Supporting Information

Combined Toxicity of Silver Nanoparticles with Hematite or Plastic Nanoparticles to Two Freshwater Algae

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15 pages, including 3 methods, 3 tables, and 8 figures

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Effects of AgNPs on the Bioaccumulation of PsNPs

Six treatments were carried out in triplicate using two concentrations of PsNPs (3 and 30 mg C/L) and three of AgNPs (0, 100, and 300 μg/L). PsNP uptake was monitored using a LSR Fortessa cell analyzer (BD, NJ, USA) at four time points (0.5, 1, 1.5, and 2 h). Each sample was recorded for the same duration and with a constant flow rate. The forward scatter (FSC) was obtained using a light filter with a bandpass of 478–498 nm. Chlorophyll and PsNP fluorescence in the samples was further excited using a 488-nm laser (50 mW). Photoluminescence was then detected using PerCP (bandpass = 655–735 nm) and FTIC (bandpass = 500–560 nm) filters. A threshold of 200 was set for the FSC and FTIC signals, and 500 for PerCP. The data were acquired and processed using the BD FACSDiva 6.0. The results from the photoluminescence measurements are expressed as arbitrary units (a.u.).

Concentration Addition (CA) and Independent Action (IA) Modeling

The CA model assumes that each toxicant in a mixture has an identical mechanism of action. This concept can be mathematically formulated as:

$$ECx_{mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i}\right)^{-1} \quad (S1)$$

where n denotes the number of mixture components, p_i is the relative fraction of chemical i in the mixture, and x is a common effect level provoked by exposure to a single substance or mixture concentration (ECx_i or ECx_{mix}, respectively).

The IA model assumes that each toxicant in a mixture has completely different modes of action. Under this condition, the combined effect can be calculated from the effects of the individual mixture components by following the statistical concept of independent random events. This can be mathematically expressed as:

$$E(C_{mix}) = 1 - \prod_{i=1}^{n} [1 - E(C_i)] \quad (S2)$$

where $E(C_{mix})$ represents the predicted effect of an n-component mixture and $E(C_i)$ the effect of the *i*th mixture component when applied alone at a concentration C_i.

Subcellular Imaging of AgNPs, HemNPs, and PsNPs

The subcellular distribution of AgNPs and HemNPs in *C. reinhardtii* and *O. danica* and the elemental composition of the respective cell areas were determined using a transmission electron microscope equipped with an energy-dispersive X-ray spectrometer (JEM-2100, JEOL, Tokyo, Japan). Briefly, after a 1-day exposure to AgNPs (200 μg/L) and HemNPs (10 mg Fe/L), the algal cells were fixed with 1.25% (w/v) glutaraldehyde in 0.2 M phosphate buffer, embedded in epoxy resin, and sectioned to obtain ~100-nm-thick sections. These were stained with 1% w/v osmium tetroxide and dehydrated in a series of 10-min incubations in graded acetone solutions of 30, 50, 70, 80, 90, and 2×100% (v/v). To better detect the NPs, the sections were not stained with uranyl acetate and lead citrate.

As PsNPs can hardly be differentiated from the cell matrix by TEM, their fluorescence-labeled counterpart (30 mg C/L) was used in the experiments with both algae. After a 2-h incubation of the PsNPs with the cells, their subcellular distribution was visualized by confocal laser scanning microscopy (CLSM, LSM710, Carl Zeiss, Oberkochen, Germany). Three channels (CH1: differential interference contrast; CH2: 488 nm laser, filter bandpass = 492–630 nm; CH3: 633 nm laser, filter bandpass = 637–747 nm) were used to obtain images of the whole cells as well as the intracellular content of PsNPs and chlorophyll. Additionally, the cells were optically sectioned (z-axis) from one side to the other at a 0.75- or 0.9-μm spacing to verify internalization of the PsNPs by *C. reinhardtii* and *O. danica*. All images were acquired and processed using the supplied software ZEN 2008 (Zeiss).

Table S1. Chemical components and their concentrations in WC* medium.

