Supporting information

Collective activation of MRI agents via encapsulation and disease-triggered release

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EXPERIMENTAL SECTION

Materials.

Gadolinium chloride hexahydrate ($GdCl_3.6H_2O$, Aldrich), sodium hydroxide (NaOH, Fisher Scientific), diethylene glycol (DEG, Aldrich), poly(lactic-co-glycolic acid) (PLGA , ratio: 50:50, Mw: 7-17 kDa, end group: alkyl ester, Aldrich), nitric acid (HNO_3, trace metal grade, Fisher Scientific), potassium dihydrogen phosphate (KH_2PO_4, Alfa Aesar), hydrogen peroxide (H_2O_2, 30%, Fisher Scientific), ethanol (EtOH, Fisher Scientific), chloroform ($CHCl_3$, EMD), phosphate buffered saline (PBS, 10X, pH 7.4, Cellgro) were used without further purification. Milli-Q water was used in the preparation of the aqueous solution of gadolinium oxide nanoparticles (Gd oxide NPs). The H_2O_2- and pH-sensitive polymers used in this study were synthesized according to procedures previously published.[1,2]

Synthesis of gadolinium oxide nanoparticles.

Ultra-small gadolinium oxide nanoparticles (Gd oxide NPs) were obtained by the polyol method published by Bridot *et al.* [3] Typically, 2.89 g of $GdCl_3.6H_2O$ was dissolved overnight in 50 mL of DEG at 60°C under vigorous stirring. Then, 3.75 mL NaOH solution (3 M) was added to the mixture and the temperature was raised to 140 °C for 1 h and 180 °C for 4 h. The colloid was purified by dialysis against ethanol to eliminate free Gd^{3+} ions and excess DEG: the colloidal solution (50 mL) was placed in a tubular membrane of cellulose (MWCO = 3.5 kDa, Spectrum) and immersed in 1 L of ethanol, which was replaced 5 times within 3 days. This long dialysis removed unreacted Gd^{3+} ions, which would hinder relaxivity measurements; since Gd^{3+} possesses a large magnetic moment due to its

seven unpaired electrons. The concentration of Gd was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after dissolution of the NPs in HNO_3. The size of the resulting Gd oxide NPs was determined by transmission electron microscopy (TEM, FEI Technai G^2 Sphera). All experiments were carried out using the same batch of Gd oxide NPs.

Encapsulation of gadolinium oxide nanoparticles in polymeric particles.

Polymer particles doped with Gd oxide NPs were obtained by electrospray. Briefly, 50 mg of polymer (PLGA, pH-sensitive polymer or H_2O_2-sensitive polymer) was dissolved in 0.4 mL of $CHCl_3$ and diluted with 50 µL of Gd oxide solution in EtOH ([Gd] = 6 mg/mL, as quantified by ICP-AES) and 50 µL of pure EtOH. The mixture was electrosprayed at 20 kV (Gamma High Voltage, ES30) at a flow rate of 0.5 mL/hr (KD Scientific) using a 25 gauge needle for 30 minutes. Samples were collected onto microscope glass slides on an aluminum plate collector at a distance of 20 cm. The particles (~2 mg/slide) were removed from their glass slide substrate by sonication in PBS, washed with PBS, and finally dispersed in 5 mL of PBS (~0.4 mg/mL). The same experimental procedure and electrospraying conditions were used to produce empty polymeric particles; the Gd-oxide solution in EtOH was replaced by pure EtOH. The size and morphology of the resulting doped polymeric particles were examined by scanning electron microscopy (SEM, Agilent 8500), TEM, and optical microscopy (Nikon, Eclipse). Size distributions were extracted from the images using *imageJ* software (NIH).

Measurement of magnetic relaxation.

