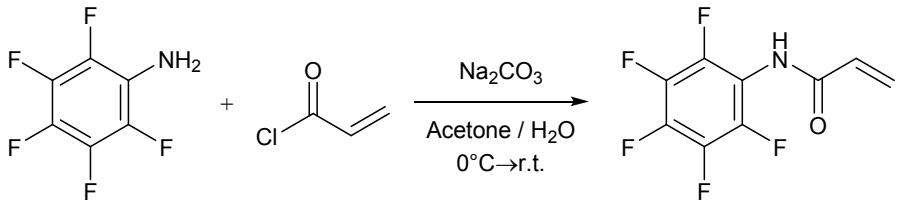
Supporting Information

◆ Supplementary Experimental

Synthesis of 2,3,4,5,6-pentafluorophenyl acrylamide (5FPAA).



The synthetic procedure is similar to that reported by Chanthamath and coworkers.[S1] Sodium carbonate (10.9 mmol, 2equiv) was suspended in acetone (6 mL, Wako Pure Chemical Industries, Ltd., 99.0%) and nanopure water (2 mL) and cooled to 0ºC. Acryloyl chloride (0.988 g, 10.9 mmol, 2equiv) was added dropwise to the stirred suspension under an argon atmosphere. Then, 2,3,4,5,6-pentafluoroaniline (1.00 g, 5.47 mmol) was added dropwise at 0ºC and allowed to come to room temperature (r.t.) over 3 h. After being concentrated to remove acetone completely under reduced pressure, the mixture was extracted 3 times with dichloromethane (3 × 10 mL, Wako Pure Chemical Industries, Ltd., 99.0%). The organic layer was dried over Na_2SO_4 (Wako Pure Chemical Industries, Ltd., 99.0%) for 1 h and concentrated *in vacuo.* The resulting white solid was purified by recrystallization from hexane / diethyl ether (Wako Pure Chemical Industries, Ltd., 99,0%; 1/2, v/v) to give 0.9 g of white crystals in 70 % yield. ^{1}H NMR (400 MHz) $CDCl_3$: δ 7.08 (s, 1H), 6.51 (dd, J = 17.0, 1.2 Hz, 1H), 6.35 (dd, J = 17.0, 10.3 Hz, 1H), 5.92 (dd, J = 10.3, 1.2 Hz, 1H).

Determination of incorporation ratios of TB-NP1 to 4

The incorporation ratios of TBAm and NIPAm in TB-NP1 to 4 were determined from the peak area in ^{1}H NMR (CD_3OD) spectrum by using eqs. 3 to 4, where A_N is the peak area of NIPAm δ 1.19 (s, 6H); and A_T, that of TBAm, δ 1.38 (s, 9H). Actual incorporation ratios of Bis were assumed to be equal to the theoretical.

NIPAm-incorporation ratio (mol %) = $(A_N/6) / [(A_N/6 + A_T/9) \times 100 / 98] \times 100$ (3)

TBAm-incorporation ratio (mol %) = $(A_T/9) / [(A_N/6 + A_T/9) \times 100 / 98] \times 100$ (4)

Determination of incorporation ratios of PA-NP1 to 4

The incorporation ratios of PAA and NIPAm in PA-NP1 to 4 were determined from the peak area in the ^{1}H NMR (DMSO-d_6) spectrum by using eq 5 to 6, where A_N is the peak area of NIPAm δ 1.19 (s, 6H); and A_P, that of PAA, δ 7.07 (s, 1H). Actual incorporation ratios of Bis were assumed to be equal to the theoretical.

NIPAm-incorporation ratio (mol %) = $(A_N/6) / [(A_N/6 + A_P) \times 100 / 98] \times 100$ (5)

PAA-incorporation ratio (mol %) = $A_P / [(A_N/6 + A_P) \times 100 / 98] \times 100$ (6)

Determination of incorporation ratios of FP-NP1 to 4

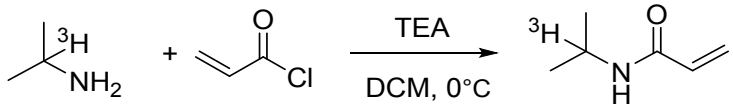
The incorporation ratios of 5FPAA and NIPAm in FP-NP1 to 4 were determined from the peak area in ^{19}F NMR (CD_3OD) and ^{1}H NMR (CD_3OD) spectrum by using eqs. 7 to 8, where A_N is the peak area of NIPAm δ 1.19 (s, 6H); and A_F, that of 5FPAA, δ -156.2 (1F). Actual

incorporation ratios of Bis were assumed to be equal to the theoretical. 1 μL of 2,2,2-triflouroethanol was used as a standard to calculate the relative incorporation ratio of each monomer in F^{19} NMR and H^{1} NMR.

