Electronic Supplementary Material (ESI) for Polymer Chemistry.

Encapsulation property of hyperbranched polyglycerols as prospective drug delivery systems

Haigang Wu[a], Ting Yin[b], Ke Li[c], Ruibin Wang[d], Yantian Chen[e] and Lili Jing[e]

[a]School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China, 200240

[b]School of Electronic Information and Electrical Engineering, Shanghai Jiao Tong University, Shanghai, China, 200240

[c]University of Michigan-Shanghai Jiao Tong University Joint Institute, Shanghai Jiao Tong University, Shanghai, China, 200240

[d]Instrumental analysis center of Shanghai Jiao Tong University, Shanghai, China, 200240

[e]School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China, 200240

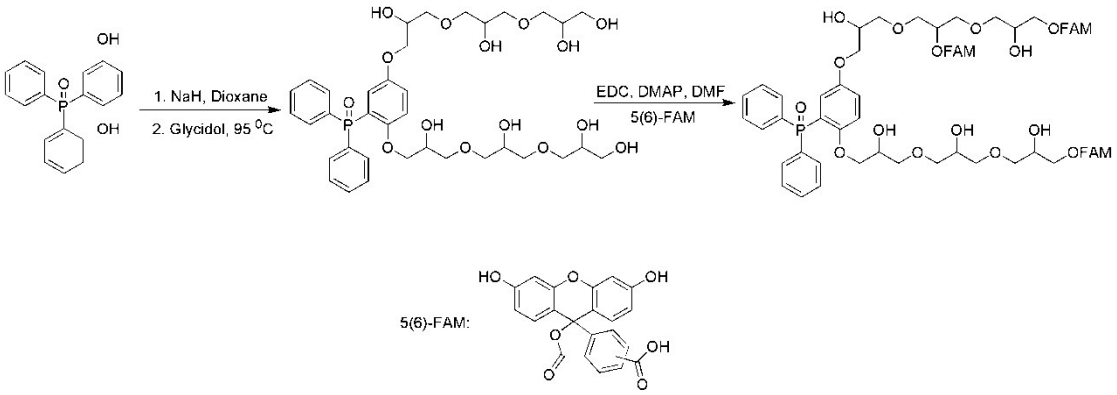


Figure S1. Synthesizing protocol of hbPG and FAM-HPG molecule. NaH is used as the de-proton reagents, (diphenylphosphinyl) hydroquinone is used as the initiated core and inner terminal marker to obtain the number-average molecular weight ($M_{n,nmr}$). And the fluorescence-labeled FAM-HPG was synthesized by using coupling reagents, 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDC), 4-dimethylaminopyridine (DMAP).[1]

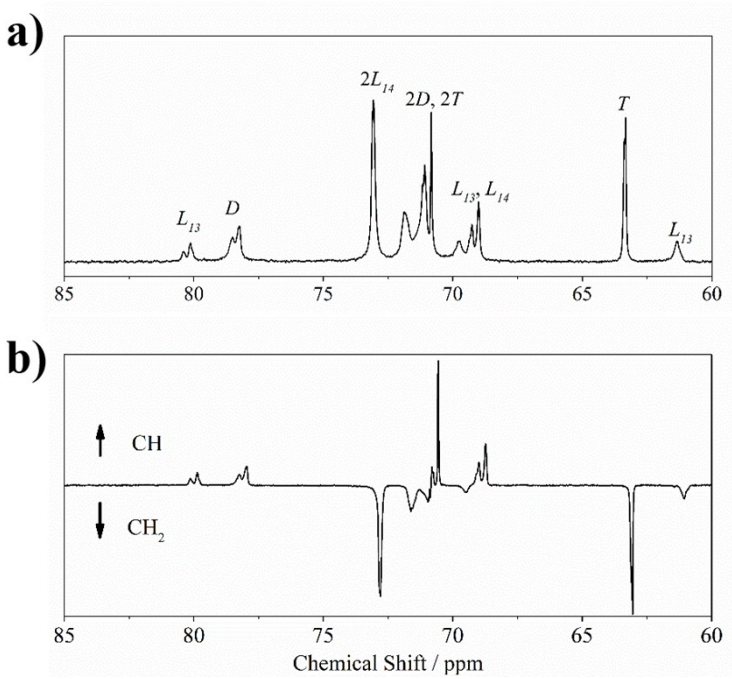


Figure S2. a). The Inverse Gated (IG) ^{13}C NMR spectrum of hbPG in d_6-DMSO. The peaks of ^{13}C in the spectra are L_{13}, D, $2L_{14}$, $2D$ with $2T$, L_{13} with L_{14}, T and L_{13}, respectively. L_{13} is the substituted site of two adjacent carbon atoms in the glycerol unit, L_{14} is the substituted site of two interval carbon atoms in the glycerol unit. Moreover, D is the fully substituted carbon atom and T is the carbon atom of terminal units. b). The DEPT 135° NMR of HPG in d_6-DMSO. All the peaks are consisted with the peaks in IG ^{13}C NMR spectra, which show the different types of carbon atom in hbPG molecules. (Note: the direction of DEPT spectra, CH2 (up) and CH (down))

Table S1. Interpretation of Inverse Gated ^{13}C NMR of hbPGs in d_6-DMSO

Region	Shift / ppm	Integral
L_{13}	80.26	1.00
D	78.37	2.61
$2L_{14}$	73.06	6.44
$2D$, $2T$	71.17-70.83	11.66
L_{13}, L_{14}	69.78-6900	4.36
T	63.34	3.45
L_{13}	61.34	1.03

Structural unit	L_{13}	L_{14}	T	D
Relative abundance (%)	9.7	31.3	33.6	25.4

The Degree of branching[2] by IG ^{13}C spectra is as follow:

$$DB = \frac{2D}{2D + L_{13} + L_{14}}$$

The DB of hbPG is approximately 0.55, and the abundance of terminal glycerol is 0.336.

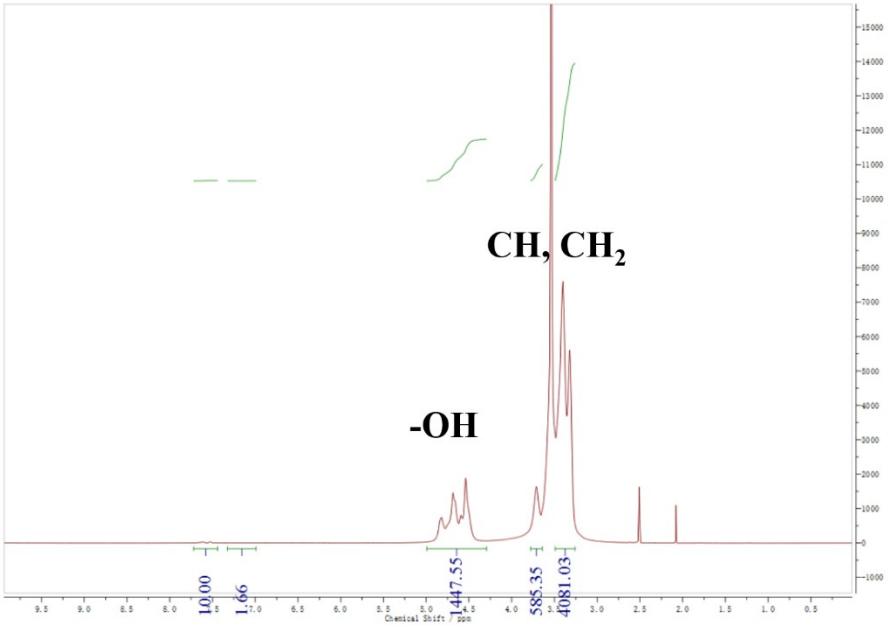


Figure S3. The number-average molecular weight ($M_{n,nmr}$) of hbPG molecules is approximately 72900 g·mol^{-1}. Calculation of $M_{n,nmr}$ is based on terminal groups, in which the phenyl rings is used as the inner marker.

