Electronic Supplementary Material (ESI) for Nanoscale.

Electronic supplementary information

Ionic Aggregation-Induced Emission Dye with Bulky Counterions for Preparation of Bright Near-Infrared Polymeric Nanoparticles

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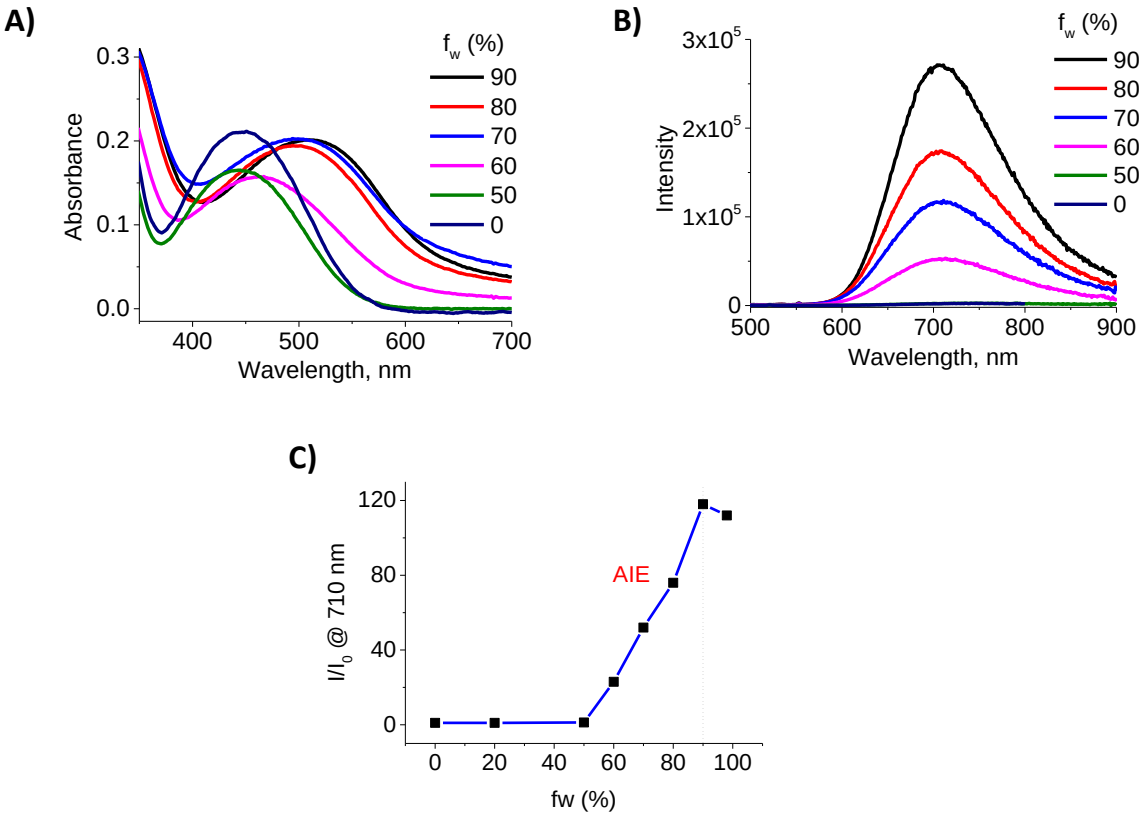

Fig. S1. AIE characteristics of **TPE-F5**. A) absorption and B) fluorescence spectra of **TPE-F5** in CH_3CN-H_2O mixtures with different water fractions (f_W). C) Plot of the relative fluorescence intensity (I/I_0) at 710 nm versus f_W of the CH_3CN-H_2O mixture of **TPE-F5**. Excitation wavelength: 450 nm.