For T_1-inversion recovery measurements, samples were transferred to NMR tubes and longitudinal relaxation times (T_1) were acquired with a contrast agent analyzer (Bruker Minispec mq60, 1.4 T, 37 °C). A fraction of the dialyzed Gd oxide NP suspension was diluted with PBS pH = 7.4 to obtain concentrations between 0 and 0.3 mM and T_1 values were measured. For PLGA particles doped with Gd oxide NPs, T_1 values reflecting the "OFF" state were obtained from various concentrations (0 - 0.4 mg/mL) of freshly prepared particles dispersed in PBS pH = 7.4. The same particle samples were then treated with a small amount of solid NaOH to bring their pH to 14 and allowed to dissolve at 37 °C for 24 hours. The pH of the samples was reverted to 7.4 using small amounts of solid KH_2PO_4 and their T_1 values were then acquired ("ON" state). The Gd concentration was quantified by linear calibration with standards containing previously determined amounts of Gd by ICP-AES and subjected to the same pH treatment. For the pH-dependent activation experiments, a suspension (200 µL, 0.4 mg/mL) of pH-sensitive poly-β-aminoester ketal-2 particles containing Gd oxide NPs was maintained at pH = 7.4 and a T_1 value was acquired every 5 min for 30 min ("OFF" state). After 30 min, 5 µL of a solution of KH_2PO_4 (1 M, pH = 4) was added to the particle suspension to lower the pH below 7.0 and trigger particle degradation; a T_1 value was acquired every 5 min for another 30 min ("ON" state). For the

ROS-dependent activation experiments, varying amounts of H_2O_2 (0 – 50 µmol) were added to a suspension of H_2O_2-responsive boronic ester protected polymer particles (500 µL, 0.4 mg/mL) in PBS pH = 7.4 and T_1 values were acquired after a 10 min equilibration time. For phantom imaging, 200 µL aliquots of composite particle suspensions in PBS (0.4 mg/mL) were placed in 700 µL microcentrifuge tubes in a 7T *Bruker Biospec* 70/20 MR scanner. FLASH scan parameters: T_R = 100 ms, T_E = 6 ms, number of excitations: 5. RARE VTR scan parameters: T_R = 1000 ms, T_E = 12.6 ms, number of excitations: 3. In order to address the diamagnetic contribution of the polymeric matrices to the observed spin-lattice relaxation, T1 relaxation values of empty particle suspensions were acquired before and after degradation. Degradation of the polymer particles were performed as previously described in this section. Interestingly, the three polymer matrices used in this study presented insignificant diamagnetic contributions as all T1 relaxation values matched that of PBS (Table S1).

Table S1. Diamagnetic contribution of polymer matrices to the T1 relaxation rate. Polymer concentration: 0.4 mg/mL. T = 37 °C. n = 3.

	PBS 1X		**PLGA**		**H_2O_2-sens. polymer**		**pH-sens. polymer**	
	T_1 (s)	***Error***	***T_1 (s)***	***Error***	***T_1 (s)***	***Error***	***T_1 (s)***	***Error***
Before degradation	3.49	0.06	3.48	0.08	3.50	0.01	3.50	0.04
After degradation	-	-	3.5	0.2	3.50	0.07	3.44	0.09

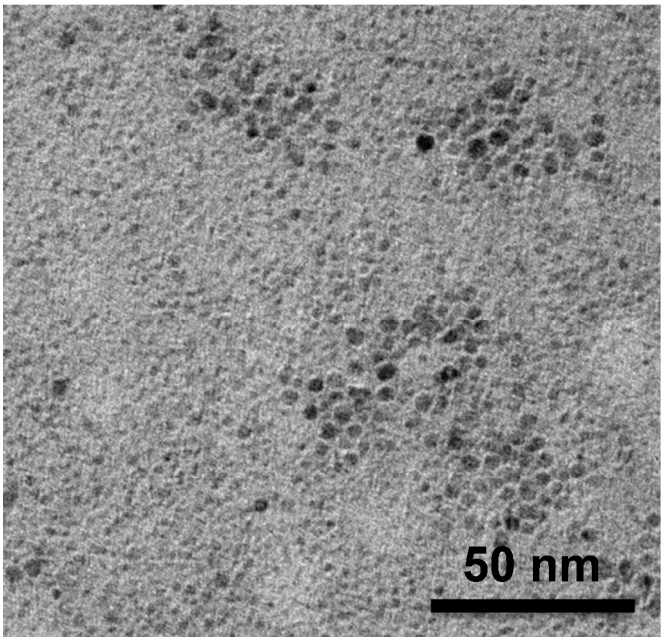


Figure S1. TEM photograph of the synthesized Gd oxide nanoparticles.

