Supplementary Information For

The synthesis of poly(ethylene glycol)-b-poly(N-2-hydroxypropyl methacrylamide) block copolymers with well-defined structure and its influence on *in vivo* circulation and biodistribution

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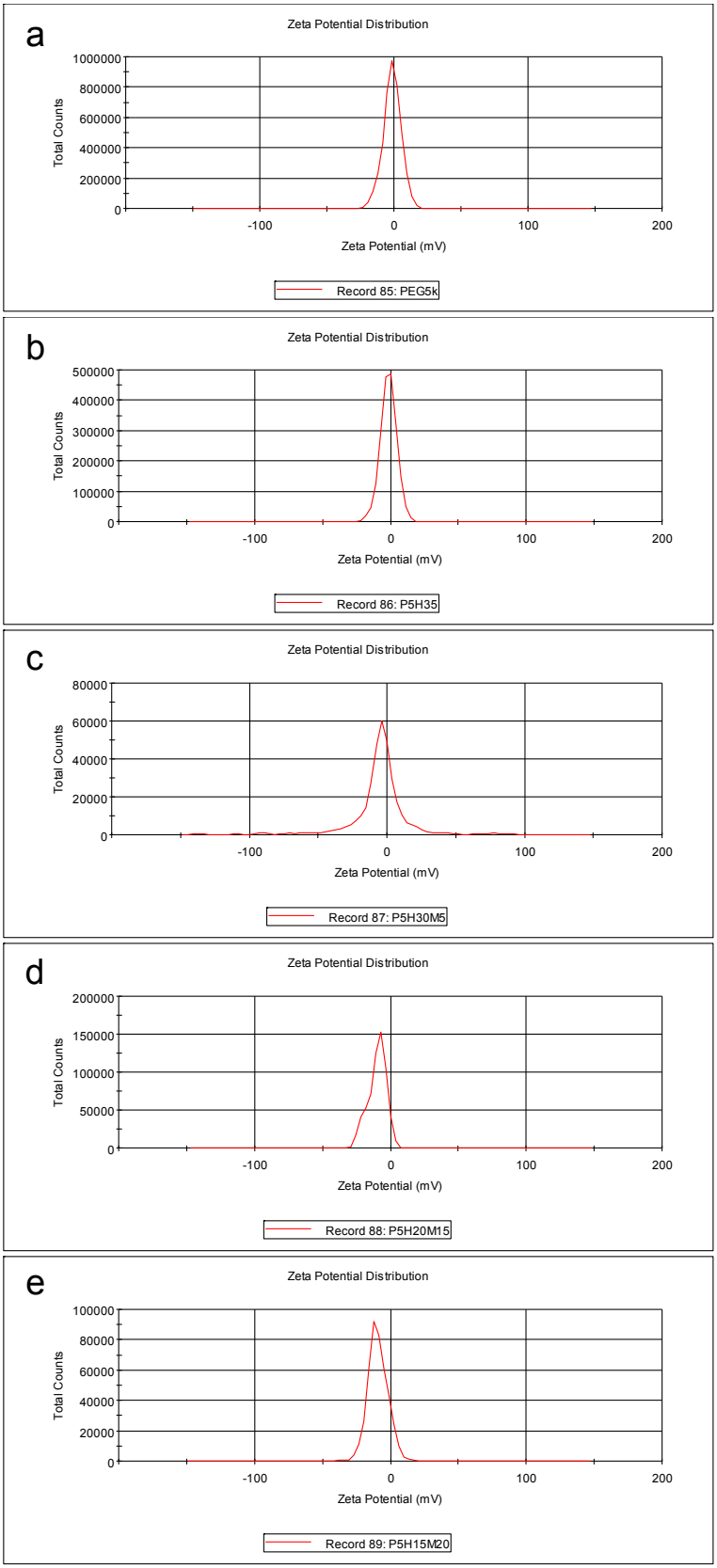
Figure S1. Zeta potentials of copolymers with different negative charge. (a)PEG5k, (b)P5H35, (c)P5H30M5, (d)P5H20M15, (e)P5H15M20, (f)P5M35, (g)P2M40, (h)P2MAA40.

Figure S2. Representative ITLC chromatograms of ^{125}I labeled P5H30M5. The original kit (a), sample after purification (b) and sample kept at room temperature in saline for 48h (c).

Figure S3. The liver cell uptake at 0h, 1h and 24h after i.v. injection of 100μl P2M40 (1mg/ml). Goat anti rabbit IgG1 PE was used as isotype control. The hepatocytes, LSEC and KC were stained by ASGR1 PE, MCAM PE and F4/80 PE, respectively.

