Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Polycation-chaperoned in-stem molecular beacon system

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Preparation of ISMBs and PLL-g-Dex and conditions for the spectroscopic measurements

Phosphoramidite monomers with perylene and anthraquinone were synthesized according to the previous reports.[1,2] Oligonucleotide syntheses (including polyacrylamide gel electrophoresis and HPLC purification) were outsourced (Nihon Techno Service Co., Ltd.). DNA sequences containing only natural nucleotides were supplied by Integrated DNA Technologies, Inc. Poly(L-lysine)-*graft*-dextran was prepared by a reductive amination reaction of poly(L-lysine) (Mw = 8×10^3, SIGMA) with dextran (Mn = 6×10^3, Dextran T-10, Amersham Pharmacia Biotech) as reported previously.[3] The dextran content of the copolymer was 90 wt%. PLL-*g*-Dex was added to the solution of MB and substrate at N/P of 2, because response speed was saturated at N/P = 2 (see Fig. S7). Here, the concentrations of $[\text{amino group}]_{\text{copolymer}}$ and $[\text{phosphate group}]_{\text{DNA}}$ were based on positively changed amino groups derived from poly(L-lysine) and negatively charged phosphate groups, respectively.

Fluorescence spectra were measured on a JASCO model FP-6500 equipped with programmable temperature controller in a microcell (3 mm x 3 mm). Fluorescence intensity was monitored at 460 nm by exciting 425 nm. Errors in F/F_0 were estimated to be within 15%. For response-measurements in Fig. 3, MB (for a final concentration of 0.2 μM) and PLL-*g*-Dex (N/P = 0 or 2) were dissolved in buffered solution (100 mM NaCl, 10 mM phosphate buffer, pH 7.0) in the cell (10 mm x 10 mm) by magnetically stirring at 20 °C, followed by the addition of **Surv** (to a final concentration of 0.4 μM) to start the reaction.

1) H. Kashida, T. Takatsu, H. Asanuma, *Tetrahedron Lett.* **2007**, *48*, 6759.
2) H. Asanuma, H. Hayashi, J. Zhao, X. G. Liang, A. Yamazawa, T. Kuramochi, D. Matsunaga, Y. Aiba, H. Kashida, M. Komiyama, *Chem. Commun.* **2006**, 5062.
3) A. Maruyama, M. Katoh, T. Ishihara, T. Akaike, *Bioconjugate Chem*. **1997**, *8*, 3.