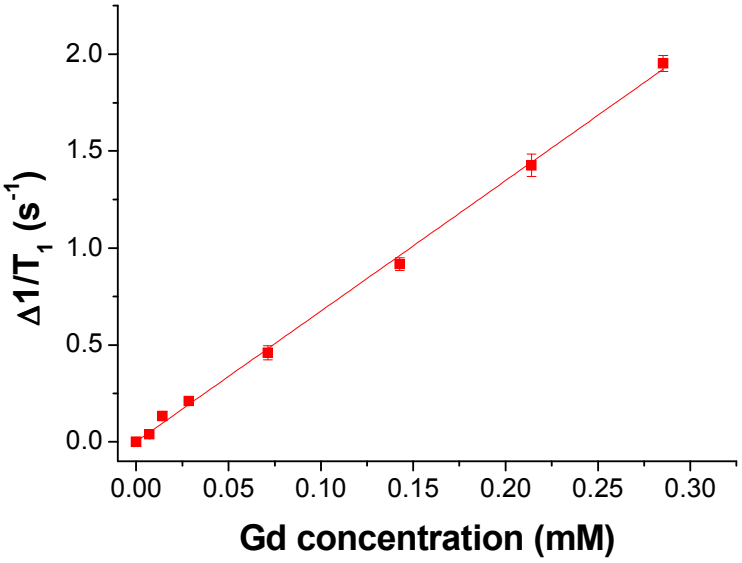
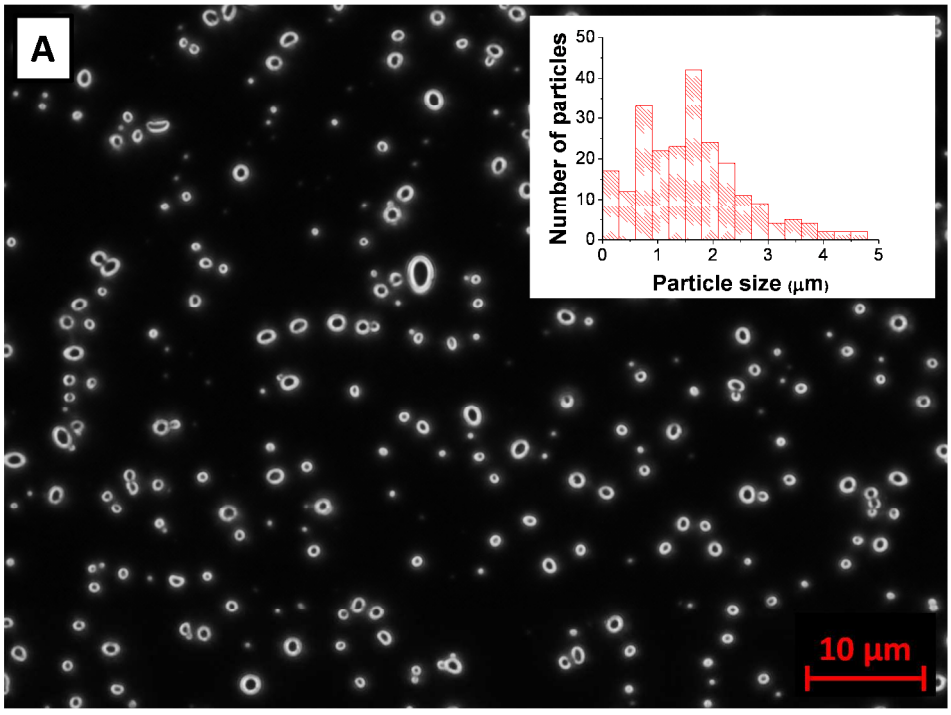


Figure S2. ^{1}H relaxation rates of varying concentrations of DEG-coated Gd oxide NPs in PBS. Relaxivity: 6.7 $s^{-1}.mM^{-1}$. n = 4.



