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Ion-Driven Cell Wall Disruption Enables Silver Nanoparticle Penetration in Freshwater Algae: Evidence from Core–Shell Dissolution Tuning

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1. The calculation of particle numbers for Au@Ag NPs was based on the following equation:

$$C_{particle} = \frac{C_{Au} \times 10^{18}}{\rho \times \frac{1}{6} \times \pi \times d^3}$$

$C_{particle}$ represents particle concentration (unit, particles per liter), C_{Au} represents Au concentration (mg/L). ρ represents the gold density, which is 19.32 g/cm^3. d represents the determined diameter of Au core, which is 15, 9, and 5.6 nm for L-, M-, and H-Au@Ag NPs, respectively.

2. The estimation of Ag species in the algae.

The internalized AgNP concentration (C_{Ag_p}) was based on the Au concentration and Ag/Au ratio, as shown in the following equation:

$$C_{Ag\_p} = C_{Au} \times R$$

C_{Au} represents Au concentration (mg/L), and R represents pristine Ag/Au ratio in the NPs.

The contributions of Ag ions to total Ag bioaccumulation could be estimated by the following equation:

$$r_{Ag\_ion} = \frac{C_{Ag\_ion}}{C_{Ag\_t}} = \frac{C_{Ag\_t} - C_{Ag\_p}}{C_{Ag\_t}}$$

C_{Ag_t} represents total Ag bioaccumulation. C_{Ag_ion} represents the internalized Ag ions concentration, which is equal to the C_{Ag_t} minus C_{Ag_p}. Since the free Ag ions concentrations could be determined, the proportion of particulate Ag concentration in the total Ag could be estimated:

$$r_p = \frac{C_p}{C_{Ag\_t}} = \frac{C_{Ag\_t} - C_{Ag\_free\_ion}}{C_{Ag\_t}}$$

$C_{Ag\_free\_ion}$ represents free Ag ion concentration in the algae. C_p represents the particulate Ag concentration, which is equal to the C_{Ag_t} minus $C_{Ag\_free\_ion}$. In the above equations, the dissolution of Au@Ag NPs within algal cells was intentionally disregarded. This is because accounting for the dissolution would lead to a higher actual r_{Ag_ion} and r_p than the estimated value. Consequently, in the main text, this proportion is consistently expressed as being greater than a certain threshold.

Table S1 Ag and Au concentration in the different exposure groups

Particle concentration	L-Au@Ag	M-Au@Ag	H-Au@Ag
5.50×10^{13} particles/L	Ag 0.94 mg/L	Ag 1.75 mg/L	Ag 1.94 mg/L
	Au 1.88 mg/L	Au 0.41 mg/L	Au 0.10 mg/L
1.10×10^{14} particles/L	Ag 1.88 mg/L	Ag 3.53 mg/L	Ag 3.88 mg/L
	Au 3.76 mg/L	Au 0.82 mg/L	Au 0.20 mg/L
1.64×10^{14} particles/L	Ag 2.82 mg/L	Ag 5.29 mg/L	Ag 5.82 mg/L
	Au 5.63 mg/L	Au 1.23 mg/L	Au 0.31 mg/L
2.19×10^{14} particles/L	Ag 3.76 mg/L	Ag 7.05 mg/L	Ag 7.79 mg/L
	Au 7.51 mg/L	Au 1.64 mg/L	Au 0.41 mg/L
2.74×10^{14} particles/L	Ag 4.70 mg/L	Ag 8.82 mg/L	Ag 9.72 mg/L
	Au 9.39 mg/L	Au 2.05 mg/L	Au 0.51 mg/L

