

Supplementary

Fast and Sensitive Bacteria Detection by Boronic Acid Modified Fluorescent Dendrimer

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1. ¹H NMR Spectra

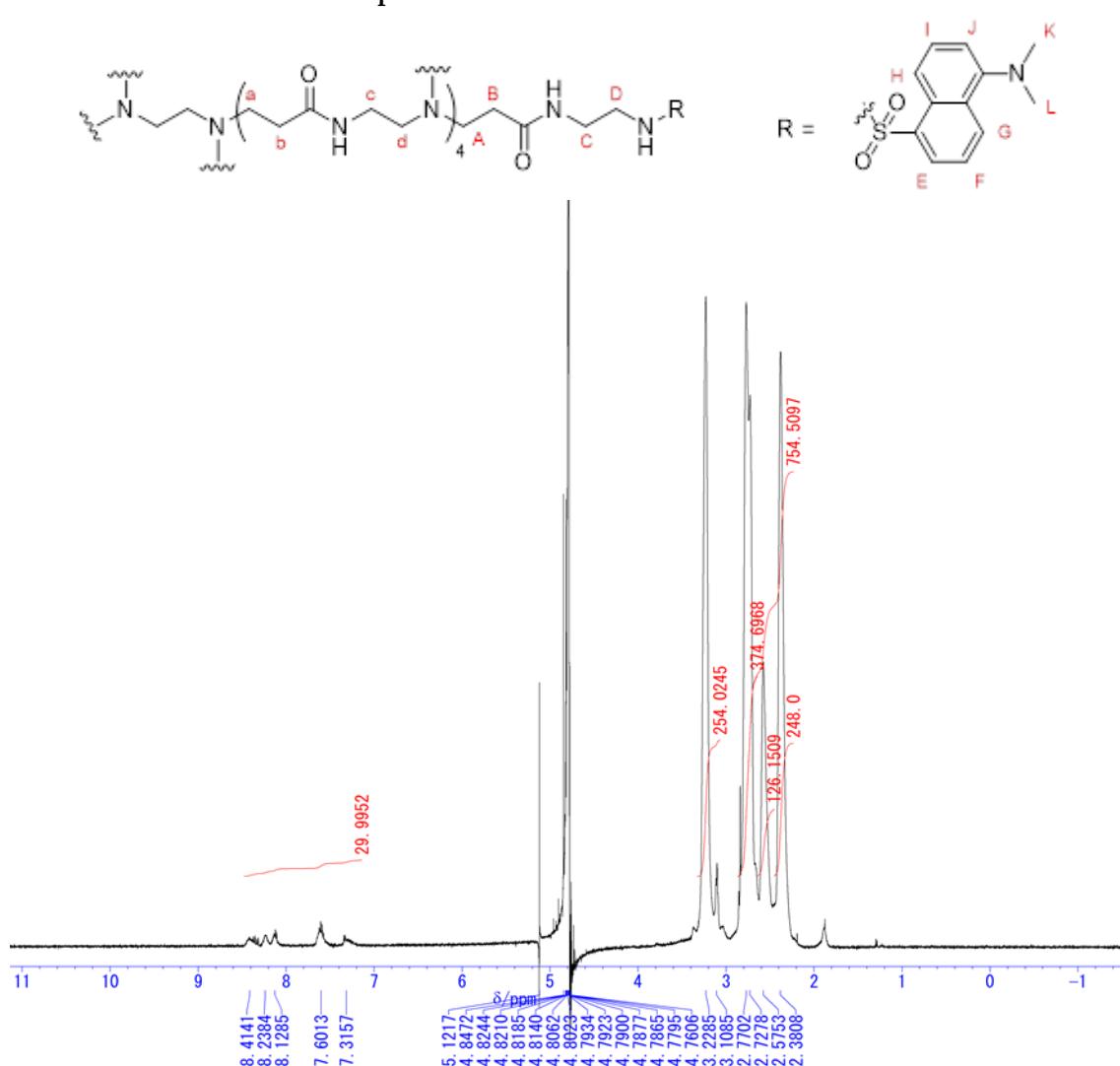


Figure S1. ¹H NMR spectrum of Dan-PAMAM (D₂O, the number of dansyl substitutions: 5). ¹H NMR (500 MHz, D₂O) δ(ppm): 8.41 (br, 30H, H_{E-j}), 8.24 (br, 30H, H_{E-j}), 8.13 (br, 30H, H_{E-j}), 7.60 (br, 30H, H_{E-j}), 7.32 (br, 30H, H_{E-j}), 3.23 (br, 248H, H_{C,c}), 2.77-2.72 (br, 496H, H_{A,a,D,d}), 2.57 (br, 496H, H_{A,a,D,d}), 2.38 (br, 248H, H_{B,b}); (E-j):(B+b) = 6M:248 (M: the number of dansyl substitutions): 29.9552:248 = 6M:248, M = 5.0.

