SUPPLEMENTARY MATERIAL

Physicochemical surface properties of *Chlorella vulgaris*: a multiscale assessment, from electrokinetic and proton uptake descriptors to intermolecular adhesion forces

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This supporting material contains 6 pages, 3 references and 7 figures. It is organized as follows:

A. Electrophoretic mobility of *C. vulgaris* as a function of pH and salinity.

B. Sequential potentiometric proton titrations on *C. vulgaris* (dark, 25°C).

C. Absorbance spectra of C. vulgaris as a function of pH and exposure time.

D. Flow cytometry measurement of the number of C. vulgaris cells and the ratio stained by propidium iodide (PI) after 0- and 2-hours exposure to $NaNO_3$ at pH 4.5 and 6.2.

E. Additional illustrative examples of maps included in each set of data presented in Figures 4, 5 and 6 of the main text.

F. CFM control measurements using AFM tips and gold surfaces both coated by -COOH and -CH_3 terminated thiols.

G. Low-resolution images associated with CFM measurements using thiol-CH_3 tip on C. vulgaris surface.

A. Electrophoretic mobility of *C. vulgaris* as a function of pH and salinity.

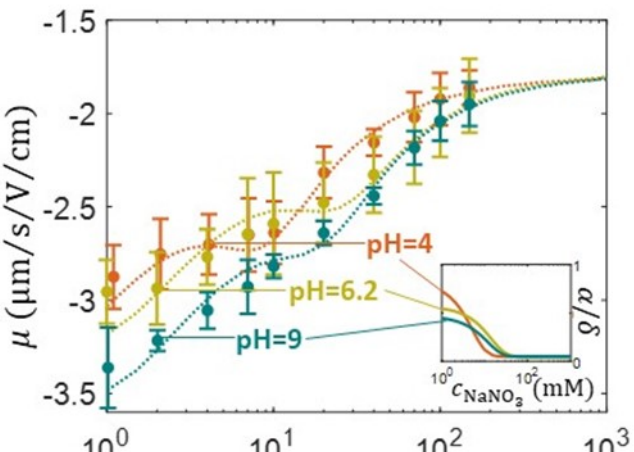

Figure S1. Electrophoretic mobility μ of *C. vulgaris* measured (symbols) as a function of $NaNO_3$ concentration at pH=4, pH=6.2 and pH=9 (indicated). Each reported data point is the average of 6 electrophoretic mobility acquisitions on 3 different batches of microalgae per tested pH condition, with one replicate per batch. The colored dotted curves represent fits of data from Duval-Ohshima model (cf. ref [42] in the main text and e.g. ref [1] at the end of this document). See text for details.

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36 **B. Sequential potentiometric proton titrations on C. vulgaris (dark, 25°C).**

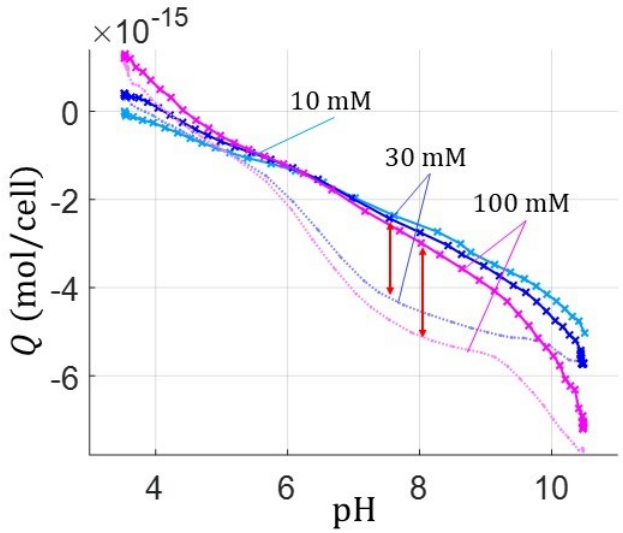

Figure S2. Titrated amounts of mean charge per microalgae cell, Q, as a function of solution pH. Data are measured upon addition of NaOH (10 mM) (solid curves, forward titration) for different $NaNO_3$ electrolyte concentrations (indicated), and addition of HNO_3 (10 mM) (dotted curves, backward titration). The sequential potentiometric titrations were performed each on a single *C. vulgaris* batch, under argon atmosphere within a thermoregulated container in dark at 25°C. The zones of the acid titration curves marked by red arrows suggest the presence of carbohydrate in solution, possibly released by the microalgae.