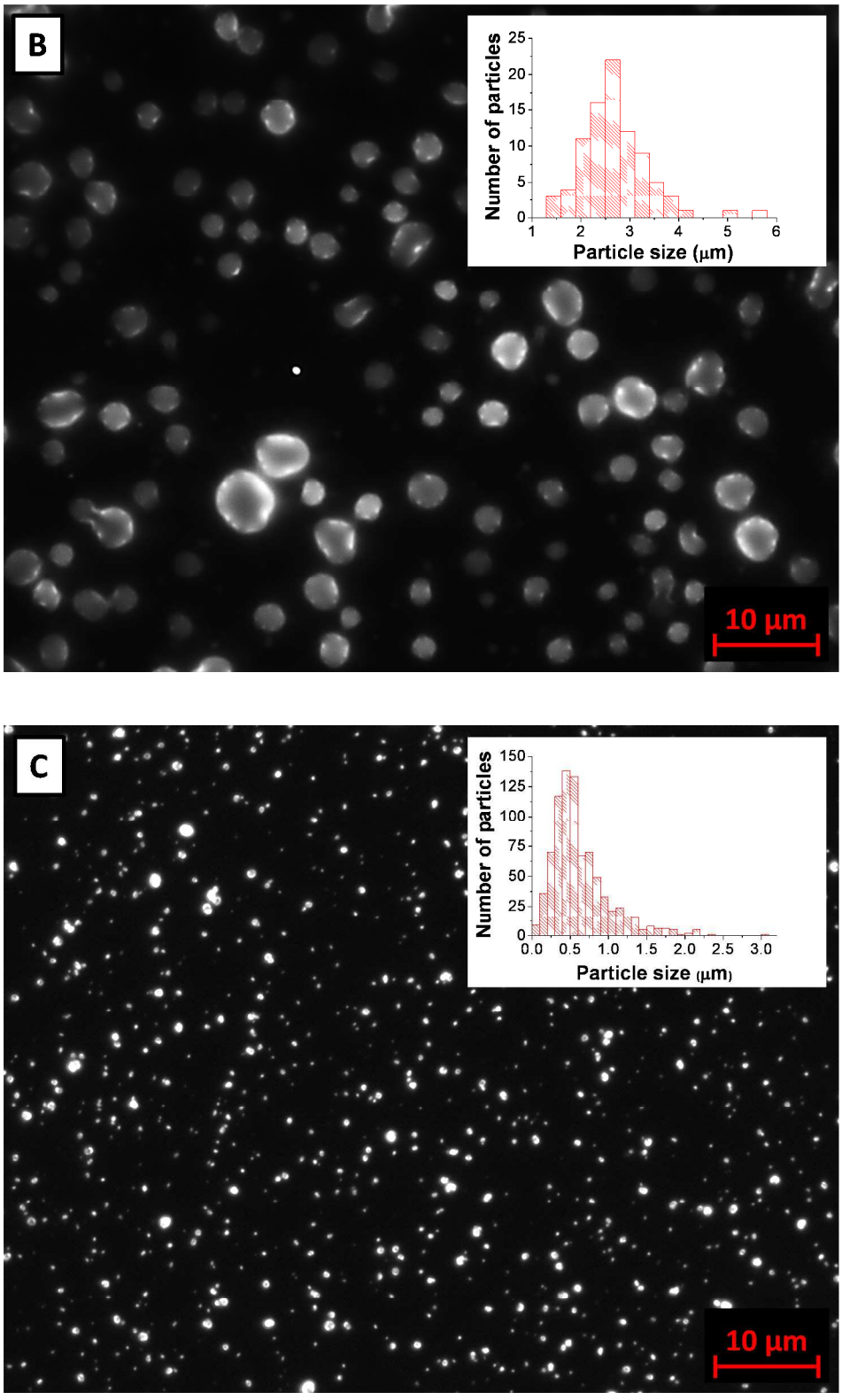


Figure S3. Dark field microscopic images and corresponding size distribution histograms of electrosprayed (A) PLGA particles, (B) pH-responsive polymer particles, and (C) H_2O_2-responsive polymer particles, all encapsulating Gd oxide NPs.

