

Supplemental File

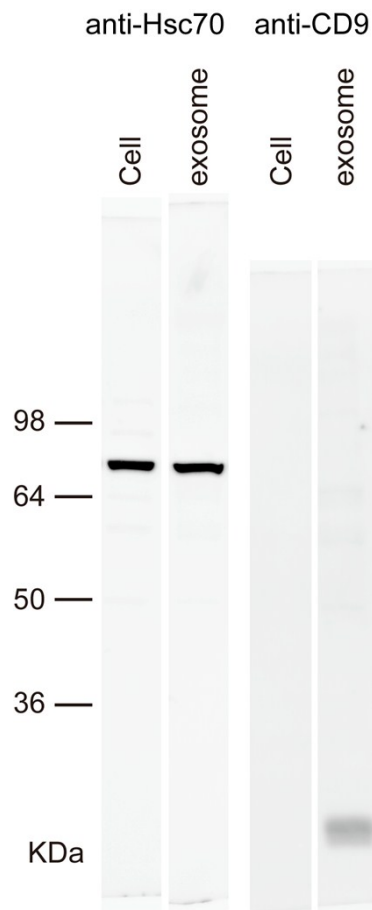
**Nanogel hybrid assembly for exosome intracellular delivery: Effects to endocytosis and fusion  
by exosome surface polymer engineering**

Shin-ichi Sawada, Yuko T. Sato, Riku Kawasaki, Jun-ichi Yasuoka, Ryosuke Mizuta, Yoshihiro  
Sasaki, and Kazunari Akiyoshi\*

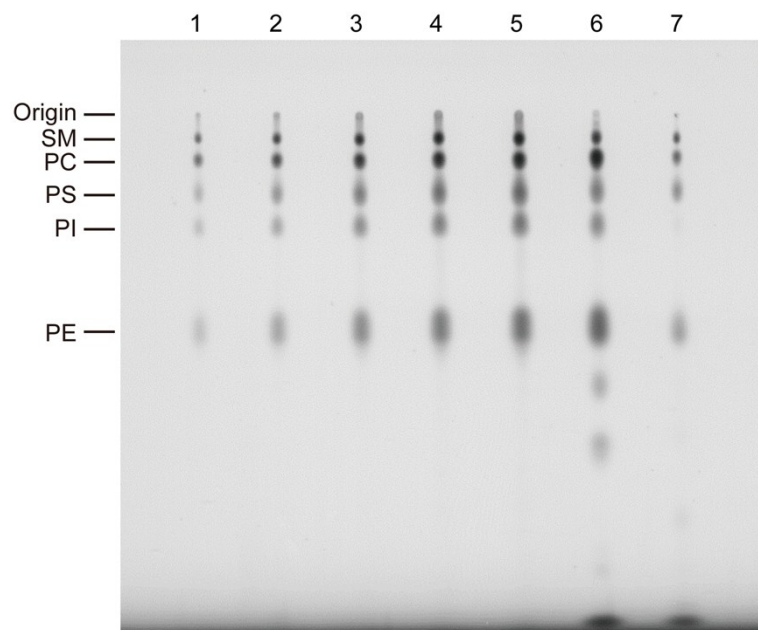
Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura,  
Nishikyo-ku, Kyoto 615-8510, Japan.

\*Address correspondence to: [akiyoshi@bio.polym.kyoto-u.ac.jp](mailto:akiyoshi@bio.polym.kyoto-u.ac.jp)