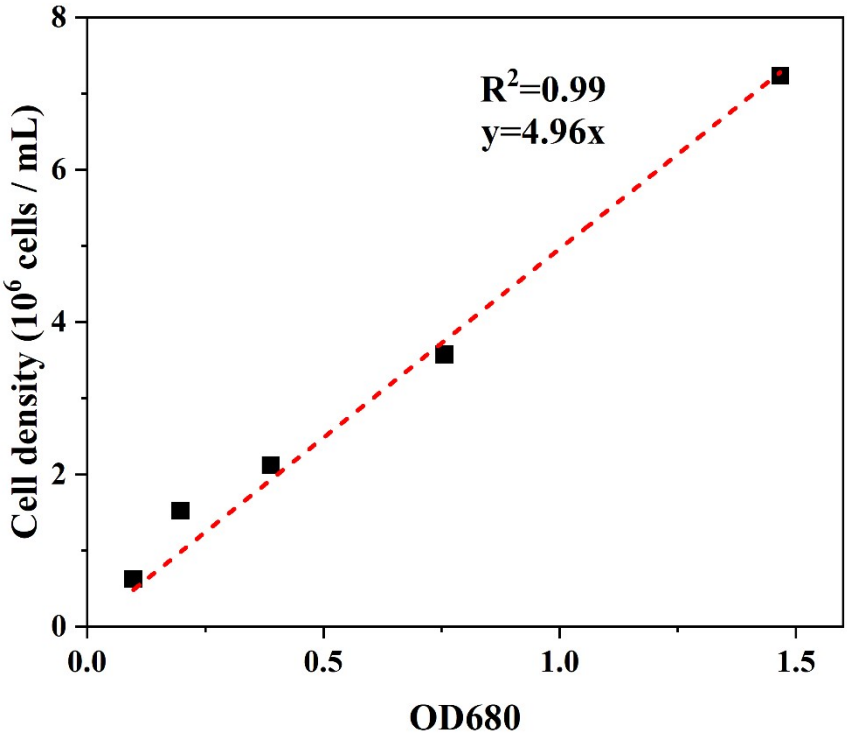

Figure S1 The linear relationship between cell density and OD680.

