

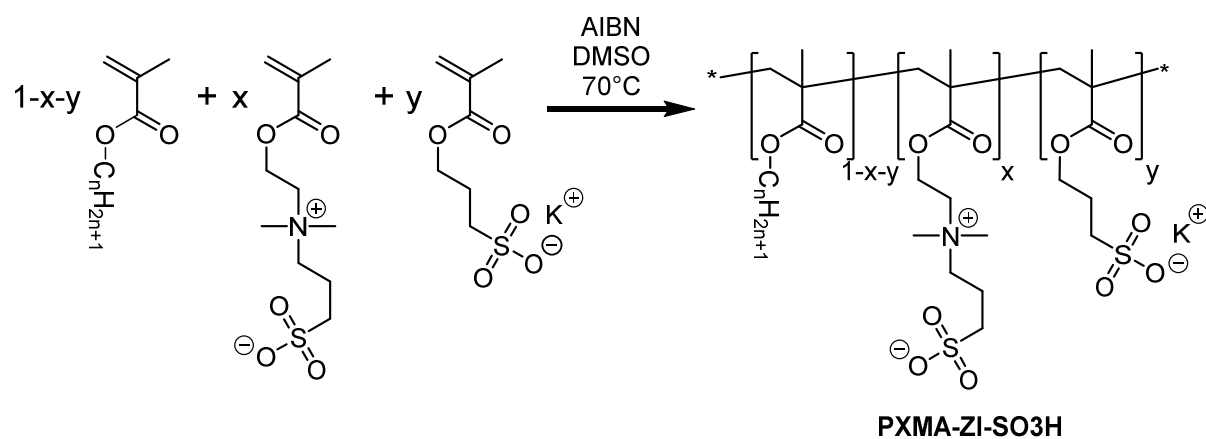
**Supporting Information for:**

# Zwitterionic Stealth Dye-Loaded Polymer Nanoparticles for Intracellular Imaging

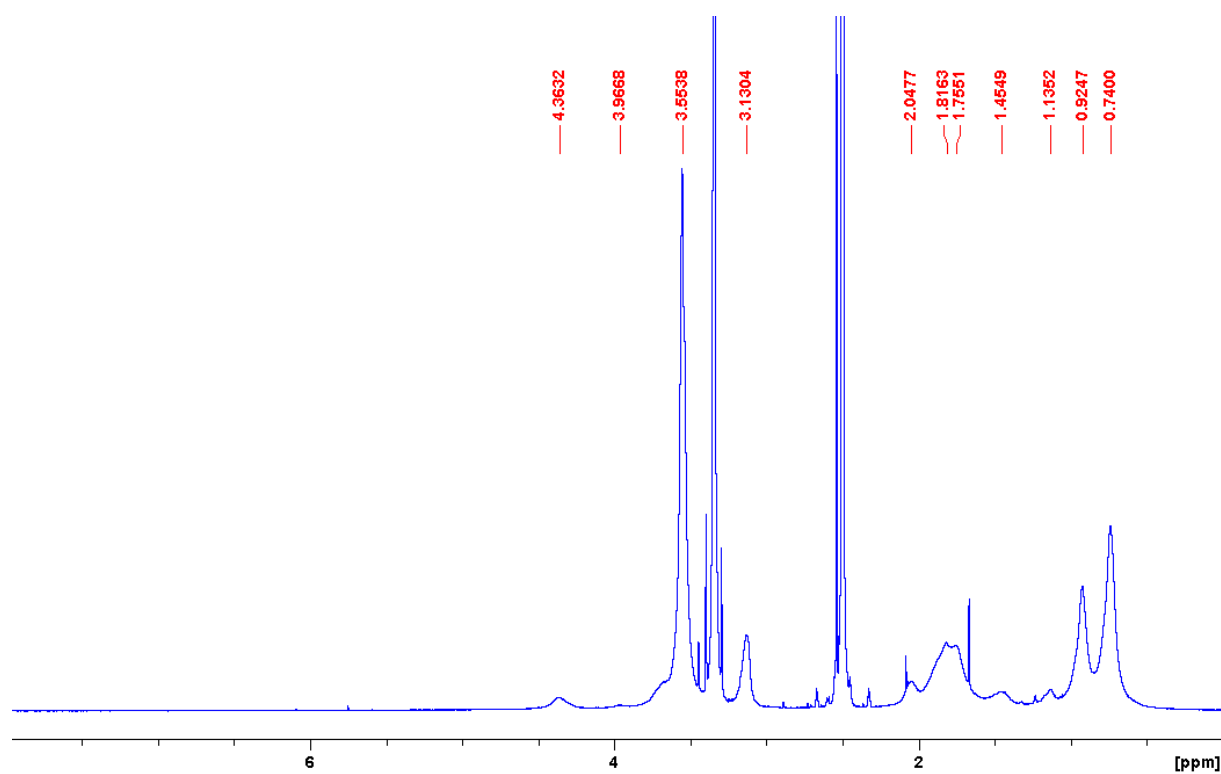
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**Scheme S1.** Polymerization of alkyl methacrylate, ZI and sulfonate monomers to obtain methacrylate polymers bearing zwitterionic and charged groups.



