

Highly Fluorescent Hyperbranched BODIPY-Based Conjugated Polymer Dots for Cellular Imaging

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Experimental Section

Instrumentation

¹H NMR spectra were recorded on Bruker AV 400 spectrometers and used tetramethylsilane (TMS) as an internal standard in CDCl₃. The molecular weight of polymers was measured by the GPC method (Waters 410 Differential Refractometer), and monodisperse polystyrene samples (THF as eluent) were used as the standard for the determination of molecular weight. The particle size of the hyperbranched BODIPY-based conjugated polymer dots in aqueous solution were characterized by dynamic light scattering (DLS). Measurements were recorded by using 1 cm glass cuvettes with a Malvern Nano ZS instrument. Transmission electron microscopy (TEM) was recorded on a transmission electron microscope (Hitachi H-600 microscope). Samples for TEM measurements were prepared by dropping one or two drops of the Pdot solution onto copper grids and allowed to evaporate at room temperature. UV–vis absorption spectra in aqueous solution were collected with a TU-1601 spectrophotometer by using a 1 cm glass cuvette. Fluorescence was obtained by using a Hitachi F-4600 fluorescence spectrophotometer. The absolute fluorescence quantum yields (QYs) of Pdot samples were measured with an absolute photoluminescence quantum yield measurement system (Hamamatsu photonic multichannel analyzer C10027).

Materials

All chemicals were purchased from Aladdin, Sigma-Aldrich, TCI, Thermo Scientific, and used directly without further purifications. The 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dioctylfluorene and triiodo-BODIPY was synthesized by following the literature methods.^{S1,S2}

Synthesis of hyperbranched polymers P1 and P2

Portions method (P1): 2,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dioctylfluorene

(160 mg, 0.25 mmol), 4,7-dibromobenzothiadiazole (71 mg, 0.24 mmol) were dissolved into toluene (6 mL)/2M aqueous K_2CO_3 (2 mL) with a few drops of A336 in a 25 mL single-neck bottom flask, and then tetrakis (triphenylphosphine)palladium $[(PPh_3)_4Pd(0)]$ (8 mg) were added. The triiodo-BODIPY (7 mg, 0.01 mmol) was dissolved into 3 mL of toluene separately. After degassing, the mixture was heated to reflux under nitrogen atmosphere, the 3 mL of triiodo-BODIPY solution in three portions (1 mL for each) was added into the mixture at 0.5, 1 and 2 hours. Then, the mixture was refluxed for two days. After that, the mixture was cooled to room temperature and the solution was poured into methanol. The collected crude polymer was dissolved into toluene and passed a short flash column, and the concentrated solution was poured methanol, and the solid was collected and dried under vacuum. GPC M_n : 30200; M_w : 63900; PDI: 2.12.

One portion method (P2): 2,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dioctylfluorene (160 mg, 0.25 mmol), 4,7-dibromobenzothiadiazole (71 mg, 0.24 mmol) and triiodo-BODIPY (7 mg, 0.01 mmol) were dissolved into toluene (6 mL)/2M aqueous K_2CO_3 (2 mL) with a few drops of A336 in a 25 mL single-neck bottom flask, and then tetrakis (triphenylphosphine)palladium $[(PPh_3)_4Pd(0)]$ (8 mg) were added. After degassing, the mixture was heated for 2 days under nitrogen atmosphere. Then, the mixture was cooled to room temperature and the solution was poured into methanol. The collected crude polymer was dissolved into toluene and passed a short flash column, and the concentrated solution was poured methanol, and the solid was collected and dried under vacuum. GPC M_n : 33510; M_w : 90100; PDI: 2.69.

Preparation of Pdots

A PSMA solution (2 mL, 1000 ppm) was added to the polymer solution of P1 or P2 in THF (2 mL, 250 ppm). The mixture solution was injected into DI water (10 mL) under ultrasonication. THF in

the aqueous solution was then evaporated under nitrogen flow at 70 °C, and the solution was concentrated to 4-5mL. The solution was then filtered through a 0.22 µm filter and stored at 4 °C until further use.

