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Two-Photon degradable supramolecular assemblies of linear-dendritic copolymers

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General Procedures and Materials. Unless otherwise noted, all chemicals were obtained from Aldrich Chemical Co. and used without further purification. 2-Diazo-1,2-naphthoquinone-5-sulfonyl chloride was supplied generously by Tokyo Ohka Kogyo Co., Ltd. and was stored in the dark. ^{1}H NMR spectra were recorded at 400 MHz. NMR chemical shifts are reported in ppm and calibrated against $CDCl_3$ (δ 7.26, 77.00) and all coupling constants are reported in Hz. Absorption spectra were recorded on a Cary 50 UV-Vis spectrometer in 10 mM aqueous buffer at pH 7.4. Fluorescence emission and excitation spectra were obtained using an ISA/SPEX Fluorolog 3.22 equipped with a 450 W Xe lamp, double excitation and double emission monochromators and a digital photon-counting photomultiplier. Slit widths were set to 5.0 nm band-pass on both excitation and emission monochromators. Correction for variations in lamp intensity over time and wavelength was achieved using a reference silicon photodiode. The spectra were further corrected for variations in photomultiplier response over wavelength

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and for the path difference between the sample and the reference by multiplication with emission and excitation curves generated on the instrument. Photoreactions were performed in a Rayonet photoreactor with two RPR-3500 bulbs (35 W) from the Southern New England Ultra Violet Company (average $\lambda \sim 350$ nm). Analytical size exclusion chromatography (SEC) in N,N-dimethylformamide (DMF) HPLC grade with 0.2% wt LiBr was performed at 70 °C at a nominal flow rate of 1.0 mL/min on a chromatography line calibrated with PEO standards and fitted with two 7.5 x 300 mm PSS SDV linear (5-μm paticle size). The SEC system used when determining PEO-equivalent molecular weights consists of a Waters 510 pump, a Waters U6K injector, and a RI Waters 410.

PEO-10K-[G4]-Polyester-OH (3) was synthesized via a literature procedure and used without further purification.[3a]

PEO-10K-[G4]-Polyester-DNQ (4) The flask was placed under yellow light. 2-Diazo-1,2-naphthoquinone-5-sulfonyl chloride (38 mg, 0.142 mmol), compound **2** (100 mg, 8.33 μmol), and 1, 4-diazabicyclo[2.2.2]octane (16 mg, 0.142 mmol) were dissolved in 1 mL of dichloromethane. The reaction mixture was stirred in the dark overnight. The reaction was concentrated *in vacuo* in the dark and then redissolved into 5 mL of water. This solution was transferred to a regenerated cellulose dialysis membrane with a molecular weight cut off of 3,500 Da. The solution was dialyzed in the dark overnight. The solution was freeze-dried to afford a powder. (109 mg, 85% yield). ^{1}H NMR

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($CDCl_3$): δ 1.19 (br, 45H), 3.64 (br, 963H), 4.25 (br, 43H), 7.00 (br, 16H), 7.55 (br, 18H), 8.30 (br, 14H), 8.62 (br, 16H); DMF SEC MALLS: M_w= 15,797 Da PDI = 1.15

Critical Micelle Concentration Studies. The following procedure was performed under yellow light. 24 μL of a 0.05 mg/mL stock solution of Nile Red in dichloromethane was added to an empty vial, which was then placed under vacuum for at least two hours to ensure complete solvent removal. To this vial was added a 4 mg/mL stock solution of **4** in 10 mM aqueous phosphate buffer at pH 7.4. More buffer was then added to bring the total solution volume to 4 mL and the solution to the desired concentration. This solution was shaken vigorously and then allowed to equilibrate at room temperature for at least six hours. Fluorescence measurements were taken at an excitation wavelength of 550 nm and the emission monitored from 560 to 700 nm.

The emission at 642 nm (λ_{max}) was plotted against the log of concentration and the linear fit (R^2 = 0.996) defined by the greatest slope was extrapolated to intersected with the baseline defined by the lowest concentration. The x-value at this intersection was used as the critical micelle concentration, which by this method was found to be 12 mg/mL.

