Supplementary Information (SI) to accompany

Control of microtubule trajectory within an electric field by altering surface charge density

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1. Supplementary figures

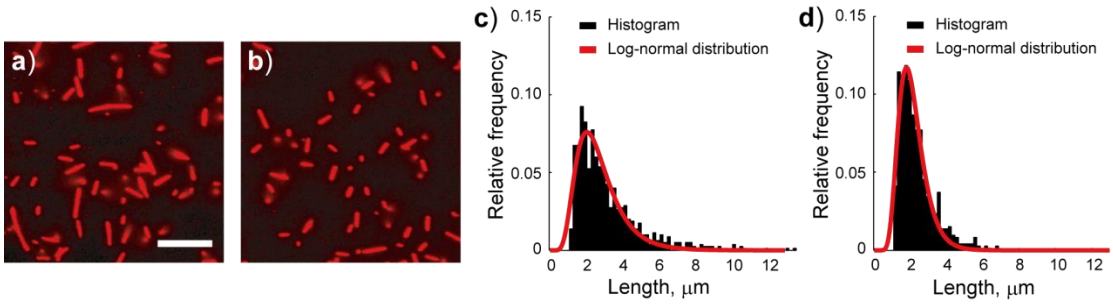

Figure S1. Effect of syringe shearing on B-seed lengths. Fluorescence images of B-R-seeds (**a**) before and (**b**) after syringe shearing. Scale bar = 10 μm. Length distribution (**c**) before (n = 801) and (**d**) after shearing (n = 726). Measured relative frequency (black bars) and fitted log normal distribution (red lines). Bin size = 0.2 μm.

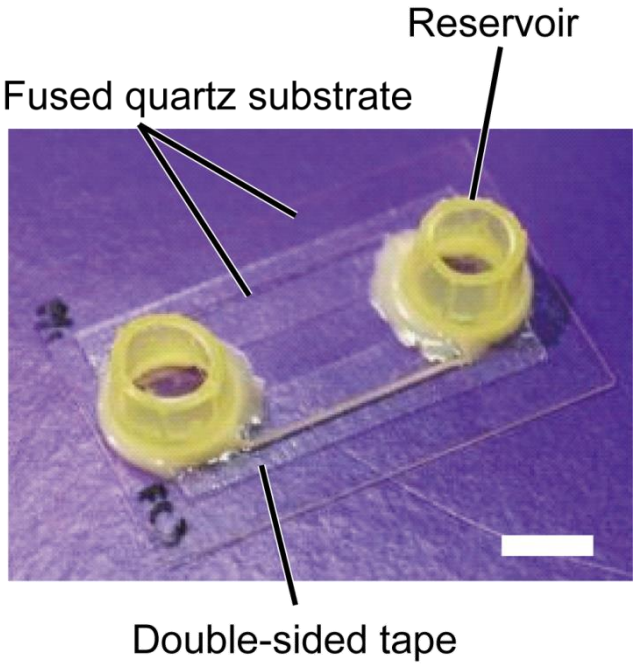

Figure S2. A FC with reservoirs. Channel width, length, and height are 3.5 mm, 15 mm, and 50 μm, respectively. The FC volume is ~2.6 μl. Scale bar = 5 mm.

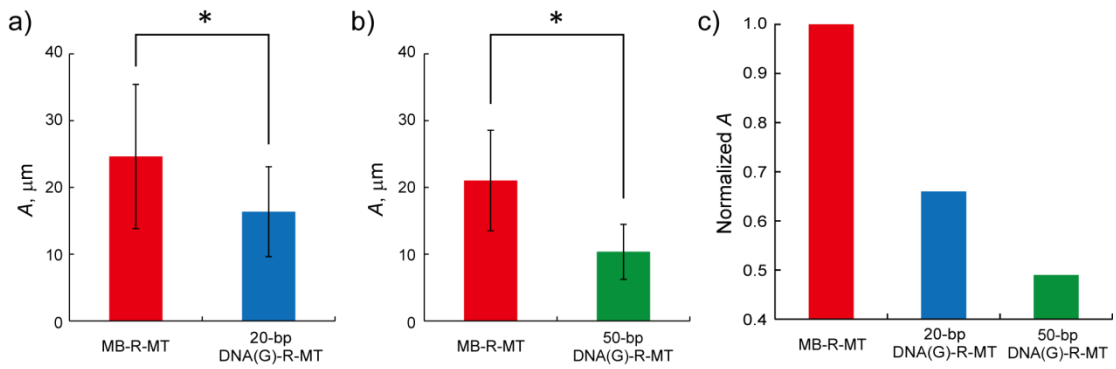

Figure S3. Evaluation of *A* values for minus end-labeled MT. *A* values measured for (**a**) MB-R-MT (n = 36) and 20-bp DNA(G)-R-MT (n = 16) in FC-20 (*p < 0.01, t test); and (**b**) MB-R-MT (n = 37) and 50-bp DNA(G)-R-MT (n = 20) in FC-50 (*p < 0.01, t test). (**c**) Ratios of mean *A* value of DNA-labeled MB-MT to that of MB-R-MT.