Components	Final concentration (μM)	Components	Final concentration (μM)
$NaNO_3$	1000	Na_2MoO_4	0.01
K_2HPO_4	50	$CoC1_2$	0.005
$MgSO_4$	150	$ZnSO_4$	0.01
$CaCl_2$	250	$FeCl_3$	0.5
$NaHCO_3$	150	$CuSO_4$	0.00005
Na_2SiO_3	100	$MnCl_2$	0.1

Table S2. Chemical components and their concentrations in DY-V* medium.

Component	Final concentration (μM)	Component	Final concentration (μM)
MES	1000	Glucose	1000
Na_2β-glycerophosphate	10	MOPs	5000
$NaNO_3$	235	$FeCl_3$	0.37
NH_4Cl	500	MnCl	0.1
KCl	40	$ZnSO_4$	0.014
$MgSO_4$	203	$CoCl_2$	0.0034
$CaCl_2$	676	Na_2MoO_4	0.0083
H_3BO_3	13	Na_3VO_4	0.00068
$Na_2SiO_3 \cdot 9H_2O$	49	Na_2SeO_3	0.0023

Table S3. The values of K_a (L/μg) and $[Ag]_{ads}^{max}$ (mg/g, mg Ag/g Fe or mg Ag/g C) determined from the adsorption of Ag ions by HemNPs and PsNPs in WC* and DY-V*, as calculated according to the Langmuir isotherm. The data are expressed as the mean ± standard deviation (n = 3).

Medium	NPs	$[Ag]_{ads}^{max}$	K_a	R^2	p
WC*	HemNPs	4.097±0.861	0.0087±0.0027	0.987	< 0.0001
	PsNPs	0.094±0.020	0.0201±0.0085	0.957	< 0.0001
DY-V*	HemNPs	0.472±0.111	0.0211±0.0075	0.967	< 0.0001
	PsNPs	0.022±0.003	0.0643±0.0174	0.963	< 0.0001

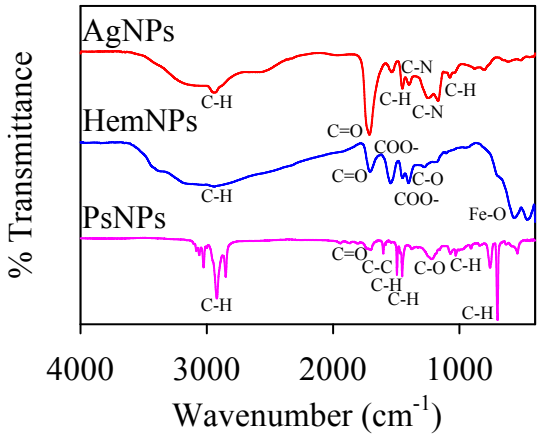


Figure S1. The FTIR spectrum of AgNPs, HemNPs, and PsNPs.