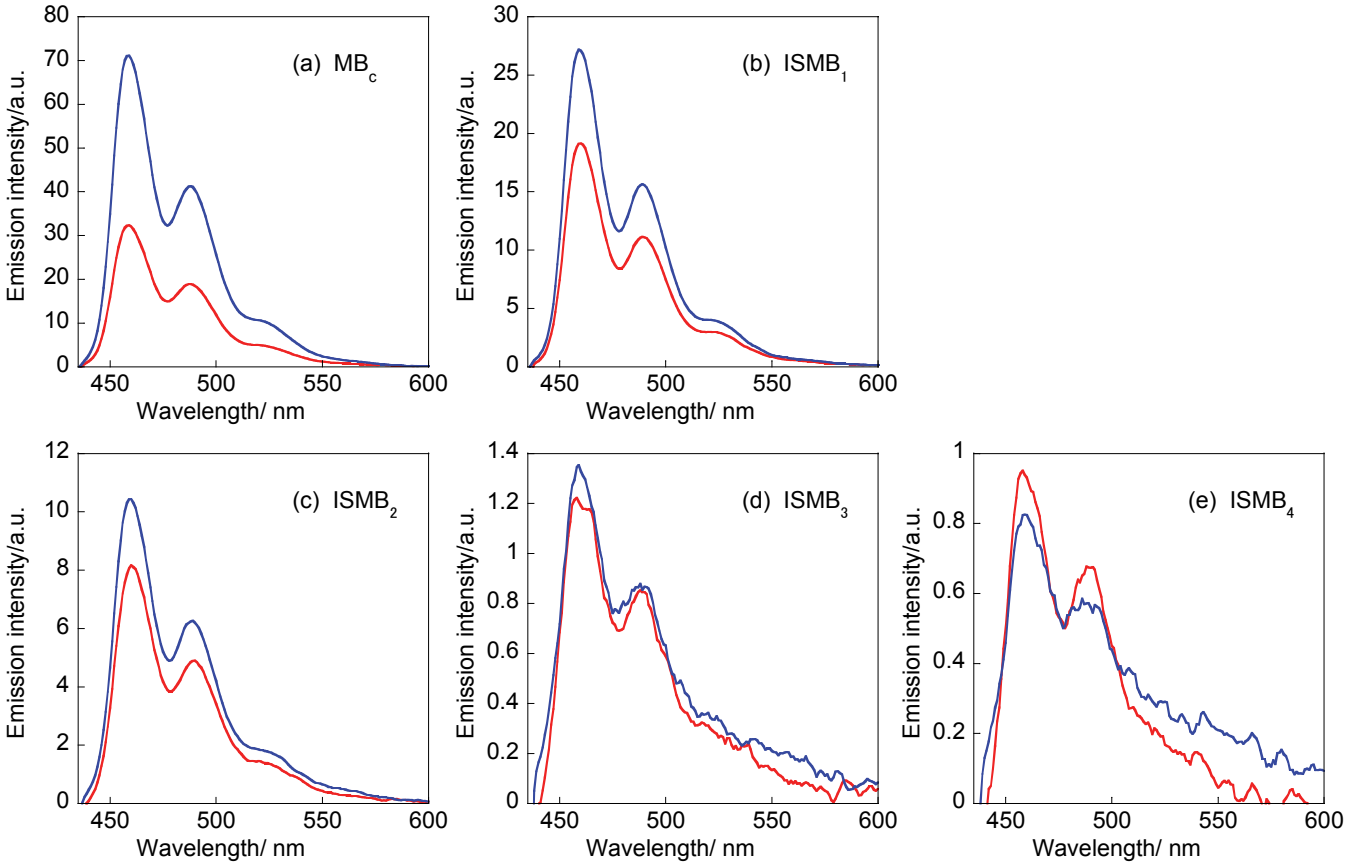
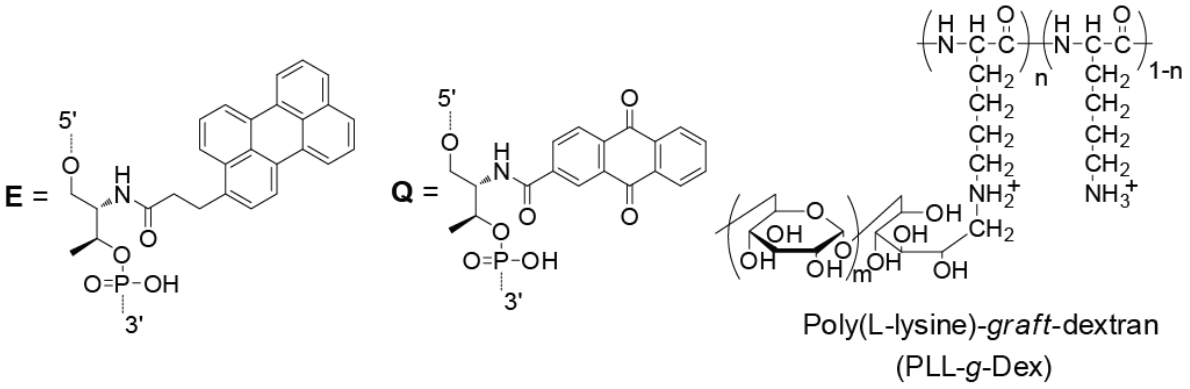


Figure S1. Fluorescence emission (Background emission) spectra of closed (a) **MB$_c$**, (b) **ISMB$_1$**, (c) **ISMB$_2$**, (d) **ISMB$_3$** and (e) **ISMB$_4$** in the presence (red line) and absence (blue line) of PLL-*g*-Dex.
Solution conditions; [MB] = 0.2 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 20 °C, N/P = 2
Measurement conditions of JASCO model FP-6500; bandwidth of excitation = 5nm, bandwidth of emission = 5 nm, Sensitivity = medium.