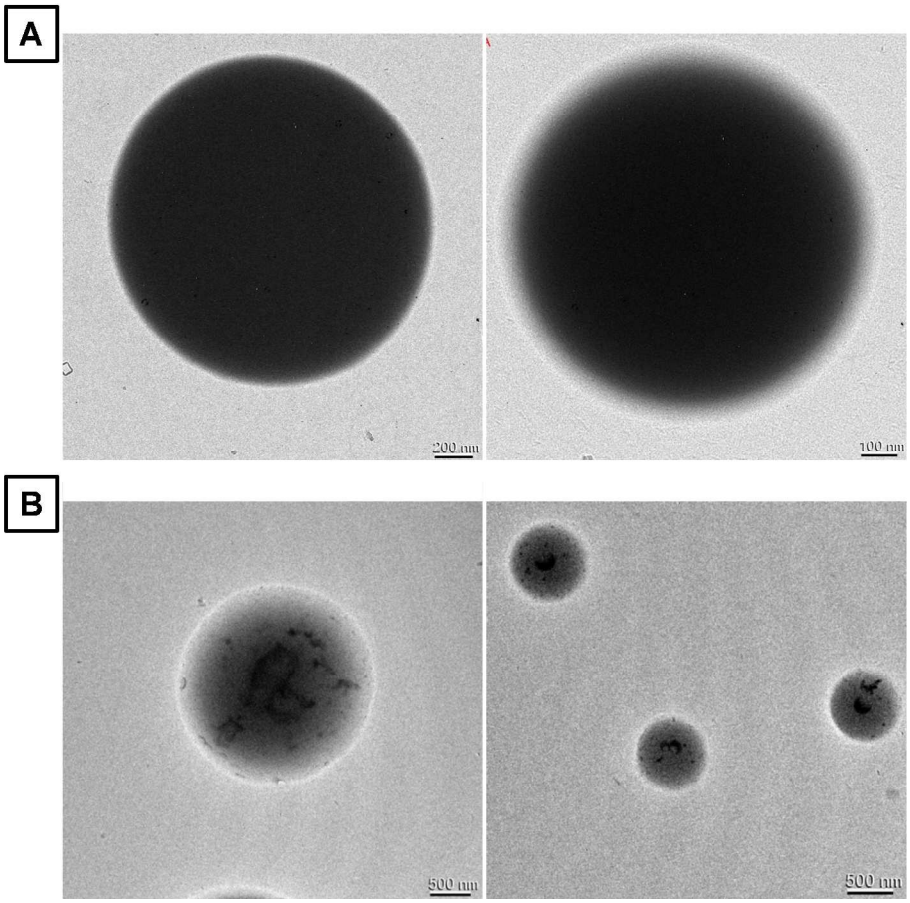


Figure S4. TEM photographs of (A) empty PLGA particles and (B) Gd oxide NP-loaded PLGA particles.

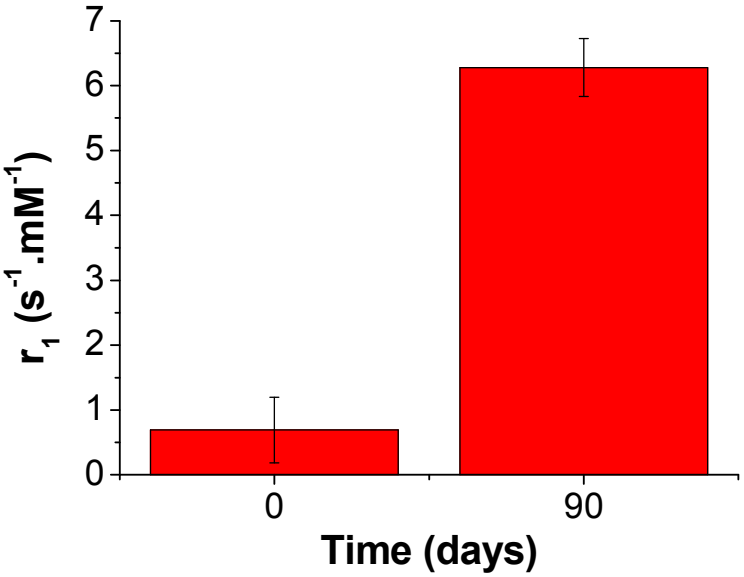


Figure S5. Relaxivity (r_1) of DEG-coated Gd oxide NPs encapsulated in PLGA particles before (t = 0) and after (t = 90 days) incubation in PBS pH = 7.4 at 37 °C. $r_{1,\ activated\ (t\ =\ 90\ days)}/r_{1,\ silenced\ (t\ =\ 0\ days)}$ = 9.1. n = 3.

