Electronic Supplementary Material (ESI) for Analytical Methods.

Preparation of polyacrylic acid surface-crosslinked strong fluorescent polymer nanoparticles and their sensitive *in vitro* imaging of cancer cells and long-life *in vivo* imaging of *in situ* tumor

Xue-Ping Cai[1,†], Huan-Huan He[1,†], Hai-Yang Ding[1], Xiao-Bo Chen[1], Tong-Hong Wei[1], Tian-mei Song[1], Si-Jia You[1], Hong-Ping Xie [1,*], Chun-Yan Min[1, 2 *]

(1.College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, China;

2. Suzhou institute for drug control, Suzhou 215104, China)

Supplementary Information

S1 Materials used in the Supplementary Information

BOC-glycine (99.0%), N, N'- dicyclohexyl carbodiimide (DCC, 99.0%), and CuCl (AR, 97%) were purchased from Aladdin in China. Pentamethyl diethylenetriamine (98%), ethyl-2-bromoisobutyrate (98%), 2-hydroxyethyl methacrylate (HEMA, 99.5%), and 5(6)-carboxy fluorescein (FAM, 97%) were purchased from J&K SCIENTIFIC. Ltd. 4-Dimethylaminopyridine (DMAP) was purchased from Sinopharm Chemical Reagent Co. Ltd. Other reagents were all in analytical grade.

S2 Synthesis of the strong hydrophilic polymer with rich branch amino groups

The method to prepare the strong hydrophilic polymer with rich branch amino groups was according to the literatures [1-2].

Synthesis of Monomer Boc-Amino-HEMA: 2.317 g of the N, N'- dicyclohexyl carbodiimide (DCC) was added into a 100 mL three-necked flask containing 20 mL ethyl

* Corresponding authors. E-mail addresses: hpxie@suda.edu.cn (H.P.Xie) and 944757490@qq.com (C.Y. Min)

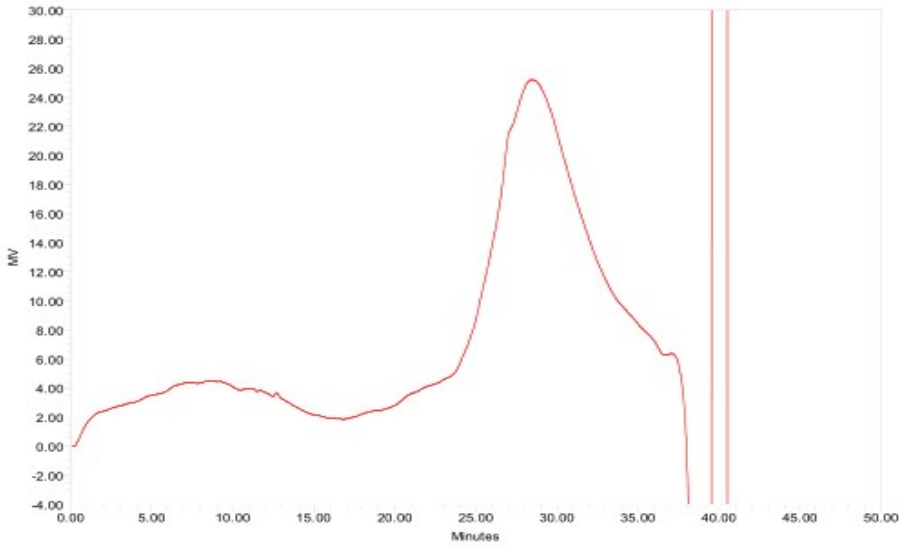
†Who had the equal contribution to this paper.

acetate and 11.232mmol HEMA under the protection of nitrogen. The flask was immersed in an ice-water bath. When the DCC completely dissolved, 1.97g Boc-Gly-OH dissolved in 10 mL ethyl acetate was added into the flask dropwise under magnetic stirring. Then 0.1374 g DMAP dissolved in 10 mL ethyl acetate was added into the above mixture. The mixture was reacted in the ice-water bath for 0.5 h and then at room temperature for 24 h. Filtered the reaction mixture and the filtrate was further purified by silica gel column chromatography using petroleum ether: ethyl acetate (2:3) as a developing solvent to get a colorless liquid, that is, the target monomer Boc-amino-HEMA.

Synthesis of Poly(Boc-amino-HEMA): This paper synthesized Poly(Boc-amino-HEMA) by atom transfer radical polymerization [3-5]. Typically, 0.74 g Boc-Amino-HEMA in 10 mL ethyl acetate was added into a 25 mL three-necked flask under continuous flow of nitrogen. Subsequently, 0.003g CuCl and 0.03mmoL PMDETA were added into the above mixture. The mixture was stirred at 25 °C for 15 min and the solution turned green and homogeneous. Then, the initiator EBIB (2.2 μL, 0.015mmol) was added via a degassed syringe. The reaction solution was kept at 80 °C for 24 h. The reaction solution was dissolved in THF and then further purified by alkali alumina column chromatography and precipitated in n-hexane subsequently. The precipitate was collected and dried under vacuum at 35 °C for 24 h to get a white powder. Then gained the polymer Poly(Boc-amino-HEMA).