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38 **C. Absorbance spectra of *C. vulgaris* as a function of pH and exposure time.**

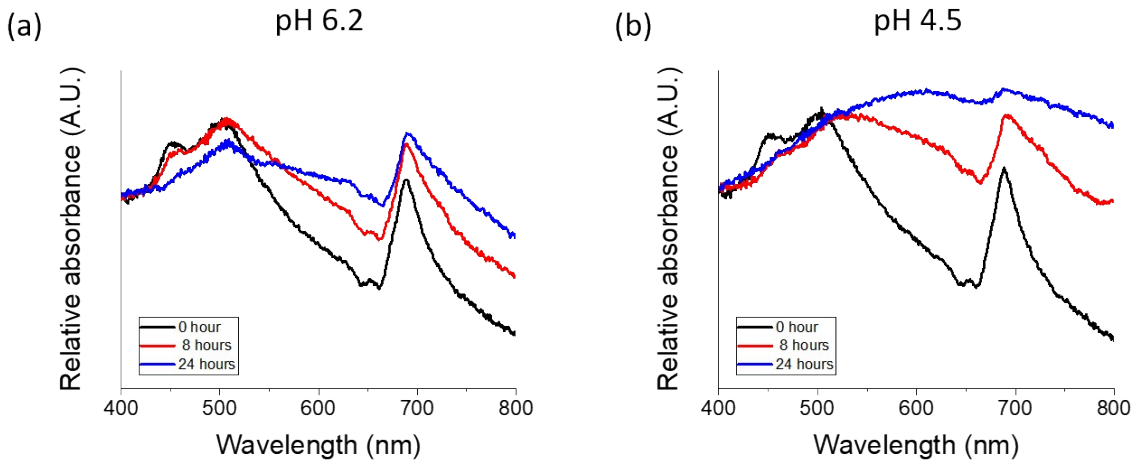

Figure S3. UV-visible spectra of *C. vulgaris* cells immersed in $NaNO_3$ suspension at **(a)** pH 6.2 and **(b)** pH 4.5 at t=0 (black curves), t=8 hours (red curves) and t=24 hours (blue curves).

UV-visible spectra have been recorded using spectrometer UV-2501PC from Shimadsu.

D. Flow cytometry measurement of the number of *C. vulgaris* cells and the ratio stained by propidium iodide (PI) after 0- and 2-hours exposure to $NaNO_3$ at pH 4.5 and 6.2.

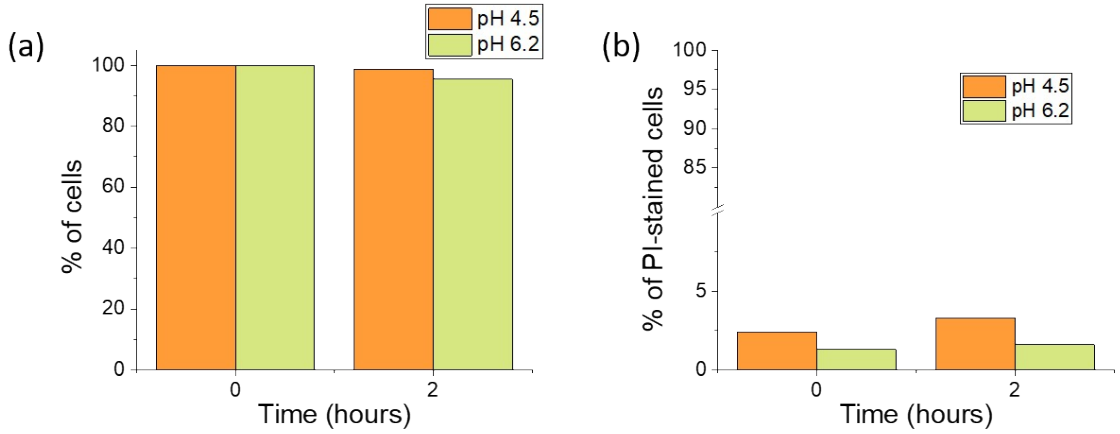

Figure S4. (a) Relative concentration of *C. vulgaris* in $NaNo_3$ suspension at pH4.5 (orange bars) and pH 6.2 (green bars). Data are expressed as percentage of cells compared with the starting cell concentration (T 0 hours). **(b)** Percentage of *C. vulgaris* cells labelled with Propidium Iodide (PI). Data are expressed as the percentage of stained cells compared with the total number of *C. vulgaris* in the sample.