Figure S4. The folw cytometric histograms of P5H35 (A), P5H30M5 (B), P5H20M15 (C), P5H15M20 (D), P5H5M30 (E), P5M35 (F), P2M40 (G) and P2MAA40 (H) internalized by NCTC-1469 cells.

Figure S5. The fluorescence spectra of copolymers with different amount of negative charge.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Zeta Potential Distribution
Total Counts
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800000
600000
400000
200000
0
-100
0
100
200
Zeta Potential (mV)
Record 85: PEG5k
b
Zeta Potential Distribution
Total Counts
500000
400000
300000
200000
100000
0
-100
0
100
200
Zeta Potential (mV)
Record 86: P5H35
c
Zeta Potential Distribution
Total Counts
80000
60000
40000
20000
0
-100
0
100
200
Zeta Potential (mV)
Record 87: P5H30M5
d
Zeta Potential Distribution
Total Counts
200000
150000
100000
50000
0
-100
0
100
200
Zeta Potential (mV)
Record 88: P5H20M15
e
Zeta Potential Distribution
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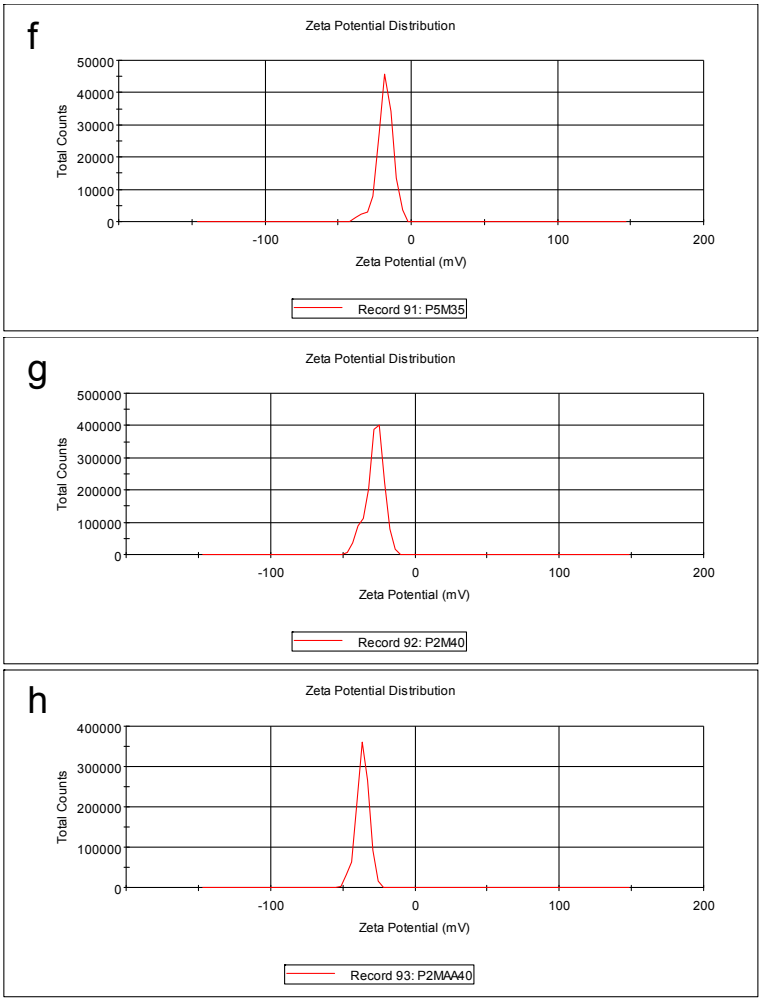

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The zeta potentials of copolymers were determined according to the literature procedures.[1] Sample concentration was 1 mg/mL in 10 mM NaCl with a pH value of 7.4. The sample was transferred to a zeta cell (DTS1060C, Malvern Instruments) and measured at 25°C using a Malvern ZetaSizer Nano ZS and an applied voltage of 120 V. From above results, we can observe the same tendency as the CE results, with the increase of MAGG content, the zeta potential became more negative accordingly. This indicated that the negative charge could be precisely controlled during the synthetic procedure.