**Figure S1.** <sup>1</sup>H-NMR spectrum of PMMA-ZI-10%-SO<sub>3</sub>H-1% in DMSO-d<sub>6</sub>.

**Table S1.** Sizes of NPs made of different polymers as obtained from TEM, DLS, and FCS.<sup>a</sup>

Polymer			Size (nm)		
Main monomer	SO <sub>3</sub> H (mol%)	ZI (mol%)	TEM <sup>b</sup>	DLS <sup>b</sup>	FCS <sup>c</sup>
MMA	1	-	13 ± 3	-	14 ± 1
MMA	1	10	11 ± 3	11 ± 1	15 ± 1
MMA	2	-	9 ± 3	-	14 ± 1
MMA	2	10	9 ± 2	-	13 ± 1
EMA	1	10	14 ± 3	10 ± 1	11 ± 1
PMA	1	10	22 ± 4	19 ± 2	13 ± 1
BMA	1	10	35 ± 7	31 ± 2	32 ± 4

<sup>a</sup> Errors correspond to width of the distribution at half maximum (FWHM) for TEM, and standard error of the mean (s.e.m.) over 3 measurements for DLS and FCS.<sup>b</sup> NPs prepared with 10 wt% R18/F5-TPB. <sup>c</sup> NPs prepared with 1 wt% R18/F5-TPB.

**Table S2.** Sizes of PMMA-ZI-10%-SO<sub>3</sub>H-1% NPs at different pH values as measured by DLS.<sup>a</sup>

pH <sup>b</sup>	Mean size (nm)
3.6	14 ± 1
5.0	15 ± 1
7.4	14 ± 1
9.0	15 ± 2

<sup>a</sup> NPs were loaded with 10 wt% of R18/F5-TPB. <sup>b</sup> For adjusting the pH we used the following buffer solutions: acetate buffer for pH 3.6 and 5; phosphate buffer for pH 7.4; borate buffer for pH 9; all at 5 mM.

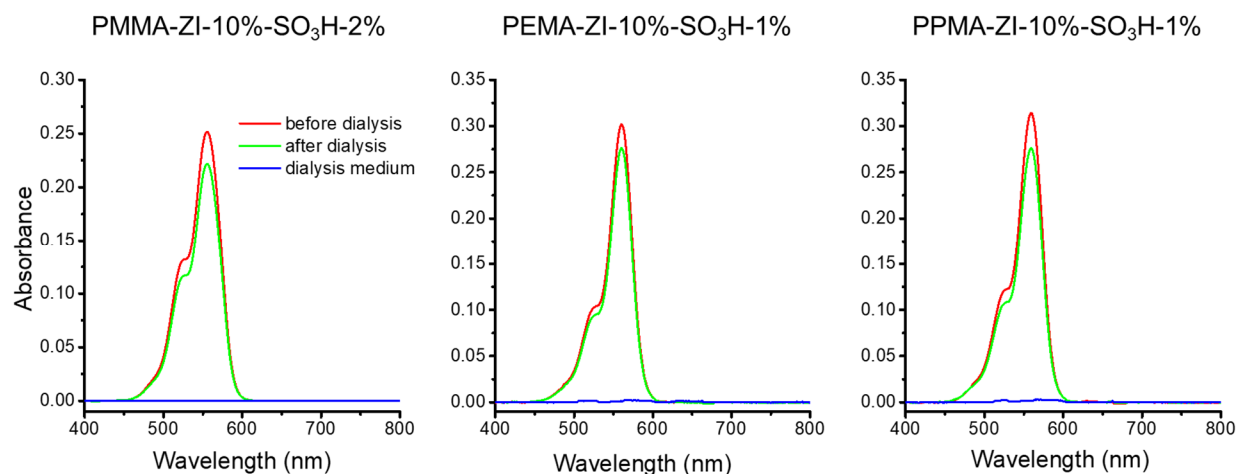
**Table S3.** Zeta potential of dye-loaded NPs made from different polymers.