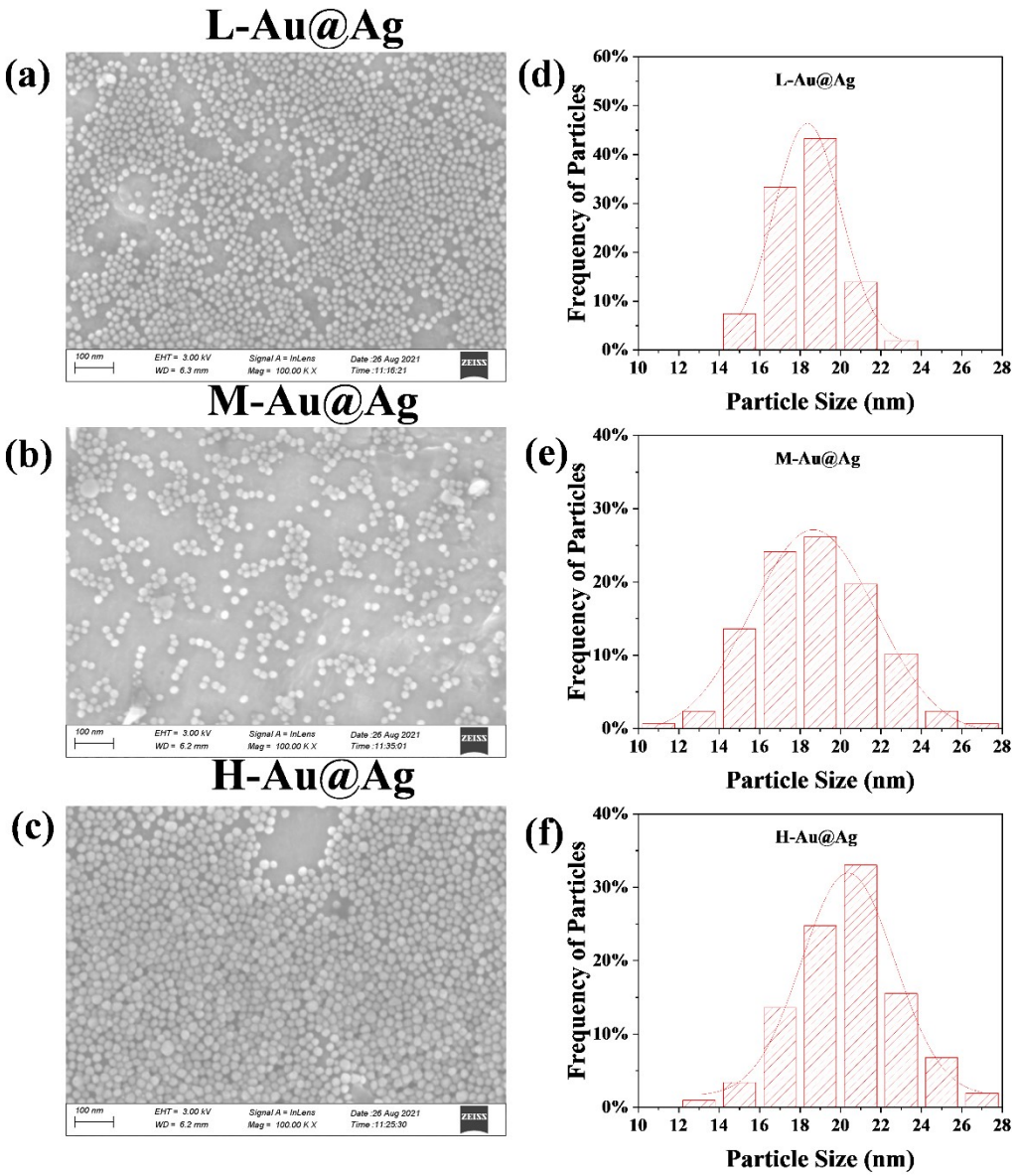

Figure S2 (a-c) Scanning electron microscope images (SEM), (d-e) size distributions of L-Au@Ag, M-Au@Ag and H-Au@Ag.