^{1}H NMR (600 MHz, DMSO-d_6): δ_H 4.96-4.64 (multiplet, C-O**H**), 3.74-3.31 (multiplet, -C**H**-, -C**H$_2$**-).

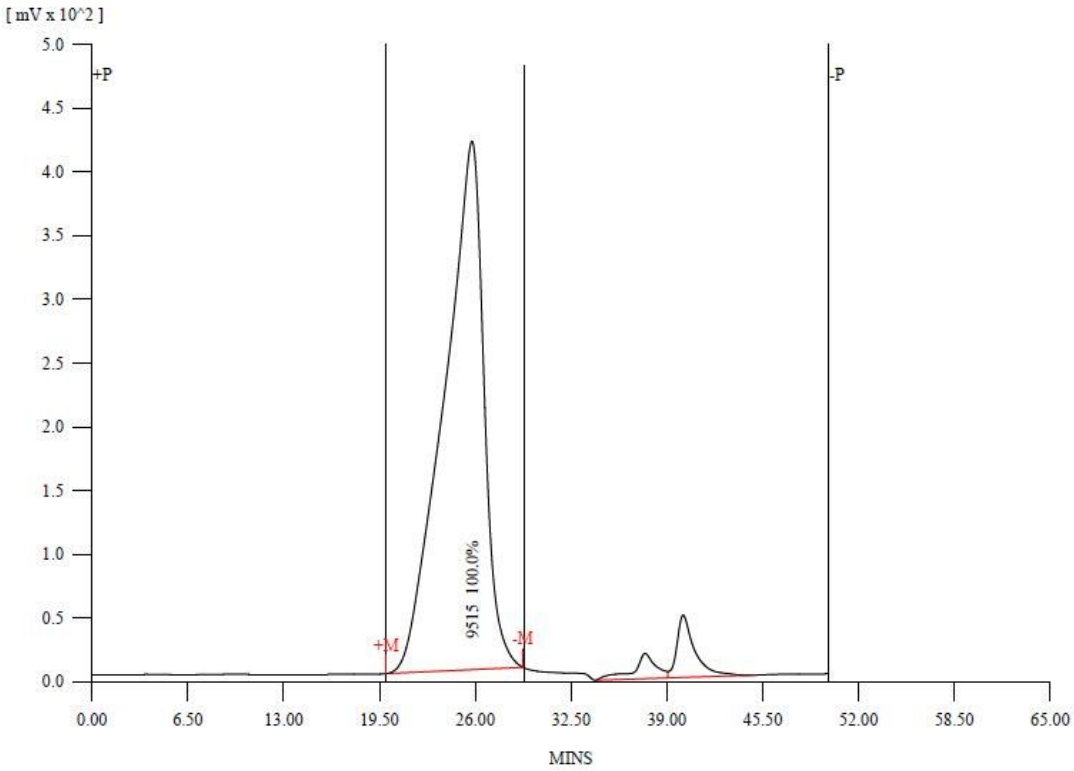


Figure S4. Gel Permeation Chromatography curve of hbPGs using DMF as flowing phase. The peaks located around 39.00 min is not attributed to the hbPGs. Here, the number-average molecular weight (M_n) and weight-average molecular weight (M_w) is 10997.7 g/mol and 29393.1

g/mol, respectively. The PDI value (Mw/Mn=2.67) indicated that the molecular weight distribution of hbPGs is moderate.

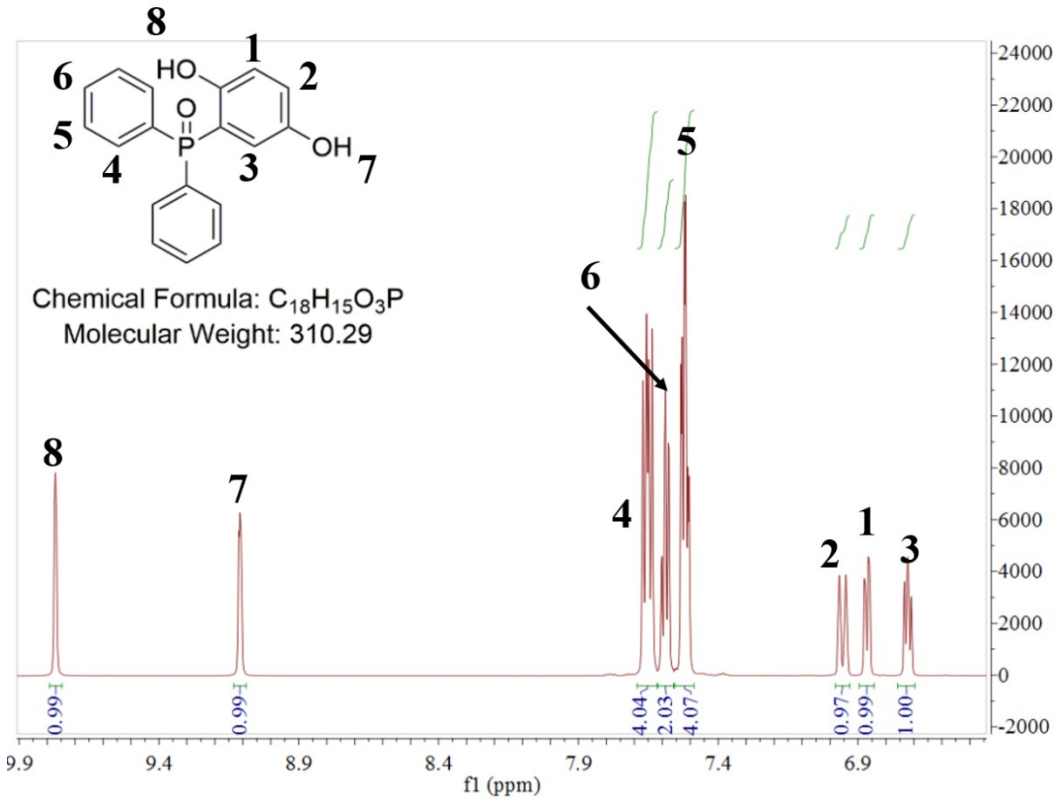


Figure S5. The ^{1}H NMR spectrum of DHTPO in d_6-DMSO.

