Supporting Information

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Fig. S1. Agarose gel electrophoresis shift assays that allow observation of **Gd3a**, **Gd3b**, **Eu3a**, and **Eu3b** binding with pDNA at increasing N/P ratios from 0 to 40. Hindrance of pDNA migration is noted with all polymers at N/P = 5; however, migration is not completely hindered until N/P = 30 for **Eu3a** and **Gd3a** and N/P = 20 for **Gd3b** and **Eu3b**. Nonchelated polymers **3a** and **3b** do not hinder to pDNA migration in agarose gel and, thus, do not bind pDNA.



Fig. 52. The ability of each polymer beacon to protect pDNA from nuclease degradation. Polyplexes are exposed to FBS for the indicated incubation times: C (control, no FBS or SDS), 0, 1, 2, 4, and 6 h, as denoted at the bottom of the gels. Samples are then exposed to SDS to release the polymer from the pDNA and electrophoresed in an agarose gel to observe pDNA integrity. Control sample gels are also shown as FBS (FBS gel, lane 1) FBS + SDS (FBS gel lanes 2–6), naked pDNA only without the addition of FBS or SDS (pDNA gel, lane 7), and naked pDNA with the addition of FBS and SDS (pDNA gel, lanes 8–12). Other controls samples of polyplex only without the addition of FBS or SDS formed with each polymer beacon are labeled as C for control in the incubation time (**Gd3a** gel, lane 1; **Gd3b** gel, lane 7; **Eu3a** gel, lane 1; **Eu3b** gel, lane 7). It should also be noted here, that band 1 is the position of the sample loading (and also shows the position of polyplexes without FBS and SDS treatment). Band 2 is intact pDNA. Band 3 results from combining both FBS and SDS. Band 4 reveals the degraded pDNA (observed in the pDNA gel lanes 9–12). As shown, the polymer beacons protect pDNA from degradation when exposed to FBS as band 4 is not observed (**Gd3a** gel, lanes 2–6; **Gd3b** gel, lanes 8–12; **Eu3a** gel, lanes 2–6; **Eu3b** gel, lanes 8–12).



Fig. S3. Cell viability after exposure to polyplexes using unlabeled pDNA. Viability is reported as a measure of the MTT conversion normalized to untreated cells. The N/P ratio of the polyplex used is indicated after the polymer name on the x axis. Polyplexes were formed using the same methodology as the DLS studies.

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Luciferase Expression **10**¹¹ 10¹⁰ Relative Light Units / mg Protein 10⁹ 10⁸ 107 **10**⁶ **10**⁵ 104 **10**³ 10² 10 0 Gd3br60 Gd3b-AD EU3DIGO Gd3aAD 6433-60 Cells Only PONA PELS 64-20 EU38-40 EU38-60 EU30-40

Fig. S4. Luciferase Expression in HeLa cells. Expression is reported as relative light units emitted by the catalyzed transformation of luciferin per milligram of protein. The N/P ratio of the polyplex used is indicated after the polymer name on the *x* axis. Polyplexes were formed using the same methodology as the DLS studies.

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Fig. 55. Two-photon confocal image of FITC-labeled pDNA delivered with **Eu3a** at an N/P of 20. Infrared red laser was tuned to 780 nm to initiate Eu(III) excitation. Slice thickness 1.2 μ m, 1.2 μ m pinhole, 63× oil-immersion objective, numerical aperture 1.4. (Scale bar, 20 μ m.) (A) FITC-pDNA fluorescence, (B) **Eu3a** luminescence, (C) FITC/**Eu3a** overlay, (D) FITC/Eu/DIC overlay. This image indicates that diffuse cytoplasm staining of **Eu3a** is similar at lower N/P ratios.

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^aConditions: (i) DMSO, 25°C, 24h; (ii) TFA, CH_2CI_2 ; (iii) [LnCI₃] = 0.1 M, H_2O , NaHCO₃ pH = 6.

Scheme S1. Polymer synthetic scheme.

Table S1. FT-IR data for the polymers

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Polymer	υ, cm ⁻¹	
2a	1,663.3; 1,712.3; 3,334.2	
2b	1,666.3; 1,692.9; 3,346.3	
3a	1,641.2; 1,692.9; 3,415.2	
3b	1,650.8; 1,667.8; 3,419.2	
Eu3a	1,585.4; 1,634.5; 3,419.4	
Eu3b	1,585.2; 1,633.8; 3,419.9	
Gd3a	1,587.2; 1,641.2; 3,433.7	
Gd3a	1,586.1; 1,633.9; 3,419.6	

Samples of each polymer (5 mg) were crushed by mortar and pestle with 20 mg of anhydrous KBr and compacted into a translucent pellet. Spectra were measured on a Perkin–Elmer Spectrum One Fourier transform infrared spectrometer.

Table S2. Ln quantification for the polymer series

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Polymer	Calculated Ln content, %	Observed Ln content, %
Eu3a	21.8	21.8
Eu3b	20.5	20.4
Gd3a	22.5	22.8
Gd3a	21.1	21.9

The percentage of Ln by mass was determined by diluting polymer samples to the ppb range and analyzed by inductively coupled plasma MS. For each polymer, the signal integration was fitted to calibration curves generated from Ln standards.

Table S3. The weight averaged molecular weight ($M_{\rm w}$), polydispersity ($M_{\rm w}/M_n$), and degree of polymerization ($n_{\rm w}$) for the polymers

Polymer	M _w , kDa	M _w /M _n	n _w
За	43	1.7	78
3b	54	1.9	91
Eu3a	64	1.7	91
Eu3b	68	1.7	92
Gd3a	67	1.9	96
Gd3a	62	2.0	89

Other Supporting Information Files

SI Appendix (PDF)

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