Pdots Bioconjugation

Pdots bioconjugation was performed via the EDC-catalyzed reaction between carboxyl groups on the Pdots' surface and the amine groups on biomolecules. In a typical bioconjugation reaction, 80 µL of polyethylene glycol (5% w/v PEG, MW 3350) and 80 µL of concentrated HEPES buffer (1 M) were added to 4 mL of Pdot solution (~50 mg/mL in DI water), resulting in a Pdot solution in 20 mM HEPES buffer with a pH of 7.3. Then, 60 µL of streptavidin (Invitrogen, Eugene, OR, USA) was added to the solution and mixed well on a vortex. 20 µL of a freshly prepared EDC solution (5 mg/mL in MiliQ water) was added to the solution, and the mixture was left on a rotary shaker. After stirring for 4 hours at room temperature, Triton-X 100 (0.25% (w/v), 80 µL) and bovine serum albumin (BSA; 2% (w/v), 80 µL) were added. The mixture was left on rotary shaker for 1 h. Finally, the resulting Pdot bioconjugates were separated from free biomolecules by gel filtration using Sephacryl HR-300 gel media.

Cell Culture

The human cervical adenocarcinoma HeLa cell and the human breast cancer cell line MCF-7 was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). HeLa cells were cultured at 37 °C, 5% CO₂ in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (5000 units/mL penicillin G, 50 µg/mL streptomycin sulfate in 0.85% NaCl). MCF-7 cells were cultured at the same condition except that 1% penicillin-streptomycin solution was changed as 50 U/mL penicillin and 50 µg/mL

streptomycin. The cells were cultured prior to experiments until confluence was reached. The cells were harvested from the culture flask by briefly rinsing with culture media followed by incubation with 5 mL of trypsin-EDTA solution (0.25 w/v % trypsin, 0.53 mM EDTA) at 37 °C for 5-15 min. After complete detachment, the cells were rinsed, centrifuged, and resuspended in labeling buffer (1× PBS, 2 mM EDTA, 1% BSA).

Cell Uptake

For the uptake experiment, a rounded coverslip was putted on the well of cell culture dishes. Then, the primarily cultured HeLa cells were seed in the cell culture dishes with 5×10^4 cells per well and allowed to grow overnight. Sterile-filtered Pdot solution was added to the well at the weight concentration of ~10 ppm and allowed to incubate for 24 h. For the control, Pdot solution was not added. Then the HeLa cells were washed with PBS(1x) buffer 3 times and a DAPI solution (1:2000) was added into the well. After placed in dark for 2 minutes, the HeLa cells were washed with PBS (1x) buffer again. Then the rounded coverslip was taken out from the well and made into loading before imaged on a confocal microscope (Zeiss LSM 510 Meta). The Pdots uptaken Hela cells were excited by a 488 nm argon laser. A Plan-Apochromat 63×/1.40 oil DIC objective lens was utilized for imaging.

Cellular Surface Labeling and Imaging

For labeling cell-surface proteins with the Pdot-streptavidin (Pdot-SA) conjugates, live MCF-7 cells in the glass-bottomed culture dish were blocked with BlockAid blocking buffer (Invitrogen, Eugene, OR, USA). Then, the MCF-7 cells were incubated sequentially with biotinylated primary anti-EpCAM antibody (used to label the cell-surface EpCAM receptors on MCF-7 cells) and 5 nM Pdot-streptavidin for 30 min each, followed by two washing steps after each incubation. As for the control

sample, biotinylated primary anti-EpCAM antibody was not added. The Pdot-tagged cells were then fixed with 4% para-formaldehyde for 15 min and imaged immediately on a fluorescence microscope (Olympus IX71) with a 0.95 NA UPLSAPO 40× objective. The excitation light was provided with a Mercury Lamp, and filtered by a band-pass filter (Semrock FF01-510/42-25). Fluorescence signal was filtered by a long-pass filter (Semrock FF01-515/LP-25), and imaged on an Andor iXon3 frame-transfer EMCCD (Andor Technology, UK).

In Vivo Imaging on Zebrafish

The transgenic zebrafish, Tg(kdrl:eGFP)^{la116} expressing eGFP in the endothelial cells, were kept at 28 °C and bred under standard conditions with approval from National SunYat-sen University Animal Care Committee. For angiography imaging, 37 nL of Pdots (125 nM) in 15 mM HEPES buffer was injected into the sinus venosus of the anaesthetized zebrafish embryos 3 day post fertilization with 5% (v/v) tricaine. After recovered for 30 min, the injected embryos were immobilized in 1.5% low melting point agarose (Life Technologies) and then imaged immediately on a fluorescence confocal microscope (Zeiss LSM 700). The green fluorescence was acquired by filtering through a 515/30 band-pass (excitation laser at 488 nm), while the red emission was acquired by using a 505 long-pass filter (excitation laser at 488 nm).