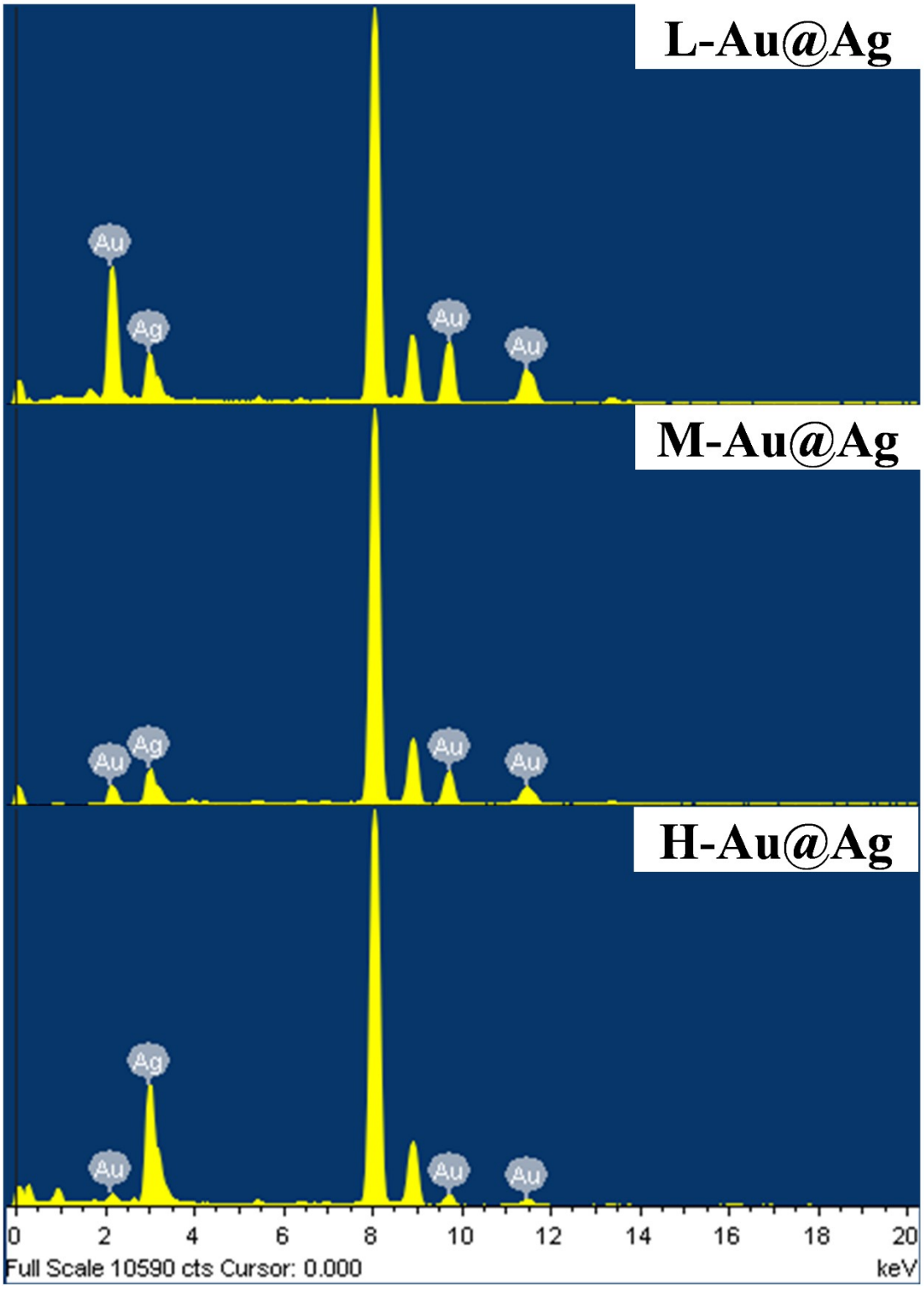

Figure S3 EDX analysis of L-Au@Ag, M-Au@Ag and H-Au@Ag

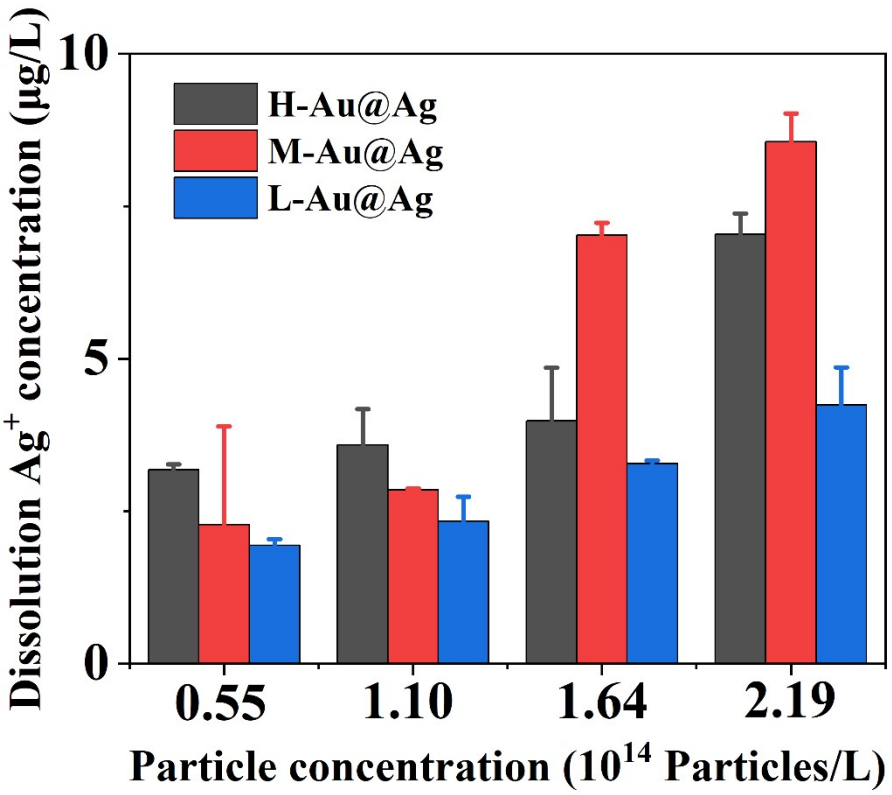

Figure S4 The released Ag ions from Au@Ag NPs at different concentrations in the SM7 medium.

