Supporting Information for

Improved atomic force microscopy stiffness measurements of nanoscale liposomes by cantilever tip shape evaluation

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Abstract of SI: Evaluation of AFM cantilever tip shapes by using line structures of the AS100P-D silicon grating (Figure S1); Comparison of statistical liposome stiffness obtained with different cantilever sets (Figure S2); Stiffness measurements of DOPC-based liposomes and DSPC-based liposomes (Figure S3); Stiffness measurements of POPC/Chol (50/50) liposomes (Figure S4).

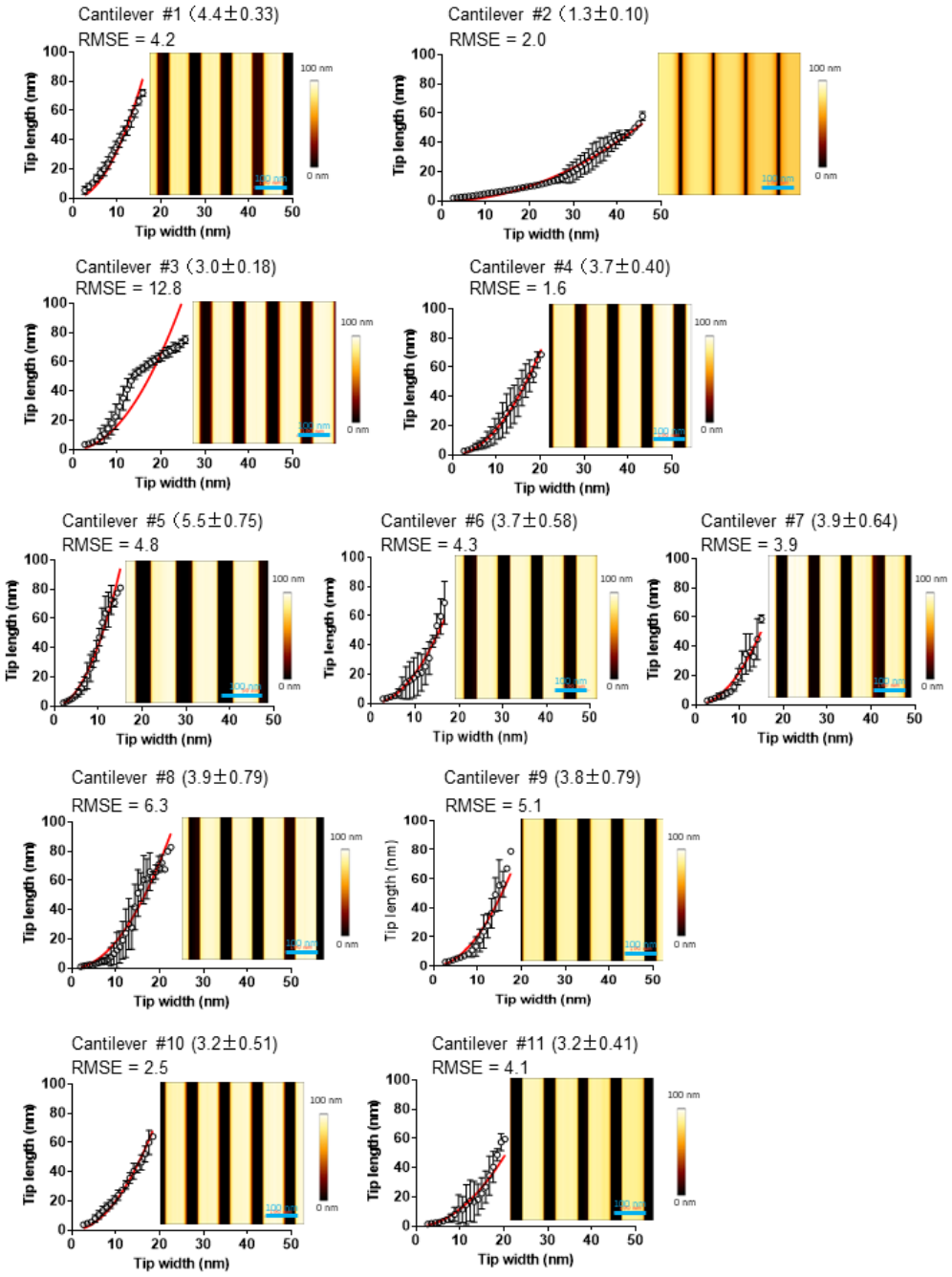


Figure S1. Evaluation of AFM cantilever tip shapes by using line structures of the AS100P-D silicon grating. Each panel shows the tip shape function and corresponding AFM image. The numerical values (mean ± SD) represent the tip aspect ratios at maximum tip width. The red lines are the best fit curves to the experimental data using a quadratic function (Tip length = constant × Tip width^2). Root mean squared errors (RMSE) with regard to the fitting were also shown.