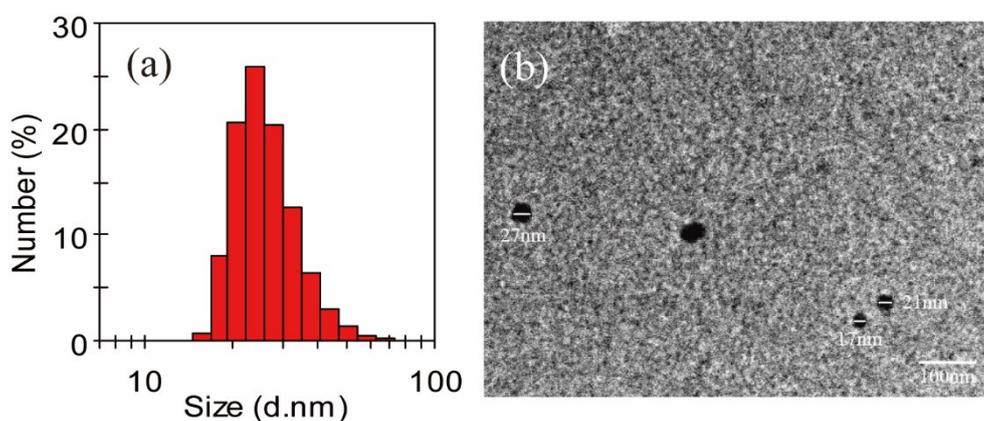


Fig. S1 The hydrodynamic diameter measured by DLS (a) and TEM (b) images of P2 Pdots.

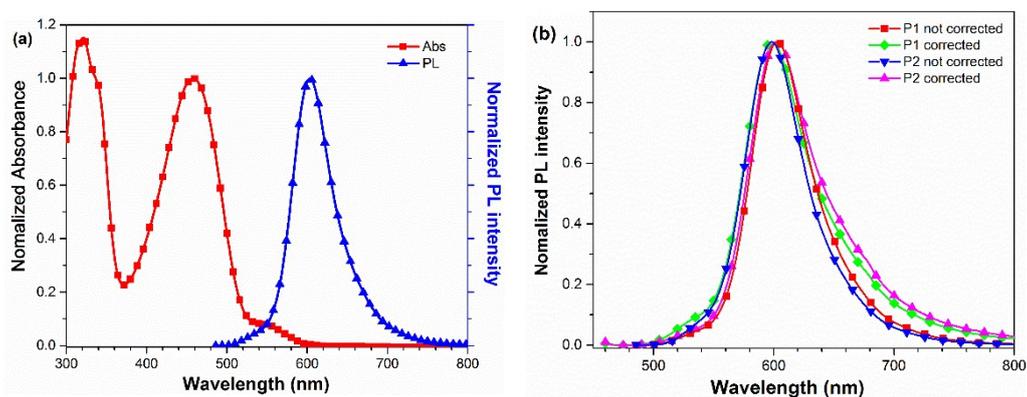


Fig. S2 The UV-Vis absorption and photoluminescence spectra of P2 in aqueous solution (a), and comparison of corrected photoluminescence spectra of P1 and P2 in aqueous solution (b).