^{1}H NMR (600 MHz, DMSO-d_6): δ 9.73 (*s*, 1H, OH), 9.07 (s, 1H, OH), 7.62-7.60 (m, 4H), 7.55 (*dd*, *J* = 1.2, 1.2 Hz, 2H), 7.49-7.46 (*m*, 4H), 6.92 (d, *J* = 13.8 Hz, 1H), 6.83 (dd, *J* = 1.8 1.8 Hz, 1H), 6.68 (t, *J*= 7.2 Hz, 1H).

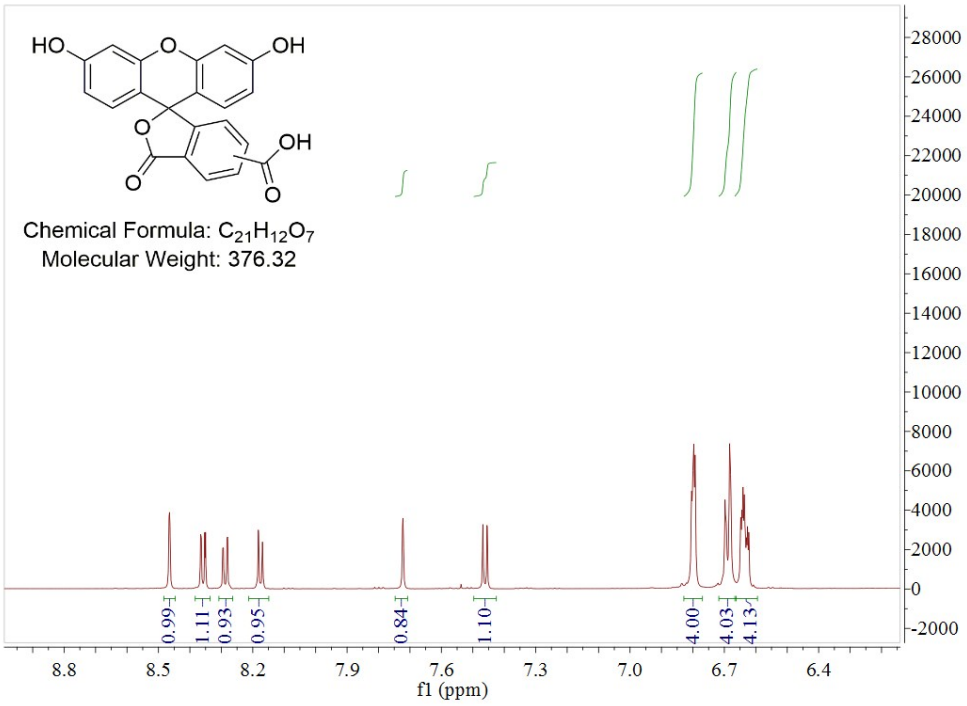


Figure S6. The ^{1}H NMR spectrum of 5(6)-FAM molecule in d_6-DMSO. Synthesis of 5(6)-FAM is according to the previous study[3]. Due to that the product is the mixture of 5-FAM and 6-FAM, the integral of hydrogen atom is double of single molecules. The peaks around 6.7 ppm belong to phenyl rings (two rings in the spectrum).

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.40 (*s*, 1H, CH_{Ar}-5-Isomer), 8.30 (*dd*, *J* = 8.0, 1.5 Hz, 1H, CH_{Ar}-5-Isomer), 8.23 (*dd*, *J* = 8.0, 1.2 Hz, 1H, CH_{Ar}-6-Isomer), 8.11 (*d*, *J* = 7.5 Hz, 1H, CH_{Ar}-6-Isomer), 7.65 (*s*, 1H, CH_{Ar}-6-Isomer), 7.39 (*d, J* = 8.3 Hz, 1H, CH_{Ar}-5- Isomer), 6.70 (*d*, *J* = 2.4 Hz, 4H, CH_{Ar}-5/6-Isomer), 6.61 (*dd*, J = 8.7, 3.8 Hz, 4H, CH_{Ar}-5/6-Isomer), 6.55 (*dd*, J = 8.7, 2.4 Hz, 4H, CH_{Ar}-5/6-Isomer);

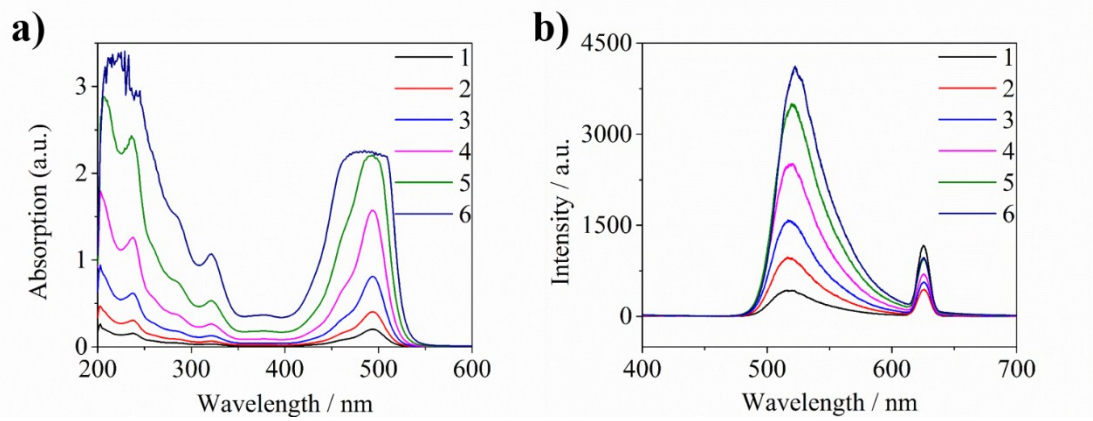


Figure S7. a). UV-vis absorption spectrum of FAM-HPG molecule. b). Fluorescence spectrum of FAM-HPG molecule.

Figure S6 a shows the absorbance spectra of FAM-HPG molecule at different concentration (list

the different concentrations for each curve). The λ_{max} is around 490 nm, and the peak at 238nm belongs to the typical benzenoid band from the aromatic rings of conjugated FAM molecule. The peak around 321 nm is the feature peak of hbPG molecule. For curves 6 and 5, the concentration is much higher than others. At low concentration range, the absorption intensity is linearly dependent on the concentration. The emission peak of FAM-HPG is at 520 nm, very close to emission peak of FAM, suggesting that hbPGs molecule does not affect the FAM ring. 520 nm is the wavelength used in confocal microscopy imaging.