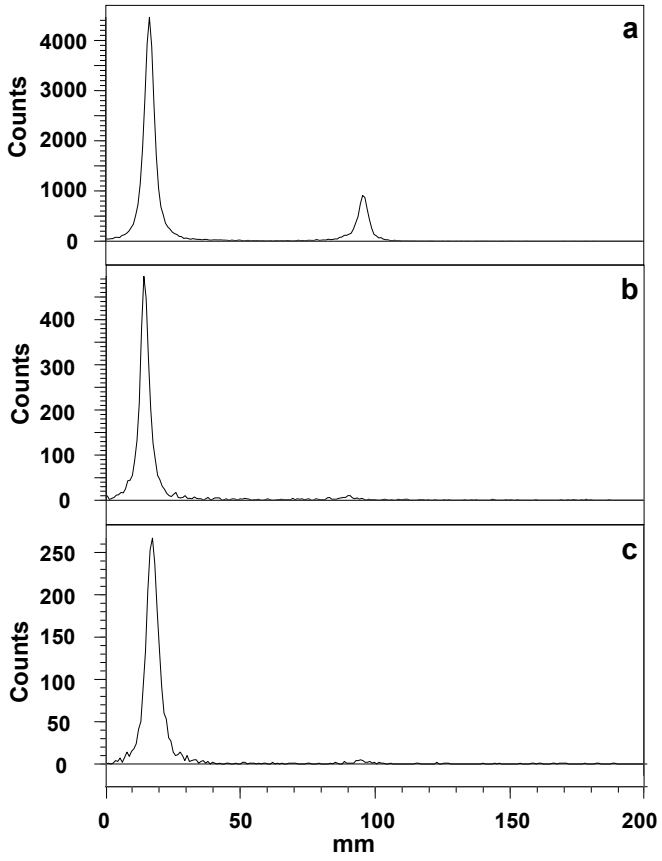


Figure S2. Representative ITLC chromatograms of ^{125}I labeled P5H30M5. The original kit (a), sample after purification (b) and sample kept at room temperature in saline for 48h (c). Free ^{125}I migrate to the solvent front whereas ^{125}I labeled copolymer remained at the origin.

The radiolabeling efficiency for all copolymers were greater than 90%, and the radiochemical purity (RCP) (for example ^{125}I labeled P5H30M5) after PD MiniTrap column purification was higher than 99%. For all radiolabeled copolymers, it was found that their RCP values were still above 90% after purification for 48h.

