Supplementary Information

Dielectric constant of flagellin proteins measured by scanning dielectric microscopy

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S1. Tip deconvolution

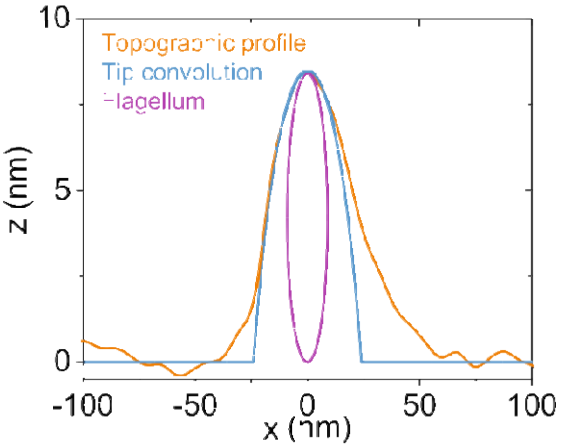

Figure S1: Example of a tip deconvolution procedure: The measured topographical profile of a S. oneidensis flagellum (orange line) is adjusted by the curve resulting from the convolution of the calibrated AFM tip with an ellipse of height equal to the topographic height and width equal to the one that best fits the topographic profile with the use of Eqs. (2) and (3) of the main text (blue line). The actual cross-section geometry obtained is shown by the magenta line. Tip radius 23.9 nm, cone angle 11.5º, flagellum height 8.4 nm and width 18 nm.

S2. Tip calibration and tip-substrate distance

According to the manufacturer's specifications, the PtSi-CONT (Nanosensors) probes are sharp silicon tips with a Platinum Silicide coating grown on the silicon lattice (conserving the geometry of the sharp silicon tips). Therefore, they have a macroscopic cone angle of 20º–25º and a microscopic angle of 10º–12º, corresponding to the last 200 nm of the tip. We have shown earlier that for this type of tips, a single cone angle model can be used if the tip-substrate distances used are below tens of nanometers (<50-70 nm depending on the tip radius) [24]. The tip geometry calibration is therefore obtained by fitting the short-range experimental capacitance gradient approach curves on the substrate with the predictions of the theoretical model, with the tip radius and the offset (representing mainly the cantilever contributions) as fitting parameters. The calibration curves for two of the tips used in the present work are shown in Fig. S2. The same curves have also been used to determine the tip-substrate distance during EFM imaging. To this end, the capacitance gradient value measured on a bare part of the substrate (symbols in Fig. S2) are compared with the calibration curves, from where the tip-substrate distances are extracted.