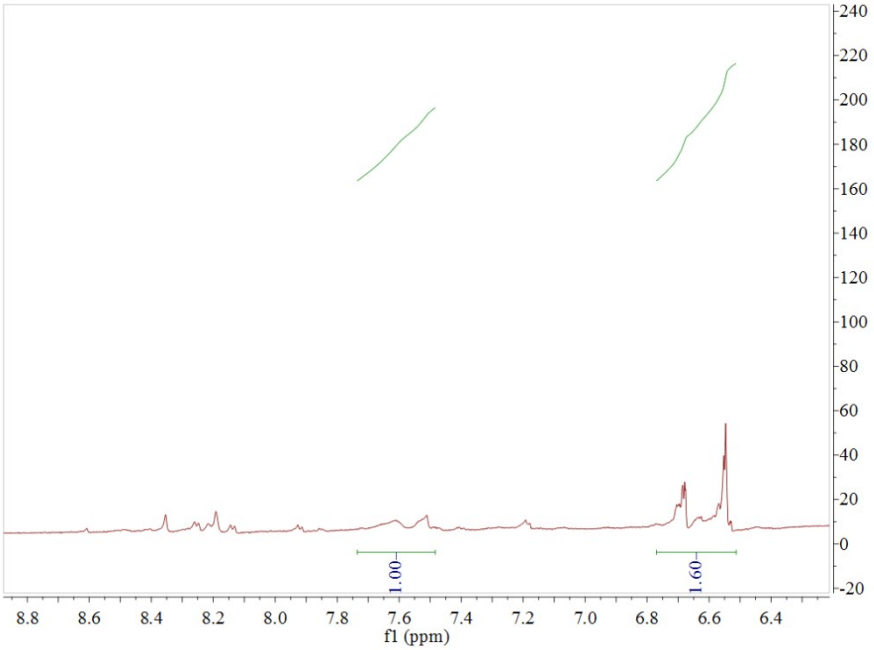


Figure S8. ^{1}H NMR spectrum of FAM-HPG at 6 ppm~9 ppm region. The integral of peaks (peak at 7.6 ppm and peak at 6.6 ppm) indicate that the average number of FAM molecules bonded to the macromolecule is about 2.7 per HPG molecule.

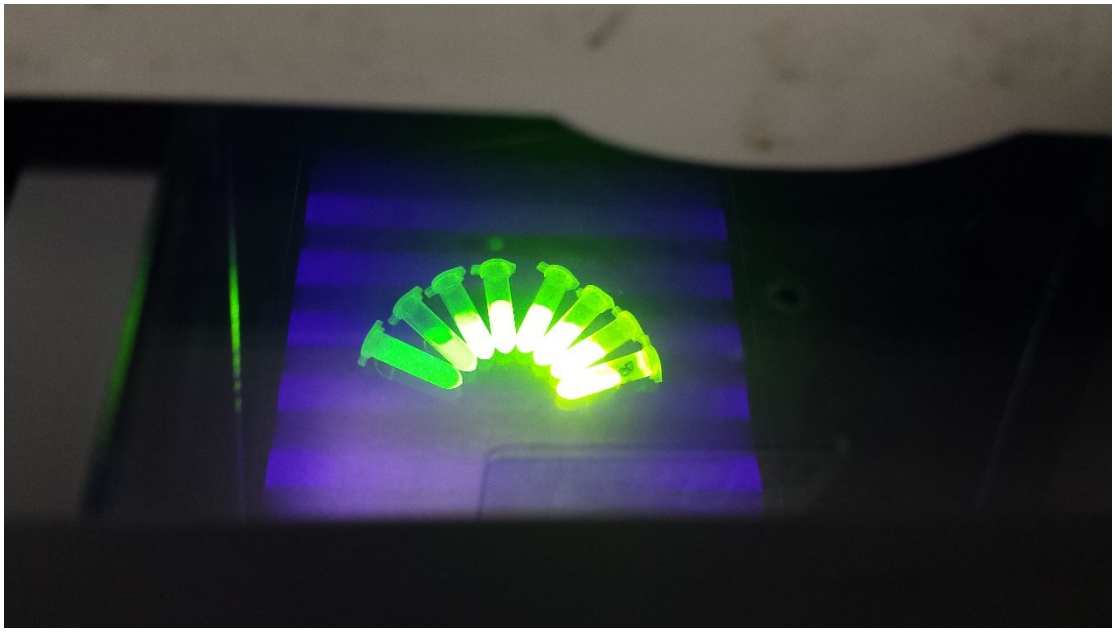


Figure S9. The fluorescence imaging of the filtrate solution under UV light. The mixed and diluted FAM-HPG@Ce6 solution was centrifuged by 10,000 rpm for 20 min using Millipore Ultra Centrifugal Filter Devices (MW cutoff > 3 kDa) for several times. After each centrifugation, PBS solution was added into residue solution to keep the constant volume of FAM-HPG@Ce6 solution[4, 5], and the resulting filtrate was put under the UV light to detect the existence of Ce6 molecules (UV λmax=257 nm) (in a right-to-left order). The left sample indicated that no free Ce6 molecules are in the FAM-HPG@Ce6 PBS solution.

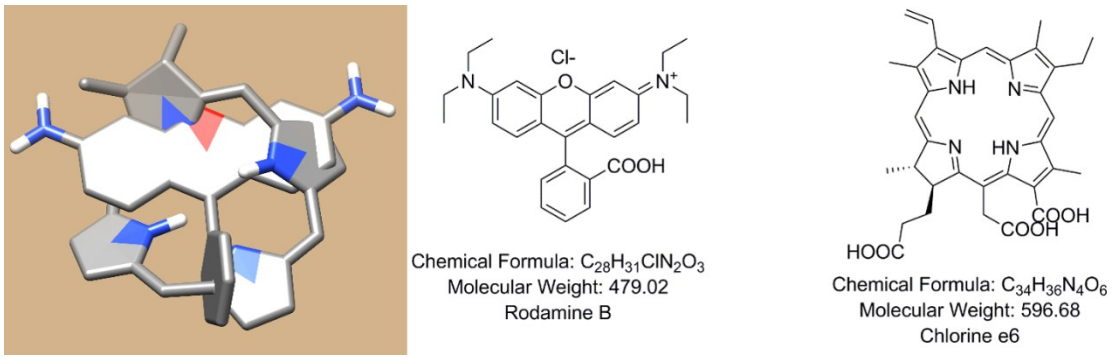


Figure S10. Overlay of Rhodamine B with chlorin e6 ring (left), and 2D structure of Rhodamine B and chlorin e6. Without the carboxyl groups, Rhodamine B molecules is structurally similar to the chlorin e6 ring.