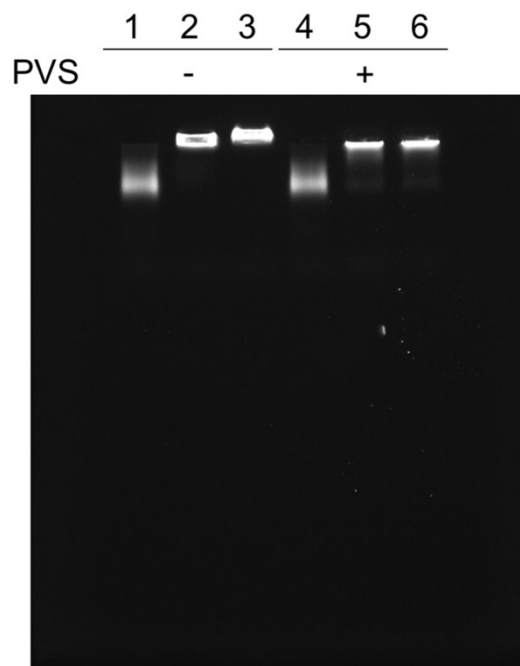
Supplementary Information



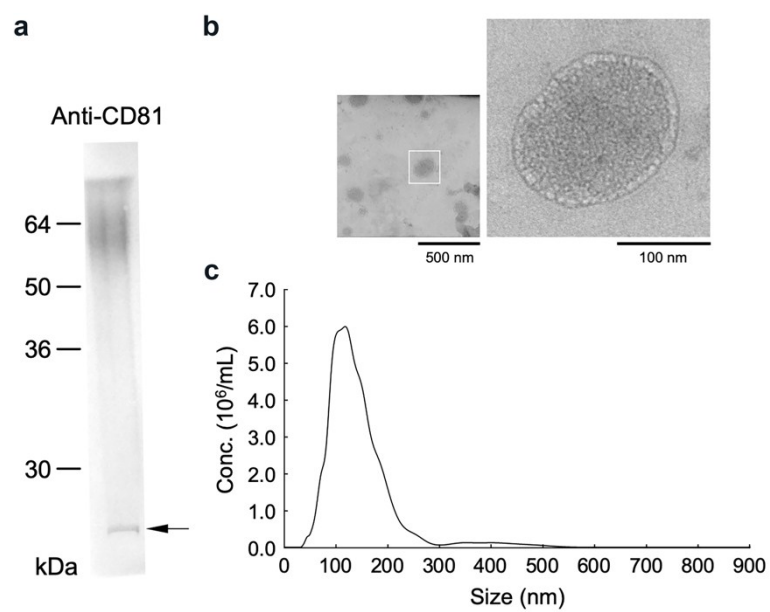
**Fig. S1** Full length of image of western blotting for Fig. 2c.



**Fig. S2** TLC chromatograms of lipids in Raw264.7 cell-derived exosomes and Raw264.7 cells. Lanes 1–5 contain lipids standard solution spotted in 0.2, 0.5, 1.0, 1.5 and 2.0  $\mu\text{g}$ , respectively. Lane 6 contain extracted sample from Raw264.7 cells. Lane 7 contain extracted sample from Raw264.7 cell-derived exosomes.



**Fig. S3** Evaluation of CHPNH<sub>2</sub> nanogel/CFSE-Raw-exosome hybrids formation by agarose gel (0.5%) electrophoresis with 40 mM Tris-acetate buffer (pH7.4) at 50V for 30 min under cooling (approximately 10–15°C), in the presence or absence of 6.7 mol % polyvinyl sulfate sodium salt (PVS) unit relative to the amino group moles of CHPNH<sub>2</sub>. Lane 1, 4: CFSE-Raw-exosome, Lane 2, 5: CHPNH<sub>2</sub> nanogel/CFSE-Raw-exosome hybrid (1wt:1wt), Lane 3, 6: CHPNH<sub>2</sub> nanogel/CFSE-Raw-exosome hybrid (2.5wt:1wt).



**Fig. S4** The characterization of PC12-derived exosomes. (a) Western blot analysis for CD81 (26 kDa) protein in PC12-derived exosomes. (b) Morphological observation by TEM. Scale bar represents 500 or 100 nm respectively. (c) Size distribution profile of exosomes determined by NTA.

**Table. S1** The size and  $\zeta$ -potential of CHPNH<sub>2</sub> nanogel/Raw264.7 cell-derived exosome hybrids.

Storage time <sup>a</sup>	Raw exosome (Raw exo)		Nanogel/Raw exo hybrid (1:1) <sup>c</sup>	
	Size $\pm$ S.D. (nm) <sup>b</sup>	$\zeta$ -potential $\pm$ S.D. (mV) <sup>b</sup>	Size $\pm$ S.D. (nm) <sup>b</sup>	$\zeta$ -potential $\pm$ S.D. (mV) <sup>b</sup>
0 h	157 $\pm$ 3	-11.9 $\pm$ 0.9	170 $\pm$ 11	-4.1 $\pm$ 0.8
4 h	162 $\pm$ 8	-11.0 $\pm$ 0.4	175 $\pm$ 10	-3.4 $\pm$ 1.1
24 h	156 $\pm$ 9	-12.1 $\pm$ 0.8	175 $\pm$ 12	-4.0 $\pm$ 0.5

<sup>a</sup> Samples were stored in PBS at 37°C. <sup>b</sup> Determined by NTA (Nano sight) and Zetasizer, respectively (n=3). <sup>c</sup> In parentheses indicates the weight ratio of nanogel to exosome.

**Table. S2** The size and  $\zeta$ -potential of CHPNH<sub>2</sub> nanogel/PC12 cell-derived exosome hybrids.

	Size $\pm$ S.D. (nm) <sup>a</sup>	$\zeta$ -potential $\pm$ S.D. (mV) <sup>a</sup>
PC12 exosome (PC12 exo)	145 $\pm$ 11	-11.5 $\pm$ 3.1
Nanogel/PC12 exo hybrid (1:1) <sup>b</sup>	161 $\pm$ 3	0.4 $\pm$ 2.2
Nanogel/PC12 exo hybrid (2.5:1) <sup>b</sup>	166 $\pm$ 7	0.4 $\pm$ 1.0

<sup>a</sup> Determined by NTA (Nano sight) and Zetasizer, respectively (n=3). <sup>b</sup> In parentheses indicates the weight ratio of nanogel to exosome.