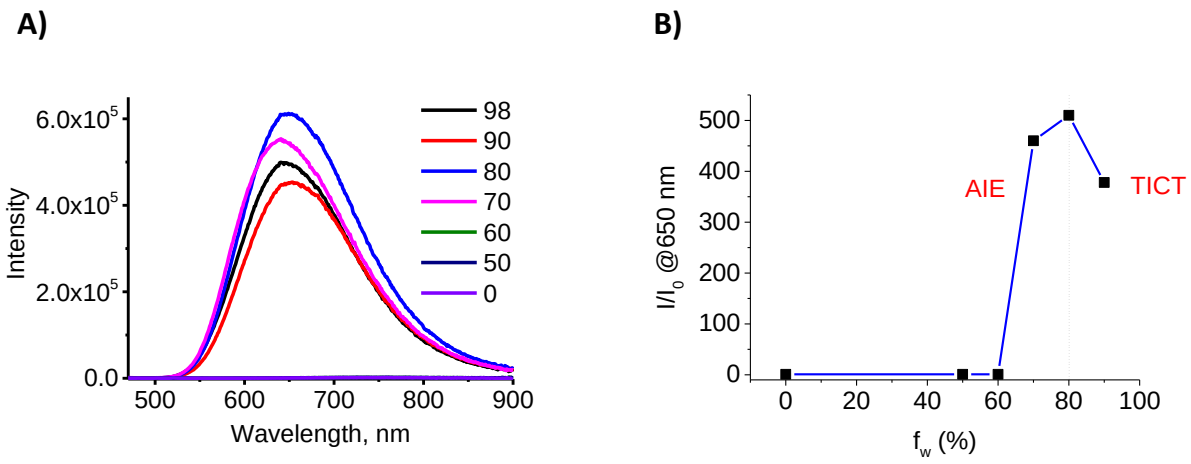

Fig. S2. AIE characteristics of **TPE-PF$_6$**. A) Fluorescence spectra of **TPE-PF$_6$** in CH_3CN-H_2O mixtures with different water fractions (f_W) B) Plot of the relative fluorescence intensity (I/I_0) at 650 nm versus f_W of the CH_3CN-H_2O mixture of **TPE-PF$_6$**. Excitation wavelength: 450 nm.

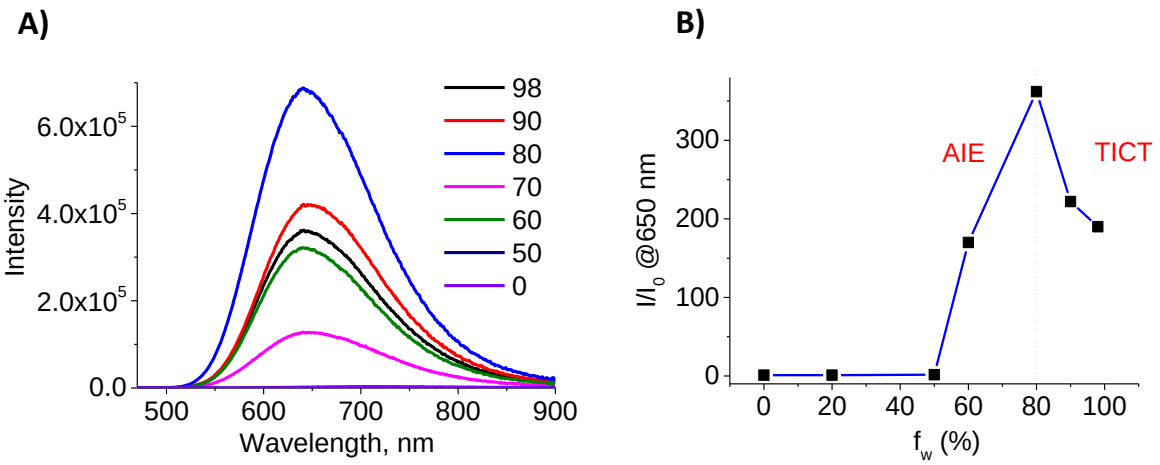

Fig. S3. AIE characteristics of **TPE-ClO$_4$**. A) Fluorescence spectra of **TPE-ClO$_4$** in CH_3CN-H_2O mixtures with different water fractions (f_W). B) Plot of the relative fluorescence intensity (I/I_0) at 650 nm versus f_W of the CH_3CN-H_2O mixture of **TPE-ClO$_4$**. Excitation wavelength: 450 nm.