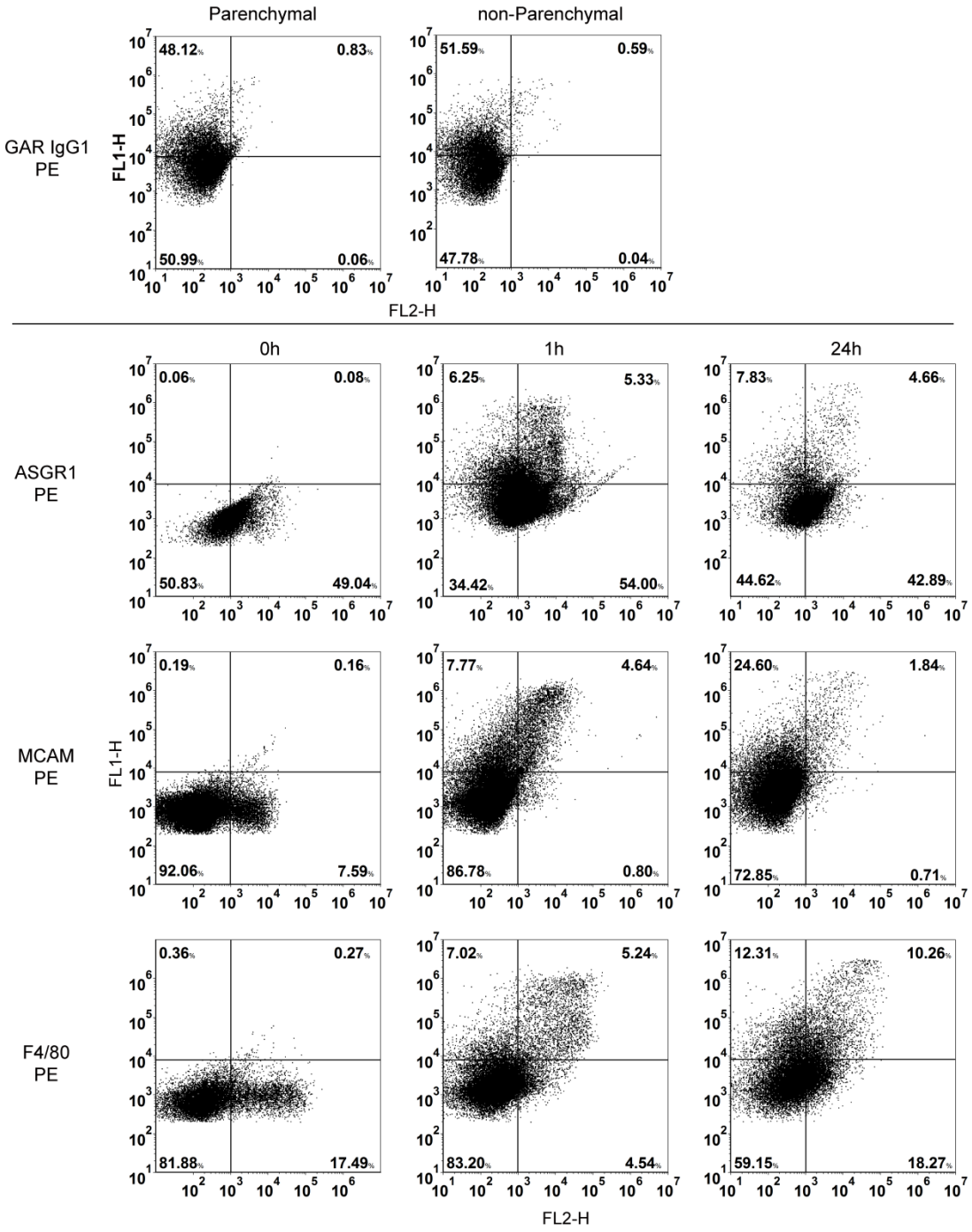
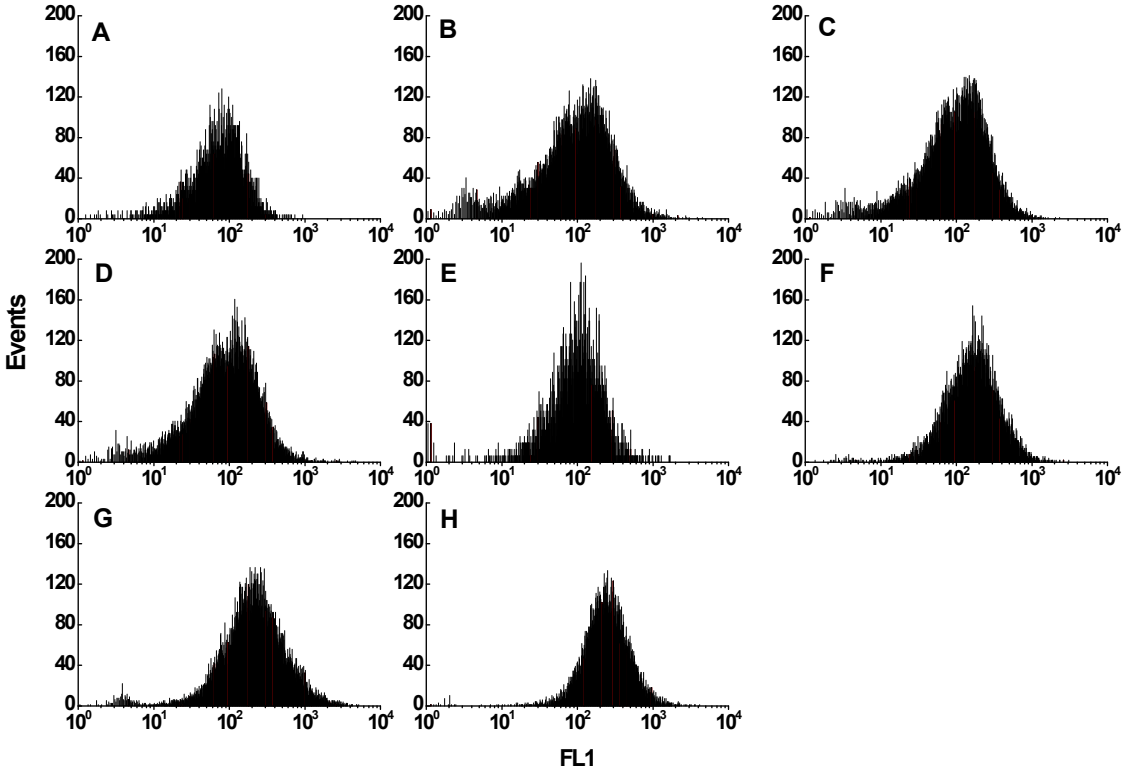


Figure S3. The liver cell uptake at 0h, 1h and 24h after i.v. injection of 100µl P2M40 (1mg/ml). Goat anti rabbit IgG1 PE was used as isotype control. The hepatocytes, LSEC and KC were stained by ASGR1 PE, MCAM PE and F4/80 PE, respectively.

