Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for:

Polydopamine-decorated Tobacco Mosaic Virus for Photoacoustic/Magnetic Resonance Bimodal Imaging and Photothermal Cancer Therapy

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Methods:

Native and denaturing gel electrophoresis: Intact native and modified TMV (10 μg per lane) were analyzed by 1% (w/v) agarose native gel electrophoresis in 0.1 M Tris-maleate running buffer (pH 6.5). Denatured protein subunits (10 μg per lane) were analyzed by polyacrylamide gel electrophoresis using 4–12% NuPAGE gels in 1x MOPS buffer (Invitrogen). Samples were denatured by boiling in SDS loading dye for 10 min. Gels were photographed under UV or white light before staining with Coomassie Brilliant Blue, and under white light after staining, using an AlphaImager system (Protein Simple, San Jose, CA, USA).

Trypan blue assay: We seeded 1×10^5 PC-3 and 4T1 cells (ATCC) into 24-well plates and cultivated them overnight in RPMI-1640 medium (Corning Life Sciences, New York, NY, USA) containing 10% (v/v) fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA, USA) and 1% (w/v) penicillin–streptomycin (Thermo Fisher Scientific). The plates were assigned to one of four groups. The experimental group was PC-3 and 4T1 cells incubated with Gd-TMV-PDA (500 μg/mL) 6 h at 37°C, washed three times with PBS to remove excess particles and then irradiated with the 808-nm laser for 10 min. The three control groups were (i) PC-3 and 4T1 cells untreated, (ii) PC-3 and 4T1 cells incubated with Gd-TMV-PDA (500 μg/mL) without irradiation, and (iii) PC-3 and 4T1 cells irradiated (1 W/cm^2) in the absence of Gd-TMV-PDA. Following treatment, all cells were incubated at 37°C for 2 h, stained with trypan blue for 15 min and observed under an IX71 optical microscope (Olympus, Tokyo, Japan).

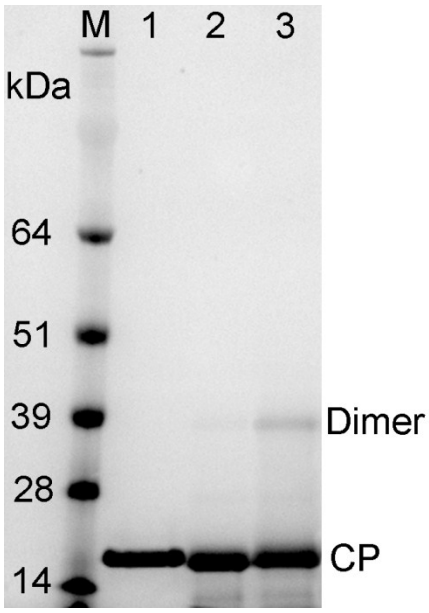

Figure S1. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) for the analysis of (1) native TMV, (2) Gd-TMV, (3) Gd-TMV-PDA; CP = coat protein.

Table S1. The longitudinal relaxivities of Gd-conjugated nanoparticles described in the literature compared to our new Gd-TMV-PDA formulation.

Compounds	size (nm)	r_1 ($mM^{-1}s^{-1}$)	field (MHz)	ref.
Gd(DOTA)-TMV-PDA	18x300	15.02/79.85	300/60	This work
Gd(DTPA)-MS2	27.4	4.3/16.9	400/60	**[1]**
Gd(DOTA)-AaLS	15	16.49/30.24	300/60	**[2]**
Gd(DOTA)-TMV	18x300	14.6	60	**[3]**
Gd(DOTA)-CPMV	28	11.9-15.5	64	**[4]**
Gd(DTPA)-P22	64	21.7	28	**[5]**
GdAAZTA-Dendrimer	25 kDa	31.4	60	**[6]**
Gd(DOTAGA)-MSN	30	28/37	60/20	**[7]**
Gd(DOTAGA)-MSN	25-220	20.3-79.1	20	**[8, 9]**
Gd(DOTA)-Dendrimer	142	22.4	60	**[10]**
Gd(DOTA)-PLGA	150 -170	17.5	60	**[11]**
Gd(DOTA)-PLGA	140	21.7	21.5	**[12]**
Gd(DTPA)-PLNP	109	6.72	50	**[13]**
Gd(HPDO3A)-PN		17	60	**[14]**
Gd(DTPA)DNA-Au	30	20	60	**[15]**
Gd(DOTA)-MSN	20-50	26.6	20	**[8]**
Gd(DTPA)-MSNR	107-535	22	20	**[16]**

