Supplementary Materials

Individual and Combined Effects of Extracellular Polymeric Substances and Whole Cell Components of *Chlamydomonas reinhardtii* on Silver Nanoparticle Synthesis and Stability

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1. Cell density

 10μ L-sample was cast on a hemocytometer (0.0025 mm², 0.1000 mm) and the cell culture density was determined by counting the number of cells per volume as observed in a Nikon Labophot-2 Light-microscope (Niko Inc., Minato, Tokyo, Japan).



Figure S1. Cell density of *C. reinhardtii* cultures used in the three synthesis routes.

2. Cell culture chlorophyll a composition

Chlorophyll *a* was extracted from the cells by diluting 1 mL of cell culture with 9 mL of 99.5% acetone, which was then vortexed for 1 min using a Fisher Scientific Vortex Mixer (Fisher Scientific, Hampton, NH, USA), sonicated for 5 min using a Cole-Parmer Ultrasonic Cleaner (Cole-Parmer, Vernon Hills, IL, USA), incubated at 34–37 °C in water for 5 min and centrifuged at 2500× g for 5 min using an Ample Scientific F-33D Centrifuge (Ample Scientific LLC, Norcross, GA, USA). The supernatant was taken in 1.00 cm path length quartz cuvettes and scanned from 500 nm to 800 nm by a Cary-Varian 100 Bio UV-Visible Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Finally, chlorophyll *a* concentration was calculated from the absorbance of the supernatant at 663 nm [1,2].



Figure S2. *Chl*-a absorbance at 663 nm and quantum efficiency of *C. reinhardtii* cultures used in the three synthesis routes.

3. Fluorescence signals and quantum efficiencies

Table S1a. Fluorescence signals (10× diluted) and quantum efficiencies (Q.E.) for WLC before and 1 h after the addition of AgNO₃.

Signal/	Initial values	Values 1 h after the addition of AgNO ₃			
Q.E.	at 0 h	0.000 mM	0.125 mM	0.625 mM	1.250 mM
Fo	8290 ± 826	1291 ± 20	1161 ± 102	1179 ± 34	1148 ± 169
Fm	16364 ± 1556	2047 ± 16	1202 ± 88	1180 ± 39	1171 ± 185
F_v/F_m	0.49 ± 0.01	0.37 ± 0.01	0.03 ± 0.03	0.00 ± 0.00	0.02 ± 0.01

Table S1b. Fluorescence signals (10× diluted) and quantum efficiencies (Q.E.) for LCFM before and 1 h after the addition of AgNO₃.

Signal/	Initial values	Values 1 h after the addition of AgNO ₃			
Q.E.	at 0 h	0.000 mM	0.125 mM	0.625 mM	1.250 mM
Fo	8772 ± 4331	11281 ± 230	8843 ± 821	6549 ± 749	6124 ± 612
\mathbf{F}_{m}	17993 ± 7789	20872 ± 873	9027 ± 781	6604 ± 710	6134 ± 595
F_v/F_m	0.49 ± 0.05	0.46 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00

Table S1c. Background noise from BBM control experiments 1 h after the addition of AgNO₃.

Signal	0 mM	0.125 mM	0.625 mM	1.250 mM
Fo	238	353	330	332
Fm	281	421	415	418

References

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- 2. Shoaf, W.T.; Lium, B.W. Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. *Limnol. Oceanogr.* **1976**, *21*, 926–928.