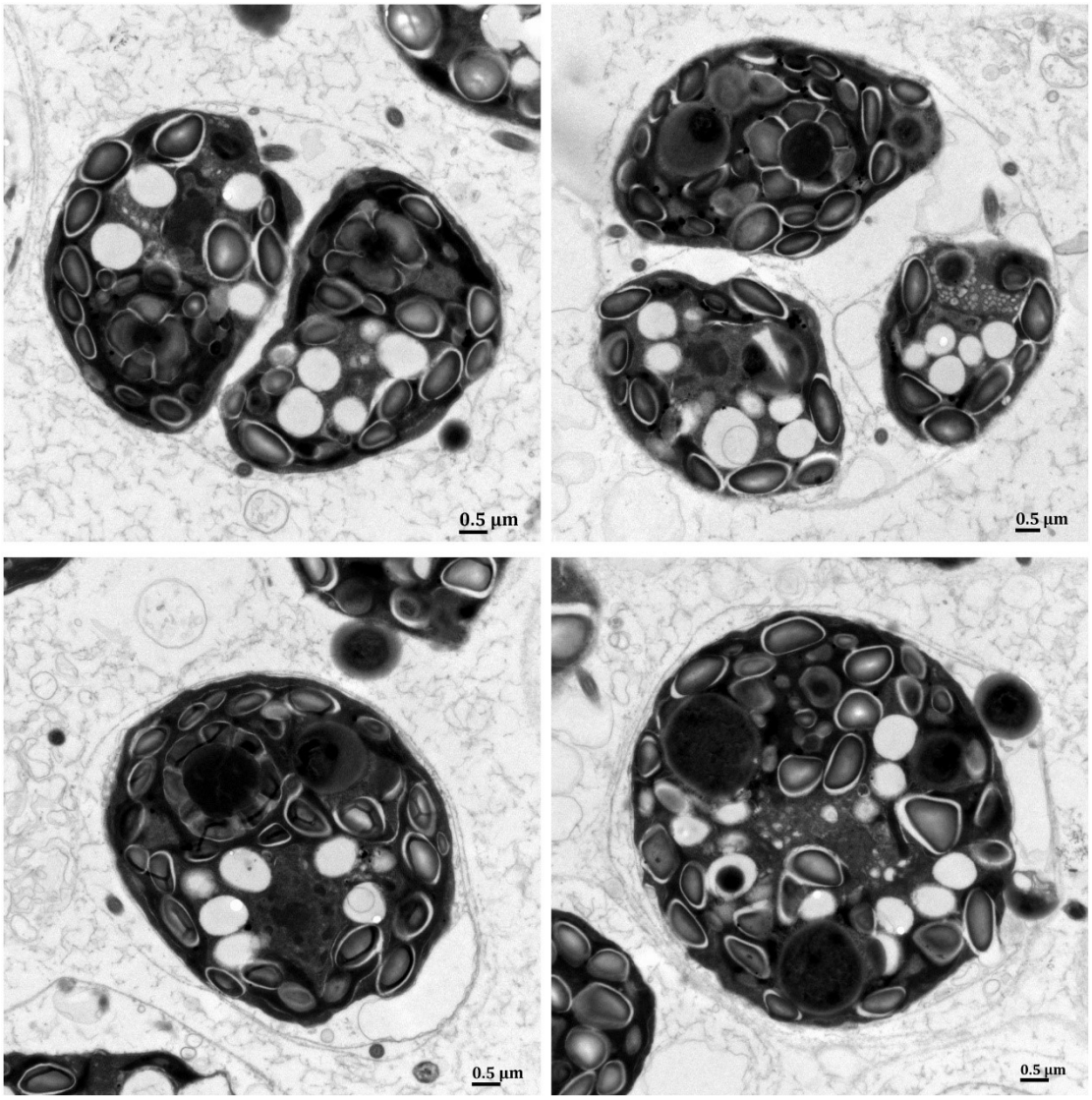

Figure S5 The morphology of algae cell without exposure of Au@Ag NPs.

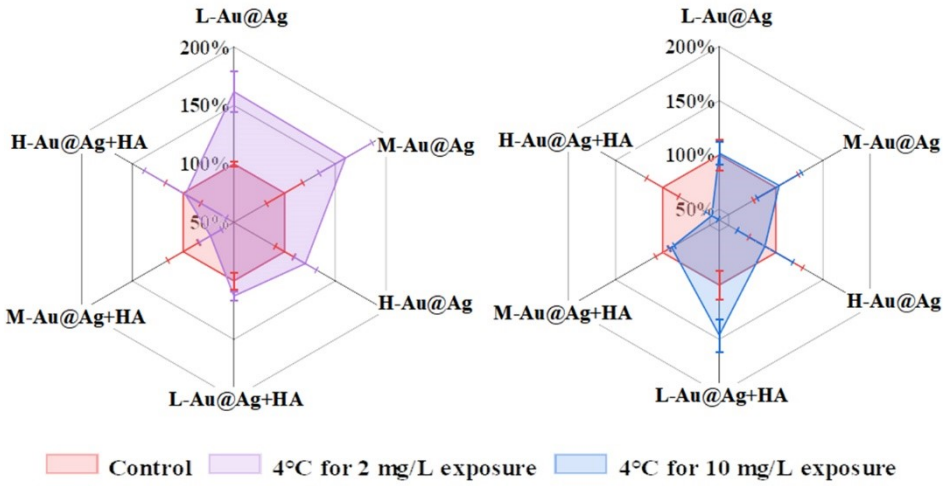

Figure S6 The influence of low temperature (4°C) on the uptake of Au@Ag NPs.