AaLS: *Aquifex aeolicus*, AAZTA: 6-amino-6-methylperhydro-1,4-diazepine tetraacetic acid, TMV: Tobacco mosaic virus, CPMV: Cowpea mosaic virus, PLNP: persistent luminescent nanoparticles; PN: peptide nanofiber; MSN: mesoporous silica nanoparticles; PLGA: poly(D,L-lactide-co-glycolide). The molecular structures of the Gd-complex are shown in Figure S2.

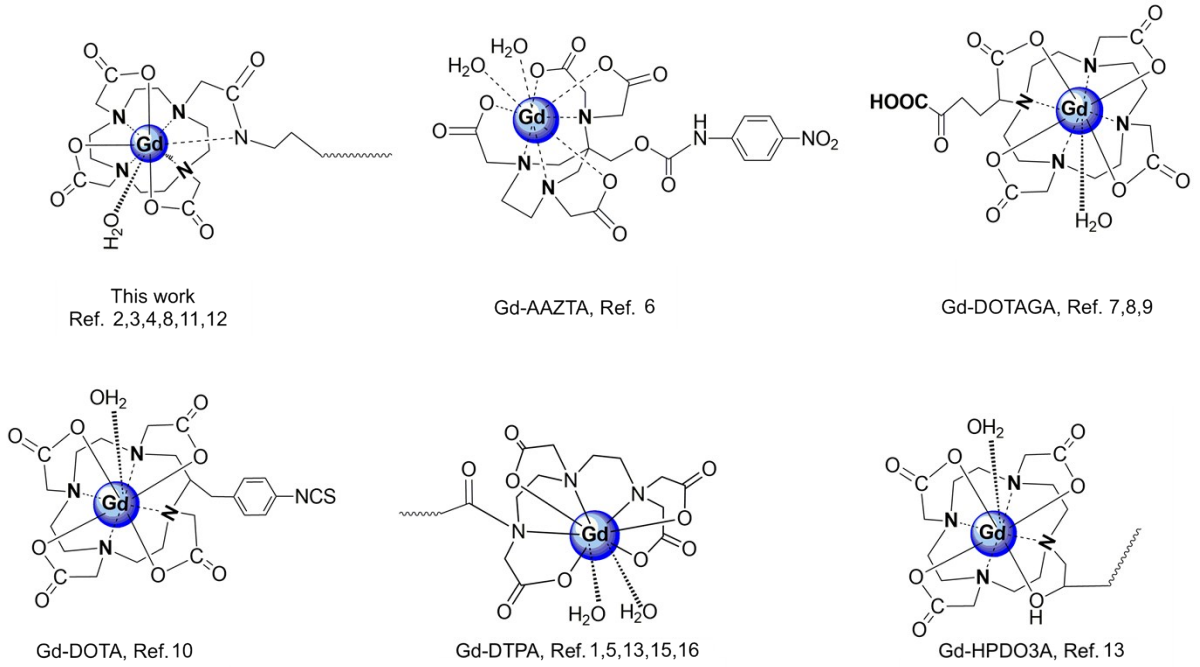

Figure S2. The molecular structure of the macrocyclic Gd complex upon which we based our MRI agent.

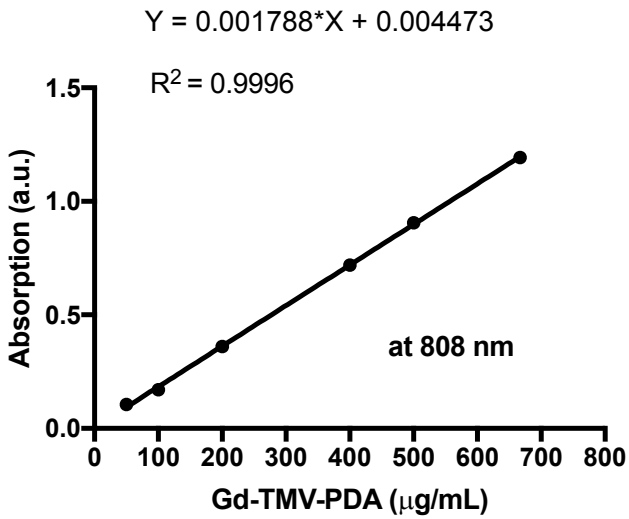

Figure S3. The absorption at 808 nm of different concentrations of Gd-TMV-PDA in PBS buffer pH 7.4