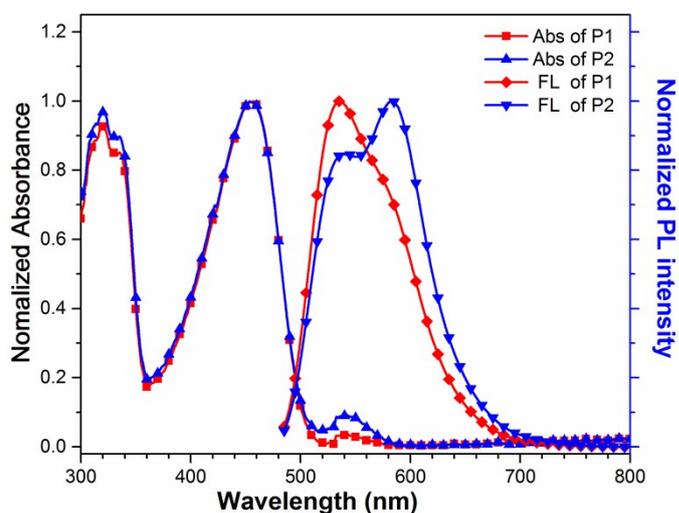


Fig. S3 The UV-Vis absorption and photoluminescence spectra of P1 and P2 in THF

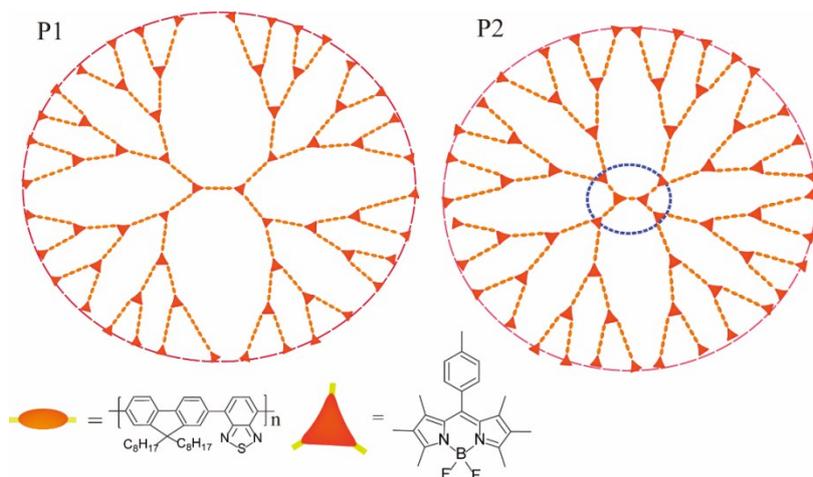


Fig. S4 Illustration of concentration quenching of BODIPY units in hyperbranched polymer

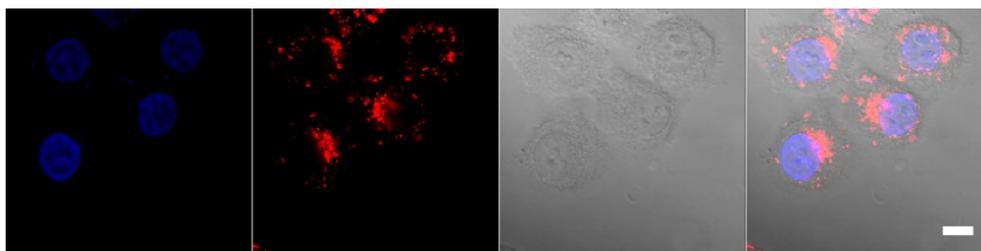


Fig. S5 Confocal fluorescence images of HeLa cells incubated with P2 Pdots. Blue fluorescence is from nuclear counterstain DAPI, while red fluorescence is from P2 Pdots. The right panel represents fluorescence overlaid with the bright-field image. Scan bar represents 10 μm .

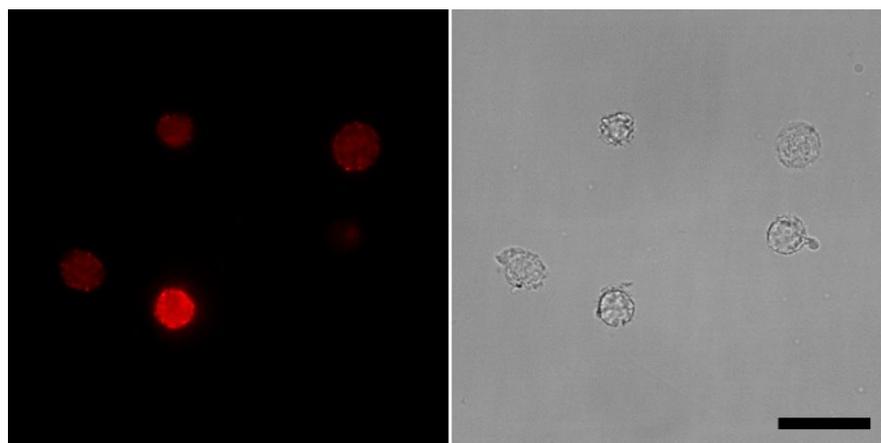


Fig. S6 Fluorescence images of MCF-7 cells labelled with P2 Pdot-streptavidin conjugates. Red fluorescence is from P2 Pdots. The right panel is bright-field image. Scan bar represents 30 μm .