polymer	Zeta potential (mV)
PMMA-SO <sub>3</sub> H-1%	-24
PMMA-ZI-10%-SO <sub>3</sub> H-1%	-21
PEMA-ZI-10%-SO <sub>3</sub> H-1%	-28
PPMA-ZI-10%-SO <sub>3</sub> H-1%	-34

**Table S4.** FCS results: concentration, diameter and brightness obtained for the different zwitterionic NP solutions before and 5 min after addition of 10% fetal bovine serum (FBS).<sup>a</sup>

	ZI %	FBS addition (vol%)	Concentration (nM)	Diameter (nm)	Brightness relative to TMR
PMMA-SO <sub>3</sub> H-2%	0	-	18.6	14.1	5.0
		10	14.7	22.6	4.3
	2	-	12.9	15.2	5.5
		10	12.2	24.6	4.4
	5	-	30.7	8.0	2.7
		10	23.3	12.4	2.5
	10	-	26.1	13.2	3.1
		10	20.2	14.6	2.9
PMMA-SO <sub>3</sub> H-1%	0	-	17.9	14.0	6.0
		10	10.8	23.5	5.9
	10	-	14.8	14.9	5.3
		10	14.5	15.4	4.6
PEMA-SO <sub>3</sub> H-1%	10	-	34.5	11.0	2.0
		10	26.4	14.3	2.4
PPMA-SO <sub>3</sub> H-1%	10	-	9.6	12.5	5.8
		10	7.8	15.9	5.7
PBMA-SO <sub>3</sub> H-1%	10	-	4.2	31.8	22.1
		10	1.6	45.5	26.4

<sup>a</sup> NPs prepared with 1 wt% R18/F5-TPB.

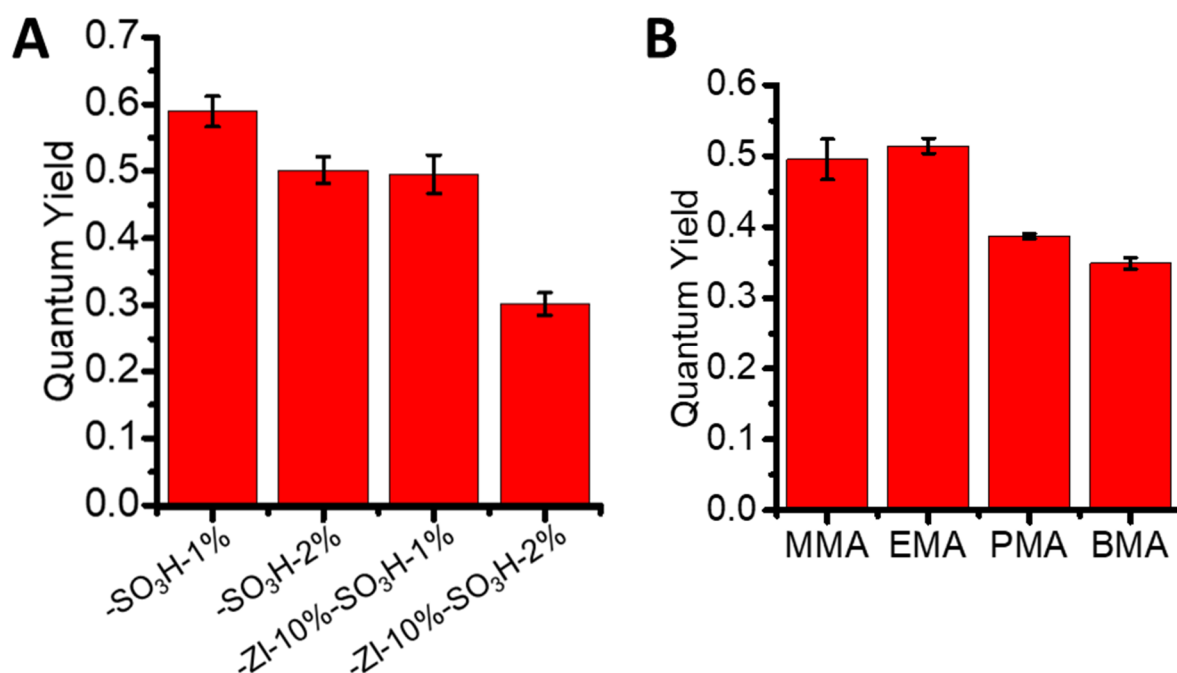


**Figure S2.** Absorbance spectra measured for solutions of PXMA-ZI-10%-SO<sub>3</sub>H-1 or 2% NPs loaded with 10 wt% of R18/F5-TPB before (red) and after (green) dialysis for 24 h. The blue curve corresponds to the dialysis medium after dialysis.