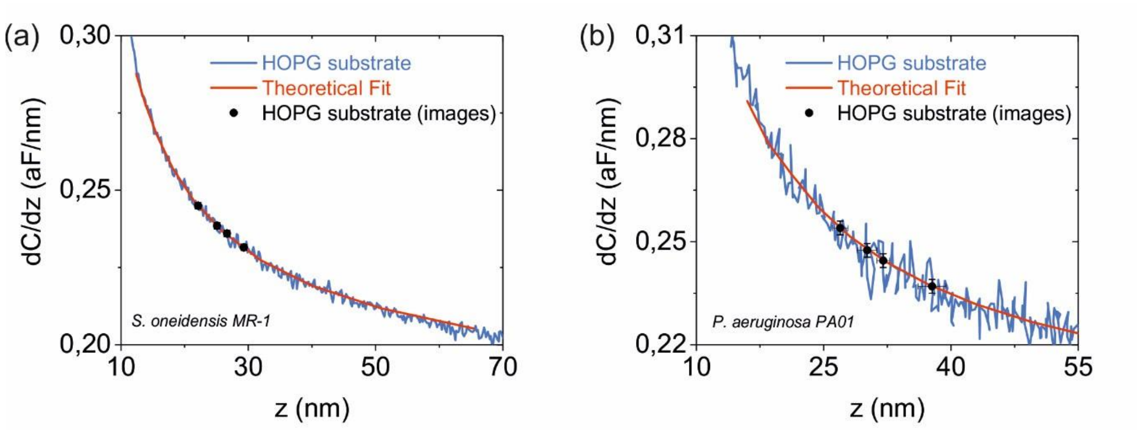

Figure S2: (a) Capacitance gradient approach curves over the HOPG substrate (blue line) for the S. oneidensis MR-1 flagellum sample. The red line corresponds to the simulated capacitance gradient curve that best fit the experimental curve, with the tip radius and capacitance gradient offset as fitting parameters. We obtain R_{So} = 23.9 ± 0.5 nm and offset$_{So}$ = 118.5 ± 0.7 zF/nm. The rest of parameters used in the calculations are set to their nominal values: cone half angle θ_{So} = 11.5º, cone height H = 12500 nm, cantilever lenght L_c = 3000 nm and width W_c = 3000 nm. The calibration curve is also used to set the tip-sample distance of the EFM images, by comparing the values of the capacitance gradient on the substrate (black dots) with those of the capacitance gradient approach curve. (b) Idem for the tip used with the P. aeruginosa PAO1 sample. We obtain R_{Pa} = 30.0 ± 0.5 nm, and offset$_{Pa}$ = 128 ± 2 zF/nm. Rest of parameters as in (a).

S3. Dependence of the capacitance gradient on flagellum length, tip radius and relative dielectric constant

