Electronic Supplementary Material (ESI) for Nanoscale.

Supplemental information for:

Diversity of physical properties of bacterial extracellular membrane vesicles revealed through atomic force microscopy phase imaging

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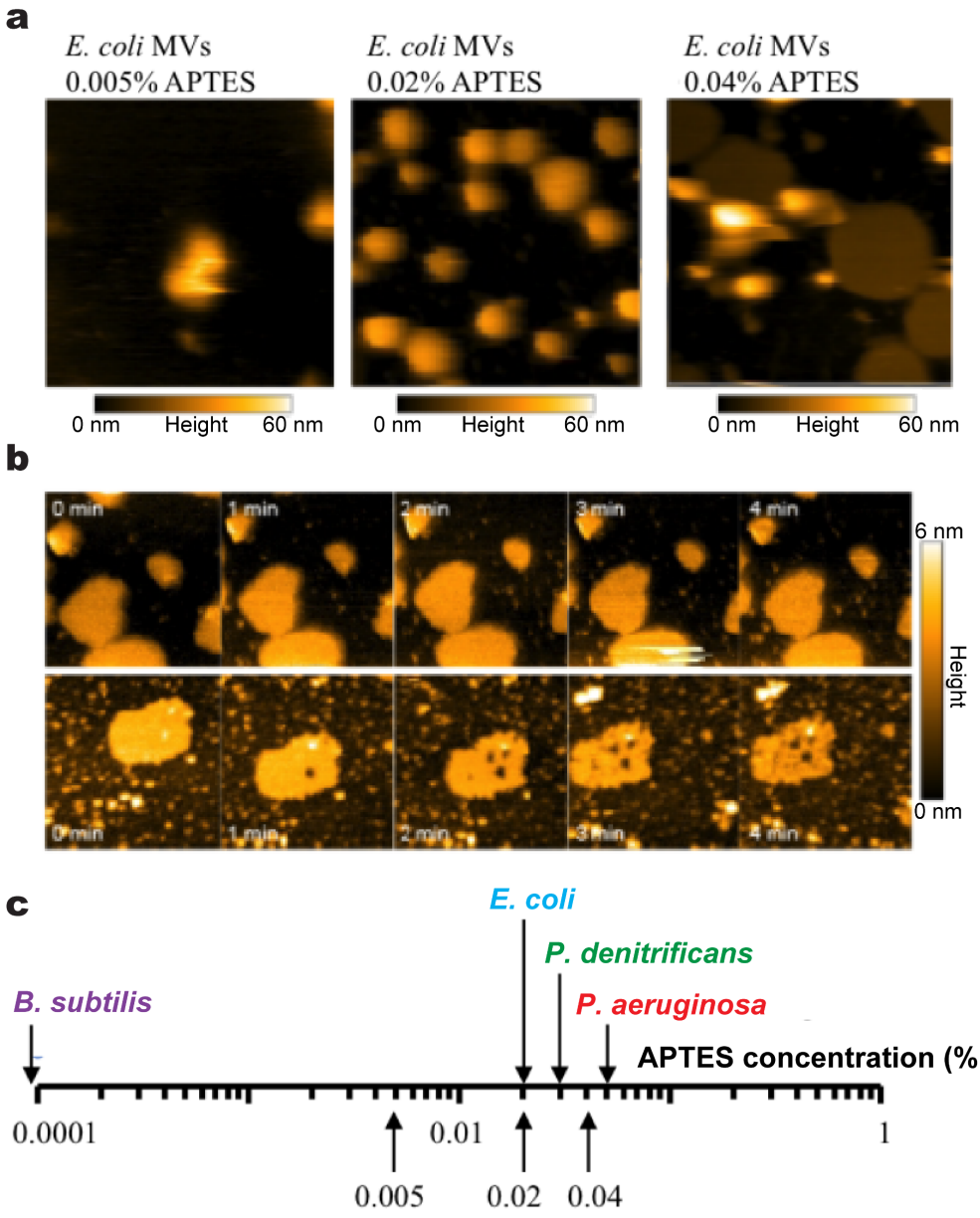


Figure S1 Adsorption of MVs on mica substrate. (a) *E. coli* MVs were immobilized on mica surfaces silanized by APTES concentrations of 0.005% (insufficient concentration), 0.02% (optimized concentration), and 0.04% (excess concentration). (b) Time-lapse still images of sheet-like *E. coli* MVs on the mica substrate treated with 0.04% APTES. Addition of 0.1% Triton X-100 surfactant into the imaging chamber at 0 min (lower images) or no addition (upper images). After adding surfactant, the sheet-like structure dissolved. (c) The optimal APTES concentrations for observing *E. coli*, *P. aeruginosa*, *P. denitrificans*, and *B. subtilis* MVs were 0.02%, 0.05%, 0.03%, and 0.0001%, respectively. AFM images were recorded at imaging rates of (a) 2.0 s/frame and 200 × 200 pixels or (d) 0.5 s/frame and 100 × 100 pixels.