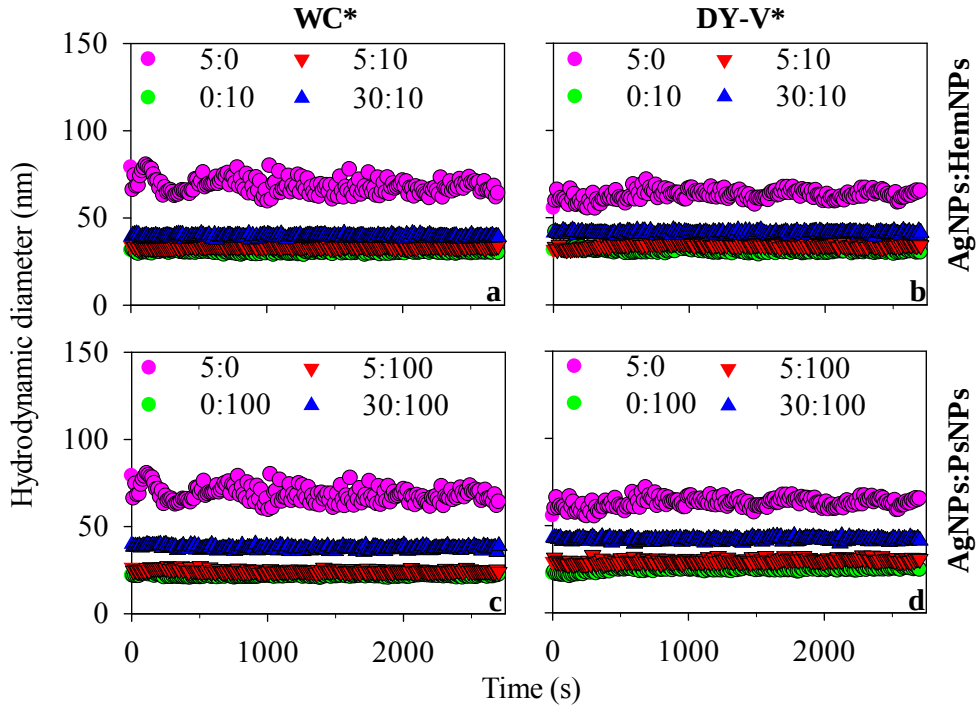


Figure S2. The hydrodynamic diameter of the particles for the mixtures of AgNPs and (a, b) HemNPs or (c, d) PsNPs with different concentration ratios in (a, c) WC* and (b, d) DY-V*. The numbers on both sides of ':' in the legends are the respective concentrations (mg/L, mg Fe/L, and mg C/L for AgNPs, HemNPs, and PsNPs, respectively) of the NPs on the right side of the figures.