$$\text{NIPAm-incorporation ratio (mol \%)} = (A_N/6) / [(A_N/6 + A_F) \times 100 / 98] \times 100 \quad (7)$$

$$\text{5FPAA-incorporation ratio (mol \%)} = A_F / [(A_N/6 + A_F) \times 100 / 98] \times 100 \quad (8)$$

Synthesis of [^{3}H]-labeled NIPAm.



The synthetic procedure was similar to that reported by Peter J. Roth and coworkers.[s2] Isopropylamine hydrochloride containing a small amount of [2-^{3}H] isopropylamine hydrochloride (21.8 mg, 0.228 mmol, 12.3 kBq) was dissolved into dry ethanol (200 μL, Wako Pure Chemical Industries, Ltd., 99.5%). Then, dry dichloromethane (2.4 mL) and distillated triethylamine (0.191 mL, 1.37mmol, 6equiv, Wako Pure Chemical Industries, Ltd., 99.0%) were added to the solution under a nitrogen atmosphere, after which the mixture was cooled to 0ºC. Acryloyl chloride (61.9 mg, 0.683 mmol, 3equiv) in dry dichloromethane (3.6 mL) was added dropwise to the stirred mixture and allowed to come to r.t. overnight. The reaction was finished by adding nanopure water (10 mL), and then the mixture was extracted 3 times with dichloromethane (5 × 5 mL). The organic layer was dried over Na_2SO_4 for 1 h and concentrated *in vacuo.* The crude products were purified by preparative thin-layer chromatography using

dichloromethane/methanol (Wako Pure Chemical Industries, Ltd., 99.8%; 20:1, v/v) to give 19 mg of white crystals in 73.6 % yield.

Cytotoxicity of NPs.

A cytotoxicity LDH Assay Kit-WST (Dojindo Co., Ltd., Kumamoto-shi, Kumamoto, Japan) was used to estimate the cytotoxicity of the NPs. This assay was conducted by using murine endothelial cells, 2H-11, according to the manufacturer's instructions. Briefly, 2H-11 cells were grown in a humidified atmosphere at 37°C under 5% CO_2. The cells were cultured in T-75 flasks using Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin. 2H-11 cells of passage numbers 40-55 were used in this test. The cells were washed with PBS and then harvested with 0.025% trypsin-ethylenediaminetetraacetic acid (EDTA) solution (Thermo Fisher Scientific, Inc.). The cell suspensions were centrifuged at 50 x *g* for 10 min to remove trypsin-EDTA and diluted to a density of 1.5×10^4 cells/mL with DMEM. The cell suspensions were seeded onto 96-well plates (Corning, Inc.) at 200 μL/well, and incubated for 24 h to allow cell adhesion. The NPs (300 μg/mL) or PBS (negative control) were added to the cells after the cells had been washed with 200 μL of PBS. At 24 h after addition of NPs, Lysis Buffer was added to the cultures to which NPs had not been added cells (positive control), and the cells were incubated further for 15 min. After the incubation, 100-μL supernatants were withdrawn from each well to a new plate, and 100-μL Waking Solution was added to each well. At 30 min after the addition, the enzymatic coloring reactions were stopped by the addition of 50-μL Stop Solution. Absorbance values at 490 nm were measured with Infinite® 200 PRO (Tecan Group Ltd., Männedorf, Switzerland).

Cytotoxicity of the samples was calculated by using eq. 9, where A_{sample} is the absorbance at 490 nm for the cells incubated with NPs, $A_{negative\ control}$ is the absorbance at 490 nm for the cells incubated with PBS, and $A_{positive\ control}$ is the absorbance at 490 nm for the cells incubated with Lysis Buffer including TritonX-100 of less than 10%.

$$\text{Cytotoxicity (\%)} = [(A_{sample} - A_{negative\ control}) / (A_{positive\ control} - A_{negative\ control})] \times 100$$

Cellular uptake of NPs.