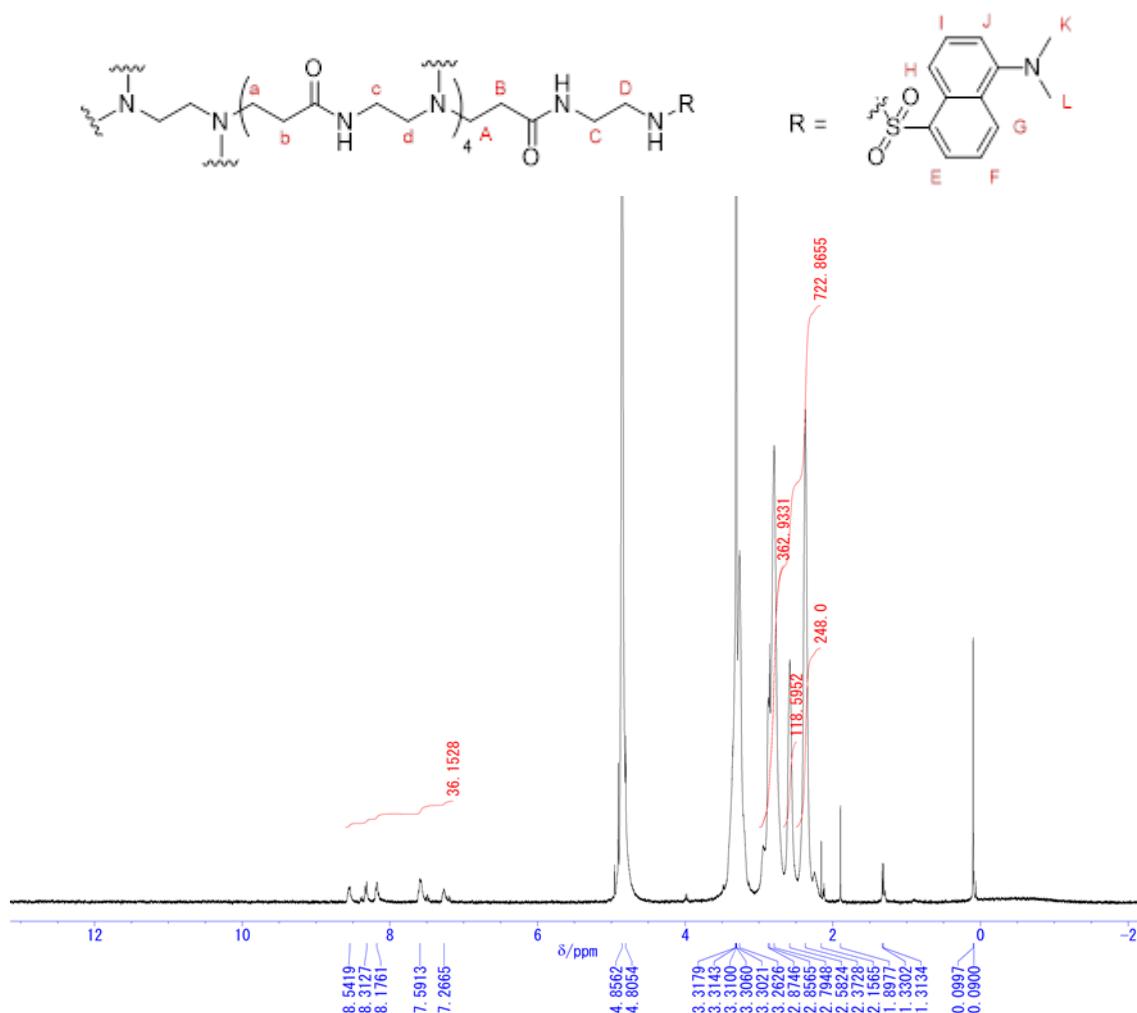


Figure S2. ¹H NMR spectrum of Dan-PAMAM (CD_3OD , the number of dansyl substitutions: 6). ¹H NMR (400 MHz, CD_3OD) δ (ppm): 8.54 (br, 36H, $\text{H}_{\text{E-J}}$), 8.31 (br, 36H, $\text{H}_{\text{E-J}}$), 8.18 (br, 36H, $\text{H}_{\text{E-J}}$), 7.59 (br, 36H, $\text{H}_{\text{E-J}}$), 7.27 (br, 36H, $\text{H}_{\text{E-J}}$), 3.26 (br, 248H, $\text{H}_{\text{C,c}}$), 2.87-2.80 (br, 496H, $\text{H}_{\text{A,a,D,d}}$), 2.58 (br, 496H, $\text{H}_{\text{A,a,D,d}}$), 2.37 (br, 248H, $\text{H}_{\text{B,b}}$); (E-J):(B+b) = 6M:248 (M: the number of dansyl substitutions): 36.1528:248 = 6M:248, M = 6.0.

