A responsive hyperbranched polymer not only can self-immolate but can also self-cross-link

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1. Materials. N,N’-cystaminebisacrylamide (CBA) were synthesized according to previous reports. p-Toluic acid (98%), N-bromosuccinimide (99%), acetic acid (99.5%), ethylenediamine (EDA, 99%), fuming nitric acid (95%) and trifluoroacetic acid (TFA, 99%) were purchased from Sinopharm Chemical Reagent Co., Ltd (SCRC, China). Di-ter-butyl dicarbonate (99%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl, 98.5%) and triethylamine (TEA, 99.5%) were purchased from Aladdin (China). Benzoyl peroxide (BPO, SCRC) was re-crystallized once from methanol and 2-Mercaptoethanol (SCRC) was distilled before used. N,N’-Dimethyldipropylenetriamine (DMDPTA, Sigma-Aldrich, 99.0%) were used as received.

2. Characterizations. $^1$H-NMR spectra were recorded on a Bruker AC 400 MHz NMR. The number average molecular weight ($M_n$), weight-average molecular weight ($M_w$), and PDI were determined from gel permeation chromatography (GPC) using DMF as the eluent and narrow polydispersity polystyrenes as the standards, the columns are two Polymer Laboratories PL gel 5 µm mixed C column. The cloud points of the polymer solutions were measured using a Beckman DU 640 UV spectrophotometer equipped with a digital temperature controller. Dynamic light scattering (DLS) was carried out on a Malvern Zetasizer Nano ZS90 with a He-Ne laser (633 nm) and 90° collecting
optics. The rheological measurements for hyperbranched polymer was performed on a rheometer (TA-AR2000, TA Instruments) to detect evolutions of storage elastic modulus (G') and loss elastic modulus (G'') versus time at different temperatures at a fixed frequency of 10 rad/s. AFM (Dimension3100, Veeco) was used.

3. **Synthesis of hyperbranched poly(amido amine) with propylenetriamine terminals.** N,N'-cystaminebisacrylamide (1303 mg, 5.0 mmol) and N,N'-dimethyldipropylenetriamine (398 mg, 2.5 mmol) were added into a vial containing 10.0 mL methanol/water mixture (7/3, v/v). Subsequently, the polymerization was carried out at 50 °C for 5 d in the vial under argon atmosphere. Subsequently, N,N'-dimethyldipropylenetriamine (796 mg, 5.0 mmol) was added into the polymerization mixture to change the vinyl terminals into N,N'-dimethyldipropylenetriamine. Then the solution was precipitated from 50 mL acetone. The polymer was collected by filtration and removal of acetone. $M_n$ is 7.8 kDa, and PDI is 1.9.
Supporting Figures

$^1$H NMR (300 MHz, d$_6$-DMSO): $\delta$ (ppm), 7.92 (d, 2H), 7.56 (d, 2H), 4.75 (s, 2H).

**Figure S1.** $^1$H-NMR spectrum of 4-(bromomethyl)benzoic acid in d$_6$-DMSO.

$^1$H NMR (300 MHz, d$_6$-DMSO): $\delta$ (ppm), 8.48 (s, 1H), 8.24 (d, 1H), 7.90 (d, 1H), 4.99 (s, 2H).

**Figure S2** $^1$H-NMR spectrum of 4-(bromomethyl)-3-nitrobenzoic acid in d$_6$-DMSO.

$^1$H NMR (300 MHz, d$_6$-DMSO): $\delta$ (ppm), 8.41 (s, 1H), 8.16 (d, 1H), 7.72 (d, 1H), 4.11 (s, 2H), 3.45 (t, 2H), 2.48 (t, 2H).
**Figure S3.** $^1$H-NMR spectrum of 4-(((2-hydroxyethyl)thio)methyl)-3-nitrobenzoic acid in d$_6$-DMSO.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm), 8.45 (s, 1H), 8.05 (d, 1H), 7.78 (s, broad, 2H), 7.56 (d, 1H), 5.04 (s, broad, 1H), 4.13 (s, 2H), 3.73 (t, 2H), 3.57 (t, 2H), 3.43 (t, 2H), 2.66 (t, 2H), 1.45 (s, 9H).