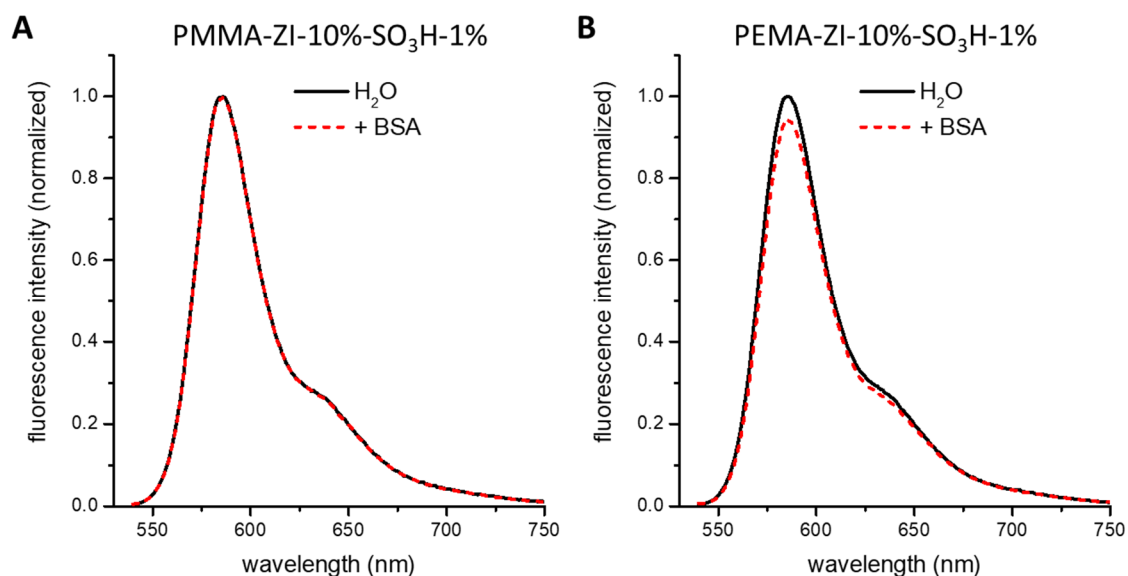
**Table S5.** Release of dye from NPs made from different polymers over 24 h.<sup>a</sup>

polymer	loss of R18/F5-TPB <sup>b</sup>
PMMA-ZI-10%-SO <sub>3</sub> H-2%	14 %
PEMA-ZI-10%-SO <sub>3</sub> H-1%	9 %
PPMA-ZI-10%-SO <sub>3</sub> H-1%	12 %

<sup>a</sup> Release was tested by dialysis for 24 h against a 1 mM solution of  $\beta$ -cyclodextrin. Absorbance spectra were recorded for the NP solution before and for the NP solution and the dialysis solution after dialysis (Figure S5). <sup>b</sup> Relative to amount of R18/F5-TPB before dialysis.



**Figure S3.** Quantum yield of dye-loaded NPs. NPs were made from (A) PMMA- $\text{SO}_3\text{H-1\%}$  and 2%, PMMA-ZI-10%- $\text{SO}_3\text{H-1\%}$  and 2% and (B) from PMA-ZI-10%- $\text{SO}_3\text{H-1\%}$  with different hydrophobic monomers. They were loaded with 10 wt% R18/F5-TPB. Given are mean values from three independent measurements. The error bars correspond to SEM.

