Electronic Supplementary Information

Imaging plasma membranes without cellular internalization: multisite membrane anchoring reagents based on glycol chitosan derivatives

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^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy analysis

The ^{1}H NMR spectra were recorded using a Bruker Avance AV-300 spectrometer operating at 300 MHz and 70^{o}C. Samples were prepared at 10 mg/mL in 2% DCl/D_2O. Chemical shifts are expressed in ppm relative to 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid (TSP) as an internal standard. Degree of deacetylation (DDA) of glycol chitosan was determined by ^{1}H NMR according to the work of Lavertu et al.,[1] and the measurement condition was set to allow the shift of solvent proton peak, leading to the visibility of the H-1 peak (Fig. S1). DDA was calculated to be 88% using the integral intensity of the proton H1 on deacetylated monomer (H1-D) and the three

protons of methyl group (-CH_3):

$$\mathrm{DDA}(\%) = \left(\frac{\mathrm{H1-D}}{\mathrm{H1-D+Me/3}}\right) \times 100\% \quad (1)$$

The calculated value of DDA is 88%, in good agreement with the result reported by Pereira et al.[2]

The successful incorporation of PEG2000-cholesterol to the glycol chitosan was confirmed by the presence of characteristic peaks between 0.85 and 1.27 ppm assigned to the high field signal of cholesterol and at 3.68 ppm for PEG protons (Fig. S2).

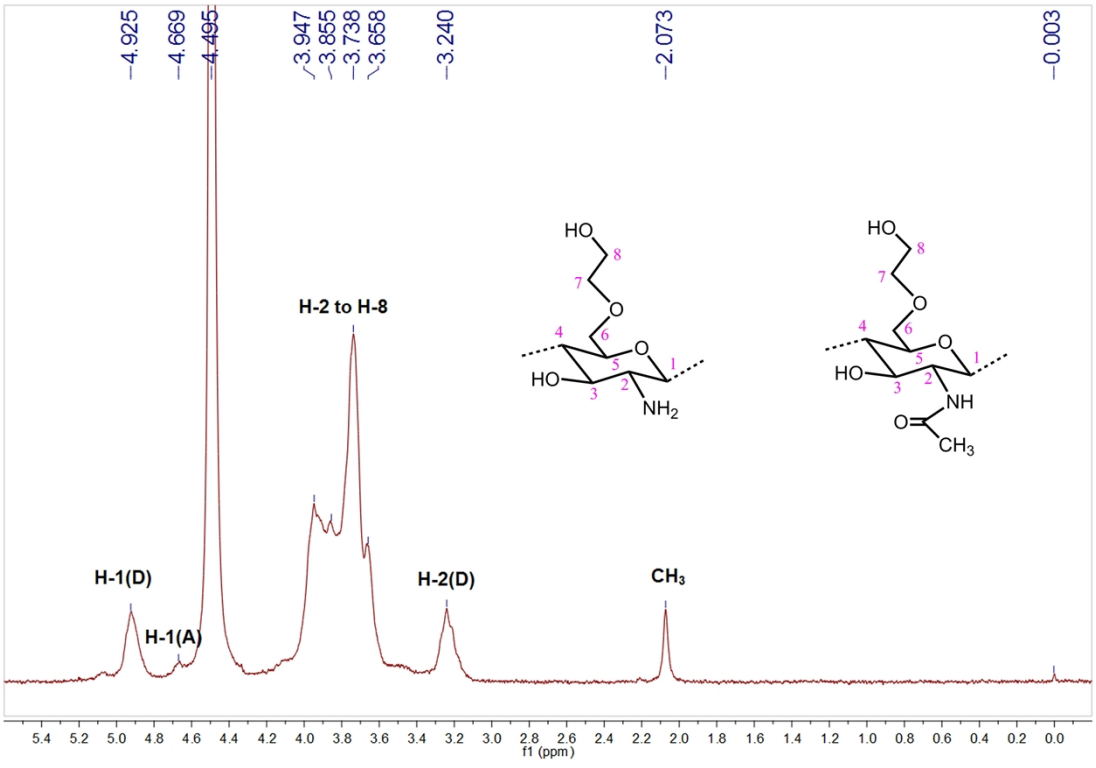

Fig. S1. ^{1}H NMR spectrum of glycol chitosan in 2% DCl/D_2O at 70°C. Acetylated and deacetylated monomers of glycol chitosan were schematically illustrated and abbreviated separately as "A" and "D", respectively.