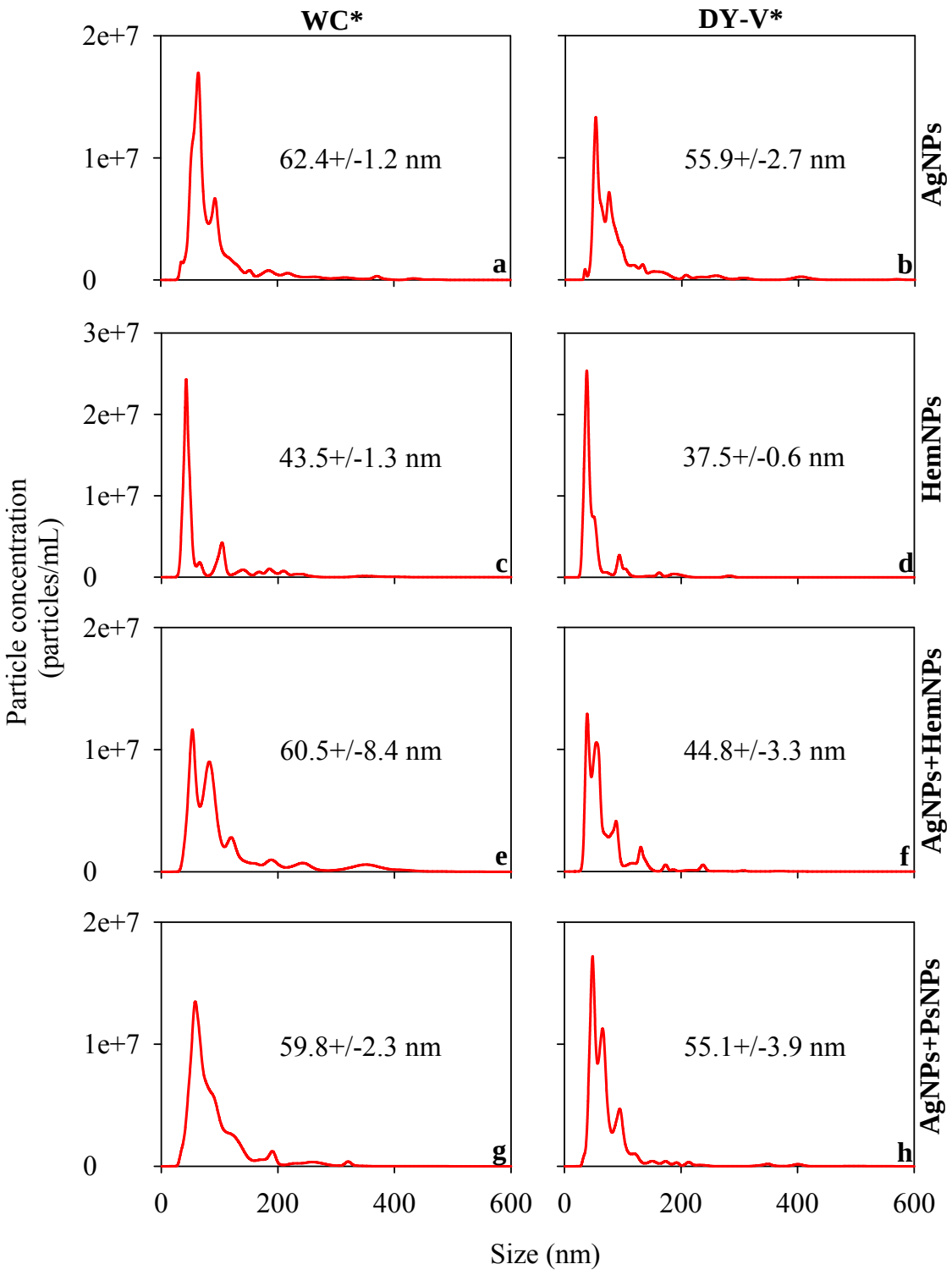


Figure S3. The size distribution of (a, b) AgNPs, (c, d) HemNPs, (e, f) AgNPs and HemNPs, (g, h) AgNPs and PsNPs in (a, c, e, g) WC* and (b, d, f, h) DY-V*, as obtained by NTA. The value in each figure represents the average size of the particles in the format of mean ± standard deviation (n = 5).

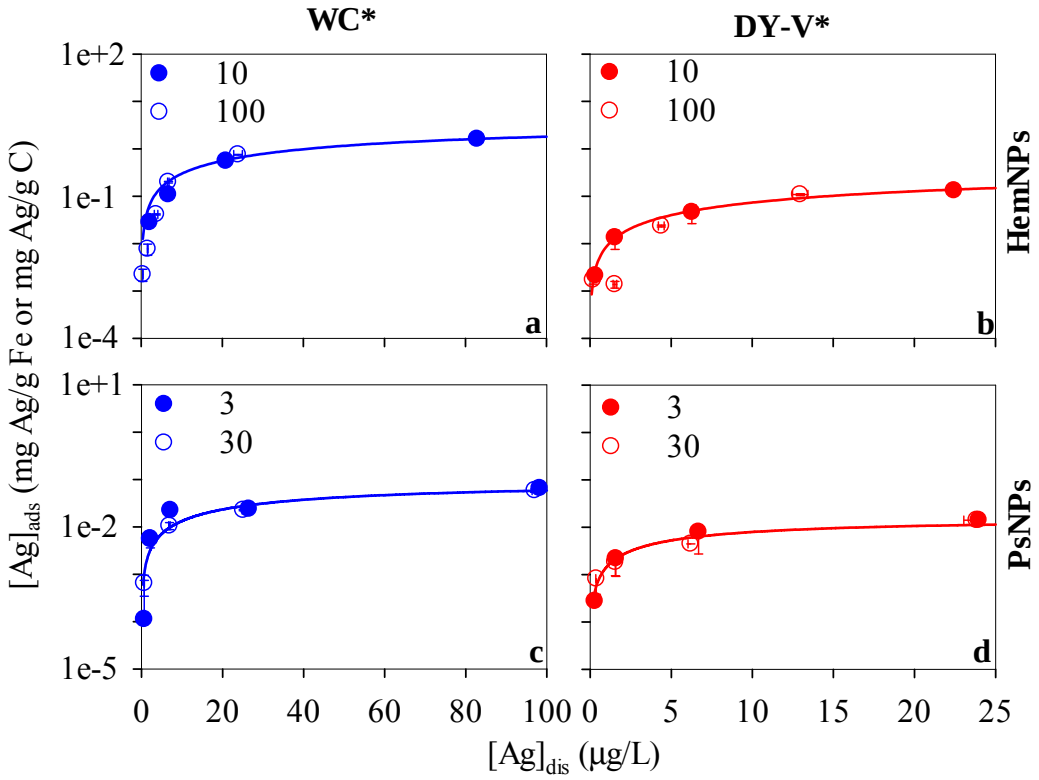


Figure S4. Ag adsorption ($[Ag]_{ads}$, mg Ag/g Fe or mg Ag/g C) on (a, b) HemNPs (10 and 100 mg Fe/L) and (c, d) PsNPs (3 and 30 mg C/L) in (a, c) WC* and (b, d) DY-V*, respectively. The data are the mean ± standard deviation (n = 3).