MB$_C$: 5'-**E**TG-GTC-CTT-GAG-AAA-GGG-C-GAC-CA**Q**-3'
ISMB$_1$: 5'-TGG-**E**TC-CTT-GAG-AAA-GGG-C-GA**Q**-CCA-3'
ISMB$_2$: 5'-T**E**G-G**E**T-C-CTT-GAG-AAA-GGG-C-GA**Q**-CC**Q**-A-3'
ISMB$_3$: 5'-G**E**G-T**E**G-G**E**T-C-CTT-GAG-AAA-GGG-C-GA**Q**-CC**Q**-AC**Q**-C-3'
ISMB$_4$: 5'-G**E**C-G**E**G-T**E**G-G**E**T-C-CTT-GAG-AAA-GGG-CGA-**Q**CC-**Q**AC-**Q**CG-**Q**C-3'
Surv: 3'-ACG-CCA-CCA-GGA-ACT-CTT-TCC-CG-5'

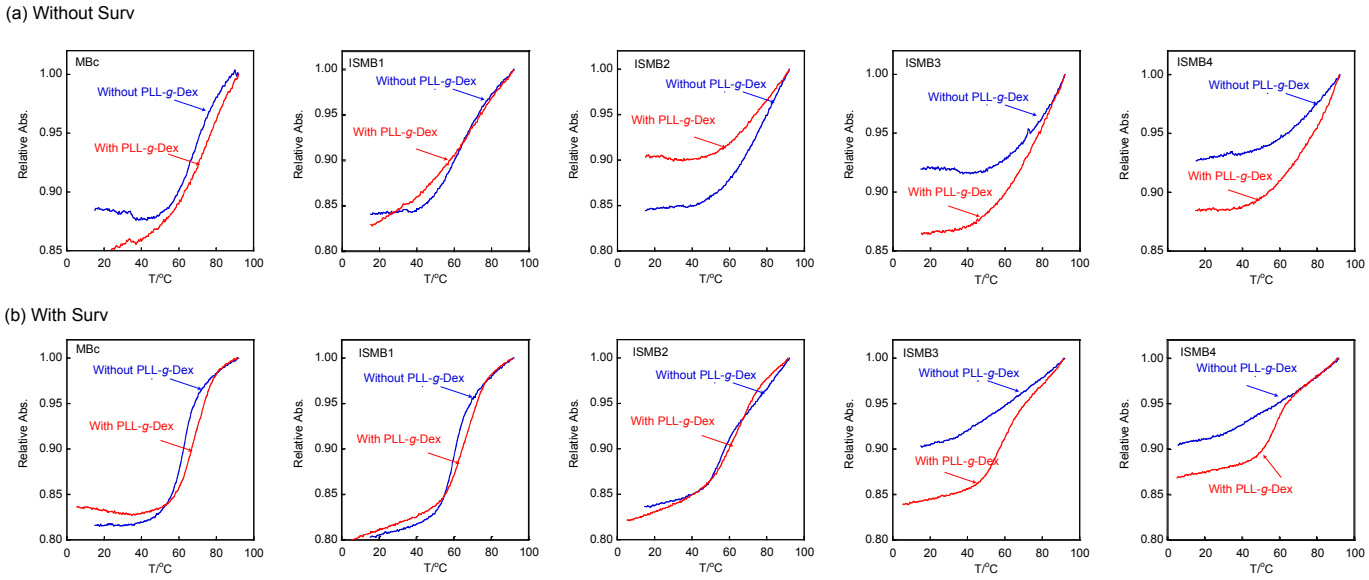


Figure S2. Melting curves of MBs (a) without and (b) with **Surv** obtained from the change of absorbance at 260 nm in the presence (red line) and absence (blue line) of PLL-*g*-Dex.
Solution conditions; [**MB**] = 1 μM, [**Surv**] = 1 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), N/P = 0 (for blue line), 2 (for red line). Temperature ramp was 0.5 °C min^{-1}.