One-Photon Kinetic Studies. The following procedure was performed under yellow or minimal light. Nile Red-containing solutions of **4** in 10 mM aqueous phosphate buffer at pH 7.4 were prepared as above. The solution was then transferred to a 1 x 1 x 3 cm quartz fluorescence cuvette. UV-Vis absorbance spectrum from 200 to 600 nm was taken, followed by a fluorescence measurement as described above. The cuvette was then transferred to a Rayonet photoreactor and irradiated for 10 s. The cuvette was

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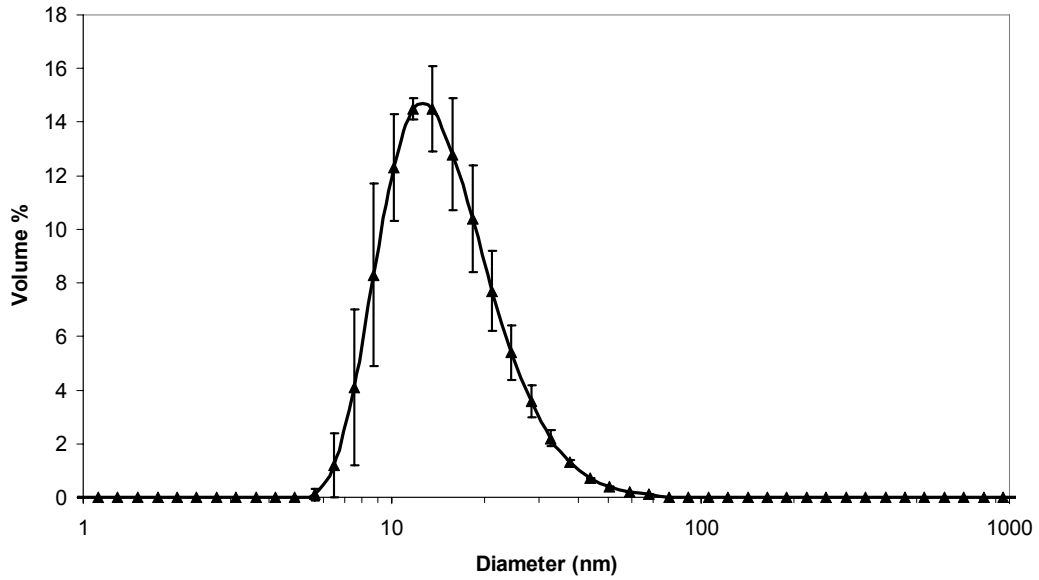
removed from the Rayonet, and absorbance and fluorescence measurements were taken. The sample was then further irradiated for a cumulative total of 20 s, and the measurements retaken, etc. A sample irradiated 640 s was used as a representative sample of the completely reacted photoproduct, and these absorbance measurements were subtracted from the experimental data to calculate the total change in absorbance, which was converted to percent relative to the initial spectra to determine reaction progress.

Two-Photon Kinetic Studies. Nile Red-containing solutions of **4** in 10 mM aqueous phosphate buffer at pH 7.4 were prepared as above. 70 μL of the solution was then transferred to a 1 x 1 x 3 cm reduced volume fluorescence cuvette with an internal sample chamber of .1 x .1 x 2 cm (manufactured by Hellma). Two-photon absorption experiments were performed using a 250 kHz regenerative Ti:sapphire amplifier, producing typically ~50 fs pulses at 795 nm. The laser beam was focused to a spot size with a diameter of ~80 μm at the sample position, and an average power of 0.86 W was used to illuminate the sample, which was reduced to 0.77 W after passing through the sample cell. After 15 min, the cuvette was removed from the beam and fluorescence measurements were taken as before. These steps were repeated to give the specified time points shown in the text.

Dynamic Light Scattering. All Dynamic Light Scattering measurements were performed on a Malvern Instruments Zetasizer Nano ZS. Size measurements were performed three times on the same sample to ensure consistency.



Supporting Figure 1. Compound **4** DLS results.

