.33	9.7	2300	7.1 (16)	0.42 (1.0)
0.50	0.43	9.4	2100	Not determined[d]	0.34 (0.72)
0.60	0.51	10	2200	Not determined[d]	0.26 (0.58)
Bz-1.8 ([MMP]/[total monomers] = 0.30)[b], e					
0.10	0.08	6.4	1600	150 (250)	150 (240)
0.20	0.12	6.1	1600	41 (63)	43 (67)
0.30	0.18	6.2	1600	20 (31)	6.6 (10)
0.40	0.26	8.1	1900	8.2 (16)	0.99 (1.9)
0.50	0.33	7.9	1900	17 (31)	0.58 (1.1)
0.60	0.40	8.7	2000	Not determined[d]	0.17 (0.35)

[a] The percentage of methyl methacrylate relative to total amount of monomers in polymerizations. [b] The ratio of methyl mercaptopropionate (MMP) to monomers in polymerizations. [c] Prepared by the method reported previously.[1] [d] MIC was not available due to the low solubility of the polymers.

Fitting parameters for MIC data in Figure 2

Table S-6. Fitting parameters for the MIC of C_1 polymer series

Polymer Series	Hydrophobic side chains	MW_{AVE}	c_1[a]	c_2	n[a]	c_{mid}
C_1-3.3	Methyl	3,300	0.93	1.2429	28	30.265
C_1-2.0	Methyl	2,000	0.93	1.8008	28	33.993
C_1-1.6	Methyl	1,600	0.93	1.8691	28	38.356

[a] Held as a global variable

Table S-7. Fitting parameters for the MIC of C_2 polymer series

Polymer Series	Hydrophobic side chains	MW_{AVE}	c_1[a]	c_2	n[a]	c_{mid}
C_2-5.0	Ethyl	5,000	0.55	1.5394	13	20.394
C_2-2.9-1	Ethyl	2,900	0.55	1.9831	13	35.437
C_2-2.9-2	Ethyl	2,900	0.55	1.6773	13	32.563
C_2-1.8	Ethyl	1,800	0.55	2.1808	13	32.563

[a] Held as a global variable

Table S-8. Fitting parameters for the MIC of C_4 polymer series

Polymer Series	Hydrophobic side chains	MW_{AVE}	c_1	c_2	$n^{[a]}$	c_{mid}
C_4-8.7	Butyl	8,700	0.70903	0.87049	3.5	13.787
C_4-5.0	Butyl	5,000	0.6514	1.3054	3.5	17.714
C_4-1.6	Butyl	1,600	0.9178	1.6747	3.5	15.914

[a] Held as a global variable

Fitting parameters for HC_{50} data in Figure 4

Table S-9. Fitting parameters for HC_{50} in Figure 4, Panels A and B.

The fitting equation: $HC50 = c_3 \times (N_{sidechains})^{c4}$

Polymer Series	c_3	c_4
C_4	58.049	-2.3704
C_6	19.555	-2.6414

Table S-10. Fitting parameters by the equation 9 for HC_{50} in Figure 4, Panels C and D.

Polymer Series	m	l	C_0´	C_0?
C_4	0.592	0.498	3.37	3.09
C_6	0.444	0.325	2.82	1.68

Emission spectra of dansyl-labeled polymers in the presence and absence of lipids.