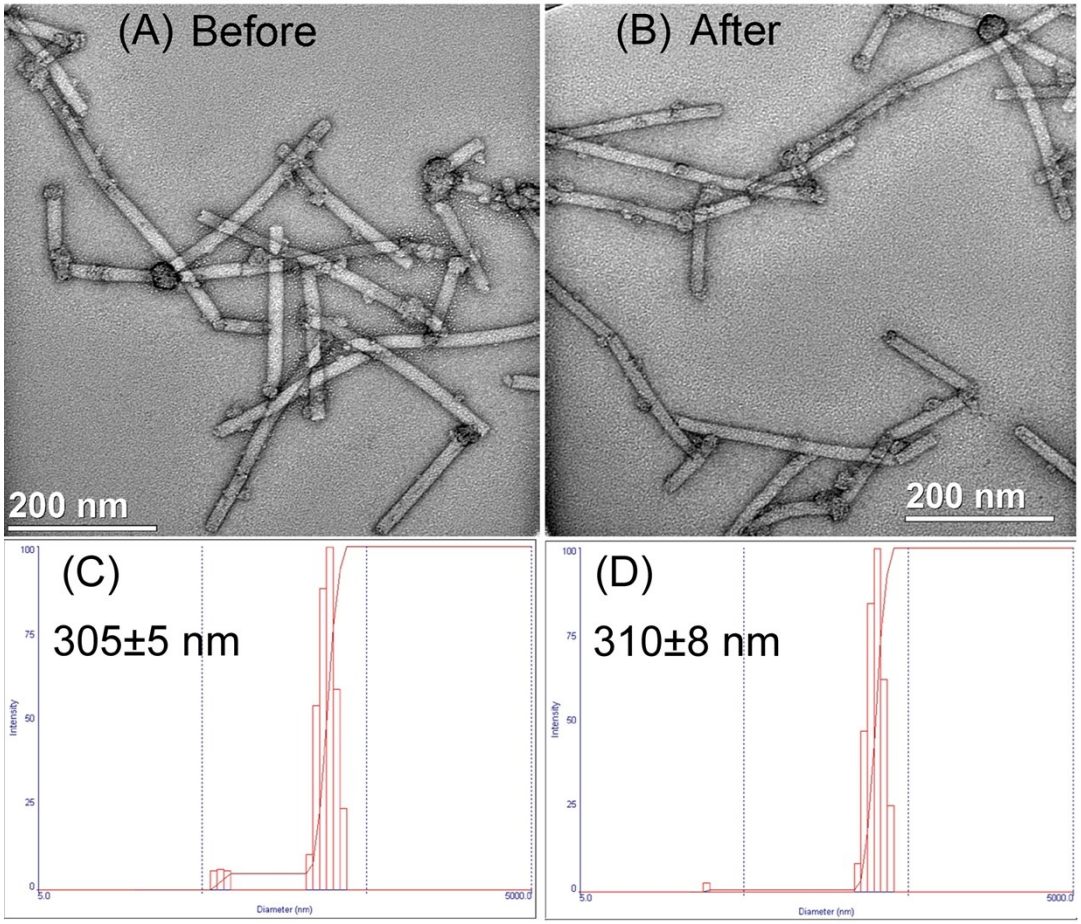

Figure S4. Transmission electron micrographs of Gd-TMA-PDA (A) before and (B) after 808 nm laser (1W/cm^2) irradiation for 10 min, and the corresponding dynamic light scattering (DLS) of the sample samples (C) before and (D) after irradiation. There were no obvious changes in the morphology and size of the Gd-TMA-PDA particles (A, C) before and (B, D) after 808-nm laser irradiation for 10 min at a power density of 1 W/cm^2 confirming the photothermal stability of the particles.