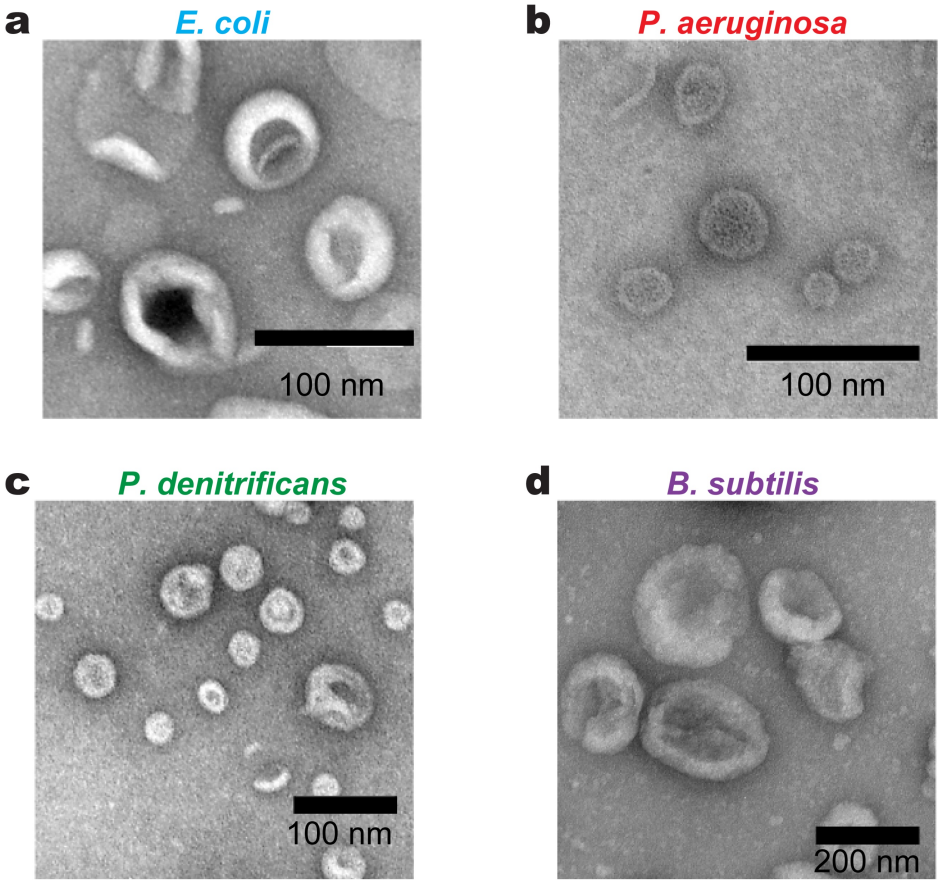


Figure S2 TEM images of negative-stained MVs. TEM images of (a) *E. coli*, (b) *P. aeruginosa*, (c) *P. denitrificans*, and (d) *B. subtilis* MVs.

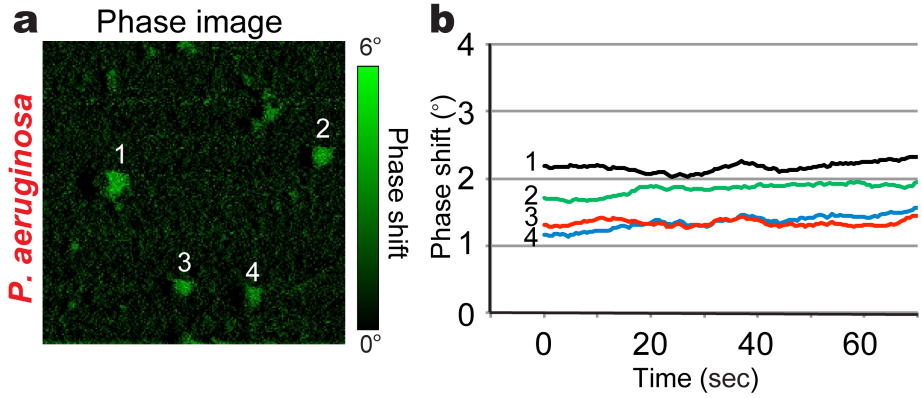
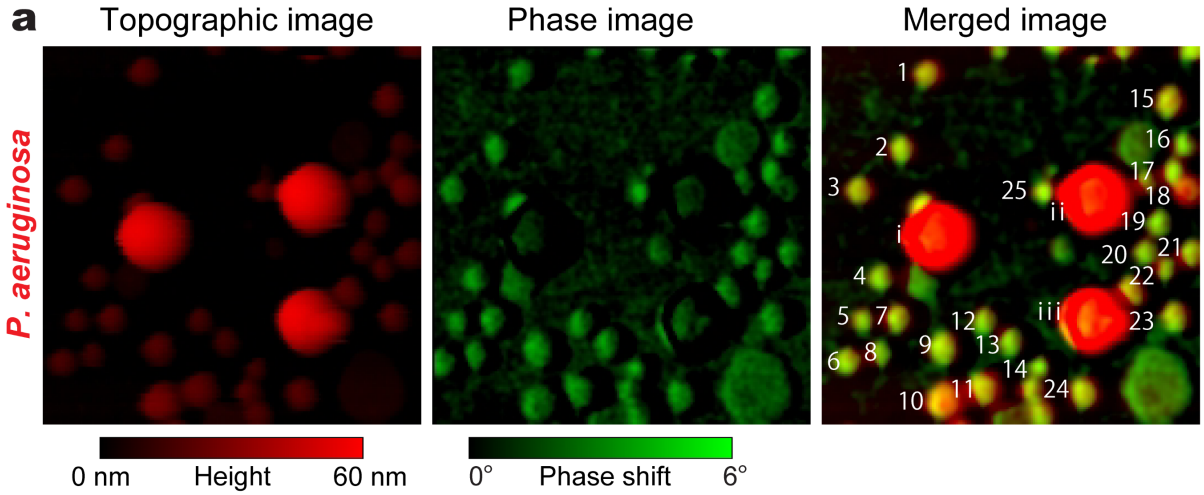