Table S1. Melting temperatures of MBs determined from Figure S2.[a)]

MB	Without Surv		With Surv	
	N/P=0	N/P=2	N/P=0	N/P=2
MB_c	67.0	74.5	62.1	68.1
$ISMB_1$	61.5	67.7	59.4	66.1
$ISMB_2$	-[b)]	-[b)]	-[b)]	64.0
$ISMB_3$	-[b)]	-[b)]	-[b)]	57.3
$ISMB_4$	-[b)]	-[b)]	-[b)]	56.9

a) Solution conditions; [**MB**] = 1 μM, [**Surv**] = 1 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), N/P = 0 (for blue line), 2 (for red line). Temperature ramp was 0.5 °C min^{-1}.
b) Not determined.

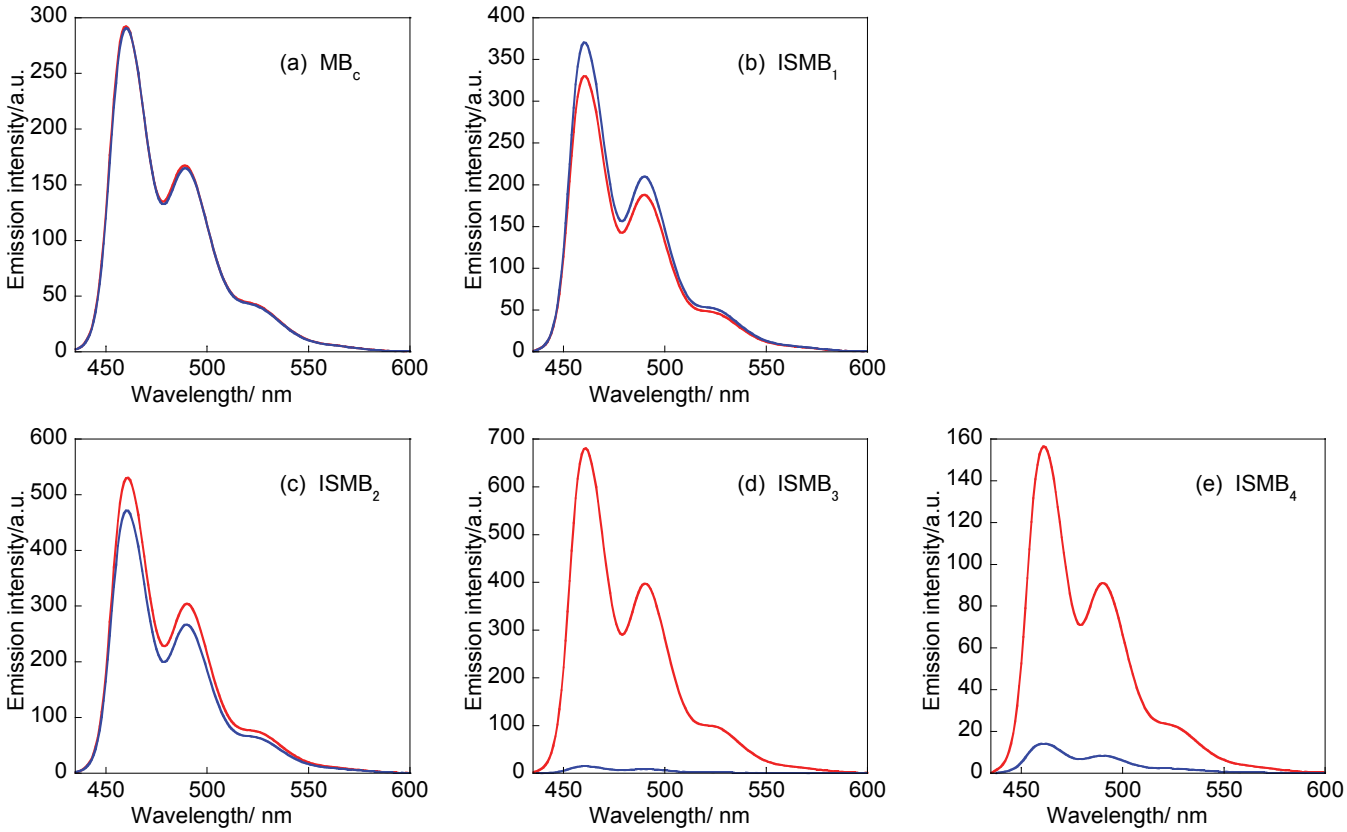


Figure S3. Fluorescence emission (signal emission) spectra of opened (a) **MB$_c$**, (b) **ISMB$_1$**, (c) **ISMB$_2$**, (d) **ISMB$_3$** and (e) **ISMB$_4$** in the presence (red line) and absence (blue line) of PLL-*g*-Dex.