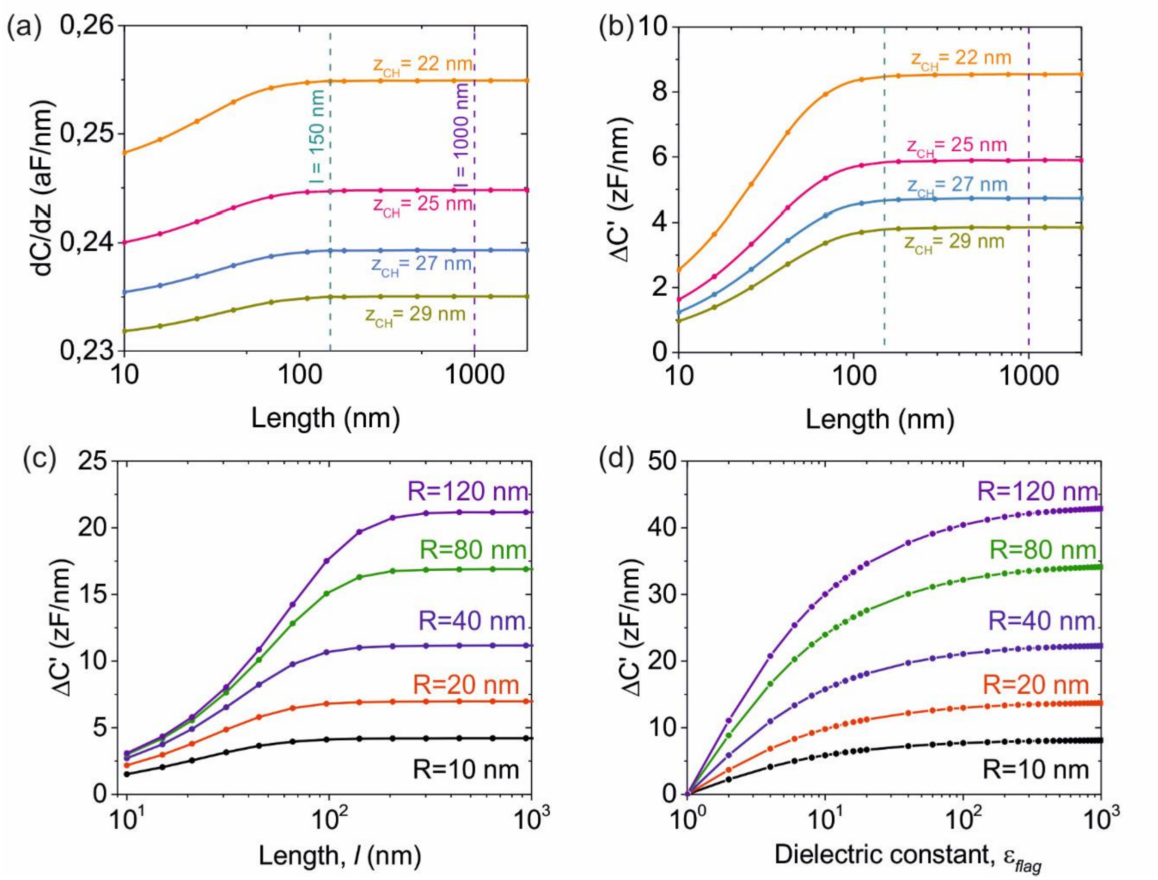

Figure S3: (a) Calculated capacitance gradient versus flagellum length at different tip-substrate distances. Parameters: R = 23.9 nm, θ = 11.5º, h = 8.4 nm, w = 18 nm, offset = 118.5 zF/nm, ε_r = 4.1, H = 12500 nm, L_c = 3000 nm, W_c = 3000 nm. (b) Same data as in (a) represented as capacitance gradient contrast. We observe that in the present case for lengths > 150 nm (indicated by a green dashed line) the capacitance gradient becomes independent from the flagellum length, indicating approximately the region of the flagellum probed by the tip. Flagella in the manuscript were several micrometers long, that we approximate for a length l = 1000 nm (violet dashed line), well beyond any finite size effect. (c) Calculated capacitance gradient contrast versus flagellum length for different tip radii at a tip substrate distance of z_{CH} = 22 nm (rest of the parameters as in (a)). The critical length at which the contrast becomes independent from the flagellum length (referred to as probed length in the main text) can be extracted from this figure (the results are plotted in Fig. 5 of the main text). (d) Calculated capacitance gradient contrast versus the flagellum dielectric constant for different tip radii at a tip substrate distance of z_{CH} = 22 nm and flagellum length l=1000 nm (rest of the parameters as in (a)). From these data one can extract the relative error in the extraction of the dielectric constant by using Eq. (1) of the main text.