2H-11 cells were seeded onto 24-well plates (BD Bioscience, SanJose, CA) at the density of 1.5×10^4 cells/well. [^{3}H]-labeled TF-NP5, TF-NP5-p or TF-NP5-n was added to the cells (300 Bq/cells, 100 μg/ml) and incubated for 24 h. After having been washed with PBS, the cells were lysed with 1 w/v% SDS. The radio activities were determined by use of a liquid scintillation counter (LSC-7400, Hitachi Aloka Medical, Tokyo, Japan).

Supplementary Figures

Supplementary Table 1. Characteristics of NPs synthesized without surfactant*

	Particle size (nm)	PdI	ζ-potential (mV)
TB-NP4b	323	0.091	-33
PA-NP4b	434	0.027	-43
FP-NP4b	172	0.009	-40

*All NPs incorporated 2-mol% Bis for cross-linking. NIPAm to make up the remaining percentages.

Supplementary Table 2. Characteristics of NPs synthesized by changing polymer density (feed ratios of Bis, a cross-linker) *

	Monomer feed (mol%)			Particle size (nm)	PdI	ζ-potential (mV)	Yied (%)
	TBAm	NIPAm	**Bis**				
T-NP5	20	70	**10**	1356	0.009	-24	78
T-NP6	40	50	**10**	168	0.048	-18	73
T-NP7	60	30	**10**	76	0.135	-27	81
T-NP8	80	10	**10**	76	0.032	-41	83
T-NP9	20	60	**20**	646	0.165	-13	79
T-NP10	40	40	**20**	504	0.237	-14	82
T-NP11	60	20	**20**	179	0.061	-34	81
T-NP12	80	0	**20**	110	0.038	-35	90
T-NP13	20	40	**40**	946	0.111	-19	89
T-NP14	40	20	**40**	1050	0.193	-9	95
T-NP15	60	0	**40**	512	0.205	-25	95

*All NPs incorporated NIPAm to make up the remaining percentages.

Supplementary Table 3. Characteristics of the NPs incorporating charged monomers*

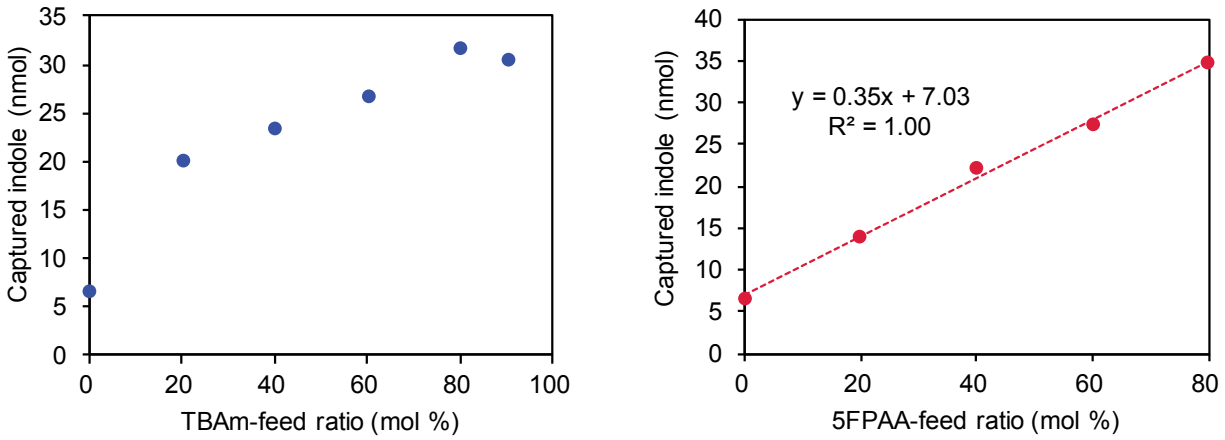
	Monomer feed ratio (mol%)				Particle size (nm)	PdI	ζ-potential (mV)	Yied (%)
	TBAm	5FPAA	APM	AAc				
TB-NP4-p	80		5		63	0.099	51	73
TB-NP4-n	80			5	81	0.268	-44	85

FP-NP4-p		80	5		75	0.268	39	76
FP-NP4-n		80		5	66	0.098	-39	82
TF-NP6-p	60	20	5		55	0.112	57	68
TF-NP6-n	60	20		5	87	0.04	-40	85