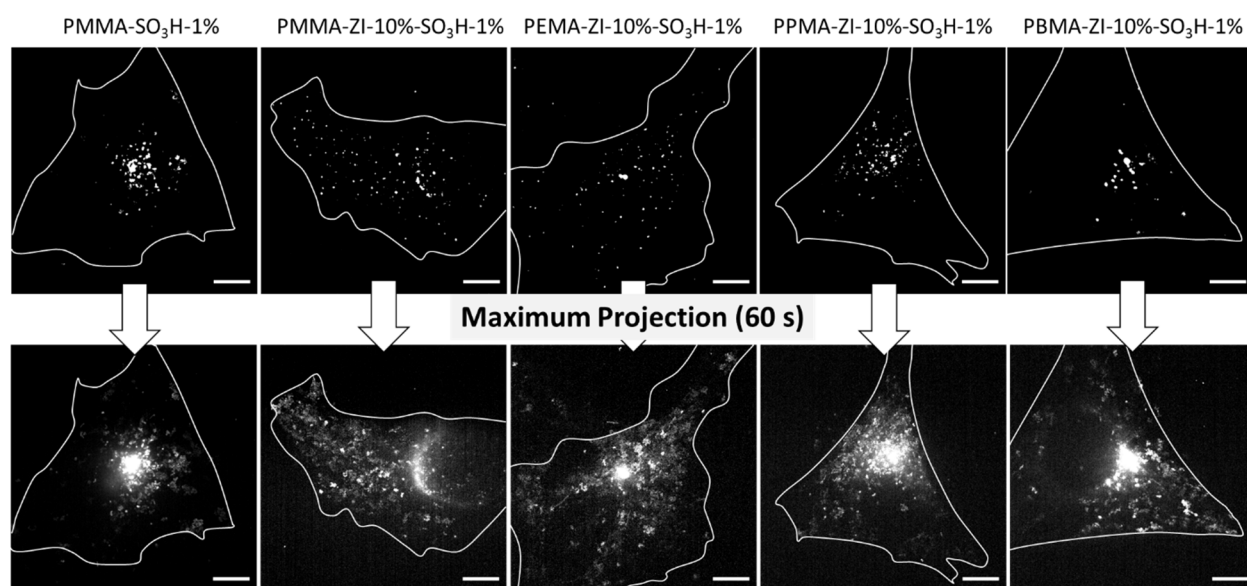


**Figure S4.** Fluorescence spectra of (A) PMMA-ZI-10%- $\text{SO}_3\text{H-1\%}$  and (B) PEMA-ZI-10%- $\text{SO}_3\text{H-1\%}$  NPs loaded with 10 wt% R18/F5-TPB in water and in the presence of 1 mg/mL bovine serum albumin (BSA).

**Table S6.** Per particle fluorescence brightness for different zwitterionic NPs loaded with 10 wt% R18/F5-TPB.<sup>a</sup>

Main monomer	SO <sub>3</sub> H, (mol%)	ZI (mol%)	TEM (nm)	QY	Brightness (M <sup>-1</sup> L <sup>-1</sup> )
MMA	1	0	13 ± 3	0.58 ± 0.04	4.2 x 10 <sup>6</sup>
MMA	1	10	11 ± 3	0.50 ± 0.02	2.2 x 10 <sup>6</sup>
MMA	2	0	9 ± 3	0.50 ± 0.03	1.2 x 10 <sup>6</sup>
MMA	2	10	9 ± 2	0.31 ± 0.03	0.9 x 10 <sup>6</sup>
EMA	1	10	14 ± 3	0.51 ± 0.04	4.6 x 10 <sup>6</sup>
PMA	1	10	22 ± 4	0.39 ± 0.03	1.4 x 10 <sup>6</sup>
BMA	1	10	35 ± 7	0.35 ± 0.04	4.9 x 10 <sup>7</sup>

<sup>a</sup> Brightness was calculated as: Brightness = N\* $\epsilon_{R18}$ \*QY, where N is the number of fluorophores based on the loading and the NP size,  $\epsilon_{R18}$  is the absorbance of R18 at 565 nm (125 000 M<sup>-1</sup>L<sup>-1</sup>) and QY is the measured quantum yield.