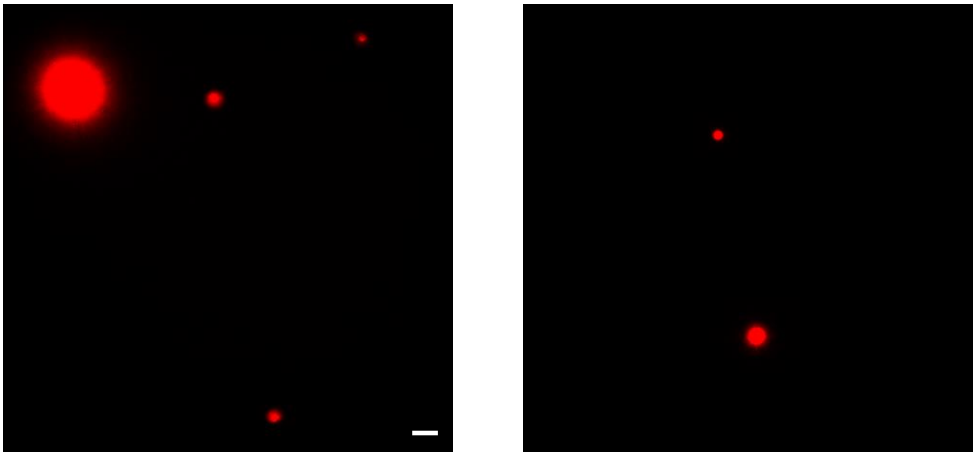

Fig. S4. Large aggregates observed for **TPE-PF6** polymeric NPs under wide-field fluorescence microscopy after immobilizing the particles on glass plates. Scale bar 10 μM.

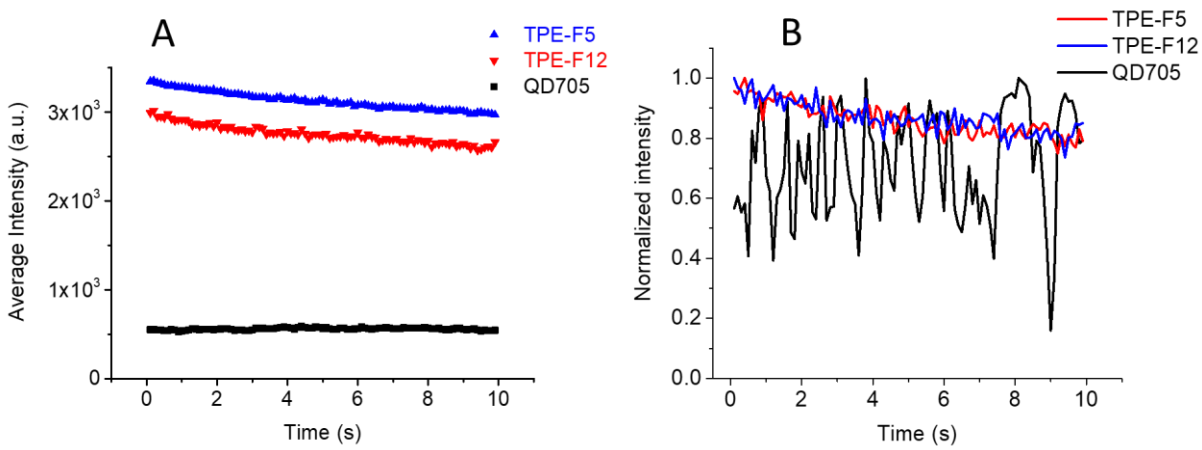

Fig. S5. Average intensity (A) and normalized single-particle intensity (B) as a function of time, recorded by a wide-field microscope. The excitation power density was 7.6 W cm^{-2} at 470 nm.