**Figure S4.** $^1$H-NMR spectrum of tert-butyl (2-((2-hydroxyethyl)thio)methyl)-3-nitrobenzamido)ethyl)carbamate in CDCl$_3$. 

(2-((2-hydroxyethyl)thio)methyl)-3-nitrobenzamido)ethyl)carbamate in CDCl$_3$. 


$^1$H NMR (300 MHz, D$_2$O): $\delta$ (ppm), 8.41 (s, 1H), 8.98 (d, 1H), 7.61 (d, 1H), 4.11 (s, 2H), 3.68 (t, 2H), 2.60 (t, 2H), 3.22 (t, 2H), 2.61 (t, 2H).

**Figure S5.** $^1$H-NMR spectrum of $N$-(2-aminoethyl)-4-((2-hydroxyethyl)thio)methyl)-3-nitrobenzamide in D$_2$O.

$^1$H NMR (300 MHz, DMSO): $\delta$ (ppm), 8.32 (s, 2H), 6.21 (q, 2H), 6.09 (d, 2H), 5.59 (d, 2H), 3.41 (t, 4H), 2.80 (t, 4H).

**Figure S6.** $^1$H-NMR spectrum of CBA in d$_6$-DMSO.
Figure S7. $^1$H-NMR spectra of hyperbranched poly(amido amine) with vinyl terminals (up) and poly(amido amine) with 2-((2-nitrobenzyl)thio)ethanol terminals (below).
Figure S8. $^{13}$C-NMR spectrum of hyperbranched poly(amido amine) with 2-((2-nitrobenzyl)thio)ethanol terminals.
Figure S9. The absorption curves of hyperbranched polymer with \(N,N'\)-dimethylpropylenetriamine terminals under UV irradiation in the presence of Ellman’s agent at different irradiation time.
Figure S10. The fluorescent curves of hyperbranched polymer with $N,N'$-dimethyldipropylenetriamine terminals under UV irradiation at different irradiation time.
Figure S11. The changes of transmittance for hyperbranched polymer with $N,N'$-dimethylidipropyleneetriamine terminals (HP-DMDPA, without 2-((2-nitrobenzyl)thio)ethanol terminals) and hyperbranched polymer with 2-((2-nitrobenzyl)thio)ethanol terminals (HP-NBTE) with pH (polymer concentration is 3.0 mg/ml).
Figure S12. The pictures of the hyperbranched polymer with $N,N'$-dimethylpropylenetriamine terminals aqueous solution upon UV activation at different time.
**In vitro transfection.** Transfection efficiency with gWiz-Luc plasmid DNA was tested in 3 Hela cell lines as follows: cells were seeded at a density of 30,000 cells per well on 48-well cell culture plates, 24 hours prior to the transfection experiment. The polyplexes were prepared as described above, and 25 µL solution was added to each well containing 150 µL of DMEM with or without serum. After 4 h of incubation, the transfection mixture was removed and the cells were cultured in fresh full DMEM media and under UV irradiation for 0, 10, 20 min. To determine level of luciferase expression, the culture medium was discarded and cell lysate was harvested after incubation of cells for 30 min at room temperature in 100 µL of cell lysis reagent buffer (Promega). To measure the luciferase content, 100 µL of luciferase assay buffer (20 mM glycylglycine (pH 8), 1 mM MgCl$_2$, 0.1 mM EDTA, 3.5 mM DTT, 0.5 mM ATP, 0.27 mM coenzyme) was automatically injected into 20 µL of cell lysate, and the luminescence was integrated over 10 s using single tube Sirius luminometer. Total cellular protein in the cell lysate was determined by the Bicinchoninic acid (BCA) protein assay using a calibration curve constructed with standard bovine serum albumin solutions (Pierce). The luciferase transfection results are expressed as Relative Light Units (RLU) per mg of cellular protein. Unless stated otherwise, the results are expressed as mean RLU/mg of protein ±SD of triplicate experiments.
**Fig. S13.** Transfection activity of the polyplexes in Hela cells at different UV irradiation time.