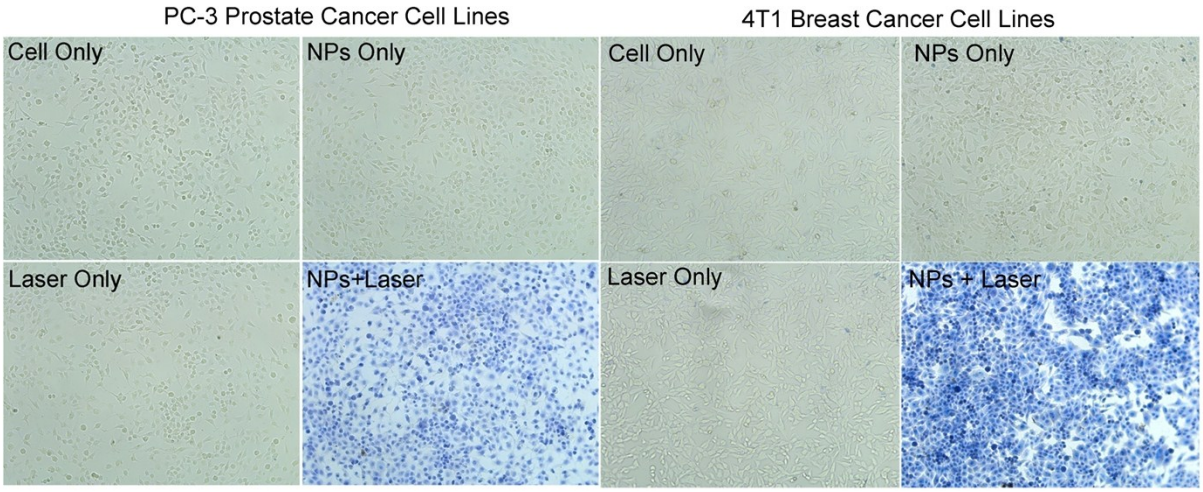

Figure S5. Trypan blue staining assay of PC-3 and 4T1 cancer cell treated with Gd-TMV-PDA. The experimental group (NPs + laser) was PC-3 and 4T1 cells incubated with Gd-TMV-PDA (500 μg/mL) 6 h at 37°C, washed three times with PBS to remove excess particles and then irradiated with the 808-nm laser for 10 min. Nearly all these cells stained blue, indicating the ablation of cancer cells by the photothermal effect of Gd-TMV-PDA. The three control groups were PC-3 and 4T1 cells untreated (cells only), PC-3 and 4T1 cells incubated with Gd-TMV-PDA (500 μg/mL) without irradiation (NPs only), PC-3 and 4T1 cells irradiated (1 W/cm^2) in the absence of Gd-TMV-PDA (laser only). Most of the control groups cells remained viable and did not take up the blue stain, therefore indicating neither the particle or laser did induce the death of cell lines. Both of the cell lines found significance death after incubated with Gd-TMV-PDA under laser irradiation that means the photothermal effect of the Gd-TMV-PDA particles killed the cell efficiently.

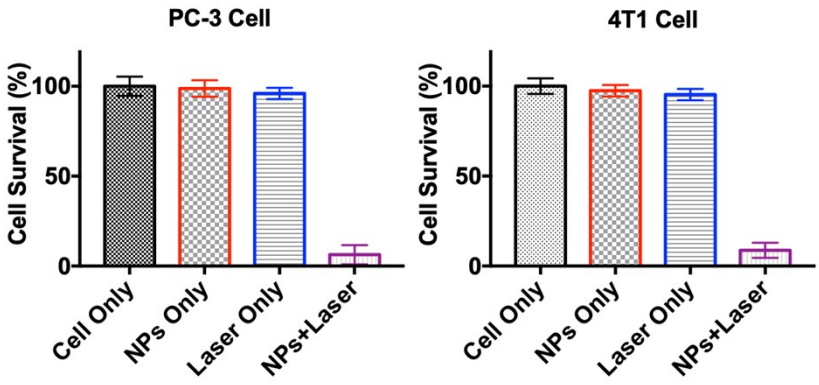

Figure S6. MTT cytotoxicity assay of PC-3 and 4T1 cells untreated (cells only), incubated with Gd-TMV-PDA (500 μg/mL) without irradiation (NPs only), irradiated under 808 nm laser (1 W/cm^2) without Gd-TMV-PDA (laser Only), and irradiated under 808 nm laser (1 W/cm^2) with incubated with Gd-TMV-PDA (NPs + laser, experimental group) 6 hours at 37^{o}C.

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