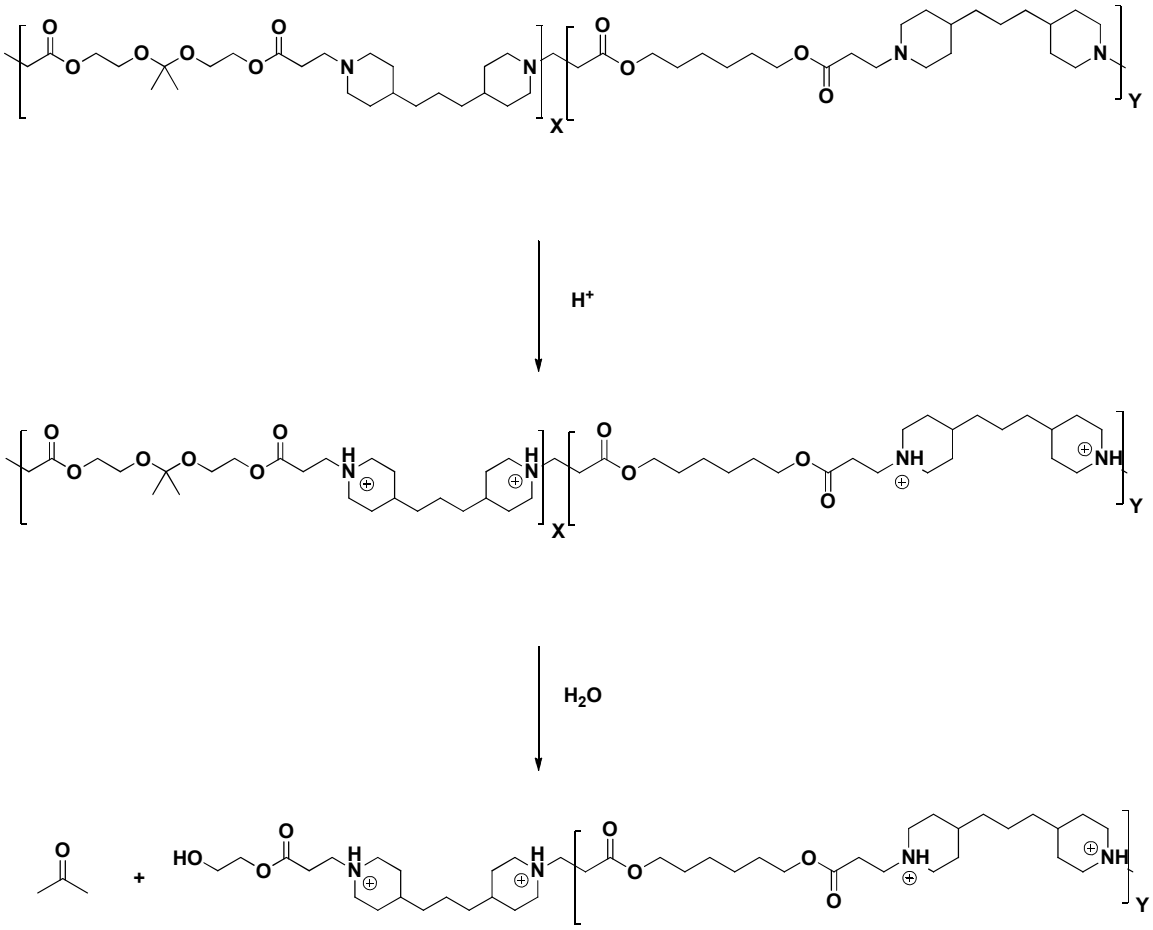


Figure S6. Mechanism of acid pH-triggered degradation of the poly-β-aminoester ketal polymer.[1]

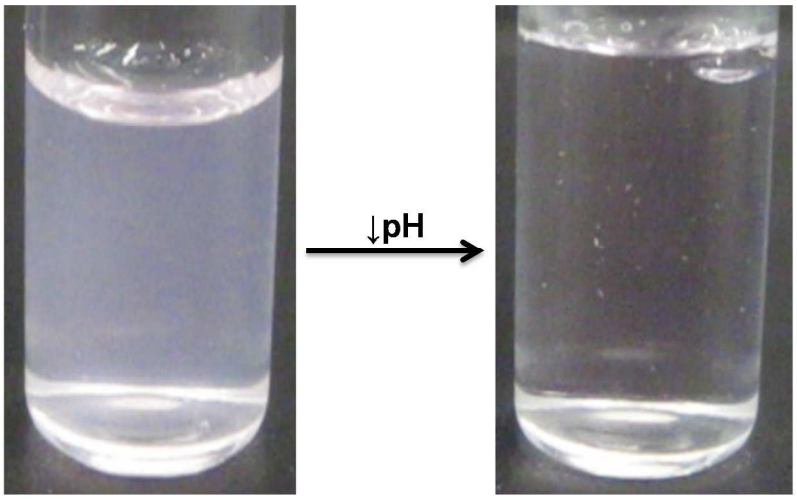


Figure S7. Lowering the pH of the aqueous particle suspension leads to an instantaneous burst degradation of the poly-β-aminoester ketal polymer particles and, hence, fast release of the encapsulated cargo. Photo taken 30 sec after addition of KH_2PO_4 solution to lower the solution pH to 6.8.