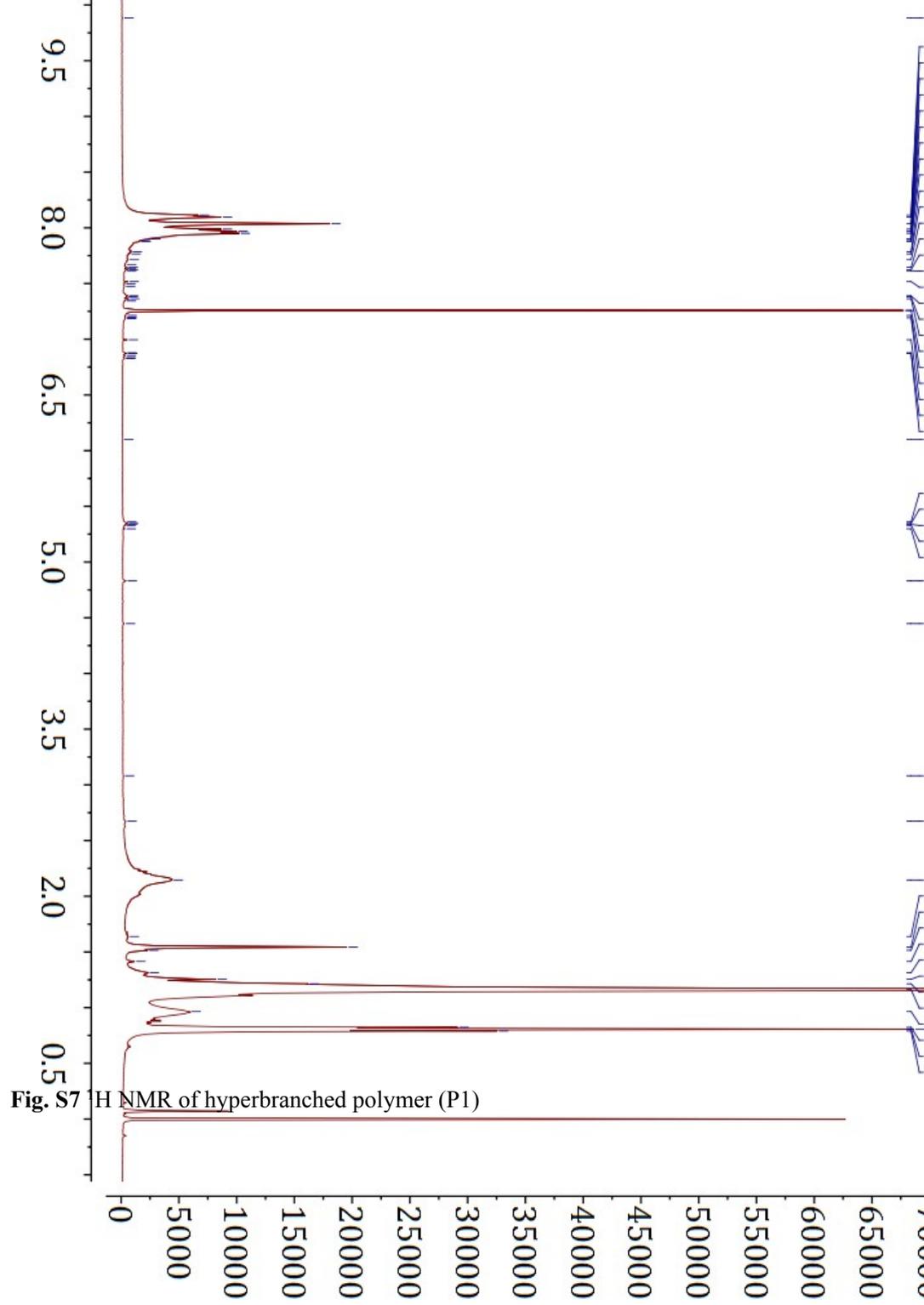
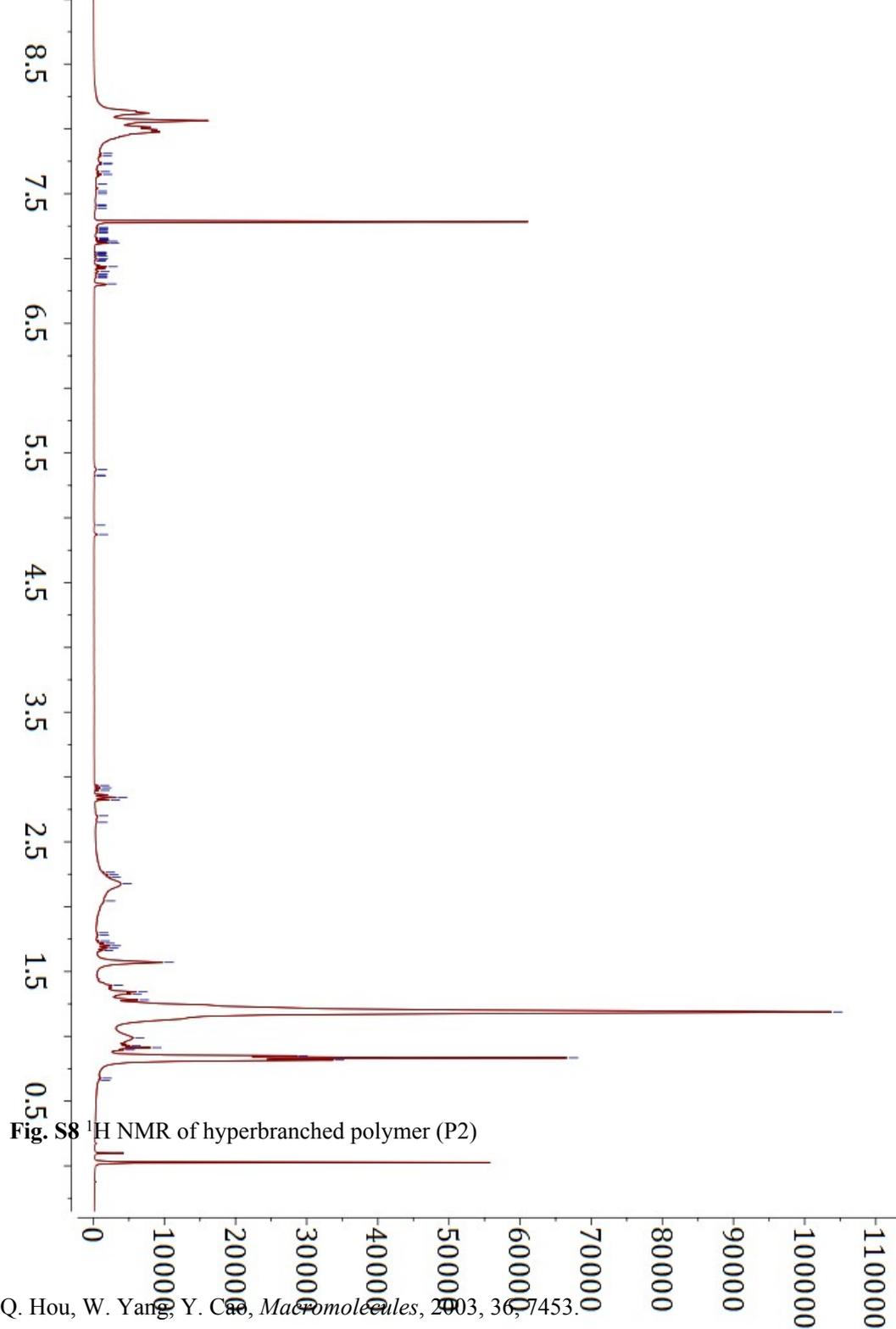


Fig. S7 ¹H NMR of hyperbranched polymer (P1)



Reference

[S1] Q. Yang, R. Y. Tian, Q. Hou, W. Yang, Y. Cao, *Macromolecules*, 2003, 36, 7453.

[S2] A. B. Nepomnyashchii, M. Bröring, J. Ahrens, A. J. Bard, *J. Am. Chem. Soc.* 2011, 133, 8633.