Solution conditions; [MB] = 0.2 μM, [Surv] = 0.8 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 20 ^{o}C, N/P = 2
Measurement conditions of JASCO model FP-6500; bandwidth of excitation = 5nm, bandwidth of emission = 5 nm, Sensitivity = medium.

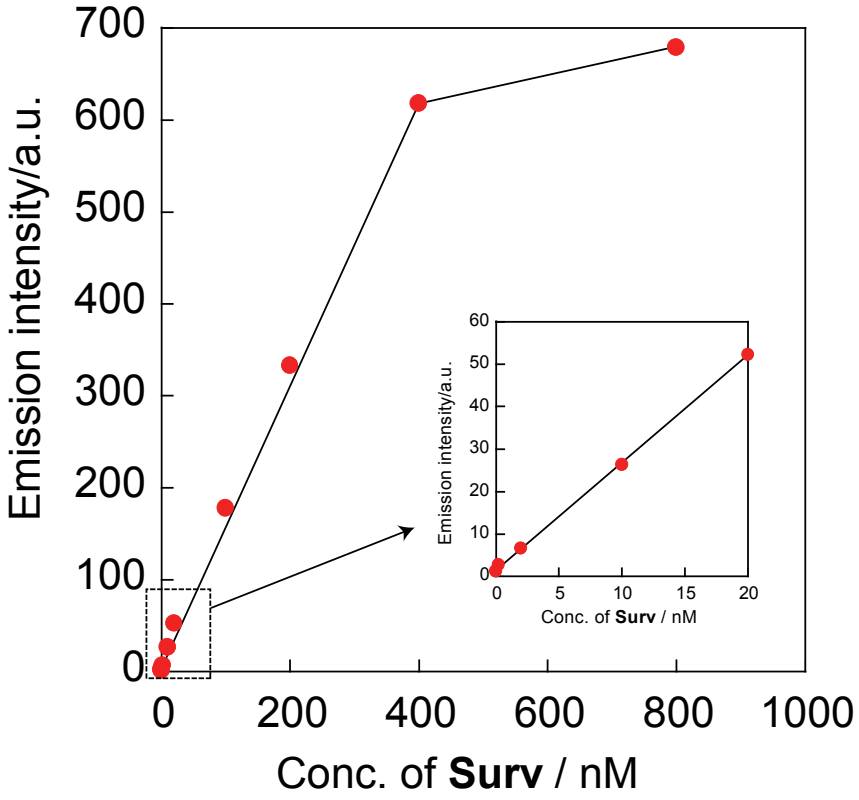


Figure S4. Linear range of **ISMB$_3$** for the detection of **Surv**. Plots at lower concentrations were magnified in inset. Solution conditions; [**ISMB$_3$**] = 200 nM, N/P = 2, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 20 ^{o}C. Measurement conditions of JASCO model FP-6500; bandwidth of excitation = 5nm, bandwidth of emission = 5 nm, Sensitivity = medium.