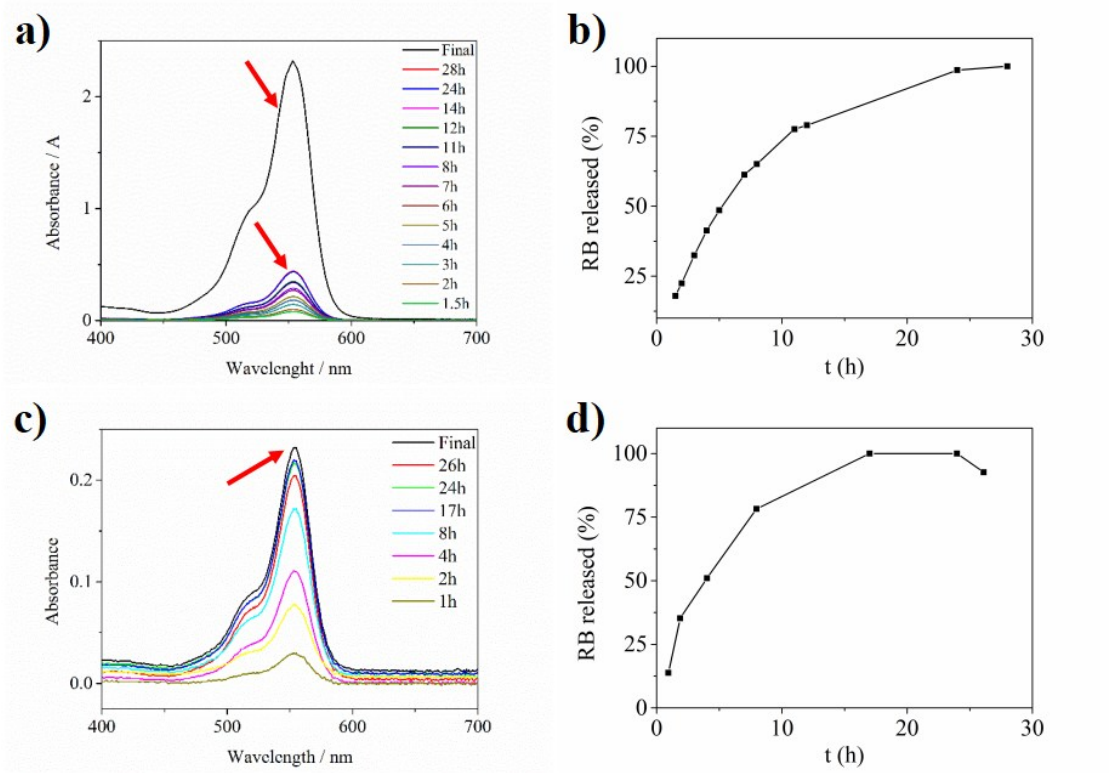


Figure S11. a and b). The figure shows that the concentration of released RB vs time in water. a) Black curve: absorbed curve of solution inside the dialysis bag, which is significantly higher than the outside dialysis bag and demonstrate that RB molecules were tightly encapsulated by hbPGs; Multi-color curves: absorbed curve of solution outside the dialysis bag after different times. c) and

d). The concentration of RB outside the dialysis bag vs time. When the time reaches to 17 h, the differential RB concentration between dialysate and the outside is small, and it likely reaches an equilibrium state. We can find that the residual binding RB is significantly more than control groups, which implied that the hbPGs can strongly hold guest molecules.

Binding constant of RB with FAM-PG in DMSO:

As literature report (*Chemical Reviews 112.7(2012):3856-91.*), the binding constant of guest molecules with host can be obtained as follows:

$$\Delta\delta_{obs} = \frac{\Delta\delta_{max}}{2}\left\{\left(1+\frac{1}{nK_a[Guest]_o}+\frac{[Host]_o}{n[Guest]_o}\right)-\left[\left(1+\frac{1}{nK_a[Guest]_o}\right.\right.\right. \quad (eq.S1)$$

And the chemical shift change of H-d is list as follows:

Table S2. Chemical shift change of H-d.

Host/Guest	0.00657	0.01313	0.02627
Chemical Shift Change / ppm	-0.0052	-0.0091	-0.0185

The $M_{n,nmr}$ of FAM-HPG is ~ 72900 g/mol. From the fitting curve of chemical shift change *vs* Host/Guest ratio, we obtained the binding constant of FAM-HPG with RB in DMSO is approximately 7.63x10^6 M^{-1}, and the binding number of RB is 38.7 per hbPG.

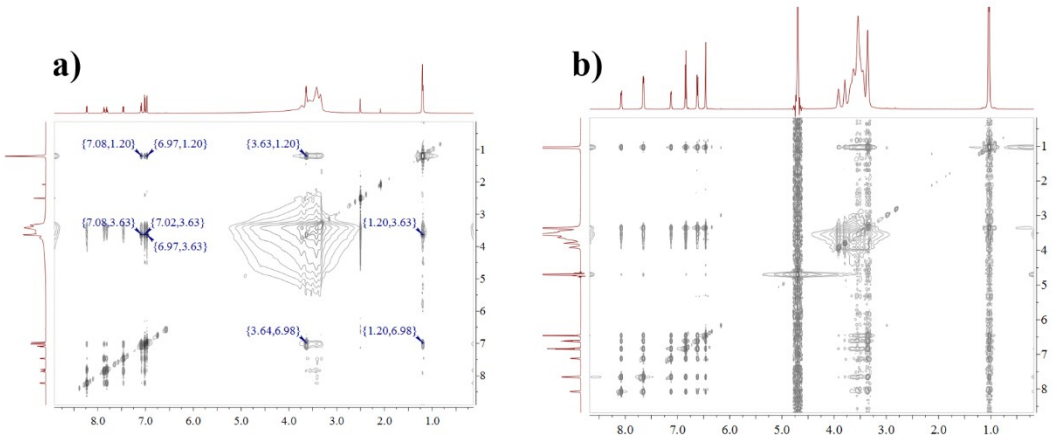


Figure S12. A). Full ^{1}H-^{1}H NOESY spectrum of FAM-HPG with Rhodamine B in d_6-DMSO. b) Full ^{1}H-^{1}H NOESY spectrum of FAM-HPG with Rhodamine B in D_2O.

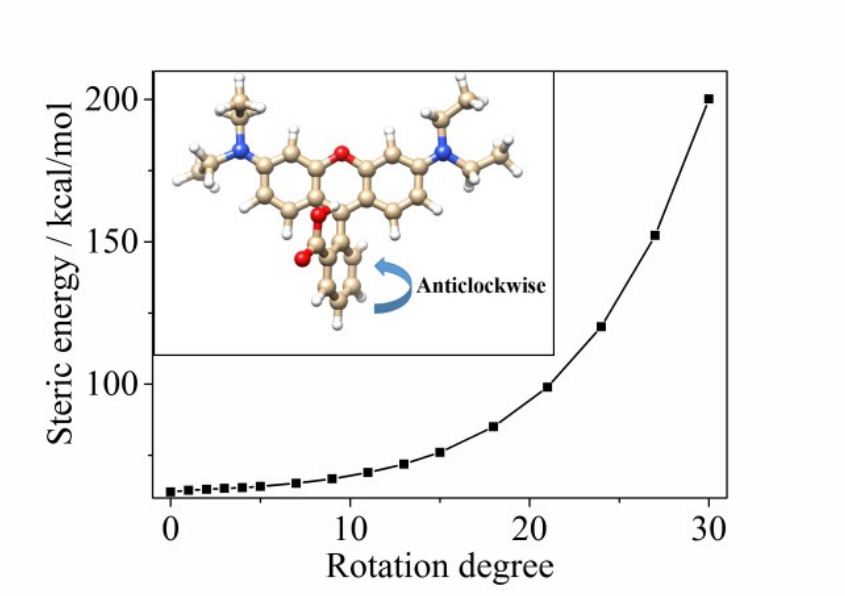


Figure S13. The steric energy of Rhodamine B (with chloride ion) depending on the rotation degree (in an anticlockwise direction). The dihedral angle of phenyl and xanthene ring is 115°, optimized by MM2 method.