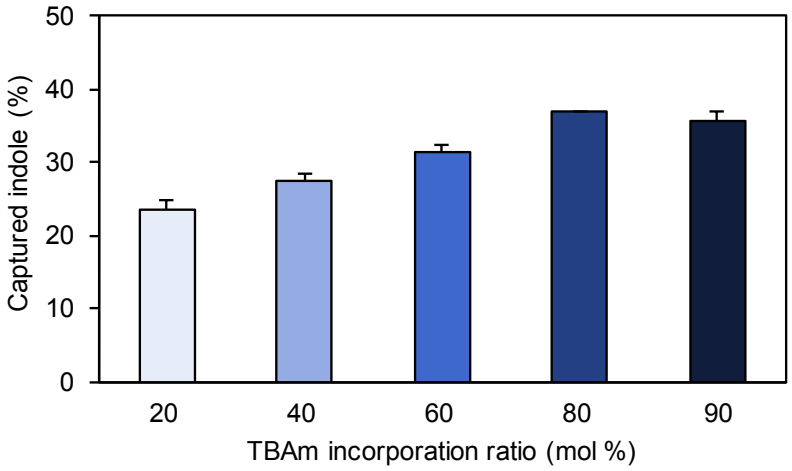
*All NPs incorporated 2-mol% Bis for cross-linking and NIPAm to make up the remaining percentages.

Supplementary Table 4. Excretion route of TF-NP5, TF-NP5-p, and TF-NP5-n

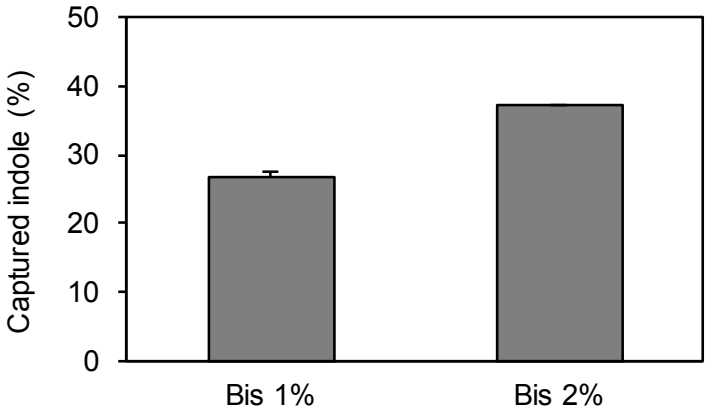
	Urine (%)	Feces (%)
TF-NP5	0.1	>95
TF-NP5-p	0.3	>95
TF-NP5-n	0.1	>95



Supplementary Figure S1. Relationship between the feed ratio of hydrophobic monomers and captured indole.