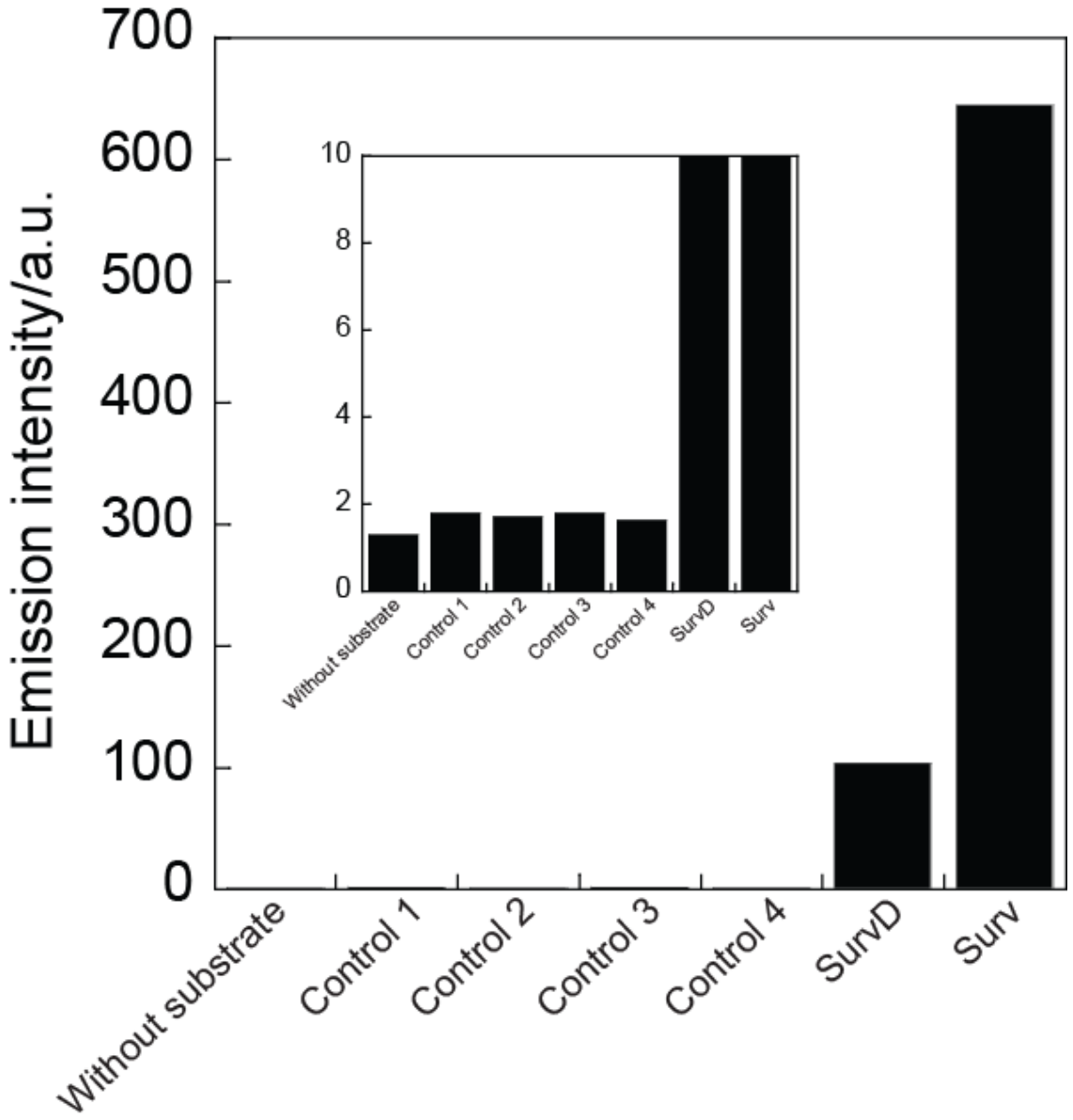


Figure S5. Change of fluorescence intensity of **ISMB$_3$** at 460 nm by the presence of DNA of various sequences excited at 425 nm with PLL-*g*-Dex. Solution conditions were as follows: [**ISMB$_3$**] = 0.2 μM, [Substrate] = 0.8 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), N/P = 2, 20 °C. Sequences of the substrates are listed as follows. All these oligonucleotides are a part of survivin gene.