Figure S3 Assessment of phase-shift degree of *P. aeruginosa* MVs over 60 s. (a) Phase images of *P. aeruginosa* MVs. Four MVs (1–4) recorded for 60 s at imaging rates of 0.5 s/frame and 100 × 100 pixels. (b) Time series of phase-shift degree at the tops of the four MVs (1–4).

a



b

MVs	φ (degree)	φ_{cal}
1	3.0	2.3
2	1.6	1.2
3	2.0	1.5
4	2.4	1.8
5	2.6	2.0
6	2.0	1.4
7	2.6	1.9
8	3.0	2.3
9	3.2	2.4
10	2.8	2.0

MVs	φ (degree)	φ_{cal}
11	2.2	1.6
12	4.0	3.0
13	3.6	2.7
14	3.4	2.5
15	2.8	2.1
16	3.0	2.2
17	3.8	2.9
18	1.8	1.3
19	2.2	1.7
20	2.6	1.9

MVs	φ (degree)	φ_{cal}
21	2.7	2.8
22	2.0	1.3
23	2.3	1.9
24	3.2	1.6
25	1.6	1.6
Beads	**φ (degree)**	**φ_{cal}**
i	1.3	1.0
ii	1.4	1.1
iii	1.3	1.0

Figure S4 Normalization of phase-shift degree. (a) AFM topographic image, phase image, and merged image of the mixture of *P. aeruginosa* MVs and polystyrene beads. (b) Obtained and normalized phase-shift values (ϕ_{cal}) of *P. aeruginosa* MVs (1–25) and polystyrene beads (i–iii) from panel a. ϕ_{cal} is the quotient of the phase-shift degree of MV particles (ϕ_{MV}) and the averaged phase-shift value of polystyrene beads (ϕ_{bead}). AFM images were recorded at imaging rates of 2.0 s/frame and 200 × 200 pixels.