Deprotection of Polymers. Typically, 0.5g poly(Boc-Gly-HEMA) was added into a 10mL flask containing 3mL trifluoroacetic acid and a magnetic stir bar. After the mixture was reacted under magnetic stirring for 1 h at room temperature, the reaction solution turned transparent and homogeneous. The product was precipitated by adding excess diethyl ether. After washing the precipitate by diethyl ether twice and drying it under vacuum at 40 °C for 8 h, the white powder was obtained, that is, the strong hydrophilic target polymers with rich branched amino groups. Its molecular weight was measured by

GPC (Fig.S1).



GPC Sample Results

	Sample Name	Inj	RT (min)	Mn	Mw	MP	Mz	Poly-dispersity
1	TBFSTM	1	28.406	17280	22577	23346	28284	1.307

图

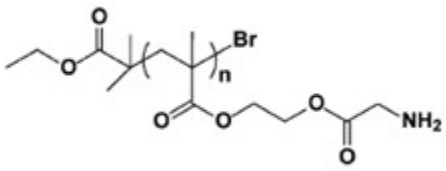


Fig. S1 GPC chromatogram and molecular structure of the synthesized branch amino polymer

S3 Optimization of conjugating ratio of FAM in the FAM-branched polymer

One fluorescent molecule used as signal tag usually has one hydrophobic fluorophore and one hydrophilic active group, which was used to label biomolecule. Here, 5(6)-carboxy fluorescein also has a similar structure. After its

hydrophilic carboxyl group was conjugated with the branch amino group of the as-synthesized hydrophilic polymer, the conjugated fluorophore would present some hydrophobicity, and then the hydrophobicity of FAM-conjugated hydrophilic polymer would be strengthened. As the FAM-conjugated polymer has enough solubility in an amphiphilic medium DMF, the more FAMs were conjugated on the as-synthesized strong hydrophilic polymer, the stronger the fluorescence of polymer was, and then the stronger ability of signal amplification the prepared fluorescent polymer nanoparticle had. Therefore, the strong hydrophilicity of the as-carrier polymer would be beneficial to conjugate a large number of FAM molecules.

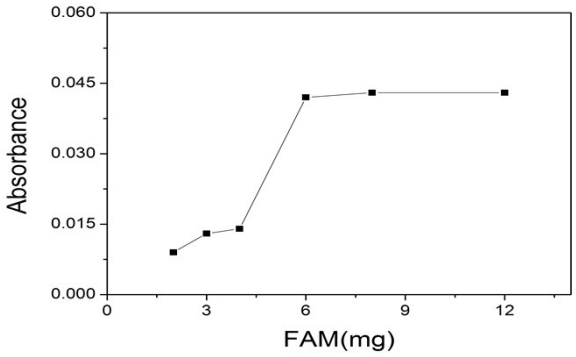


Fig.S2 Absorbance of the fluorescent polymers at 452nm
for different amount of the conjugated FAM

Based on the chemical reaction between the carboxylic groups of FAM and the branch amino groups of the polymers, the strong fluorescent polymer with some extent hydrophobicity was synthesized. As the FAM-conjugated polymer has enough solubility in DMF, the conjugating ratio of FAM has been optimized based on the characteristic absorption of the fluorophore in the FAM-conjugated polymer at 452 nm (Fig.S2). As shown in Fig.S2, for a certain amount of the strong

hydrophilic polymers, the absorptions of the as-synthesized fluorescent polymers at 452 nm gradually increased with the conjugated amount of FAM. After the conjugated amount was over 6mg, there was no further change of the absorptions at 452 nm. It suggested that the conjugating ratios of FAM reached a maximum. At this time, the FAM-conjugated polymer still has a good solubility in DMF. Therefore, the optimum conjugating ratio of FAM in fluorescent polymer has been obtained, and the FAM-branched polymer was prepared.

References

[1] Sun, H. Y.; Gao, C. Biomacromolecules **2010**, 11, 3609-3616.

[2] Ji, W. H.; Panus, D.; Palumbo, P. N.; Tang, R. P.; Wang, C. Biomacromolecules **2011**, 12, 4373-4385.

[3] Matyjaszewski, K. Macromolecules **2012**, 45, 4015-4039.

[4] Li, J. H.; Tan, D. S.; Zhang, X. Q.; Tan, H.; Ding, M. M.; Wan, C. X.; Fu, Q. Colloids Surf. B. **2010**,78, 343-350.5

[5] Erkoc, S.; Mathias, L. J.; Acar, A. E. Macromolecules **2006**, 39, 8936-8942.