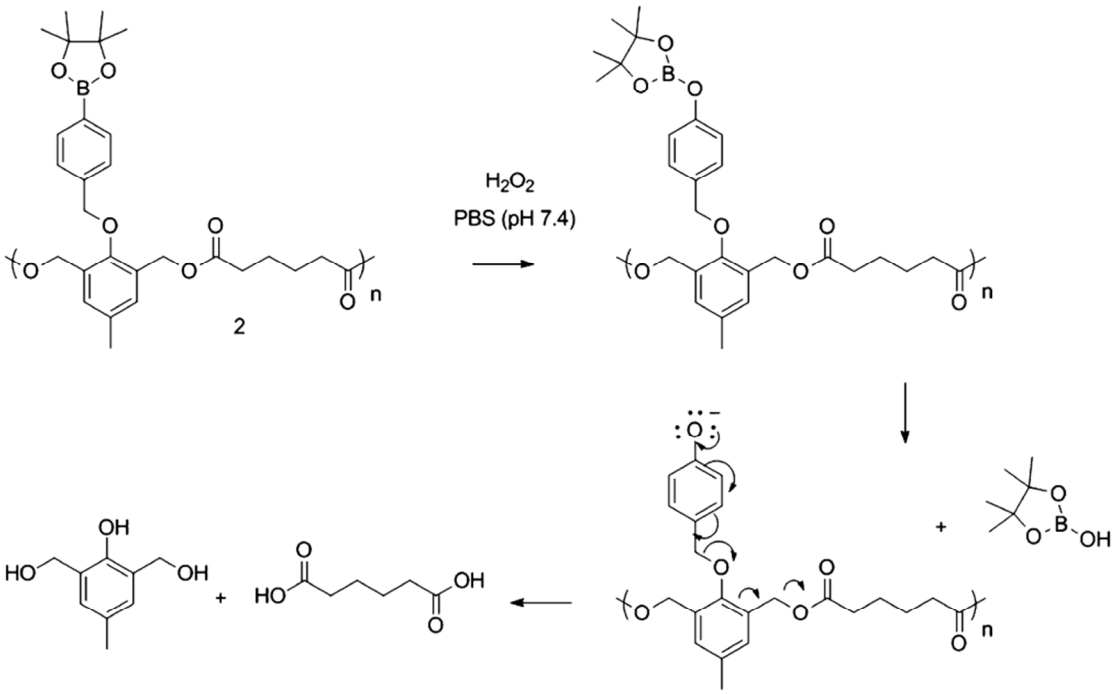


Figure S8. Mechanism of degradation of the aryl boronic ester protected polyester polymer upon exposure to hydrogen peroxide.[2]

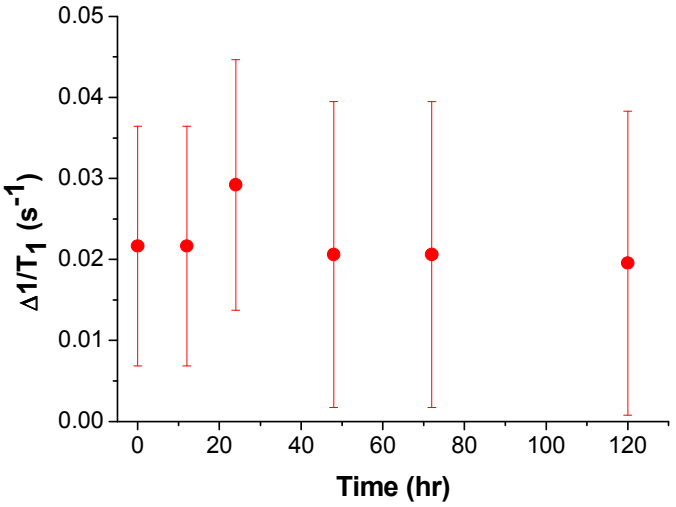


Figure S9. Longitudinal relaxation rates ($\Delta 1/T_1$) of Gd oxide NPs encapsulated in H_2O_2-responsive polymer particles before and after varying incubation times at pH=7.4 in the absence of H_2O_2.