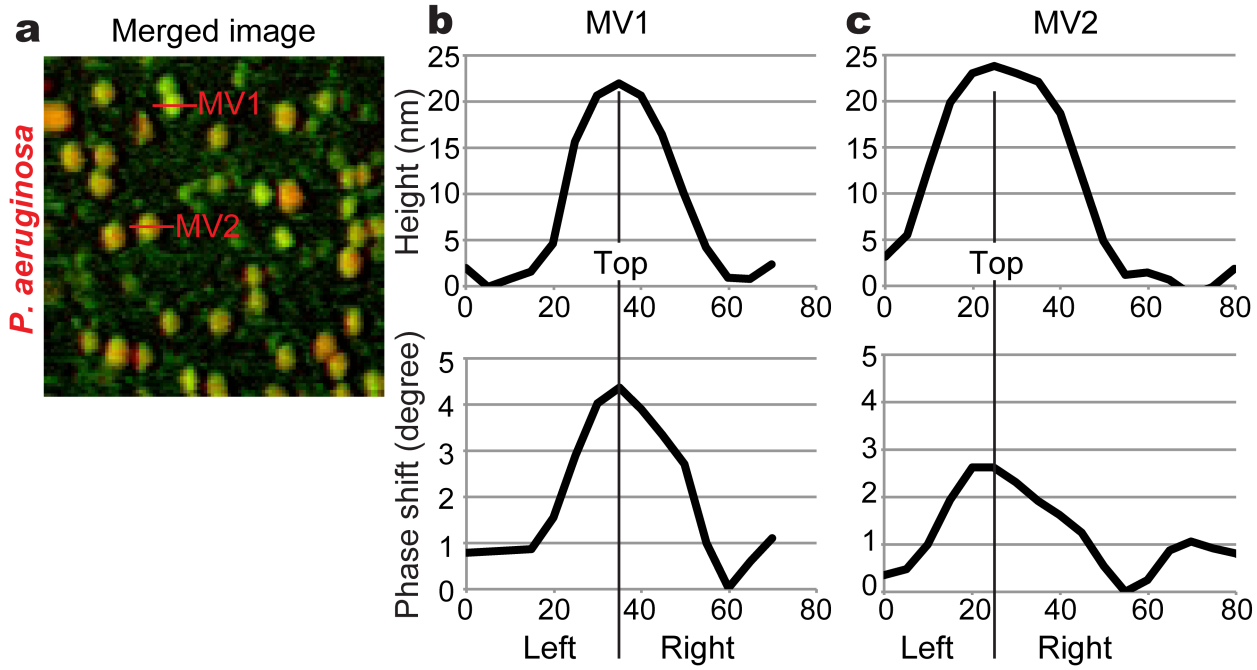


Figure S5 Effect of parachuting on phase image. (a) The merged image of the AFM topographic and phase images from *P. aeruginosa* MVs. (b–c) Profiles of height (upper) and phase-shift (lower) along red solid lines at MV1 and MV2 in panel a. Left–right asymmetry in all profiles was caused by parachuting effect.

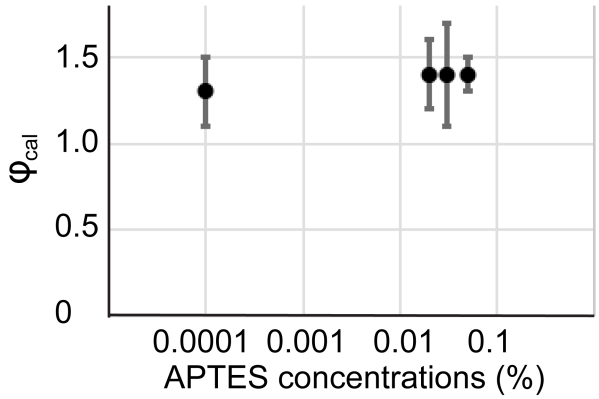


Figure S6 Effect on ϕ_{cal} of interaction between mica surface and samples. ϕ_{cal} was averaged across carboxylate polybeads immobilized on the mica coated by APTES at a given concentration. Studied APTES concentrations were 0.05% (n = 23), 0.03% (n = 28), 0.02% (n = 40), and 0.0001% (n = 30). The error bars indicate standard deviation.

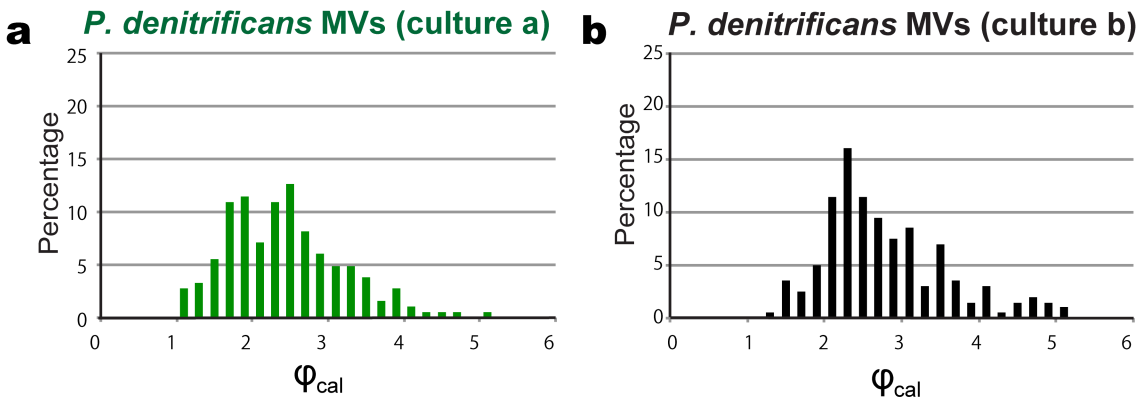


Figure S7 Comparison of ϕ_{cal} distributions of two *P. denitrificans* MV samples isolated from independent cultures. The ϕ_{cal} distributions of *P. denitrificans* MVs from (a) culture a and (b) culture b exhibit no significant differences (χ-square test $p = 0.3$).