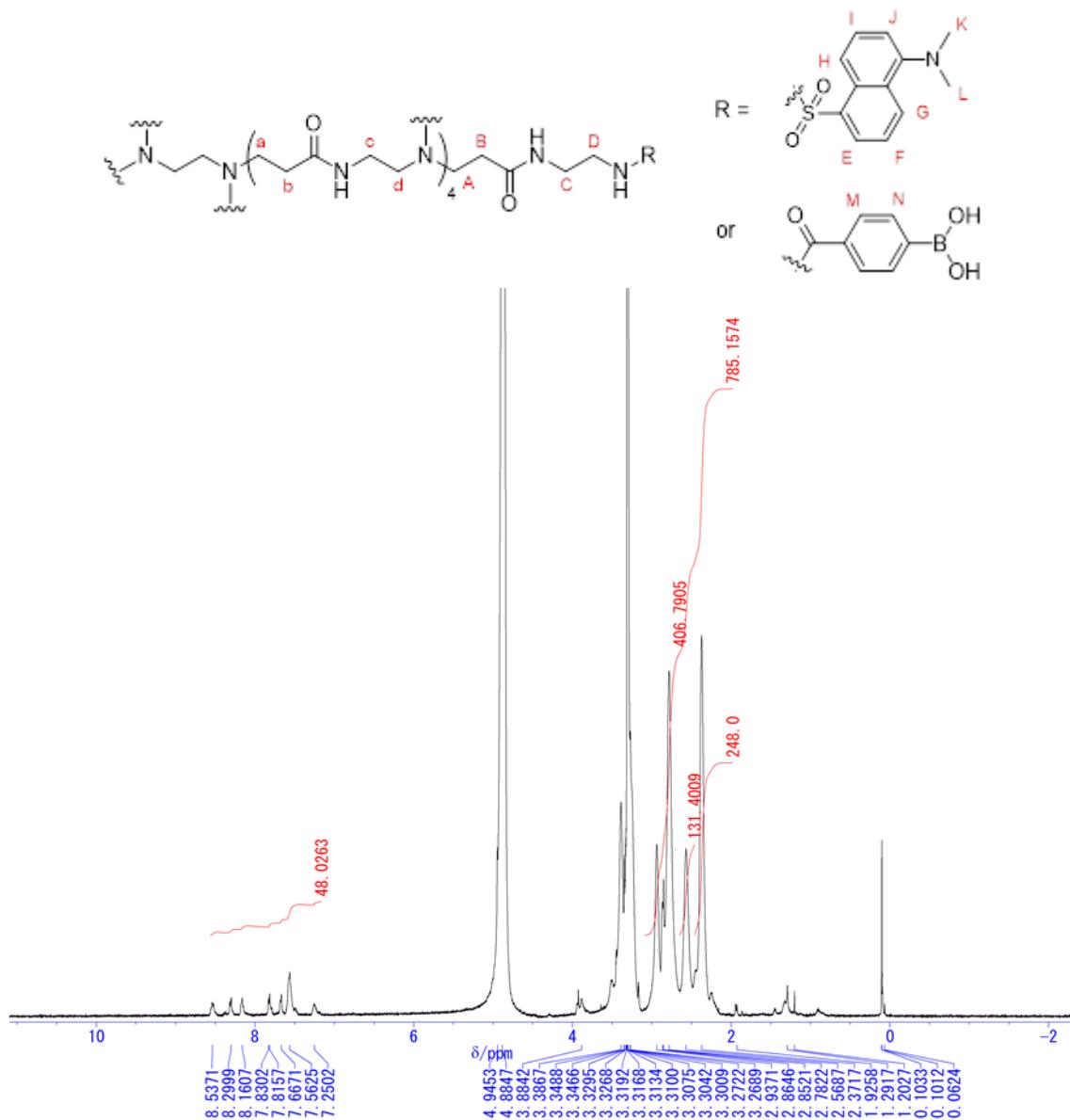


Figure S3. ^1H NMR spectrum of Dan-B3-PAMAM (CD_3OD). ^1H NMR (500 MHz, CD_3OD) δ (ppm): 8.54 (br, 42H, He-J,M,N), 8.30 (br, 42H, He-J,M,N), 8.16 (br, 42H, He-J,M,N), 7.83 (br, 42H, He-J,M,N), 7.67 (br, 42H, He-J,M,N), 7.56 (br, 42H, He-J,M,N), 7.25 (br, 42H, He-J,M,N), 3.35 (br, 248H, Hc,c), 2.93–2.78 (br, 496H, Ha,a,D,d), 2.57 (br, 496H, Ha,a,D,d), 2.37 (br, 248H, Hb,b); (E~J,M,N):(B+b) = (4M+36):248 (M: the number of phenylboronic acid substitutions): 48.0623:248 = (4M+36):248, M = 3.0.