ISMB$_3$: 5'-GEGTEGGETC-CTTGAGAAAGGGC-GAQCCQACQC-3'
Control 1: 3'-AACTTACATCTCTACGCC-5'
Control 2: 3'-GAGGCCCCACGTCCGCGT-3'
Control 3: 3'-ATAAAAATCACCTCTGCC-5'
Control 4: 3'-AACTCTGCCTCAGAGTAA-5'
SurvD : 3'-ACGCCACAGGAACTCTTTCCCG-5' (One-base deletion mutant, underlined base of Surv is deleted)
Surv: 3'-ACGCCACCAGGAACTCTTTCCCG-5' (Full-match)

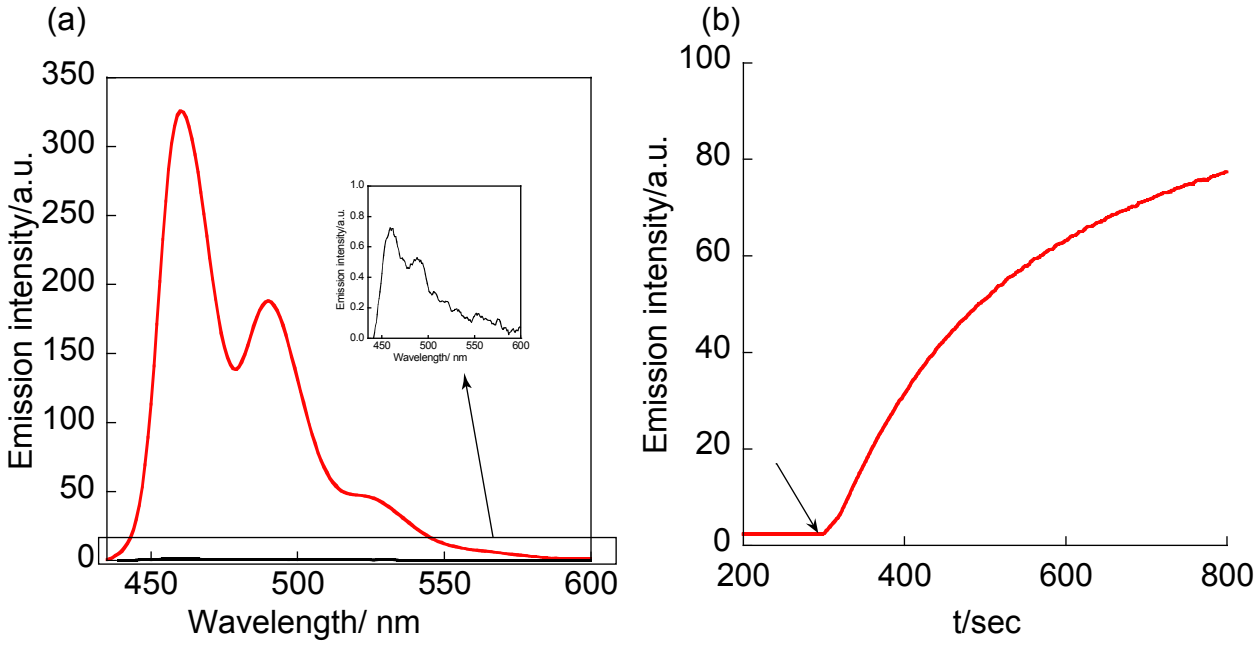


Figure S6. (a) Fluorescence emission spectra of **ISMB$_3$** in the absence (black line) and presence (red line) of **Surv** with 1 M NaCl at 20 ^{o}C. Solution conditions were 0.2 μM **ISMB$_3$**, 0.8 μM **Surv**, 1M NaCl, 10 mM phosphate buffer (pH 7.0).
(b) Change in fluorescence intensity of **ISMB$_3$** with 1M NaCl at 20 ^{o}C. Arrow shows the point when **Surv** was added (300 sec). Solution conditions were 0.2 μM **ISMB$_3$**, 0.4 μM **Surv**, 1M NaCl, 10 mM phosphate buffer (pH 7.0), 20 ^{o}C.