In **Figure S4**, propidium iodide (PI) was used to evaluate the cell viability as cells are labelled with PI when they have a compromised membrane integrity. C. vulgaris were suspended at 10^5 cells/ml in $NaNO_3$ medium (pH 4.5 and pH6.2). At 0 and 2 hours, cells were incubated for 15 min with 10 µg/mL of propidium iodide (Invitrogen, MA, USA) in the dark at room temperature. Cell concentration and PI fluorescence (PI) were recorded on an Attune® NxT flow cytometer (Life technologies, CA, USA). C. vulgaris cells were detected with their chlorophyll autofluorescence. Chlorophyll fluorescence was collected on the BL3 detector (laser 488 nm, filter 590/40 nm). PI signal was collected on the YL2 detector (laser 561 nm, filter 620/15 nm). A positive control for PI labeling was done by heating C. vulgaris at 65°C for 7 min before PI staining. Pi labeled cells were gated as BL3+/YL2+. For each sample, 100 µL and at least 20 000 signals of chlorophyll positive cells were recorded. Data analysis was done on the Attune Nxt software. Cell concentration was normalized to cell concentration measured at 0 hours. PI labeled cells were expressed in percentage of labeled cells compared to the total number of cells at the same time.

E. Additional illustrative examples of maps included in each set of data presented in Figures 4, 5 and 6 of the main text.

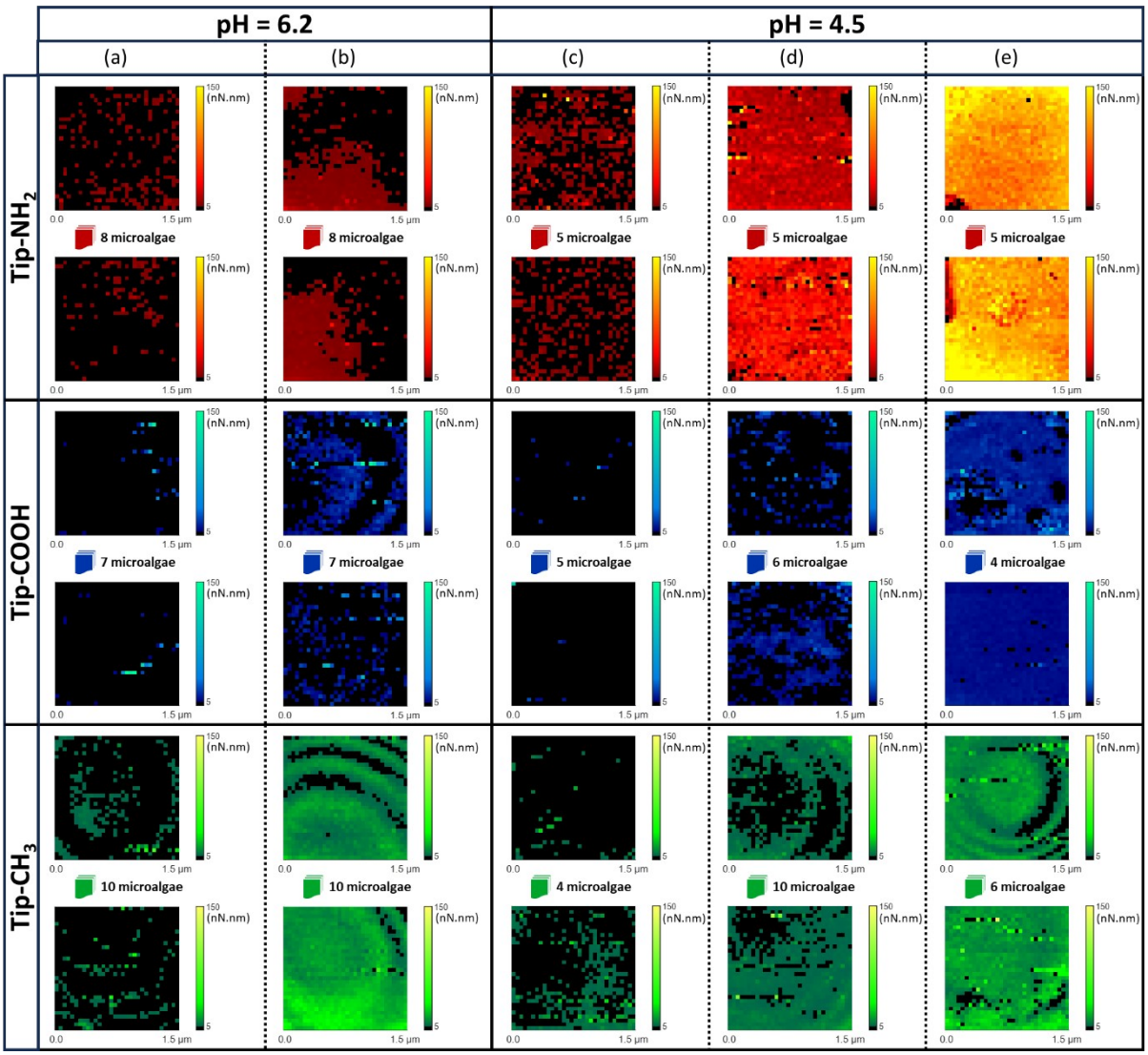

Figure S5. Work of adhesion W_A of AFM tips coated by thiol-NH_2 (top), thiol-COOH (middle) or thiol-CH_3 (bottom) on *C. vulgaris* surface, in 10 mM $NaNO_3$ solution, for **(a,b)** pH=6.2 and **(c,d,e)** pH=4.5.