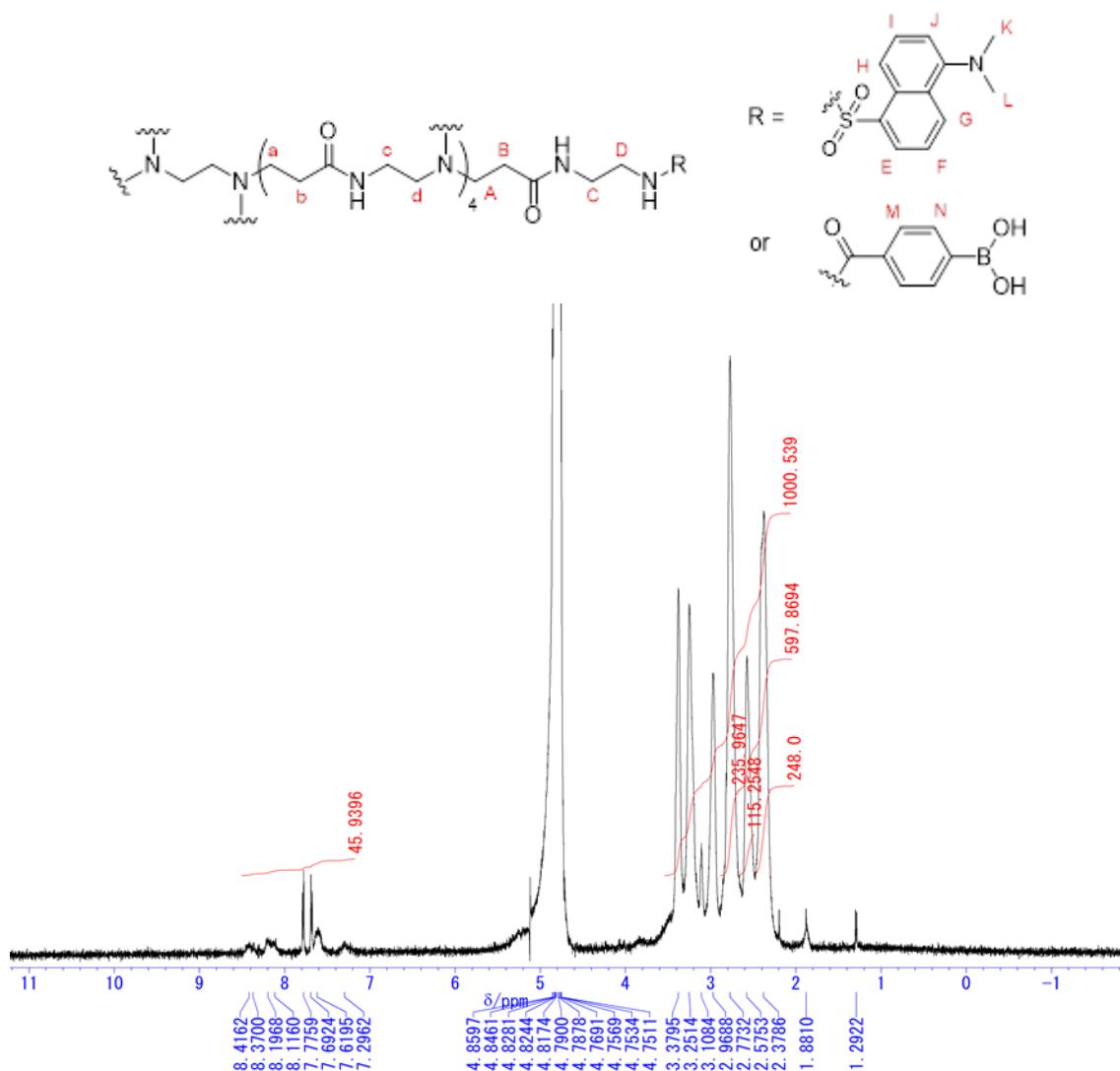


Figure S4. ^1H NMR spectrum of Dan-B4-PAMAM (D_2O). ^1H NMR (500 MHz, D_2O) δ (ppm): 8.42 (br, 46H, $\text{H}_{\text{E-J,M,N}}$), 8.37 (br, 46H, $\text{H}_{\text{E-J,M,N}}$), 8.20 (br, 46H, $\text{H}_{\text{E-J,M,N}}$), 8.12 (br, 46H, $\text{H}_{\text{E-J,M,N}}$), 7.78 (br, 46H, $\text{H}_{\text{E-J,M,N}}$), 7.69 (br, 46H, $\text{H}_{\text{E-J,M,N}}$), 7.62 (br, 46H, $\text{H}_{\text{E-J,M,N}}$), 7.30 (br, 46H, $\text{H}_{\text{E-J,M,N}}$), 3.38 (br, 744H, $\text{H}_{\text{A,a,C,c,D,d}}$), 3.25 (br, 744H, $\text{H}_{\text{A,a,C,c,D,d}}$), 3.11 (br, 744H, $\text{H}_{\text{A,a,C,c,D,d}}$), 2.93–2.78 (br, 744H, $\text{H}_{\text{A,a,C,c,D,d}}$), 2.57 (br, 744H, $\text{H}_{\text{A,a,C,c,D,d}}$), 2.37 (br, 248H, $\text{H}_{\text{B,b}}$); (E-J,M,N):(B+b) = (4M+30):248 (M: the number of phenylboronic acid substitutions): 45.9336:248 = (4M+30):248, M = 4.0.