Figure S1. Continued

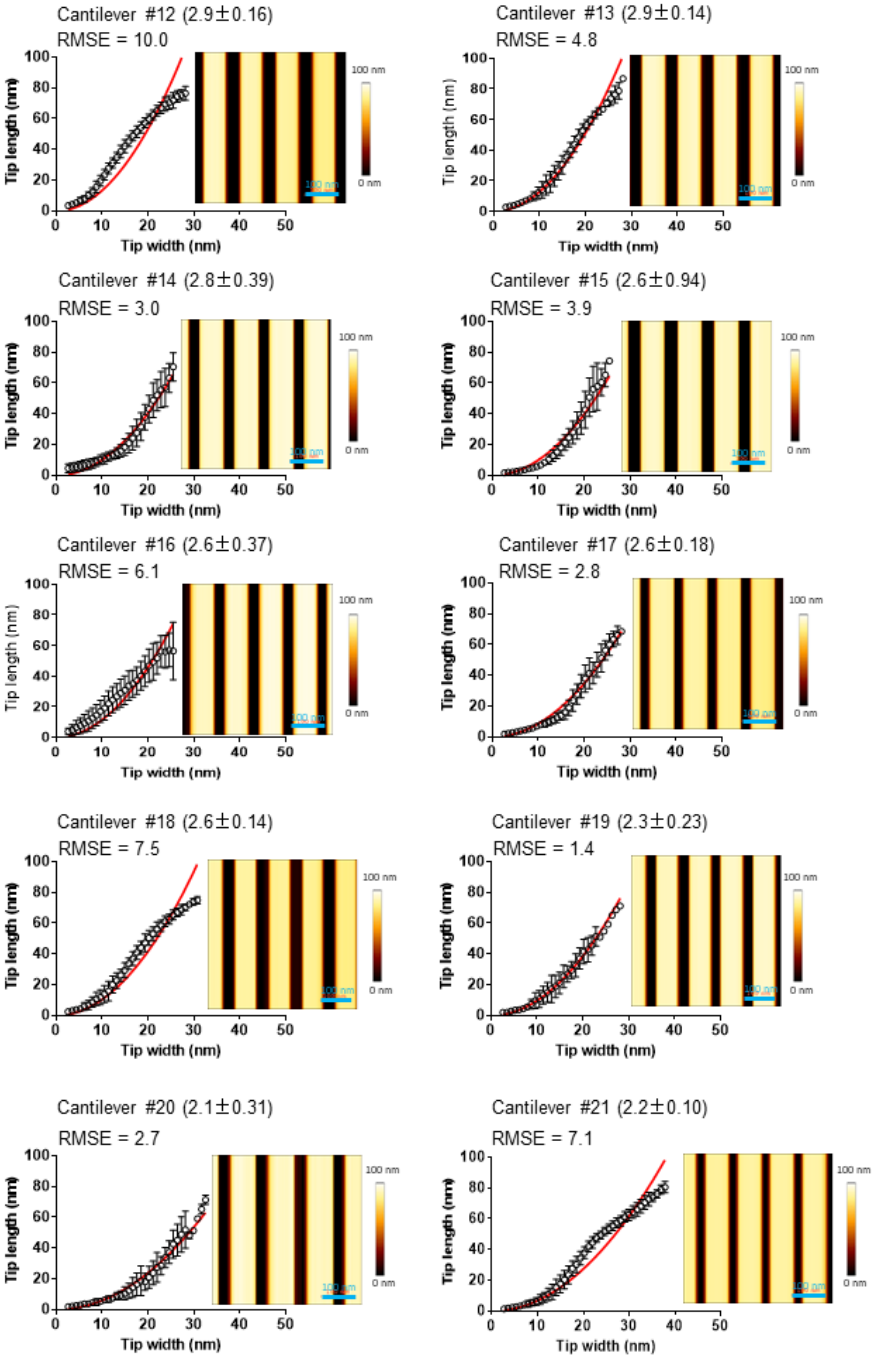