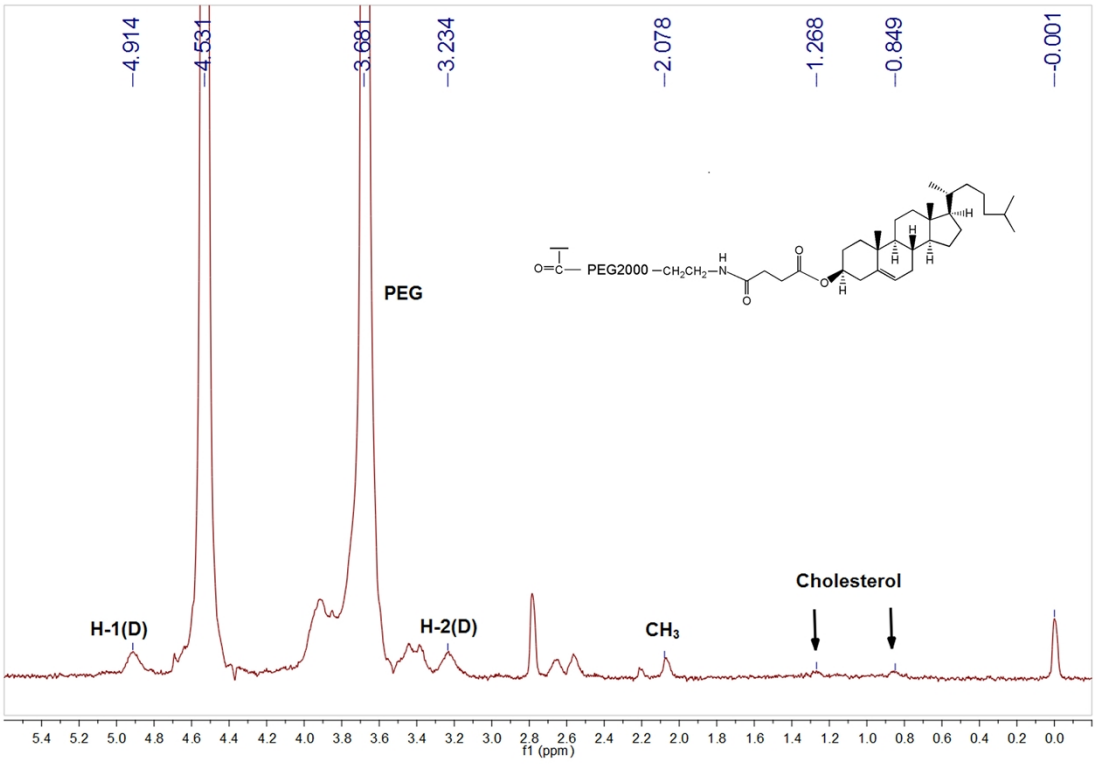

Fig. S2. ^{1}H NMR spectrum of glycol chitosan-30% cholesterol in 2% DCl/D_2O at 70^{o}C.

Size calculation of glycol chitosan

According to a reference (*Carbohydrate Polymers* **2011**, *84*, 1237−1243), for molecular weight 67 K, (highest M 90 K, lowest M 49 K):

Kuhn length: $l_k = 16.0\,\text{nm}$

Persistence length: $L_p = 8.0\,\text{nm}$

The molar mass per unit of contour length:

$$M_L = \frac{F_A \cdot M_{GlcNAc} + (1 - F_A) \cdot M_{GlcN}}{b} = \frac{0.12 \times 203 + 0.88 \times 221}{0.515} = 391\,\text{g/(mol} \cdot \text{nm)}$$

where M_{GlcNAc} and M_{GlcN} are the monomer weights of intra-chain *N*-acetyl glucosamine (203 g/mol) and glucosamine (acetate salt, 221 g/mol) residues, respectively; F_A is the mole fraction of acetylation; b is the average bond length.

Or according to the textbook (Rubinstein, M.; Colby, R. H. Polymer Physics. Oxford

University Press, USA 2003).