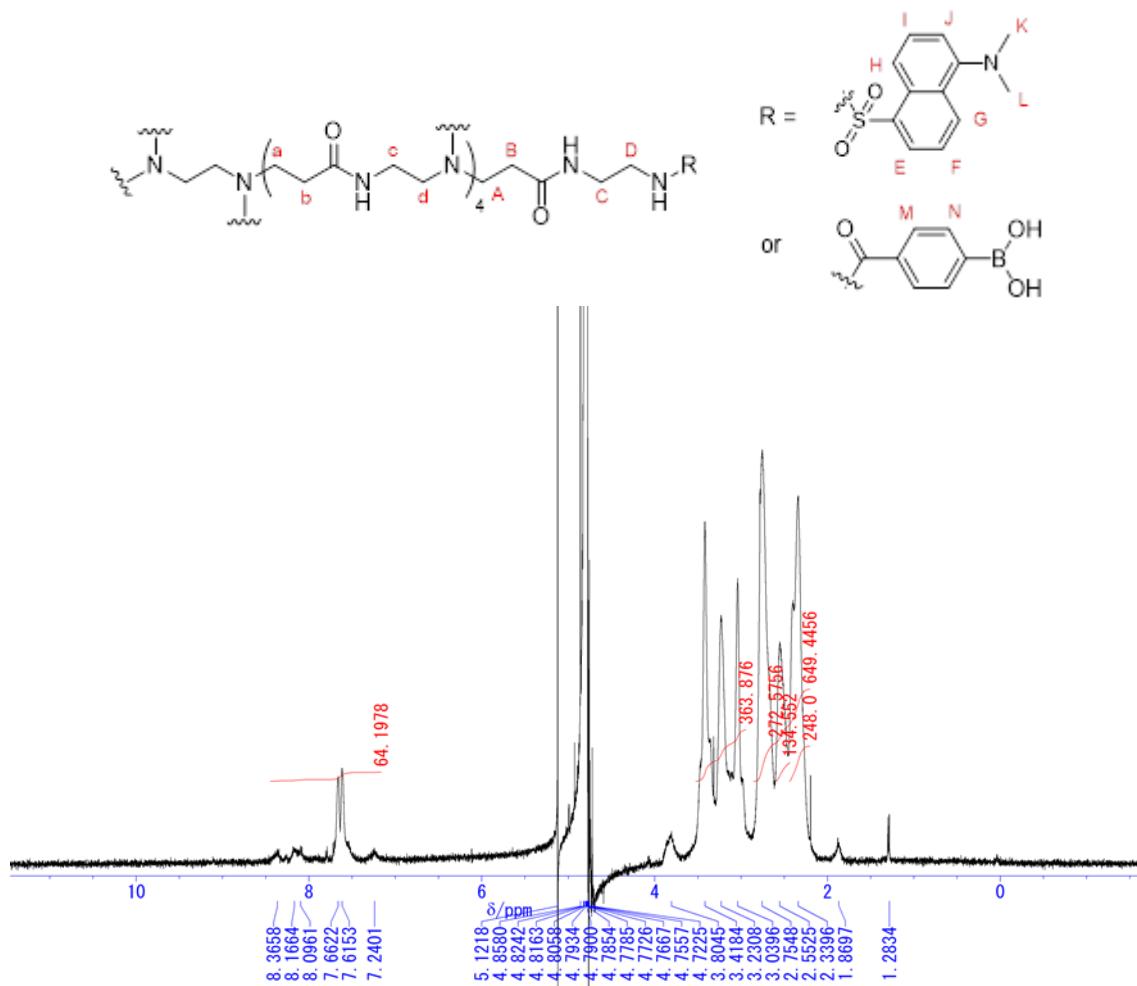


Figure S5. ¹H NMR spectrum of Dan-B8.5-PAMAM (D₂O). ¹H NMR (500 MHz, D₂O) δ (ppm): 8.37 (br, 46H, H_{E-J,M,N}), 8.17 (br, 46H, H_{E-J,M,N}), 8.10 (br, 46H, H_{E-J,M,N}), 7.66 (br, 46H, H_{E-J,M,N}), 7.62 (br, 46H, H_{E-J,M,N}), 7.24 (br, 46H, H_{E-J,M,N}), 3.42 (br, 744H, H_{A,a,C,c,D,d}), 3.23 (br, 744H, H_{A,a,C,c,D,d}), 3.04 (br, 744H, H_{A,a,C,c,D,d}), 2.75 (br, 744H, H_{A,a,C,c,D,d}), 2.55 (br, 744H, H_{A,a,C,c,D,d}), 2.34 (br, 248H, H_{B,b}); (E~J,M,N):(B+b) = (4M+30):248 (M: the number of phenylboronic acid substitutions): 64.1978:248 = (4M+30):248, M = 8.5.

2. Analytical Data

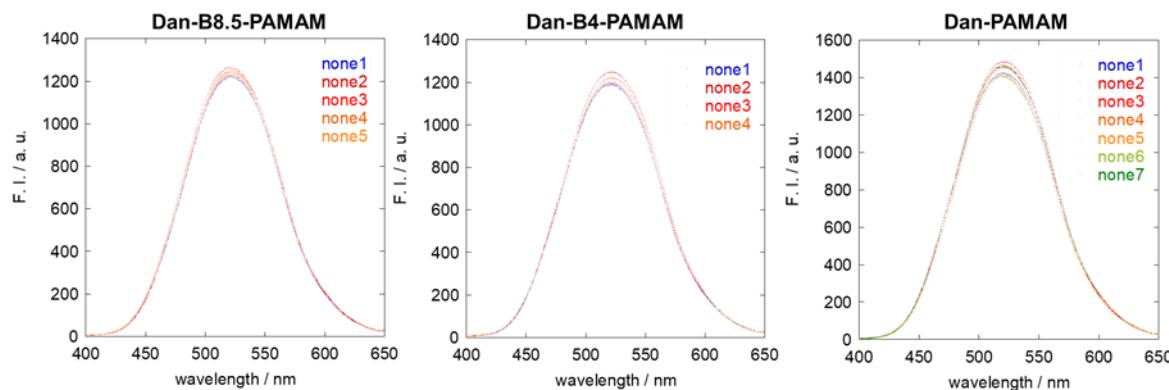


Figure S6. Fluorescence measurements of control samples in PBS buffer pH 7.4 ($\lambda_{\text{ex}} = 330 \text{ nm}$, $\lambda_{\text{em}} = 522 \text{ nm}$, [probe] = 3.3 μM).

Table S1. Fluorescence intensity of control samples at 522 nm in PBS buffer pH 7.4 ($\lambda_{\text{ex}} = 330 \text{ nm}$, $\lambda_{\text{em}} = 522 \text{ nm}$, [probe] = 3.3 μM). The entire spectra: see Figure S6.

	Dan-PAMAM	Dan-B4-PAMAM	Dan-B8.5-PAMAM
none1	1421	1188	1221
none2	1459	1249	1229
none3	1484	1197	1261
none4	1404	1219	1241
none5	1454		1248
none6	1408		
none7	1464		
F_{none} average	1442	1213	1240
SD	28.59	23.52	14.06

Table S2. Fluorescence intensity of Dan-B-PAMAMs in PBS buffer pH 7.4 ($\lambda_{\text{ex}} = 330 \text{ nm}$, $\lambda_{\text{em}} = 522 \text{ nm}$, [probe] = 3.3 μM , [bacteria] = $10^8 \text{ CFU} \cdot \text{mL}^{-1}$).