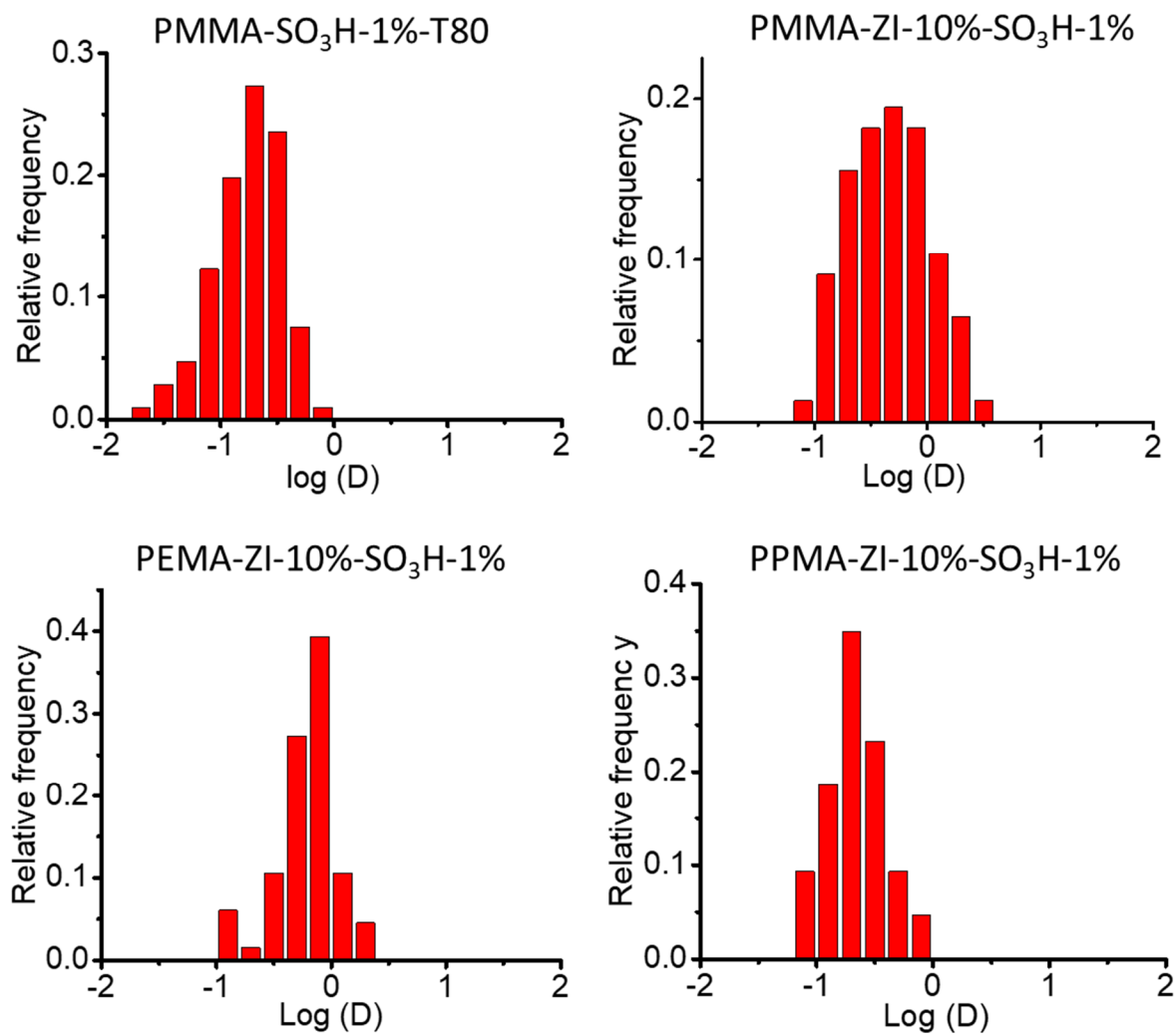
**Figure S5.** Approximate outline of cells obtained from phase-contrast images marked on representative epi-fluorescence micrographs of HeLa cells microinjected with different types of NPs loaded with 10 wt% R18/F5-TPB. Top: single frame (50 ms exposure); bottom: maximum projections over 60 s. Scale bars correspond to 10  $\mu\text{m}$ .

**Table S7.** Percentage of fluorescence at the injection point to total NP fluorescence in cells microinjected with different types of NPs loaded with 10 wt% R18/F5-TPB.

polymer	Fluorescence at the injection point (%) <sup>a</sup>
PMMA-SO <sub>3</sub> H-1%	39 $\pm$ 10
PMMA-ZI-10%-SO <sub>3</sub> H-1%	6 $\pm$ 2
PEMA-ZI-10%-SO <sub>3</sub> H-1%	10 $\pm$ 1
PPMA-ZI-10%-SO <sub>3</sub> H-1%	7 $\pm$ 2
PBMA-ZI-10%-SO <sub>3</sub> H-1%	35 $\pm$ 12

<sup>a</sup> The fluorescence in the entire cell and the fluorescence in a circle of about 5  $\mu\text{m}$  around the injection point were measured for at least 3 different fields of view per condition. Given is the ratio of fluorescence at the injection point to the total fluorescence in the NP channel in the cell.





**Figure S6.** Distribution of diffusion coefficients ( $\mu\text{m}^2.\text{s}^{-1}$ ) of NPs in the cytosol obtained from MSD curves.