S4. Dependence of the capacitance gradient on flagellum curvature

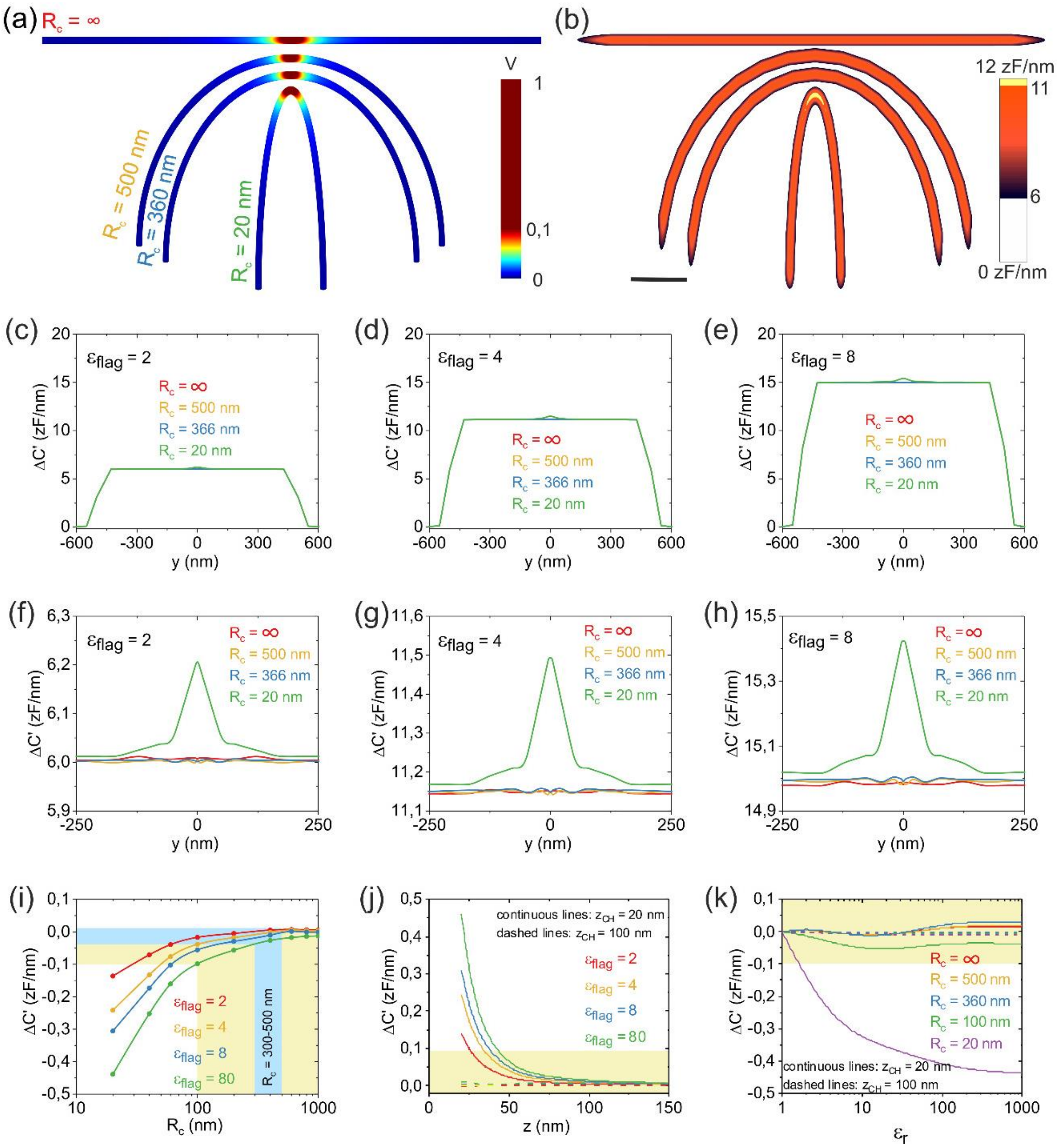

Figure S4. Numerical calculations to quantify the contribution of the curvature of the flagellum to the measured/calculated electric forces. Parameters of the flagellum: h = 8.4 nm, w = 18 nm, l = 1 µm. The probe dimensions and flagellum properties, if not otherwise stated, are the same than those used for the quantification of the S. oneidensis MR- 1 flagellum in the main text, that is: R = 23.9 nm, θ = 11.5, h = 8.4 nm, w = 18 nm, ε_{flag} = 4, offset = 118.5 zF/nm, H = 12500 nm, L_c = 3000 nm W_c = 3000 nm. (a) Electric potential distribution on the flagellum surface for four flagella having radii of curvature $R_c = \infty$ (straight), 500 nm, 360 nm and 20 nm, for the tip located at the centre of the flagellum at a tip-substrate distance z_{CH} = 20 nm. (b) Corresponding capacitance gradient images. (c)-(e) Longitudinal capacitance gradient profiles taken along the flagellum length for three different dielectric constants of the flagellum ε_{flag} = 2, 4, 8, respectively. (f)- (h) Zoom-ins on the profiles shown in (c)-(e) around the centre of the flagellum. (i) Variation of the capacitance *gradient values in the longitudinal profiles with respect to a straight flagellum (R_c = ∞) on top of the vertex of the flagellum as a function of the radius of curvature of the flagellum for ε_{flag} = 2, 4, 8, 80. The blue shadowed zone corresponds to the experimental radii of curvature (R_c = 360-500 nm) and the yellow shadowed region corresponds to the part that is not experimentally detectable (noise sensitivity assumed to be 0.1 zF/nm). Note that the vertical axis is here in zF/nm. (j) Variation of the capacitance gradient approach curves on top of the vertex of a flagellum with radius of curvature R_c=20 nm with respect to the straight flagellum for ε_{flag} = 2, 4, 8, 80 and two different flagellum curvatures. (k) Variation of the capacitance gradient as a function of the dielectric constant of the flagellum ε_{flag} with the tip fixed on top of the vertex of the flagellum with respect to the straight flagellum (R_c = ∞), for different tip sample distances and curvatures. For the experimental radii of curvature (R_c = 360-500 nm), tip radii (~30 nm) and tip sample distances (below 70 nm), we conclude that there is no effect of the flagella curvature on the calculated capacitance gradients This justifies using a straight flagellum model in the calculations, as we did in the main text.*