Contour length can be calculated as $L = \frac{Mw}{M_L} = \frac{67000}{391} = 171\,\text{nm}$

Kuhn unit number can be calculated as $N_k = \frac{L}{l_k} = 10.7$

Supposing a random-walk conformation, the radius of gyration is:

$\left\langle R_g^2 \right\rangle = \frac{N_k l_k^2}{6} = \frac{10.7 \times 16^2}{6} = 456.5\,\text{nm}^2$, $\left\langle R_g \right\rangle = 21.3\,\text{nm}$

Supposing the chain is semi-flexible, the radius of gyration is:

$$\left\langle R_g^2 \right\rangle = \frac{l_k L}{6} - \frac{l_k^2}{4} + \frac{l_k^3}{4L} \cdot \left[1 - \frac{l_k}{2L} \cdot \left(1 - e^{-\frac{2L}{l_k}} \right) \right]$$

$$= \frac{16 \times 171}{6} - \frac{16^2}{4} + \frac{16^3}{4 \times 171} \times \left[1 - \frac{16}{2 \times 171} \cdot \left(1 - e^{-\frac{2 \times 171}{16}} \right) \right] = 456 - 64 + 6.0 \times \left[1 - 0.05 \right] = 398\,\text{nm}$$

$\left\langle R_g \right\rangle = 19.9\,\text{nm}$

Thus the diameter (2 $\langle R_g \rangle$) of glycol chitosan is around 40 nm, which is in good agreement of the DLS result of glycol chitosan as shown in Fig. S3 below:

Fig. S3. Hydrodynamic diameter of glycol chitosan measured by DLS.

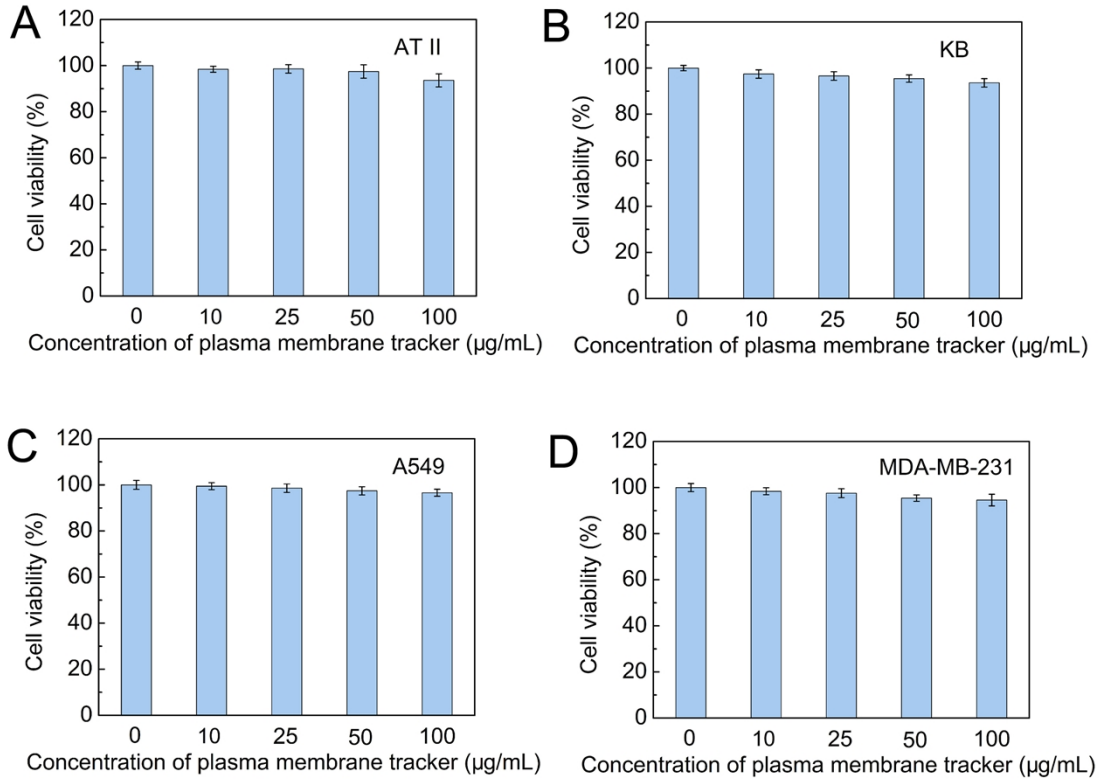

Fig. S4. Cytotoxicity of glycol chitosan-30% cholesterol-2% FITC on AT II (A), KB (B), A549 (C) and MDA-MB-231 (D) cells determined by MTT assay. The cells were exposed with tested reagents for 36 h.