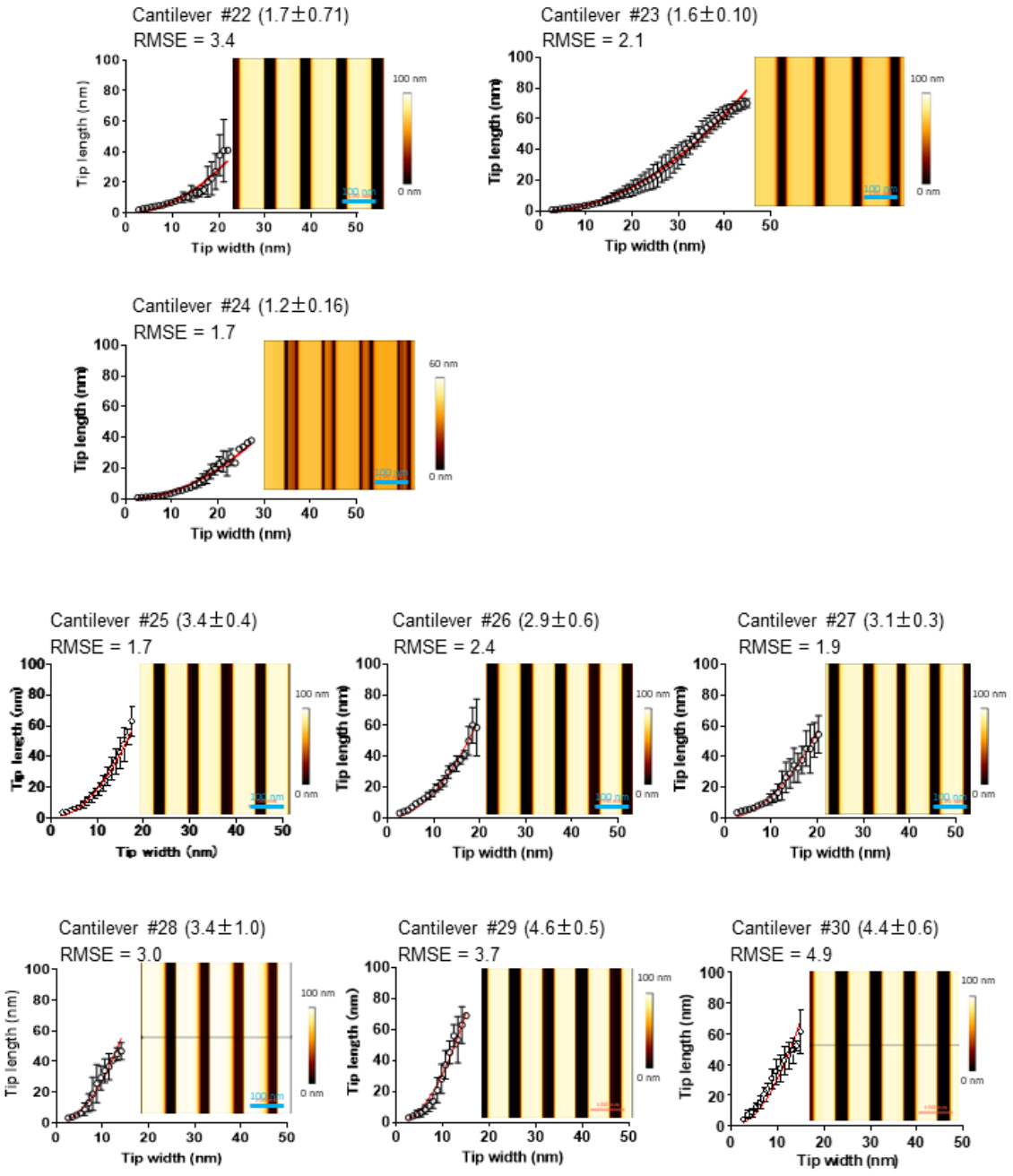