2. Supplementary methods

The derivation of Debye length in a FC and effective charges of tubulin dimers and labeled DNA molecules is described. At pH 6.8, the BRB80 buffer solution contained 38 mM $PIPES^-$, 42 mM $PIPES^{2-}$, 122 mM K^+, 1 mM Mg^{2+}, and 2 mM Cl^-, given that the pK_{a1} and pK_{a2} of PIPES are < 3.0 and 6.76, respectively, at 25°C. Debye length λ_D was expressed as

$$\lambda_D = \sqrt{\frac{\varepsilon k_B T}{2N_A e^2 \sum_i c_i z_i^2}} \quad \text{(S1)}$$

where k_B is the Boltzmann constant, T is temperature, N_A is the Avogadro constant, and e is the elementary charge,[1] and was calculated as a summation over all ion species i with valence z_i and molar concentration c_i. Using the dielectric constant of water ($\varepsilon = 6.93 \times 10^{-10}$ C V^{-1} m^{-1}) for BRB80 buffer solution and constants $k_B = 1.38 \times 10^{-23}$ J K^{-1}, $N_A = 6.02 \times 10^{23}$ mol^{-1}, $e = 1.60 \times 10^{-19}$ C, and $T = 298$ K, λ_D was calculated as 0.74 nm from equation S1. As discussed in the main text, λ_D provides zeta potential ζ and surface charge density σ of seed MTs from equations 1 and 2 (Table 1). When there is no DNA molecule labeled on a tubulin dimer, an effective charge of 10 e^- and 9.7 e^- per bare tubulin dimer is calculated with σ for R-seed and G-seed, respectively, assuming a surface area of 50 nm^2.[2] Surface areas of 20- and 50-bp dsDNAs were calculated as 47 and 117 nm^2, respectively, assuming that DNA has a cylindrical structure with a diameter and length of 2.2 nm and 0.34 nm per bp, respectively.[3] Given that SA binds to 80% of the biotin on MTs,[4] 200% of the SA was bound to biotinylated DNA[5] and 3.2 biotin molecules were bound to a tubulin dimer (for a biotinylation ratio of 320%), and the number of DNA molecules conjugated to a tubulin dimer was calculated as 5.1. The total surface area of 20-bp DNA(G)-R-seed was the sum of the DNA and tubulin dimer surfaces (47 × 5.1 nm^2 and 50 nm^2,

respectively). Thus, the total effective charge of 20-bp DNA(G)-R-seed per tubulin dimer was calculated as 73.7 e^- by multiplying σ (= 0.25 e^- nm^{-2}, Table 1) by the total surface area (47 × 5.1 + 50 nm^2). Since the effective charge of a bare tubulin dimer was 10 e^-, the total effective charge of 20-bp DNA on a tubulin dimer was 63.7 e^-. It was divided by the number of DNA molecules on a tubulin dimer (5.1) to obtain the net effective charge per single 20-bp DNA of 12.4 e^- (0.62 e^- per bp). The corresponding value per single 50-bp DNA was also calculated as 34.5 e^- (0.69 e^- per bp). The effective charge for 50-bp DNA was constant regardless of their tagged-fluorophores.

3. Supplementary movies

Movie S1. Gliding of 20-bp DNA(G)-R-MTs and MB-R-MTs under an average electric field of E = 7 kV m^{-1} (from right to left) in FC-20 (40× actual speed). Scale bar = 10 μm.

Movie S2. Gliding of 50-bp DNA(G)-R-MTs and MB-R-MTs under an average electric field of E = 7 kV m^{-1} (from right to left) in FC-50 (40× actual speed). Scale bar = 10 μm.

Movie S3. Gliding of 50-bp DNA(R)-G-MTs, 20-bp DNA(G)-R-MTs, and MB-R-MTs under an average electric field of E = 7 kV m^{-1} (from right to left) in FC-mix (40× actual speed). Scale bar = 10 μm.

References

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