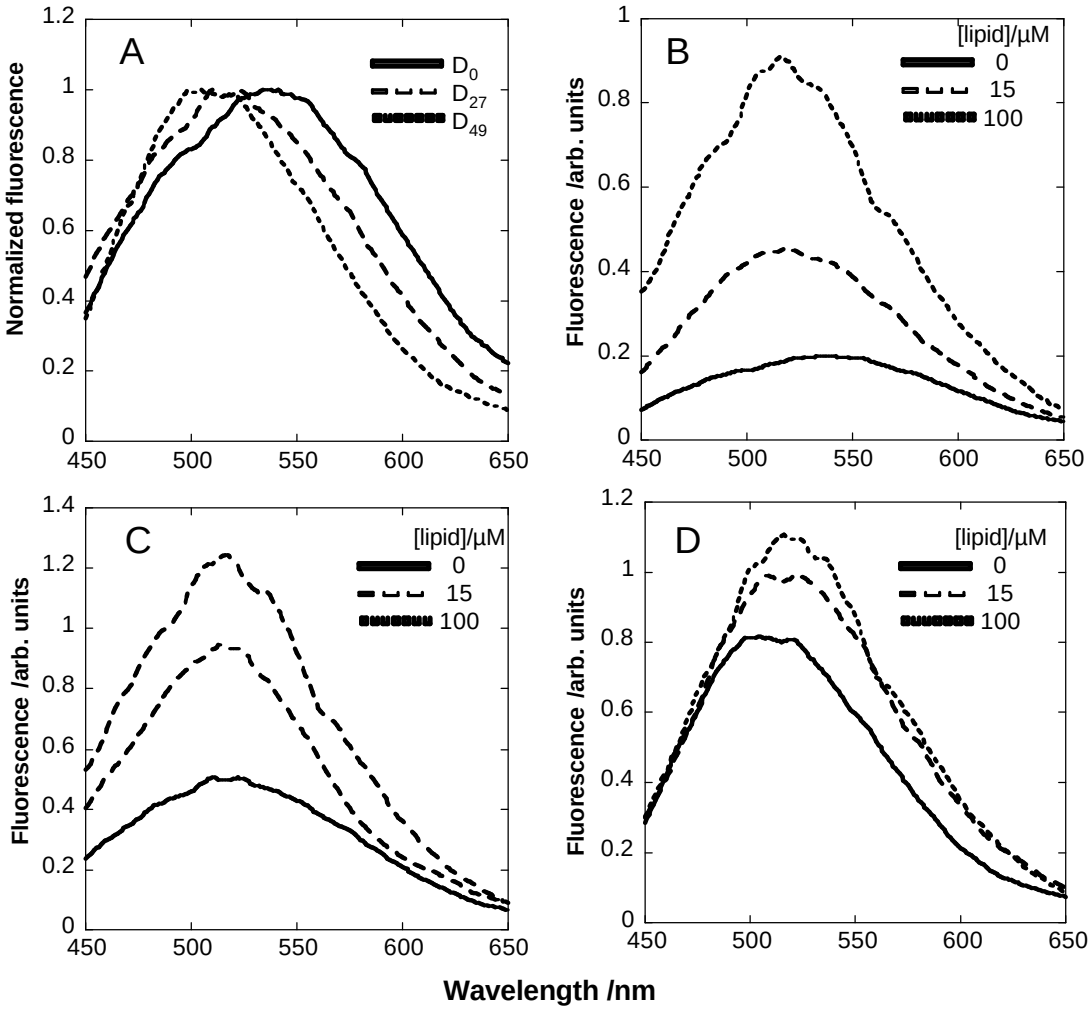


Figure S-3. Emission spectra of dansyl-labeled polymers in the absence or presence of POPC/POPG (8:2) LUVs. Normalized fluorescence spectra of $\mathbf{D_0}$, $\mathbf{D_{27}}$, and $\mathbf{D_{49}}$ in the absence of LUVs (A). Emission spectra of $\mathbf{D_0}$, (B), $\mathbf{D_{27}}$ (C), and $\mathbf{D_{49}}$ (D) in the presence of 0, 15, and 100μM lipids.