S5. Dielectric constant extraction from capacitance gradient approach curves and validation with full EFM dielectric profiles

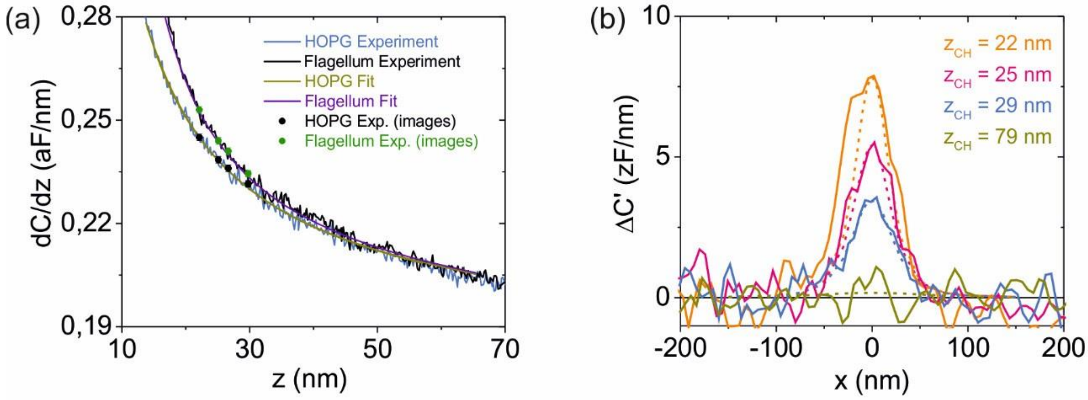

Figure S5: (a) Experimental capacitance gradient approach curves measured on the HOPG substrate (blue line) and on top of the S. oneidensis MR-1 flagellum (black line). Continuous green and violet lines represent the theoretical fitted curves for the HOPG and flagellum approach curves, respectively, used to calibrate the tip geometry and capacitance gradient offset (tip radius R_{So} = 23.9 ± 0.5 nm, $offset_{So}$=118.5±0.7 zF/nm) and the dielectric constant of the flagellum, ε_{So}=4.3 ± 0.6. The obtained dielectric constant value agrees, within the experimental error, with the value obtained in the main text from the data extracted from the EFM images profiles at different heights. The black and green symbols represent the capacitance gradient values extracted from the EFM images on the HOPG substrate (used to extract the tip sample distance) and on the flagellum (used to extract the dielectric constant of the flagellum in the main text). (b) Experimental electric profiles of the S. oneidensis MR-1 EFM images (continuous lines) and simulated profiles (dashed lines) at four tip-substrate distances. Parameters used in the calculations: R = 23.9 nm, θ = 11.5, h = 8.4 nm, w = 18 nm, l = 1000 µm, offset = 118.5 zF/nm and ε_r = 4.3, H = 12500 nm, L_c = 3000 nm W_c = 3000 nm. The different ways to obtain the dielectric constant provide consistent results among them.

S6. Table of parameters for the different flagella analyzed

Table S1: Table of parameters of the different flagella analyzed in Fig. 3g of the main text, indicating the tip radius, cone angle, flagellum height and width, the dielectric constant, and the type of bacterial cell.

# flagellum	Tip radius (nm)	Cone angle (deg)	Height (nm)	Width (nm)	Dielectric constant	Bacteria
Flagellum	23.9 ± 0.5	11.5	8.4	18	4.3 ± 0.6	*S. oneidensis*
Flagellum 2	24.5 ± 0.5	11.5	10.8	14	4.5 ± 0.3	*S. oneidensis*
Flagellum	26.0 ± 0.5	11.5	10.7	13.8	3.6 ± 0.5	*S. oneidensis*
Flagellum 4	27.8 ± 0.5	11.5	9.4	19	4.0 ± 0.9	*S. oneidensis*
Flagellum	30.0 ± 0.5	11.5	13	27	4.5 ± 0.7	*P. aeruginosa*