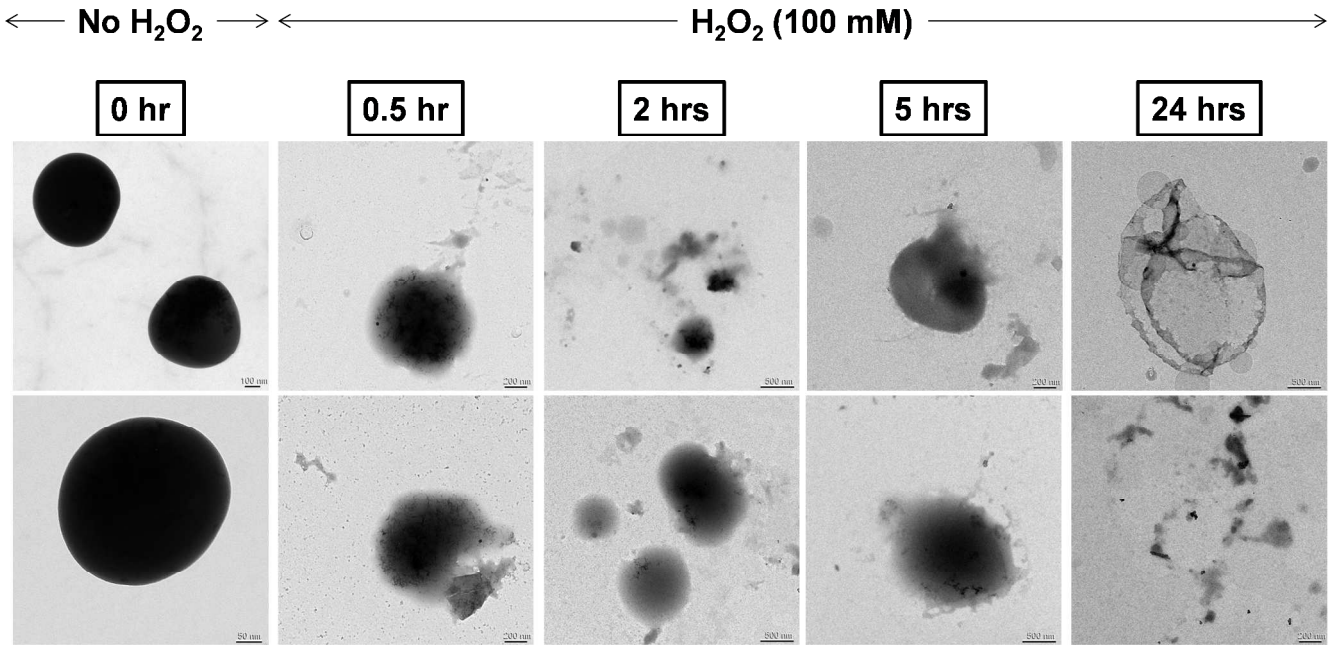


Figure S10. TEM images of H_2O_2-degradable polymer particles encapsulating Gd oxide NPs taken before (0 hr) and after addition of H_2O_2 (100 mM) at varying incubation times (0.5, 2, 5, and 24 hrs) at 37 °C.

REFERENCES

(1) Sankaranarayanan, J.; Mahmoud, E. A.; Kim, G.; Morachis, J. M.; Almutairi, A. *ACS Nano* **2010**, *4*, 5930.

(2) De Gracia-Lux, C.; Joshi-Barr, S.; Nguyen, T.; Mahmoud, E.; Schopf, E.; Fomina, N.; Almutairi, A. *J Am Chem Soc* **2012**, *134*, 15758.

(3) Bridot, J. L.; Faure, A. C.; Laurent, S.; Riviere, C.; Billotey, C.; Hiba, B.; Janier, M.; Josserand, V.; Coll, J. L.; Vander Elst, L.; Muller, R.; Roux, S.; Perriat, P.; Tillement, O. *J Am Chem Soc* **2007**, *129*, 5076.