Figure S1. Continued



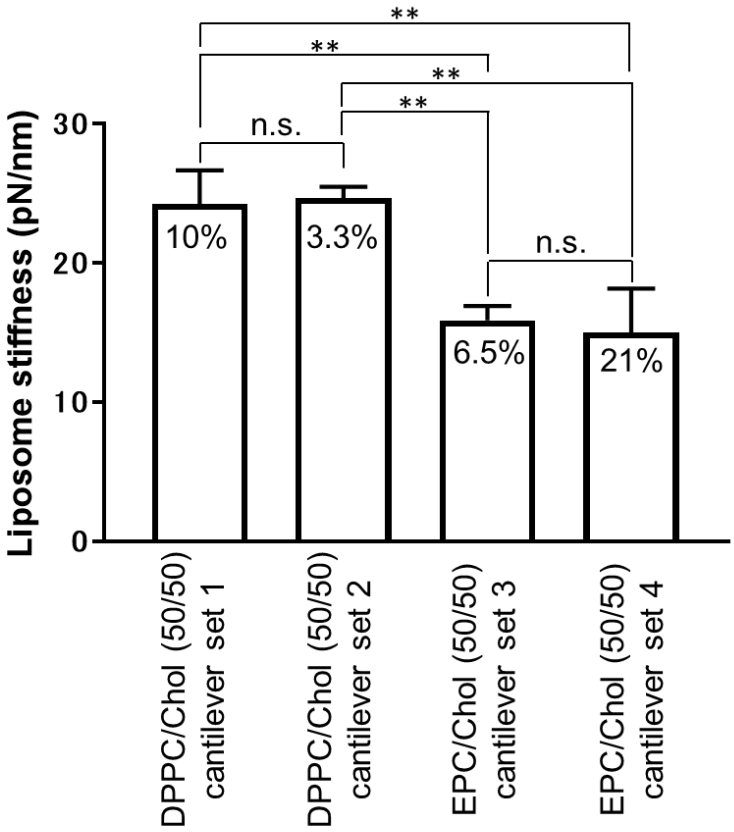


Figure S2. Comparison of statistical liposome stiffness obtained with different cantilever sets (mean ± SDs). DPPC/Chol (50/50) and EPC/Chol (50/50) liposomes on AP-mica in 5% w/w aqueous glucose solution at 25 °C. Cantilevers #6, #9, #10 were used for cantilever set 1 (data from Fig. 6B), Cantilevers #7, #8, #11 were used for cantilever set 2, cantilevers #1, #8, #11 were used for cantilever set 3 (data from Fig. 6B), and cantilevers #6, #7, #10 were used for cantilever set 4. The percentage shown in each column shows the relative standard deviation. Heights (means ± SDs) of DPPC/Chol (50/50) liposomes analyzed by cantilever set 1 and set 2 were 76±3.7 nm and 77±7.3 nm, respectively. Heights (means ± SDs) of EPC/Chol (50/50) liposomes analyzed by cantilever set 3 and set 4 were 72±5.9 nm and 76±10 nm, respectively. Total number (*N*) of analyzed liposomes: *N*=189 for cantilever set 1, *N*=127 for cantilever set 2, *N*=188 for cantilever set 3, *N*=117 for cantilever set 4. ns, not significant. $^{**}P < 0.01$.