F. CFM control measurements using AFM tips and gold surfaces both coated by -COOH and -CH_3 terminated thiols.

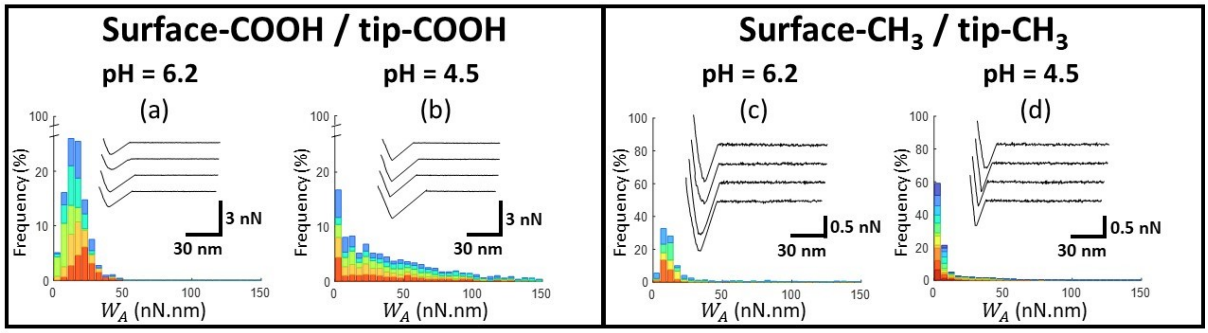

Figure S6. Work of adhesion W_A of AFM tips coated by thiol-COOH **(a,b)** and thiol-CH_3 **(c,d)** on gold surfaces coated with thiol-COOH **(a,b)** and thiol-CH_3 **(c,d)**, in 10 mM $NaNO_3$ electrolyte, at pH=6.2 and pH=4.5 (indicated). The histograms in **(a)**, **(b)**, **(c)** and **(d)** represent the cumulative statistical distribution of W_A values from sets of 5, 5, 4 and 11 W_A-maps, respectively. Each color in the histograms corresponds to the contribution of one W_A-map measured on a given functionalized gold surface to the overall W_A-histogram (cf. **Figure 4f** in the main text). From **(a)** to **(b)**, the increase of the adhesion is attributed to the formation of hydrogen bonds between protonated -COOH tip and -COOH gold surface [2]. For hydrophobicity control measurements **(c,d)**, the decrease in the adhesion when lowering pH is attributed to proton binding or absorption on the thiols coating both the tip and the gold surface [3].

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61 **G. Low-resolution images associated with CFM measurements using thiol-CH_3 tip on *C.***

62 **vulgaris surface.**

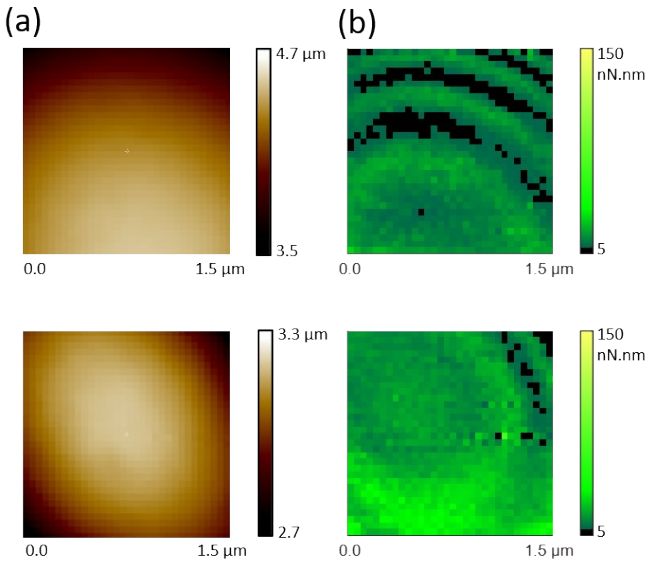

Figure S7. **(a)** Low resolution topographic maps associated with the FV measurements using CH_3-thiol-coated AFM tips on *C. vulgaris* surface, in 10 mM $NaNO_3$ at pH 6.2 (cf. **Figure 6b**) and **(b)** corresponding maps of work of adhesion generated from the force-distance curves.

63

References

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2 F. Ahimou, F. A. Denis, A. Touhami and Y. F. Dufrêne, *Langmuir*, 2002, **18**, 9937–9941.

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