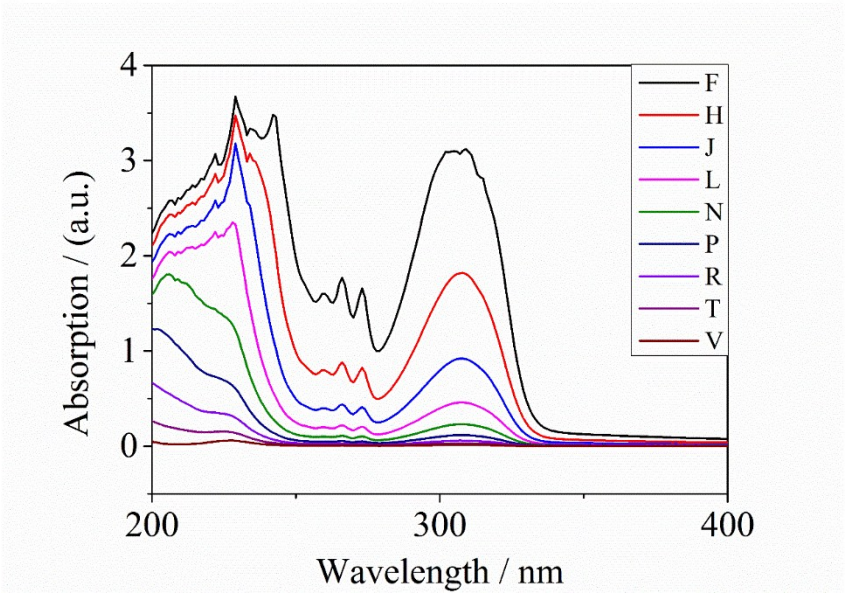


Figure S14. The UV/vis spectra of HPG aqueous from 50 mg/mL to 0.38 mg/mL. (F to V).

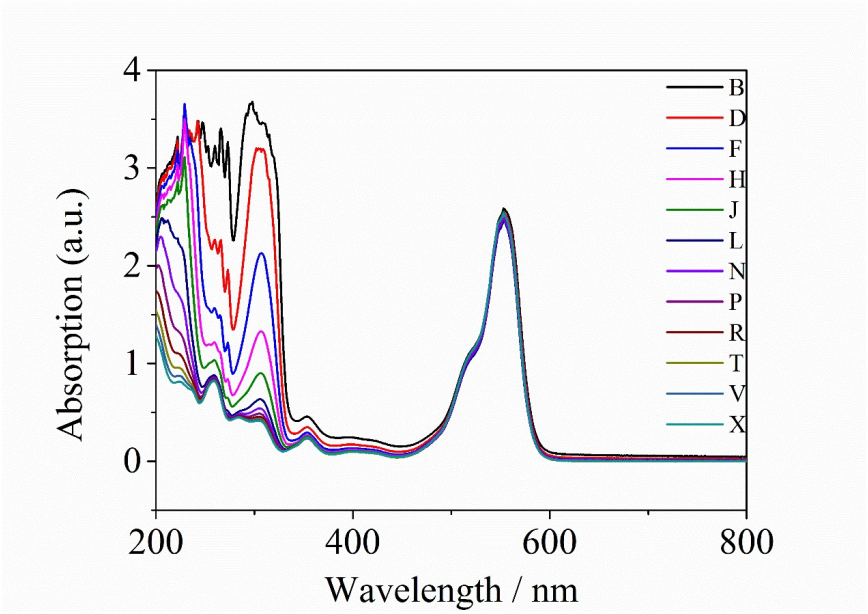


Figure S15. The UV/vis spectra of 8 ug/mL RB mixed with hbPGs solution (Concentration of hbPGs is from 50 mg/mL to 0.05 mg/mL, B to X).

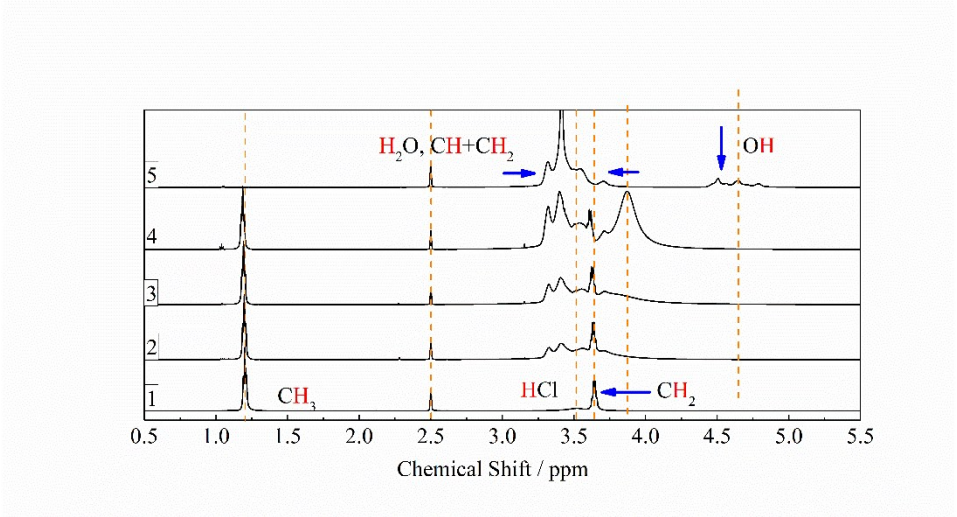


Figure S16. ^{1}H NMR titration of hbPGs with Rhodamine B in d_6-DMSO. hbPGs: Rhodamine B = Curve 1 (0 mg : 20 mg); Curve 2 (20 mg : 20mg); Curve 3 (40 mg : 20 mg); Curve 4 (80 mg:20 mg); Curve 5 (20 mg : 0 mg). Chemical shift region from 0.5 ppm – 5.5 ppm.