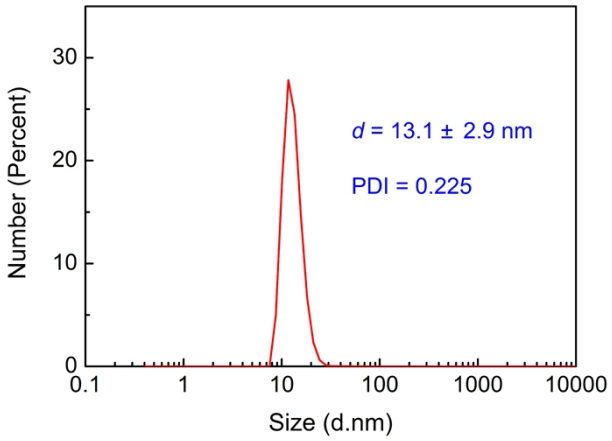

Fig. S5. Hydrodynamic diameter of glycol chitosan-2% FITC measured by DLS.

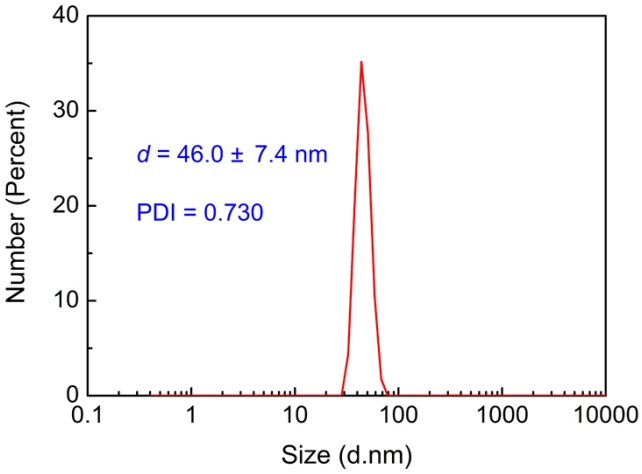

Fig. S6. Hydrodynamic diameter of glycol chitosan-30% PEG-2% FITC measured by DLS.

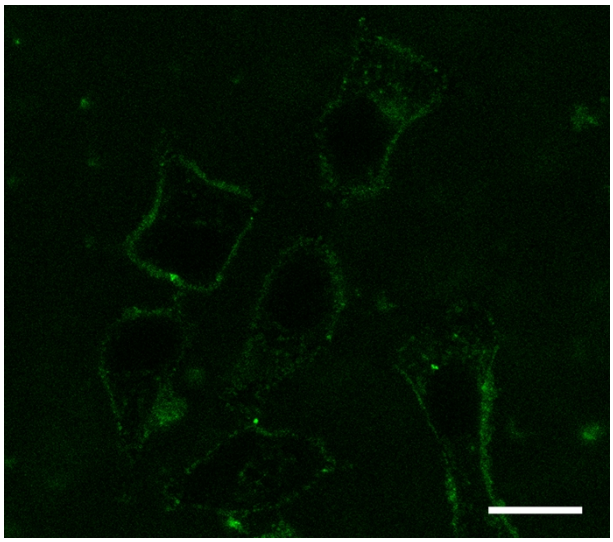

Fig. S7. Confocal fluorescence image of AT II cells incubated with cholesterol-free compound glycol chitosan-30% PEG-2% FITC for 5 min. Scale bar is 20 μm.

Fig. S8. Hydrodynamic diameter of glycol chitosan-30% cholesterol-10% FITC measured by DLS.

References

(1) M. Lavertu, Z. Xia, A. N. Serreqi, M. Berrada, A. Rodrigues, D. Wang, M. D. Buschmann and A. Gupta, *J. Pharm. Biomed. Anal.*, 2003, **32**, 1149–1158.

(2) P. Pereira, D. Morgado, A. Crepet, L. David and F. M. Gama, *Macromol. Biosci.*, 2013, **13**, 1369–1378.