In order to determine the intracellular distribution of the negatively charged copolymer, the liver tissue was digested by collagenase type IV and incubated with fluorescent antibodies. These cells were analyzed by flow cytometer. Figure S2 shows the proportion of P2M40 within hepatocytes, Kupffer (KC) and endothelial (LSEC) cells over a period of 24h after a 10mg/kg i.v. bolus dose. The upper quadrant of FL1 fluorescence reflected the uptake of P2M40. The right quadrant of FL2 in ASGR1 PE, MACM PE and F4/80 PE rows represented the hepatocytes,[2] LSEC[3] and KC,[4]

respectively.

From Figure S2, it was found that P2M40 was taken up by both parenchymal and nonparenchymal cells after 1h dosing. The substantial differences in the proportion of cells containing P2M40 (the ratio of the upper right quadrant to the right quadrant) were evident, i.e. only 9% of the hepatocytes endocytosed the copolymer. In the contrast, LSEC contained a greater proportion of 85% while KC showed a proportion of 53%. The proportion decreased to 72% and 36%, respectively for LSEC and KC after 24 h. These results indicated that the RES system played an important role in the uptake of P2M40. It has been reported that clearance capacity of KCs, LSEC, and parenchymal cells is heavily dependent on the nature of the substance used.[5] Although the uptake of P2M40 by mouse heptocytes is lower than KC and LSEC, it may also make an important contribution to the clearance of P2M40 if considering the amount of the hepatocytes in liver tissue.



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To investigate the influence of negative charge on the hepatocytes endocytosis, the copolymers with different net charge were coincubated with murine hepatocytes (NCTC-1469). Then the cells were analyzed by flow cytometers. Figure S3 represents the flow cytometric histograms of NCTC-1469 cells treated by copolymers with different charge (A-H). The mean mean values of cell associated fluorescence from three parallel experiments calibrated by the fluorescence spectra as shown in Figure S4

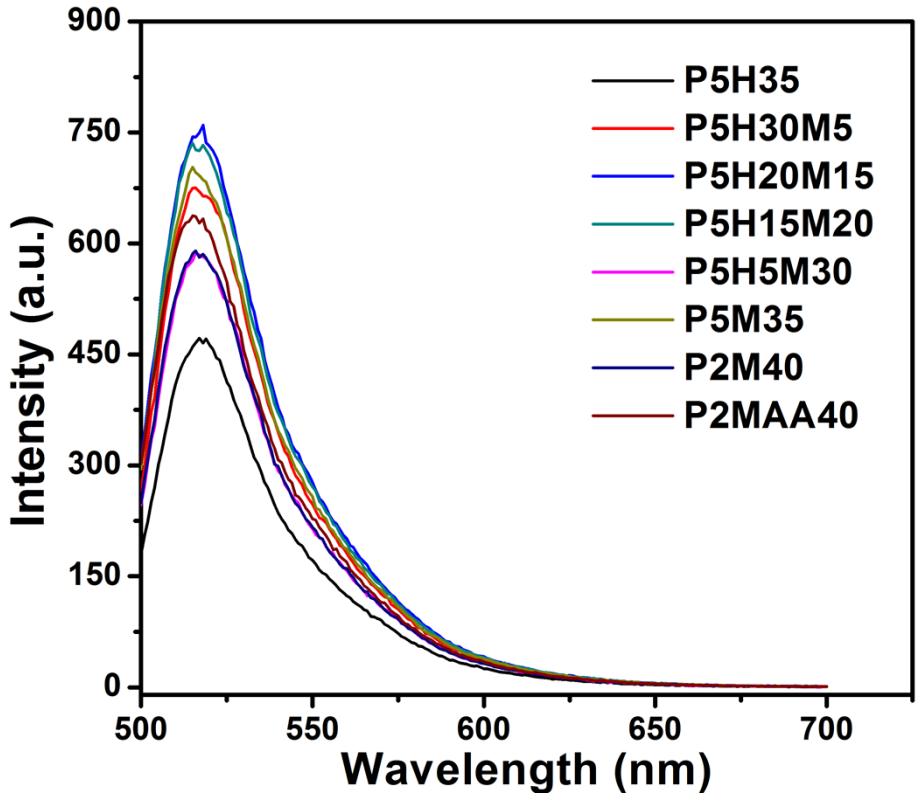

Figure S5. The fluorescence spectra of copolymers with different amount of negative charge.

References

1. J. D. Clogston and A. K. Patri, in *Characterization of Nanoparticles Intended for Drug Delivery*, Springer, 2011, pp. 63-70.
2. P. H. Weigel, *Journal of Biological Chemistry*, 1980, **255**, 6111-6120.
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