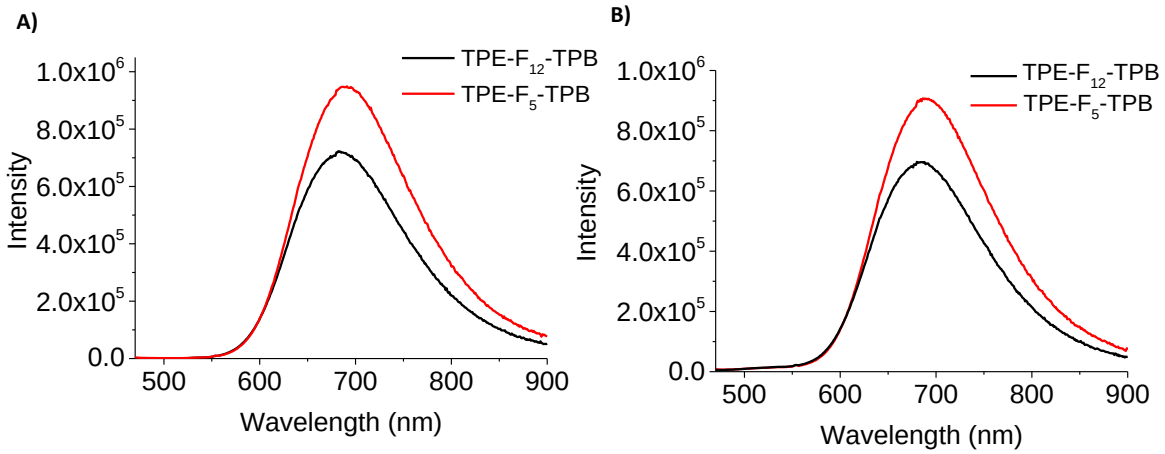

Fig. S6. Stability of emission properties of TPE NPs in Serum. Fluorescence spectra of **TPE-F12** and **TPE-F5** NPs in A) phosphate buffer (pH 7.4) and B) 10% FBS-Phosphate buffer (pH 7.4) after incubation for 1 h. Excitation wavelength: 450 nm.