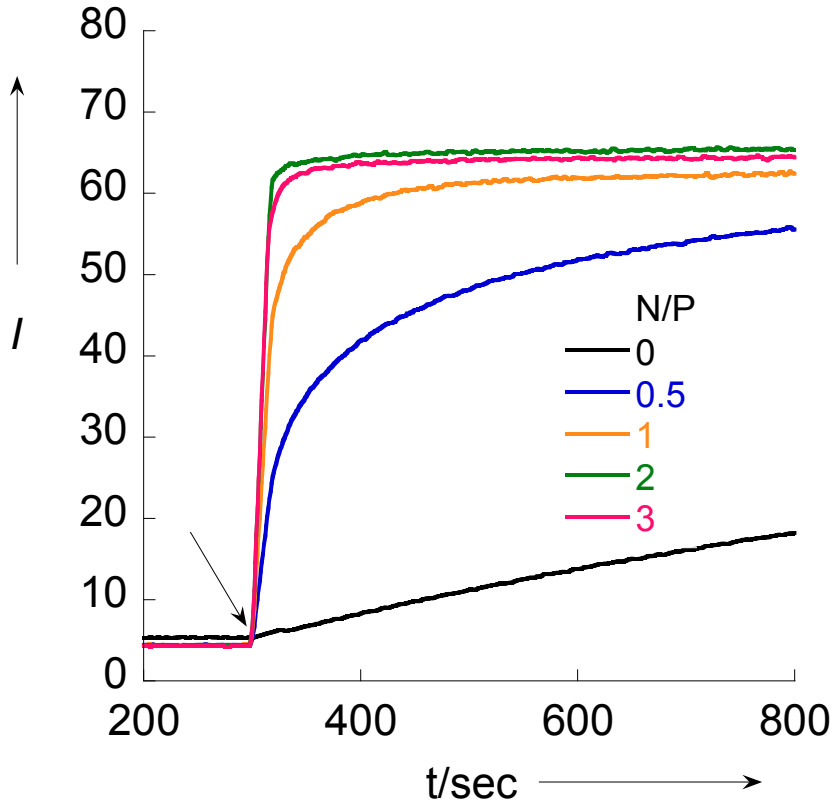


Figure S7. Response of **ISMB$_1$** in the presence of PLL-*g*-Dex at various N/P ratios at 20 °C.
Black line, N/P = 0 (without PLL-*g*-Dex); blue line, N/P = 0.5; orange line, N/P = 1; red line, N/P = 2; green line, N/P =3.
Arrows show the point when **Surv** was added (300 sec). Solution conditions are as follows: [**ISMB$_1$**] = 0.2 μM, [**Surv**] = 0.4 μM, [NaCl] = 100 mM, pH 7.0 (10 mM phosphate buffer), 20 °C.

Table S2. Signal/background (S/B) ratio of each MB in the presence and absence of PLL-g-Dex.[a]

MB	w/o PLL-*g*-Dex[b]			with PLL-*g*-Dex[c]		
	S[d]	B[e]	S/B[f]	S[d]	B[e]	S/B[f]
MB_c	291.0	70.4	4.1	292	32.1	9.1
$ISMB_1$	370.0	27.1	13.7	330	19.1	17.1
$ISMB_2$	471.0	10.4	45.4	530	8.17	64.9
$ISMB_3$	14.7	1.33	11.1	679	1.19	571.0
$ISMB_4$	14.2	0.82	17.2	155	0.93	168.0

[a]Solution conditions: 0.2 μM MB, 0.8 μM **Surv**, 100 mM NaCl, 10 mM phosphate buffer (pH 7), 20 ºC. [b]N/P = 0. [c]N/P = 2. [d]Emission intensity at 460 nm in the presence of **Surv**. [e]Emission intensity at 460 nm in the absence of **Surv**. [f]The ratio of S with respect to B.