S. aureus

	Dan-PAMAM	Dan-B4-PAMAM	Dan-B8.5-PAMAM
1	1356	1112	1058
2	1366	1133	1077
3	1360	1122	1065
Average	1361	1122	1067
SD	4.110	8.576	7.846

E. coli

	Dan-PAMAM	Dan-B4-PAMAM	Dan-B8.5-PAMAM
1	1315	1178	1088
2	1342	1120	1089
3	1349	1143	1113
Average	1335	1147	1097
SD	14.66	13.77	11.56

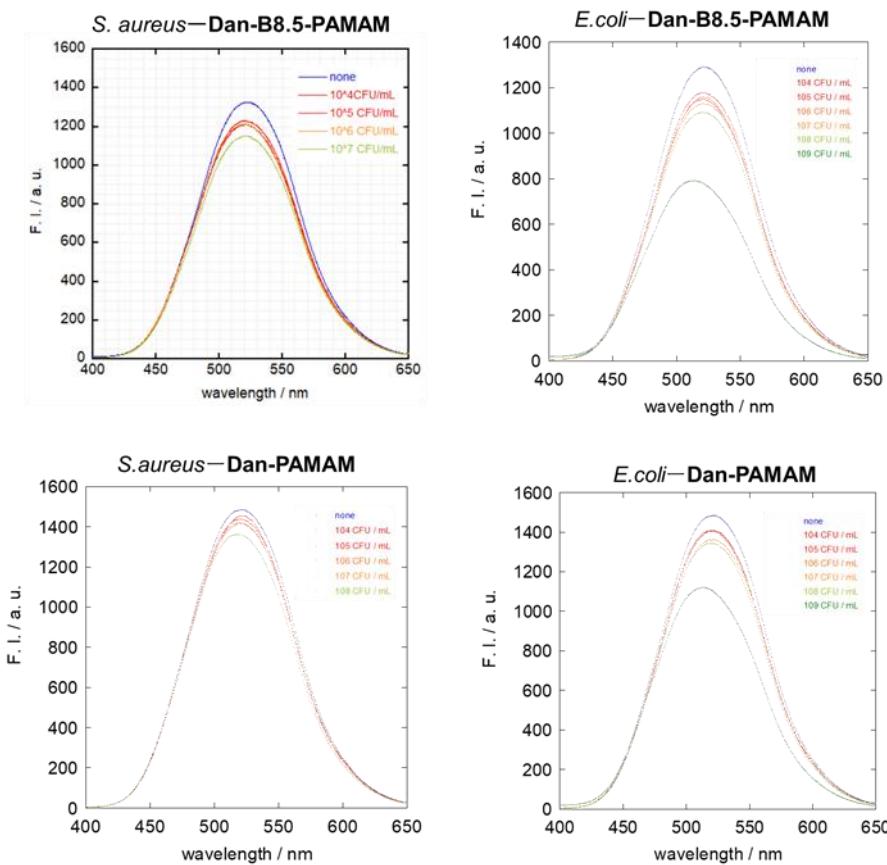


Figure S7. Examples of fluorescence spectra of the mixture between the probe and bacteria in PBS buffer pH 7.4 ($\lambda_{\text{ex}} = 330$ nm). [probe] = 3.3 μM , [*S. aureus*] = 10^4 - 10^8 CFU·mL $^{-1}$ (Since Dan-B8.5-PAMAM showed visible aggregates in 10^8 CFU·mL $^{-1}$, it was not obtained.), [*E. coli*] = 10^4 - 10^9 CFU·mL $^{-1}$. Entire lists of fluorescent intensity: see Table S1-S3.

Table S3. Fluorescence intensity of the mixture between the probe and bacteria in PBS buffer pH 7.4 ($\lambda_{\text{ex}} = 330 \text{ nm}$, $\lambda_{\text{em}} = 522 \text{ nm}$). [probe] = 3.3 μM , [*S. aureus*] = 10^4 - 10^7 or $10^8 \text{ CFU} \cdot \text{mL}^{-1}$, [*E. coli*] = 10^4 - $10^9 \text{ CFU} \cdot \text{mL}^{-1}$. Results about F_{none} : see Table S1, $10^8 \text{ CFU} \cdot \text{mL}^{-1}$: see Table S2. Welch's t-test was used to compare F_{none} and each concentration. Differences were calculated with two-side test with an alpha level of 0.05. Asterisk was considered as significant difference ($p < 0.05$).

Dan-B8.5-PAMAM with *S. aureus*

	F_{none}	$10^4 \text{ CFU} \cdot \text{mL}^{-1}$	$10^5 \text{ CFU} \cdot \text{mL}^{-1}$	$10^6 \text{ CFU} \cdot \text{mL}^{-1}$	$10^7 \text{ CFU} \cdot \text{mL}^{-1}$
1	1325	1209	1227	1212	1151
2	1299	1254	1239	1253	1139
3	1282	1229	1243	1245	1161
4	1279				
5	1284				
Average	1294	1231	1236	1237	1150
SD	17.06	18.41	6.799	17.75	8.994
<i>p</i> value	-	0.018*	0.001*	0.021*	0.00001*

Since the measurements of Dan-B8.5-PAMAM with *S. aureus* were conducted on another day, control (F_{none}) was newly obtained for the experiments.