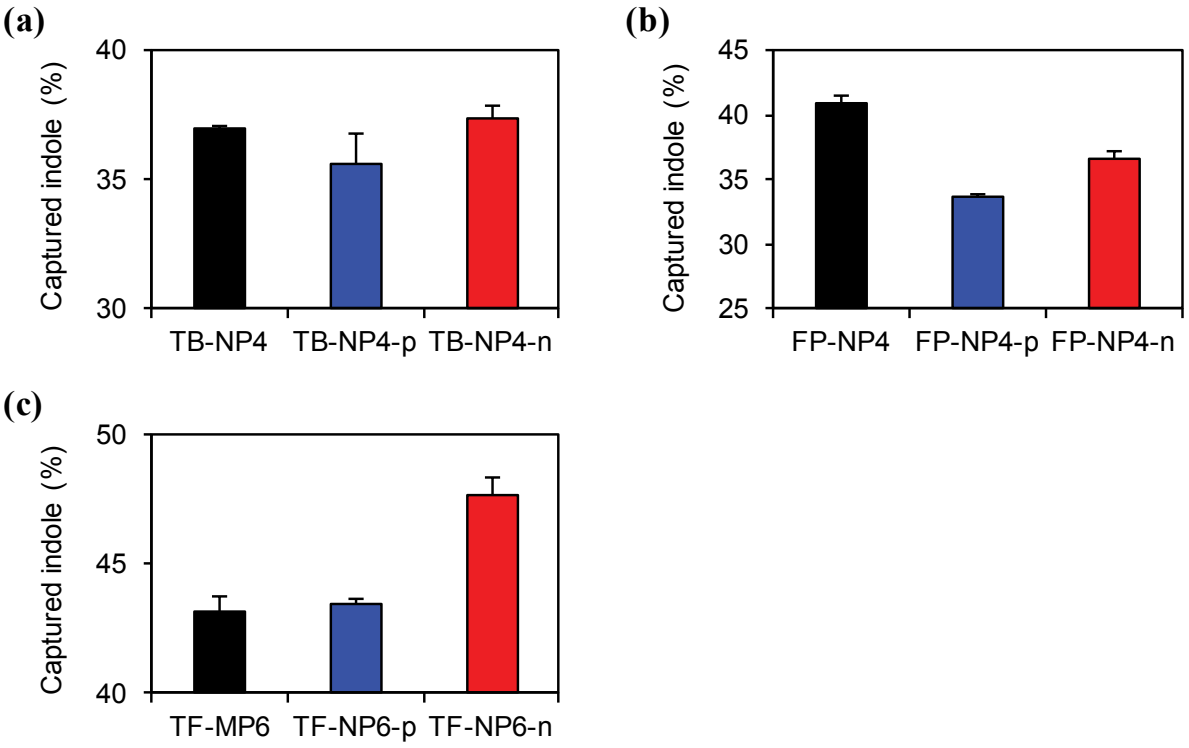


Supplementary Figure S2. Amounts of indole captured by TBAm-incorporating NPs following a 10-sec incubation.



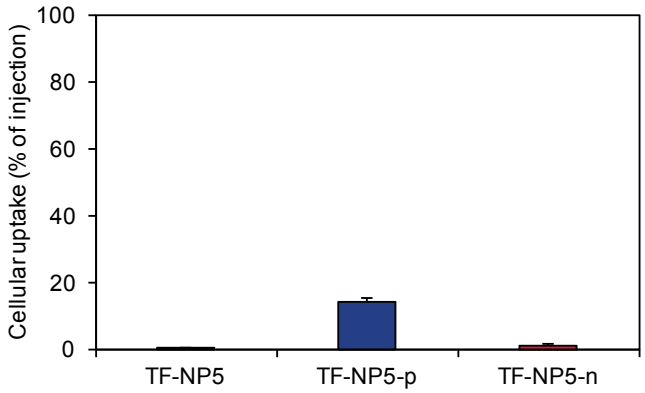
Supplementary Figure S3. The amounts of indole captured by lightly cross-linked NPs incorporating 80-mol% TBAm following a 10-sec incubation.

Data are shown as the mean ± standard deviation.



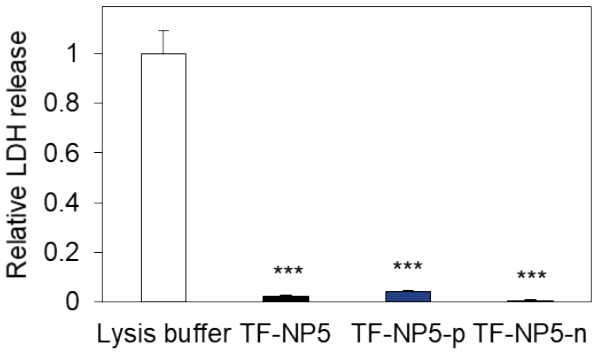
Supplementary Figure S4. Effect of charged-monomer incorporation into NPs on capturing indole.

Indole-capturing rates of TB-NP4 (a), FP-NP4 (b), or TF-NP6 (c) and these synthesized with APM (blue) or AAc (red).



Supplementary Figure S5. Cellular uptake of NPs into the normal cells.

Cells were incubated with [^{3}H]-labeled TF-NP5, TF-NP5-p or TF-NP5-n for 24. Then, cells were lysed and measured radio activities.



Supplementary Figure S6. Relative amount of LDH released from 2H-11 cells after incubation with TF-NP5, TF-NP5-p or TF-NP5-n for 24-h.

Data represent the mean release relative to that of the positive control (lysis buffer) (n=6). Significant differences between Lysis buffer and NP-injected groups are indicated by *** ($P < 0.001$, Dunnet's test subsequent to ANOVA).

◆Supplementary References

S1. Chanthamath, S.; Takaki, S.; Shibatomi, K.; Iwasa, S., Highly Stereoselective Cyclopropanation of α,β-Unsaturated Carbonyl Compounds with Methyl (Diazoacetoxy)acetate Catalyzed by a Chiral Ruthenium(II) Complex. *Angew. Chem., Int. Ed.* **2013,** *52* (22), 5818-5821.

S2. Roth, P. J.; Wiss, K. T.; Zentel, R.; Theato, P., Synthesis of Reactive Telechelic Polymers Based on Pentafluorophenyl Esters. *Macromolecules (Washington, DC, U. S.)* **2008**, 41 (22), 8513-8519.