S7. 3D numerical calculation details and optimization

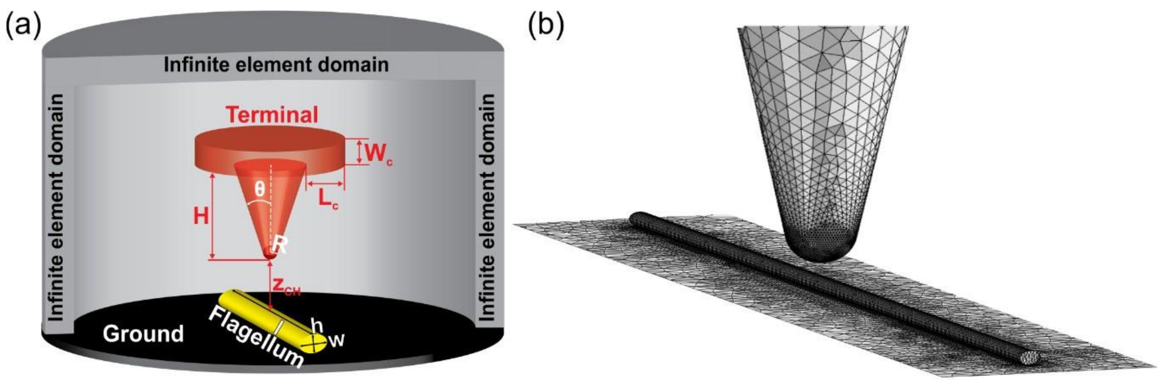

Figure S6: (a) Schematic representation of the simulated geometry and simulation box to calculate the theoretical values of the electric force acting on the tip, with the parameters defining the structure used (not to scale). (b) Image of the real meshing used for the calculation. The mesh used was set to a minimum of $6 \cdot 10^5$ elements.