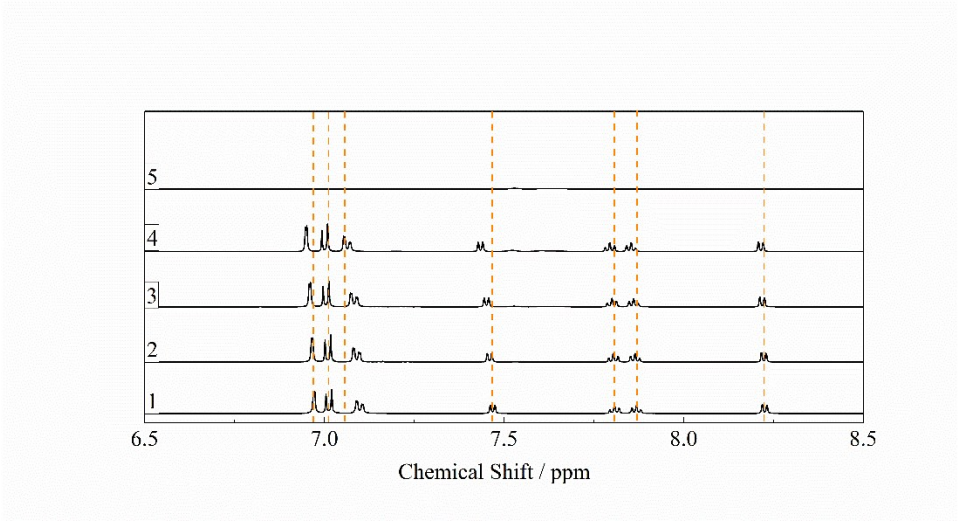


Figure S17. ^{1}H NMR titration of hbPGs with Rhodamine B in d_6-DMSO solution. hbPGs: Rhodamine B = Curve 1(0 mg: 20 mg); Curve 2 (20 mg : 20mg); Curve 3 (40 mg: 20 mg); Curve 4 (80 mg:20 mg); Curve 5 (20 mg : 0 mg). Chemical shift region from 6.5 ppm - 8.5 ppm.

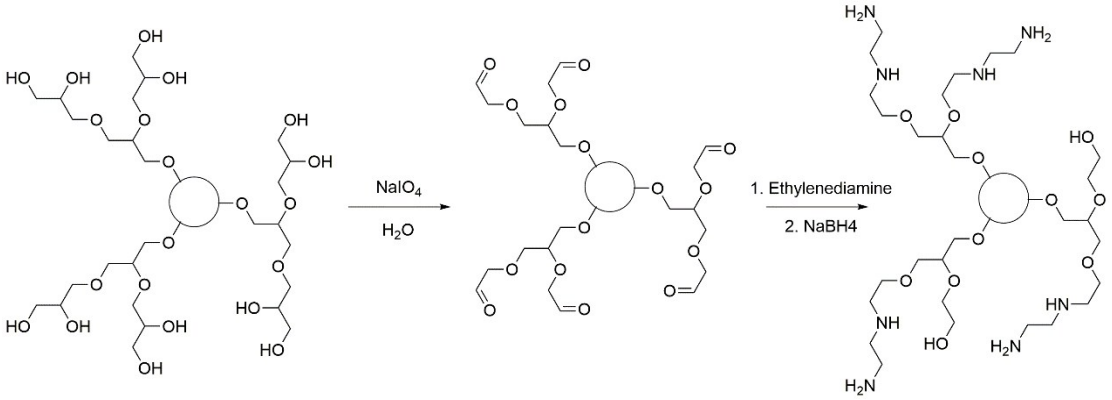


Figure S18. The synthesis protocol of surface modification through $NaIO_4$ oxidation and Borch reduction with ethylenediamine and $NaBH_4$. Through this process, the surface glycerol units are completely removed.

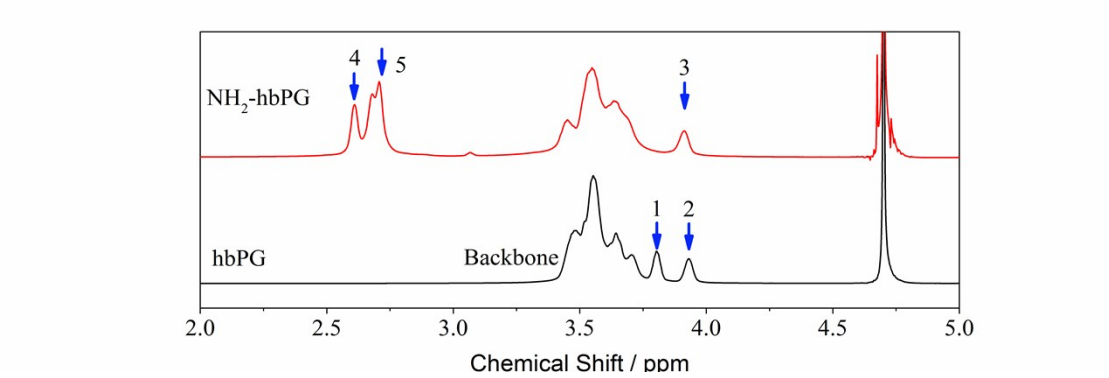


Figure S19. The ^{1}H NMR spectra of hbPGs and NH_2-hbPG in D_2O. The peak 1 and 2 are attributed to the surface glycerol units. The peak 3 are attributed to the primary alcohol which is originated from aldehyde reduction. Peak 5 are attributed to the methylene groups which is near

the secondary amine and peak 4 is attributed to the methylene groups which is near the primary amine. Note: The backbone of hbPG molecules is not modified by the process in Fig.S17.

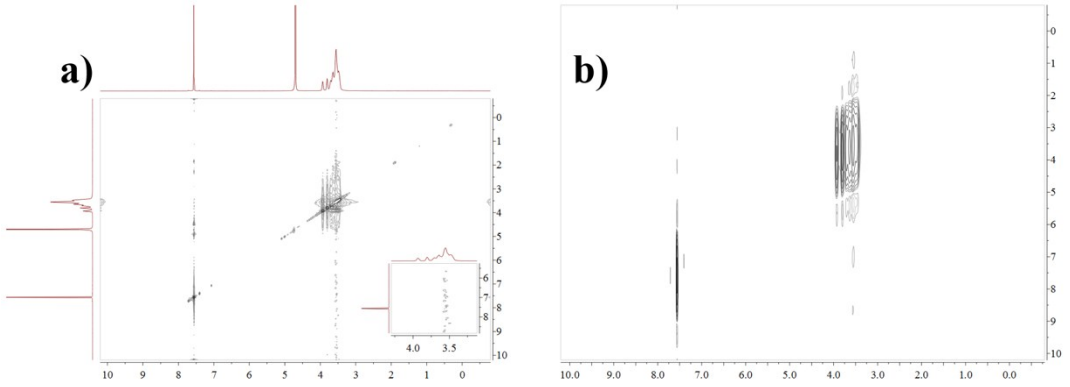


Figure S20. a). The ^{1}H-^{1}H NOESY spectrum of hbPGs with 5-Fu in D_2O, and the noise NOE signal indicates that few 5-Fu molecules are encapsulated by hbPGs molecules. b). The diffusion spectrum of hbPGs with 5-Fu.

The diffusion of hbPGs is not associated with 5-Fu, suggesting that the complex between 5-Fu and hbPGs is not formed.