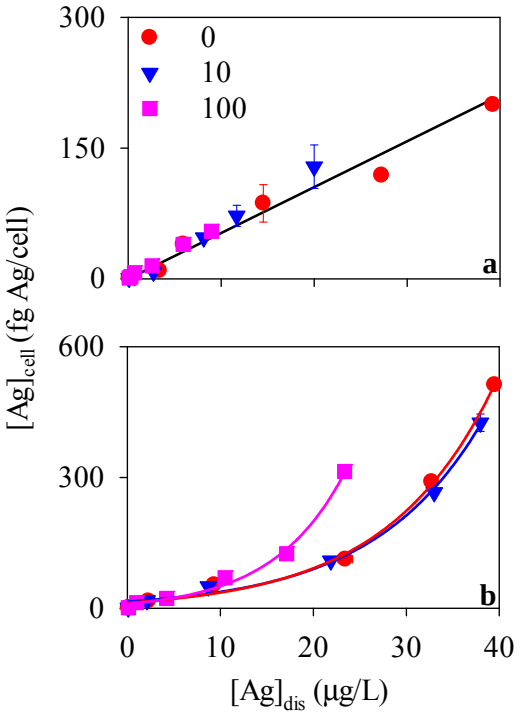
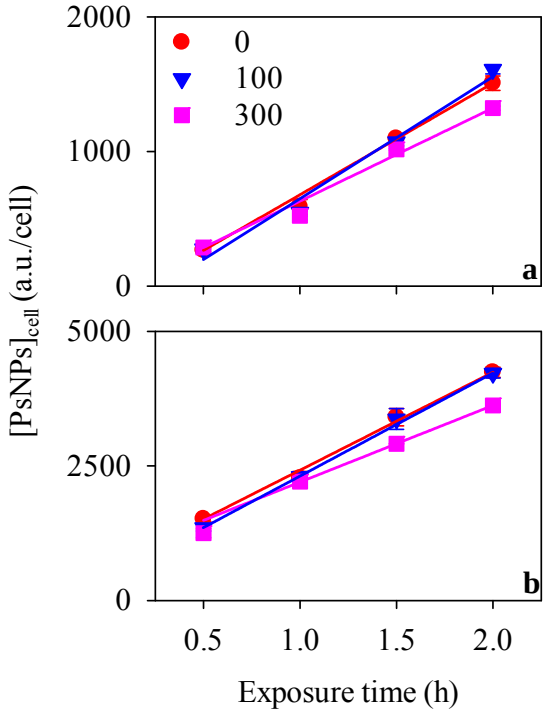


Figure S5. Variation of cellular concentration of Ag ($[Ag]_{cell}$, fg Ag/cell) with the concentration of dissolved Ag ($[Ag]_{dis}$, µg/L) for (a) *C. reinhardtii* and (b) *O. danica* in the presence of 0, 10, and 100 mg Fe/L of HemNPs, respectively. Solid lines are the linear or biphasic regressions between $[Ag]_{cell}$ and $[Ag]_{dis}$. The data are the mean ± standard deviation (n = 3).



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107 **Figure S6.** Variation of cellular concentration of PsNPs ($[PsNPs]_{cell}$, a.u./cell) in *O. danica*

108 with exposure time in the presence of 0, 100, and 300 μg/L of AgNPs. The concentration of

109 PsNPs in the experimental medium was fixed at (a) 3 and (b) 30 mg C/L, respectively. The

110 data are the mean ± standard deviation (n = 3).