Cytotoxicity testing. To compare the *in vitro* cytotoxicity of the linear (**1**) and dendrimer DNQ systems (**4**), the relative proliferation of HEK293T cells was determined after incubation with each compound at varying concentrations. To avoid photoreaction of DNQ, all handling of solutions containing the tested compounds was performed under a red-filtered light. HEK293T cells (passage 19) were added to the wells of a 96-well microtiter plate at a density of 1 x 10^4 cells/well (100 μL/well). After overnight incubation at 37 ^oC/5% CO_2, 100 μL of fresh medium was added to the existing medium in each well to further prevent non-specific binding of the compounds tested to the wells. After 4 h of incubation, the medium was aspirated from each well and dilutions of each DNQ-containing compound were prepared in fresh medium consisting of Dulbecco's Modified Eagle Medium (Gibco) with 10% fetal bovine serum (HyClone) and 100 units/100 μg of penicillin/streptomycin (Gibco). Eight replicates of each dilution were added to the plate at 100 μL/well. After 24 h of incubation, the medium was aspirated

and each well was washed 5 x 150 μL with fresh medium. Following the last wash, the medium was aspirated, 100 μL of fresh medium was added to each well, and the cells were incubated for 22 h. The relative cell count was determined using an MTS-based assay (Promega, CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay). The medium was aspirated and 120 μL of assay solution (consisting of 100 μL of fresh medium and 20 μL of MTS solution) was added to each well. After 3.5 h of incubation the absorbance in each well was read at 490 nm using a microplate reader (SpectraMax M2, Molecular Devices). The reported results represent the average within one standard deviation of eight replicates.

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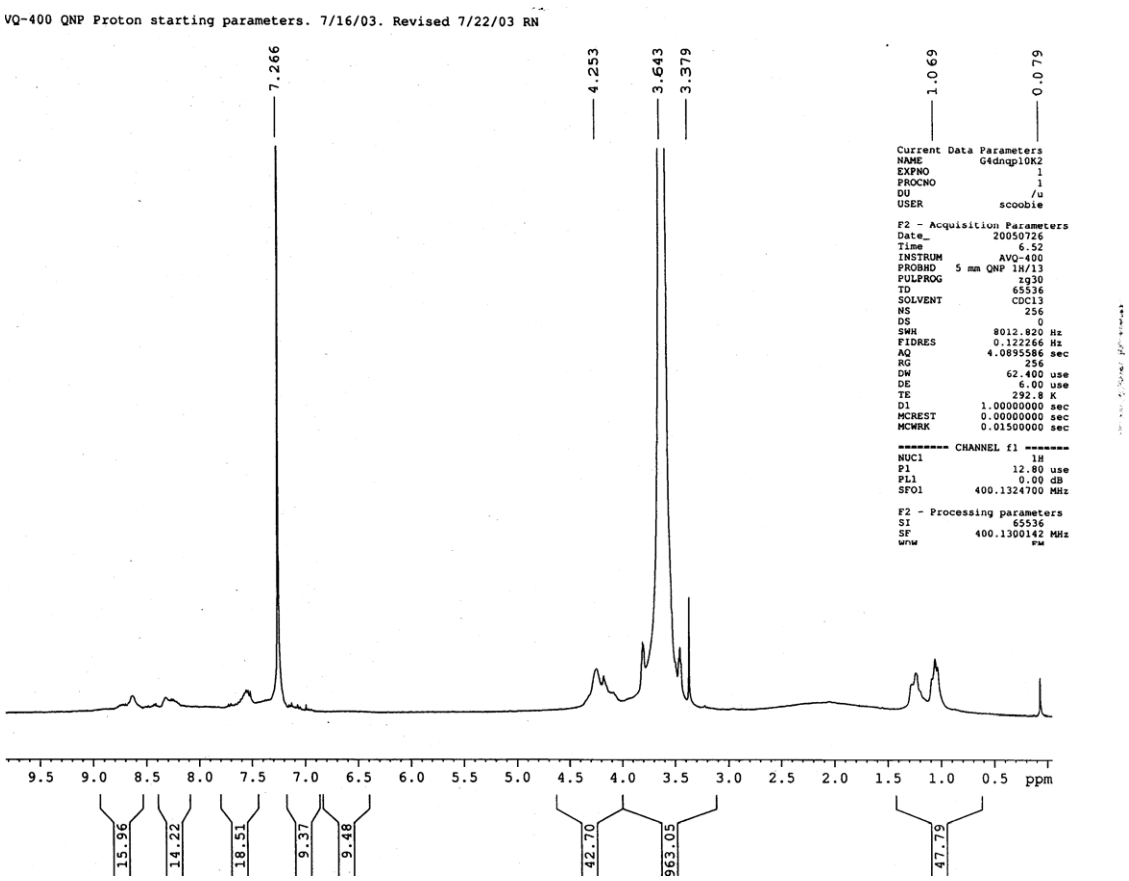

Compound **4** NMR