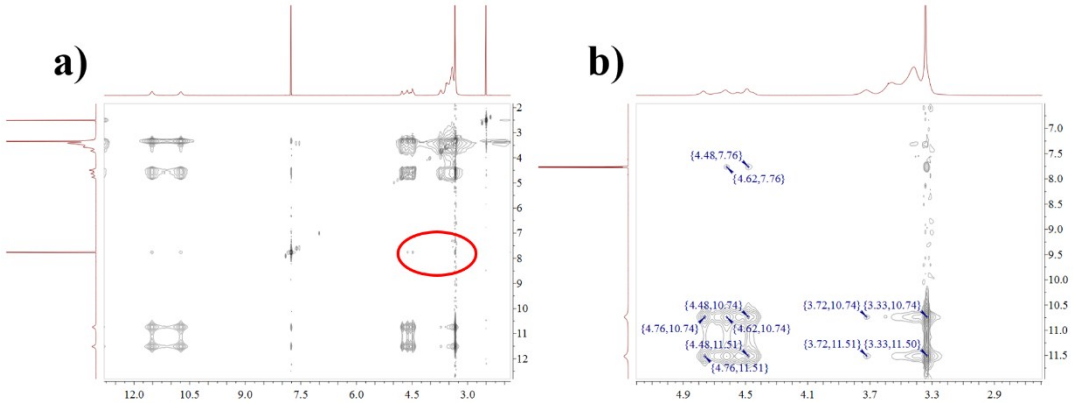
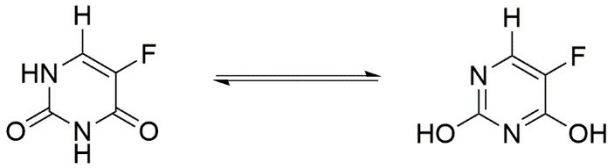


Figure S21. The ^{1}H-^{1}H NOESY spectra of hbPGs with 5-Fu in d_6-DMSO. The crossing peaks between amide groups with the backbone of hbPGs molecules indicate that 5-Fu molecules are uptaken by hbPGs molecules, and the encapsulated complex are formed. The interaction between hydrogen from the aromatic ring and the backbone of hbPGs molecules is weaker than amide groups, which is labeled in figure a (red circle). The interaction between amide groups and backbone of hbPGs molecules is not directly through NH bond since one of amide groups is very close to the aromatic hydrogen atom. As the previous literature reported[6], the isomer of 5-Fu is as follow:



The hydroxyl groups can strongly interact with oxygen atom from ether bond, and this interaction model is the main interaction type.

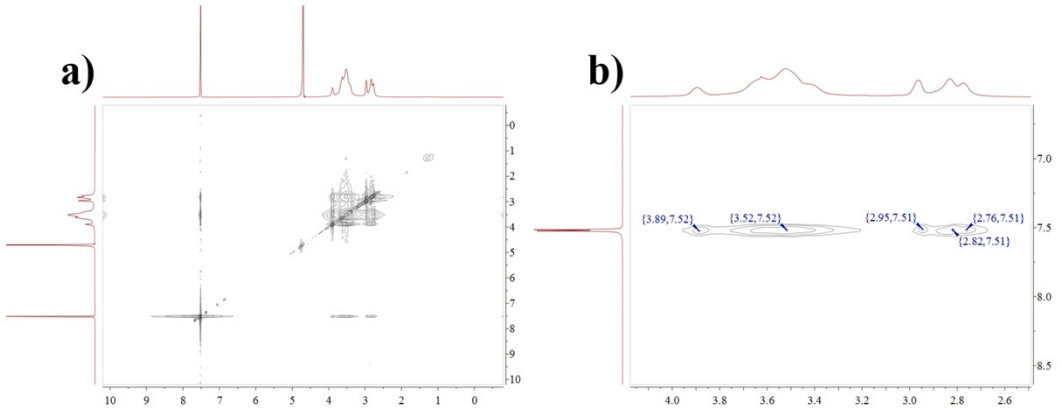


Figure S22. The ^{1}H-^{1}H NOESY spectra of 5-Fu with NH_2-hbPG in D_2O (Figure b is the expanded image between 5-Fu and backbone of hbPGs). The crossing peaks between aromatic hydrogen indicate that the 5-Fu molecules are up-taken by NH_2-hbPG molecules. Compared to the SI Fig.S19, the removing of surface glycerol units break the shell structure and induce the uptake of 5-Fu molecules.

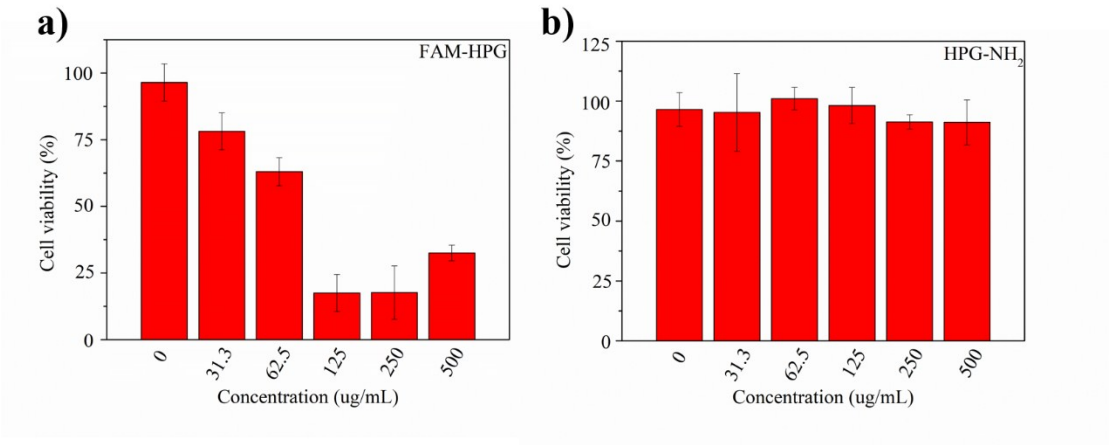


Figure S23. *In vivo* cytotoxicity measurements of HPG-NH_2 (a) and FAM-HPG (b).

Reference

[1] G. Kasza, G. Gyulai, Á. Ábrahám, G. Szarka, B. Iván, É. Kiss, *Rsc Advances* **2017**, *7*, 4348-4352.

[2] D. Hölter, A. Burgath, H. Frey, *Acta Polymerica* **1997**, *48*, 30-35.

[3] J. Schulte-Zweckel, F. Rosi, D. Sreenu, H. Schröder, C. M. Niemeyer, G. Triola, *Chemical Communications* **2014**, *50*, 12761-12764.

[4] T. Yin, Q. Zhang, H. Wu, G. Gao, J. G. Shapter, Y. Shen, Q. He, P. Huang, W. Qi, D. Cui, *NPG Asia Materials* **2017**, *9*, e383.

[5] T. Yin, H. Wu, Q. Zhang, G. Gao, J. G. Shapter, Y. Shen, Q. He, P. Huang, W. Qi, C. Zhang, *NPG Asia Materials* **2017**, *9*, e408.

[6] H. Lee, T. Ooya, *The Journal of Physical Chemistry B* **2012**, *116*, 12263-12267.