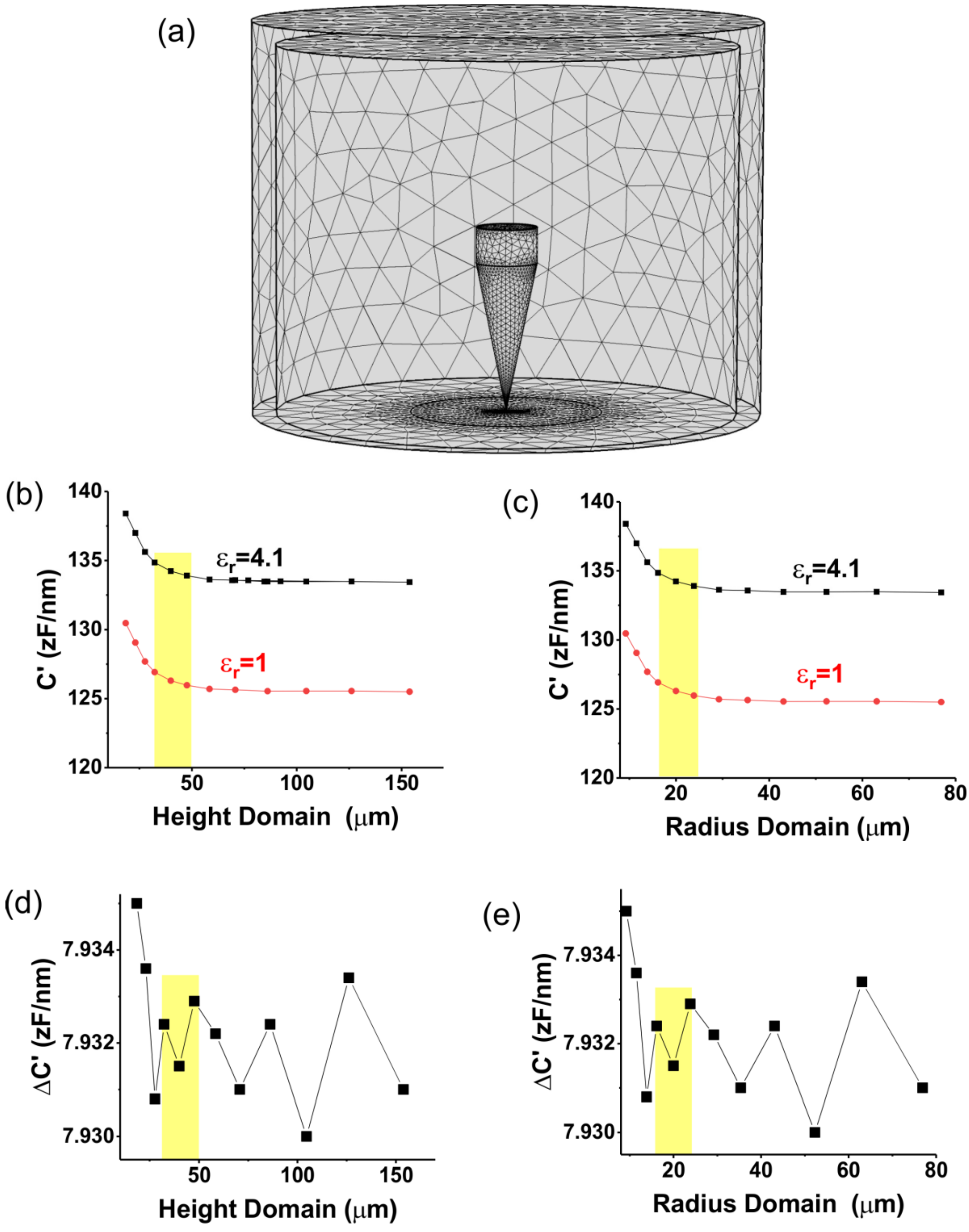

Figure S7: (a) Simulation domain with the meshing scheme used in the numerical calculations. Absolute value of the calculated capacitance gradient versus (b) simulation domain height and (c) domain radius for two dielectric constants of the nanofilament. Capacitance gradient contrast as a function of (d) domain height and (e) domain radius. The yellow bands represent the range of values considered in the calculations. In all cases, the numerical noise due to the meshing is below 0.1 zF/nm as intended.

S8. Dielectric probed length in force gradient detection.

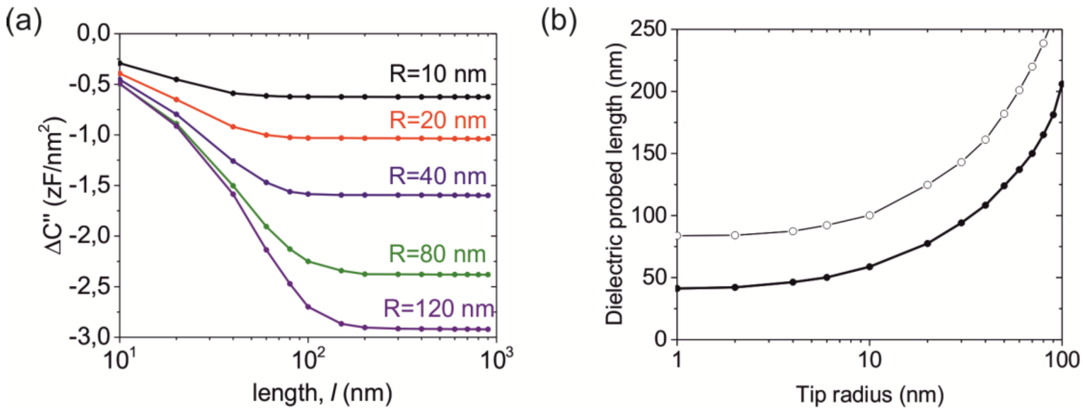

Figure S8: (a) Calculated force gradient (second order capacitance derivative) contrast versus flagellum length for different tip radii at a tip substrate distance of z_{CH} = 22 nm. Parameters: R = 23.9 nm, θ = 11.5º, h = 8.4 nm, w = 18 nm, offset = 118.5 zF/nm, ε_r = 4.1, H = 12500 nm, L_c = 3000 nm, W_c = 3000 nm. (b) (Thick line, solid black filled symbols) Critical length at which the force gradient contrast becomes independent from the flagellum length (referred to as dielectric probed length in force gradient detection in the main text) as a function of the tip radius. For comparison we also show the dielectric probed length values in force detection (thin line, empty symbols, same as in Figure 4). Force gradient detection improves the locality of the measurement by a factor ~0.5-0.7, depending on tip radius.