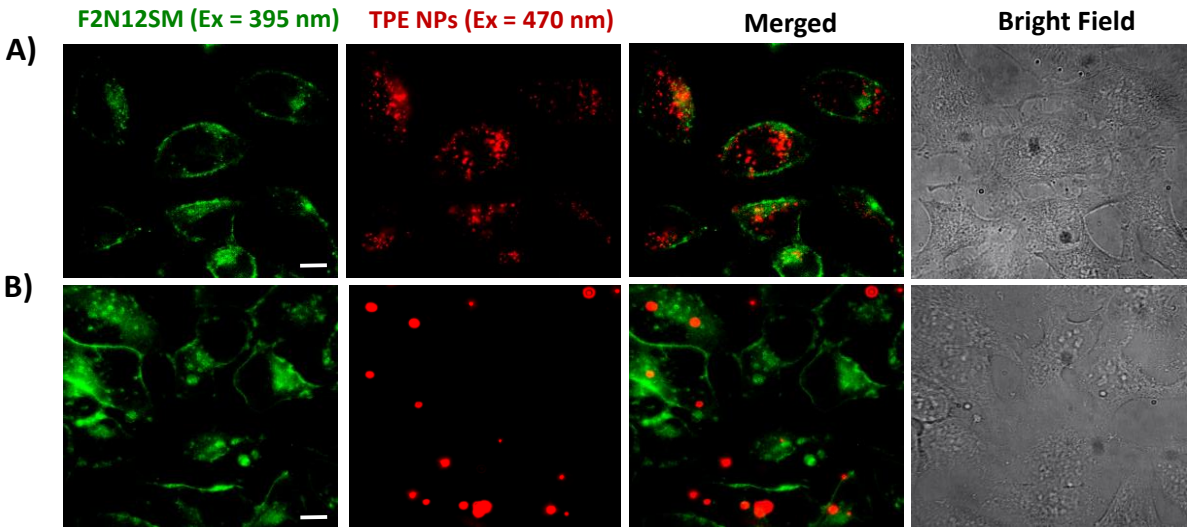

Fig. S7. Fluorescence imaging of HeLa cells incubated with **TPE-F5** PMMA-MA NPs or **TPE-PF6** NPs without polymer. The images correspond to A) **TPE-F5** polymeric NPs (PMMA-MA, 350 mM dye loading, pH 7.4), B) **TPE-PF6** (without polymer, pH 7.4) were incubated with HeLa cells for 3 h at 37 °C. The first column (in green) represents images of NPs after co-staining with a membrane marker **F2N12SM** using an excitation at 405 nm and a detection range of 450–500 nm, while second panels (red) present images of TPE polymeric NPs with an excitation of 488 nm and a detection range of 670-750 nm. Scale bar, 10 µM. NPs were added to a final concentration corresponding to 210 nM of the loaded dye salt.