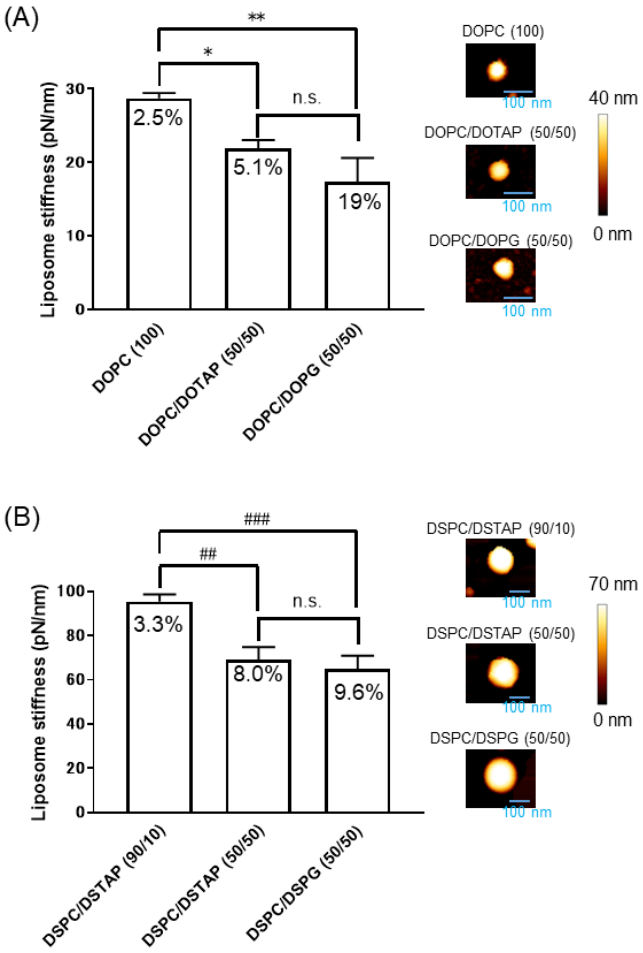


Figure S3. Stiffness measurements of (A) DOPC-based liposomes and (B) DSPC-based liposomes on BSA-glass in 5% w/w aqueous glucose solution at 25 °C. The liposomes were also measured in our previous study (Langmuir, 34, 7805-7812, 2018). Each statistical value for liposome stiffness was obtained with three cantilevers (mean ± SD), and the percentage in each column shows the relative standard deviation. Cantilevers #25, #26, ad #27 were used for DOPC-based liposomes, and cantilevers #28, #29, and #30 were used for DSPC-based liposomes. The right panels show AFM images of liposomes. The scale bar is 200 nm. Heights (means ± SDs) of liposomes analyzed by the cantilever set: 48±2.3 nm for DOPC (100) liposomes, 43±3.0 nm for DOPC/DOTAP (50/50) liposomes, 47±7.0 nm for DOPC/DOPG (50/50) liposomes, 72±6.9 nm for DSPC/DSTAP (90/10) liposomes, 68±16 nm for DSPC/DSTAP (50/50) liposomes, 63±1.0 nm for DSPC/DSPG (50/50) liposomes. Total number (N) of analyzed liposomes: N=69 for DOPC (100) liposomes, N=33 for DOPC/DOTAP (50/50) liposomes, N=29 for DOPC/DOPG (50/50) liposomes, N=64 nm for DSPC/DSTAP (90/10) liposomes, N=28 for DSPC/DSTAP (50/50) liposomes, N=38 for DSPC/DSPG (50/50) liposomes. *$P < 0.05$, compared with DOPC (100) liposomes; **$P < 0.01$, compared with DOPC (100) liposomes; $^{\#\#}P < 0.01$, compared with DSPC/DSTAP (90/10) liposomes; $^{\#\#\#}P < 0.001$, compared with DSPC/DSTAP (90/10) liposomes. ns, not significant.

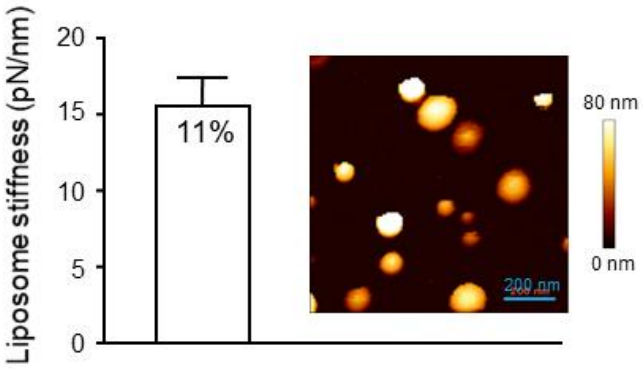


Figure S4. Stiffness measurements of POPC/Chol (50/50) liposomes on AP-mica in 5% w/w aqueous glucose solution at 25 °C. The data was expressed by mean ± SD, and the percentage in the column shows the relative standard deviation. A cantilever set (Cantilevers #14, #15, #17) was used. The inset shows an AFM image of POPC/Chol (50/50) liposomes. The scale bar is 200 nm. Height (mean ± SD) of POPC/Chol (50/50) liposomes analyzed by the cantilever set was 63±5.6 nm. Total number of analyzed liposomes was 189. By using the shell theory, stiffness of liposomes obtained by AFM can be deduced from the membrane bending modulus (K_c) using by the following equation: $K_c = \sqrt{3}khH/96\sqrt{(1-\nu^2)}$, where k is liposome stiffness, h is the membrane thickness, H is the height of the liposome, and ν is the Poisson ratio (*Phys. Rev. E* **2006**, *74*, 030901). On the basis of a Poisson ratio of 0.5 and a membrane thickness of ~about 4 nm (*Phys. Rev. E* **2009**, *80*, 021931), K_c of POPC/Chol (50/50) liposomes was calculated to be 0.83×10^{-19} J, which is similar to the value of 1.6×10^{-19} J for POPC/Chol (50/50) liposomes determined using neutron spin echo spectroscopic method (*Biophys. J.*, **2009**, *96*, 3629–3637).