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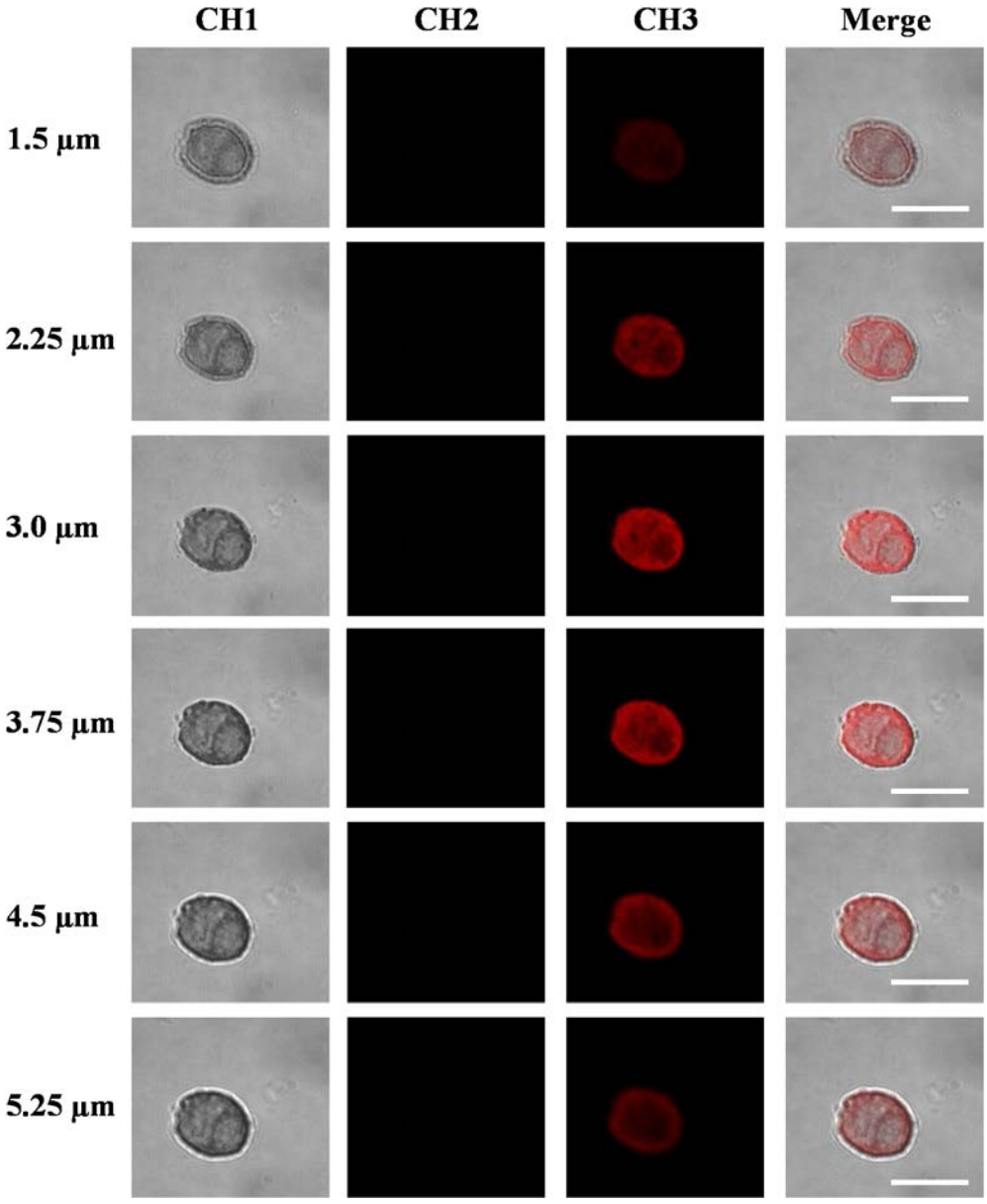

Figure S7. The confocal laser scanning microscopy images of *C. reinhardtii* as obtained *via* different channels (CH1: differential interference contrast; CH2: 488 nm laser, filter bandpass = 492–630 nm; CH3: 633 nm laser, filter bandpass = 637–747 nm; Merge: combinative images from CH1 to 3) under the z-scanning mode at a 0.75 μm spacing after exposed to 30 mg C/L of PsNPs for 2 h. Scale bars are 10 μm.