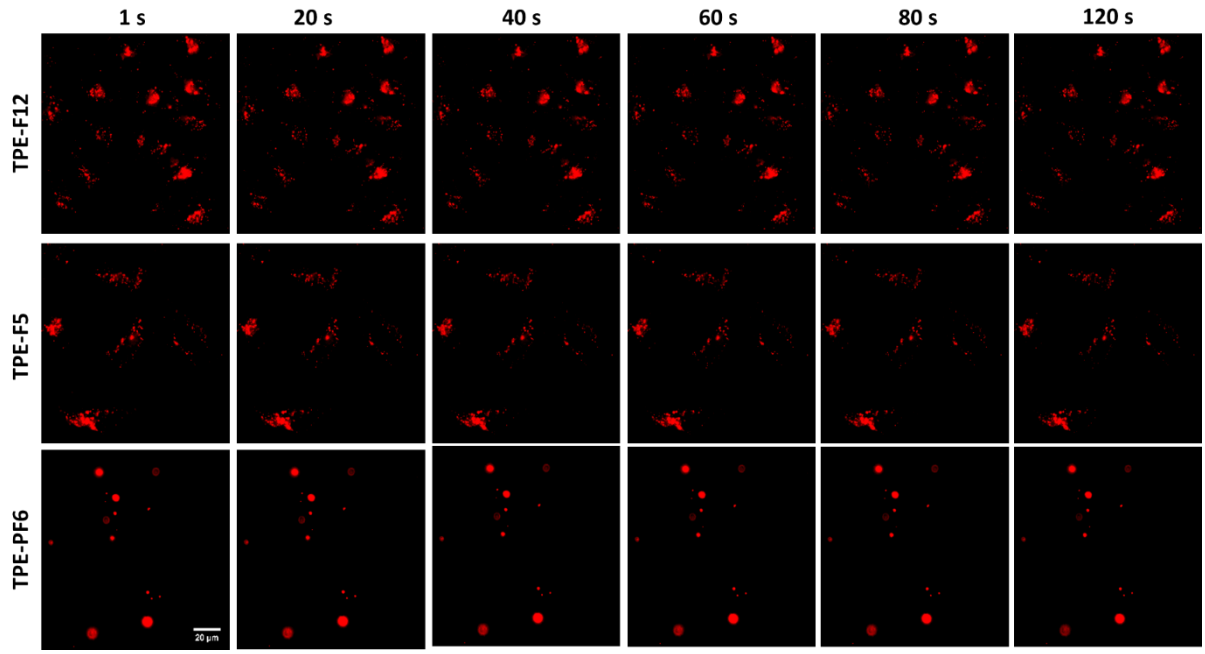

Fig. S8. Photostability of the TPE NPs in HeLa cells under continuous irradiation under wide-field microscope. The images correspond to polymeric NPs loaded with **TPE-F12** (top), **TPE-F5** (middle) and **TPE-PF6** (bottom) incubated with HeLa cells for 3 h at 37 °C taken during the continuous irradiation at 470 nm (60x objective, power density 2.5 W cm^{-2}) and a detection range of 670-750 nm. Scale bar, 20 µM. NPs were added to a final concentration corresponding to 210 nM of the loaded dye salt.

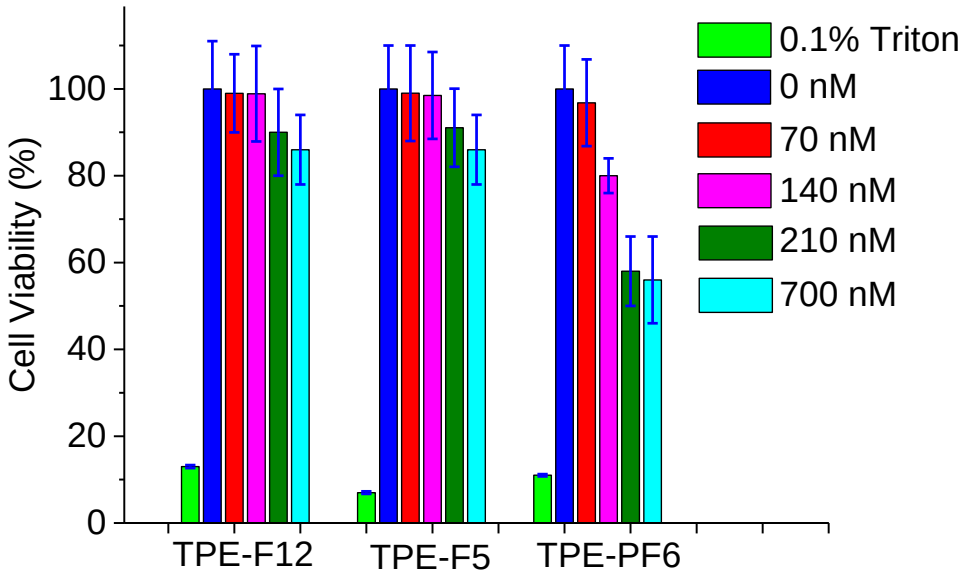

Fig. S9. Cytotoxicity of the TPE NPs in HeLa cells after 24 h incubation, using MTT assay. Final concentration of the loaded dye salt is presented. Triton X-100 (0.1%) was used as a positive control. The error is the standard deviation of the mean (n = 6).

Table S1. DLS data obtained for different AIEgens in water-acetonitrile mixtures.[a]

f_w (%)	DLS Measurements					
	TPE-F12		TPE-F5		TPE-PF6	
	Size (nm)	PDI	Size (nm)	PDI	Size (nm)	PDI
90	115±2	0.120	240±10	0.143	60-5000[b]	0.628
80	116±4	0.105	230±15	0.150	150-5000[b]	0.543
70	130±4	0.097	680±30	0.080	70-400[b]	0.780
60	300±10	0.081	370±30	0.230	130-4000[b]	0.300
50	185±5	0.078	200-5000[b]	0.165	185±10	0.090
30	160±5	0.137	30-5000[b]	0.300	100-4000[b]	0.300
10[c]	-	-	-	-	-	-

[a] The values are corrected for the viscosity of each solvent mixture. f_w (%) is water fraction in the mixtures. [b] Broad range of sizes indicates a largely polydisperse sample with different populations observed in this range. [c] The signal was poor indicating absence of aggregates.