Dan-B8.5-PAMAM with *E. coli*

	$10^4 \text{ CFU} \cdot \text{mL}^{-1}$	$10^5 \text{ CFU} \cdot \text{mL}^{-1}$	$10^6 \text{ CFU} \cdot \text{mL}^{-1}$	$10^7 \text{ CFU} \cdot \text{mL}^{-1}$	$10^9 \text{ CFU} \cdot \text{mL}^{-1}$
1	1127	1131	1090	1130	777.1
2	1149	1177	1157	1128	819.6
3	1136	1157	1119	1149	764.3
Average	1137	1155	1122	1136	787.0
SD	9.031	18.83	27.43	9.463	23.64
<i>p</i> value	0.00006*	0.0010*	0.016*	0.00007*	0.0002*

p value of $10^8 \text{ CFU} \cdot \text{mL}^{-1}$: 0.00006*.

Dan-PAMAM with *S. aureus*

	$10^4 \text{ CFU} \cdot \text{mL}^{-1}$	$10^5 \text{ CFU} \cdot \text{mL}^{-1}$	$10^6 \text{ CFU} \cdot \text{mL}^{-1}$	$10^7 \text{ CFU} \cdot \text{mL}^{-1}$
1	1436	1427	1420	1429
2	1419	1453	1407	1453
3	1313	1400	1418	1412
Average	1389	1427	1415	1431
SD	54.42	21.64	5.715	16.82
<i>p</i> value	0.302	0.466	0.064	0.546

Dan-PAMAM with *E. coli*

	$10^4 \text{ CFU} \cdot \text{mL}^{-1}$	$10^5 \text{ CFU} \cdot \text{mL}^{-1}$	$10^6 \text{ CFU} \cdot \text{mL}^{-1}$	$10^7 \text{ CFU} \cdot \text{mL}^{-1}$	$10^9 \text{ CFU} \cdot \text{mL}^{-1}$
1	1465	1423	1408	1401	988.9
2	1397	1420	1399	1383	1065
3	1408	1410	1404	1362	1097
Average	1423	1418	1404	1382	1050
SD	29.80	5.558	3.682	15.94	45.34
<i>p</i> value	0.490	0.088	0.016*	0.010*	0.003*

p value of $10^8 \text{ CFU} \cdot \text{mL}^{-1}$: 0.0003*.

Table S4. Absorbance changes by MTT assay ($\lambda_{abs} = 560$ nm), [probe] = 3.3 μ M, [*S. aureus*] = 3.0×10^8 CFU·mL $^{-1}$, [*E. coli*] = 3.0×10^8 CFU·mL $^{-1}$. Welch's t-test was used to compare control and each probe. Differences were calculated with two-side test with an alpha level of 0.05. Asterisk was considered as significant difference ($p < 0.05$).

S. aureus

	control (PBS)	PAMAM (COOH)	PAMAM (NH ₂)	Dan-PAMAM	Dan-B4-PA-MAM	Dan-B8.5-PA-MAM
1	2.6933	2.6966	2.6962	2.7133	2.7125	2.6991
2	2.6752	2.6796	2.6752	2.6814	2.6756	2.6764
3	2.6806	2.6786	2.6740	2.6822	2.6803	2.6770
Average	2.6830	2.6849	2.6818	2.6923	2.6895	2.6842
SD	7.5870×10^{-3}	8.2597×10^{-3}	0.010194	0.014853	0.016400	0.010562
<i>p</i> value	-	0.823	0.898	0.490	0.651	0.908

E. coli

	control (PBS)	PAMAM (COOH)	PAMAM (NH ₂)	Dan-PAMAM	Dan-B4-PAMAM	Dan-B8.5-PA-MAM
1	3.2752	3.1024	3.0644	3.2952	3.3053	1.7634
2	2.5414	2.5516	1.8659	2.4474	2.2686	0.8538
3	2.6580	2.5772	2.0177	2.4674	2.4305	1.2944
Average	2.8249	2.7437	2.3160	2.7367	2.6681	1.3039
SD	0.32198	0.25383	0.53281	0.39503	0.45537	0.37140
<i>p</i> value	-	0.794	0.325	0.819	0.713	0.012*



Figure S8. Fluorescence microscopy images of *S. aureus* with Dan-PAMAM. *S. aureus* (10^8 CFU·mL $^{-1}$) was stained with AO and PI, AO: $\lambda_{ex} = 500$ nm, PI: $\lambda_{ex} = 530$ nm, Dan-PAMAM: $\lambda_{ex} = 330$ nm. [Dan-PAMAM] = 3.3 μ M. The images suggested that Dan-PAMAM did not form bonds with bacteria and any damaged bacteria were not observed.