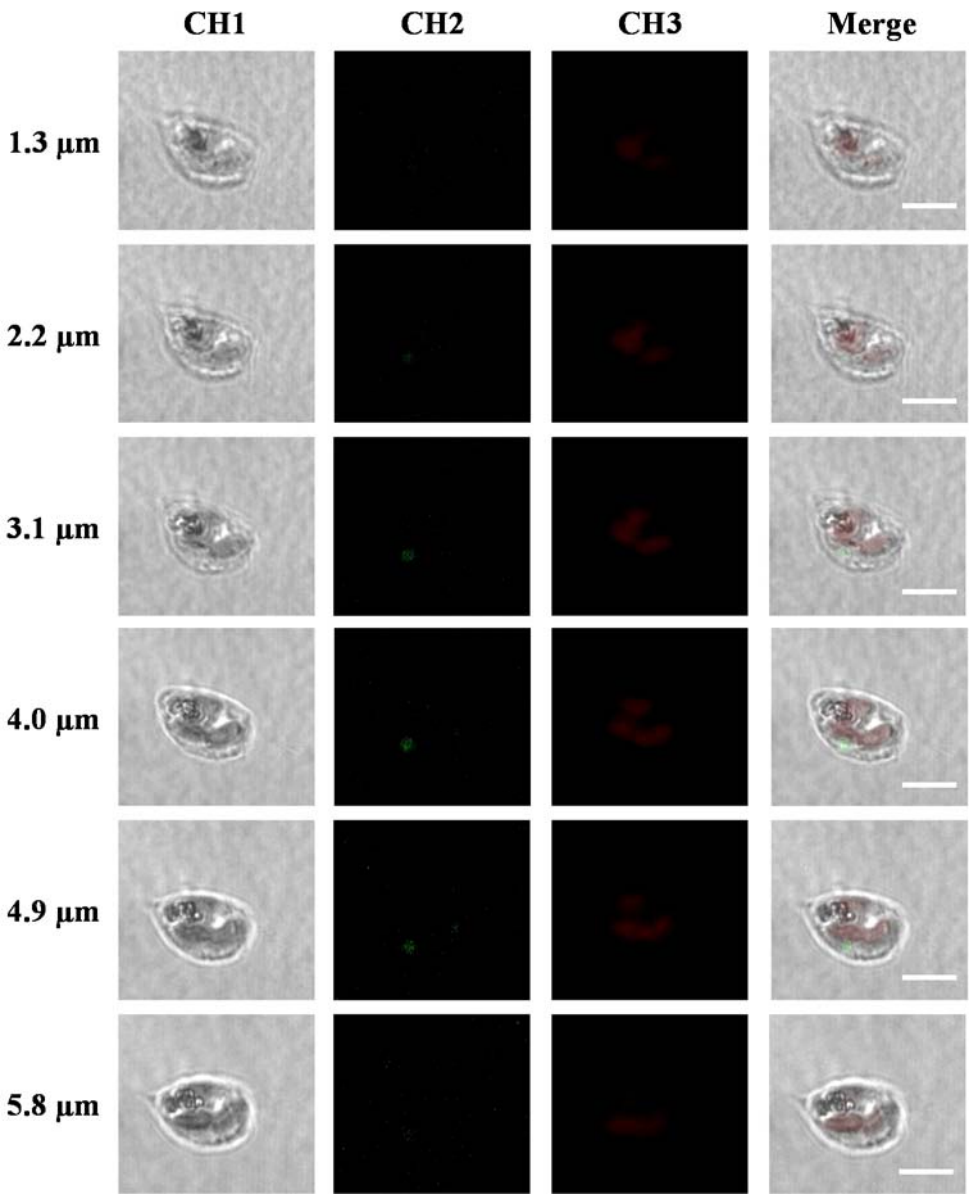

Figure S8. The confocal laser scanning microscopy images of *O. danica* as obtained *via* different channels (CH1: differential interference contrast; CH2: 488 nm laser, filter bandpass = 492–630 nm; CH3: 633 nm laser, filter bandpass = 637–747 nm; Merge: combinative images from CH1 to 3) under the z-scanning mode at a 0.9 μm spacing after exposed to 30 mg C/L of PsNPs for 2 h. Scale bars are 10 μm.