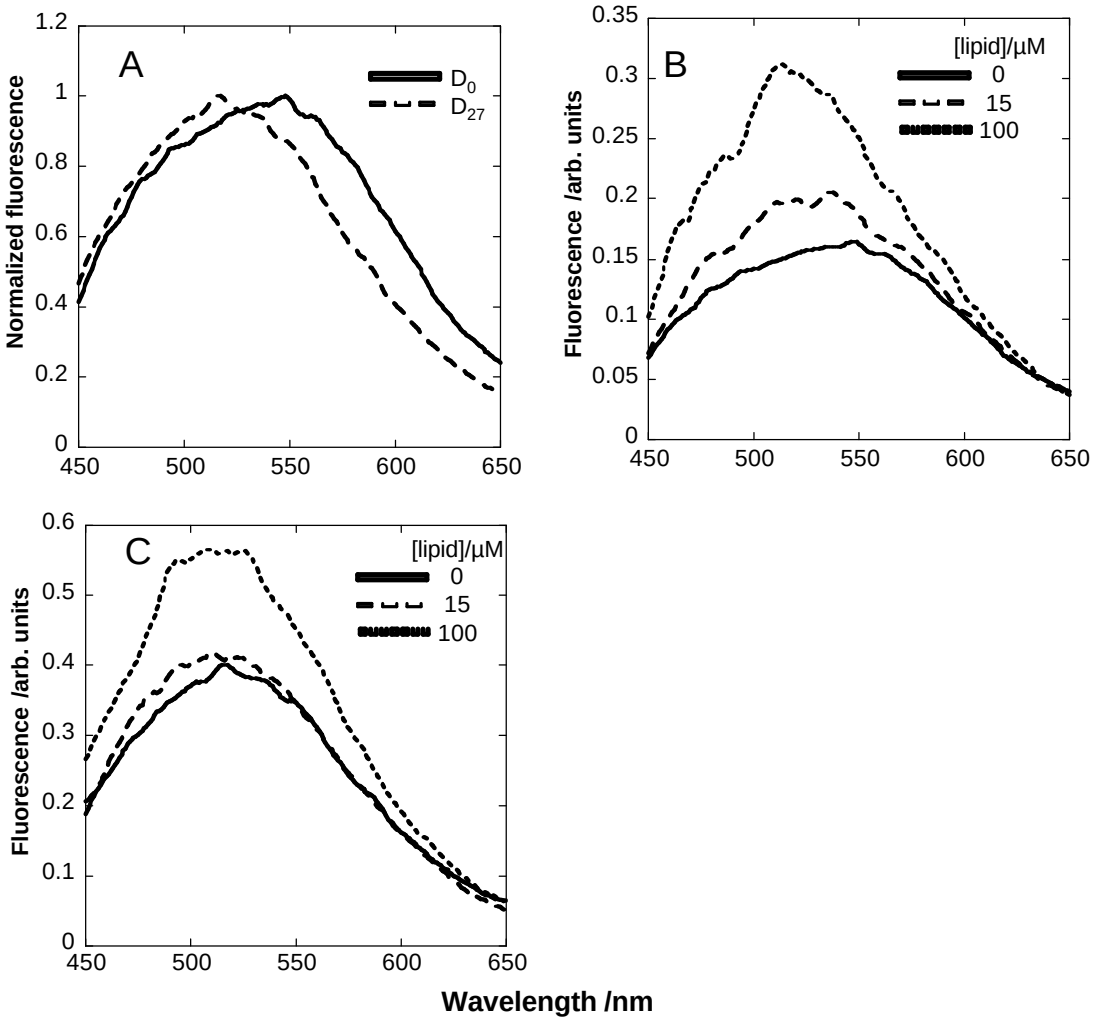


Figure S-4. Emission spectra of dansyl-labeled polymers in the absence or presence of POPC/POPE (8:2) LUVs. Normalized fluorescence spectra of $\mathbf{D_0}$, and $\mathbf{D_{27}}$ in the absence of LUVs (A). Emission spectra of $\mathbf{D_0}$, (B), and $\mathbf{D_{27}}$ (C) in the presence of 0, 15, and 100µM lipids.

Membrane-binding of polymers

Table S-11. Enhancement of emission from dansyl groups.

Polymer	F_{inf}/F_0 [a]			
	POPC	POPC/POPG	POPE/POPC	POPE/POPG
D_0	2.00	4.00	2.67	8.86
D_{27}	1.90	2.79	n.d.[b]	2.70
D_{49}	1.81	1.86	n.d.[b]	n.d.[b]

a) F_{inf} and F_0 are fluorescence intensity at [lipid] = infinite and 0, respectively. b) not determined.

References:

[1] K. Kuroda, W. F. DeGrado, *J. Am. Chem. Soc.* **2005**, *127*, 4128-4129.