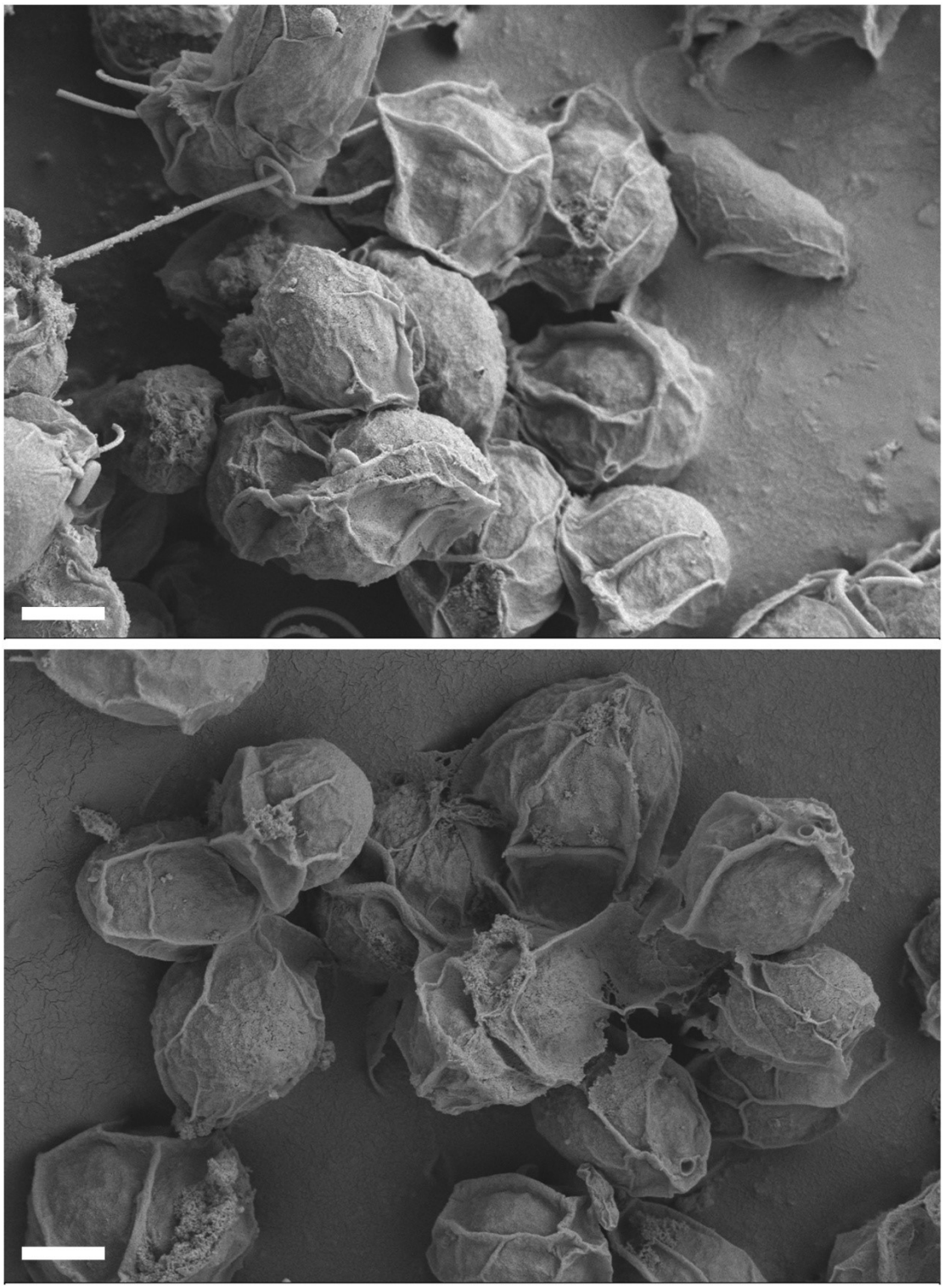

Figure S7 The SEM image of algal cells in the control group exhibited intact morphology. The white bar represents 2 μm.

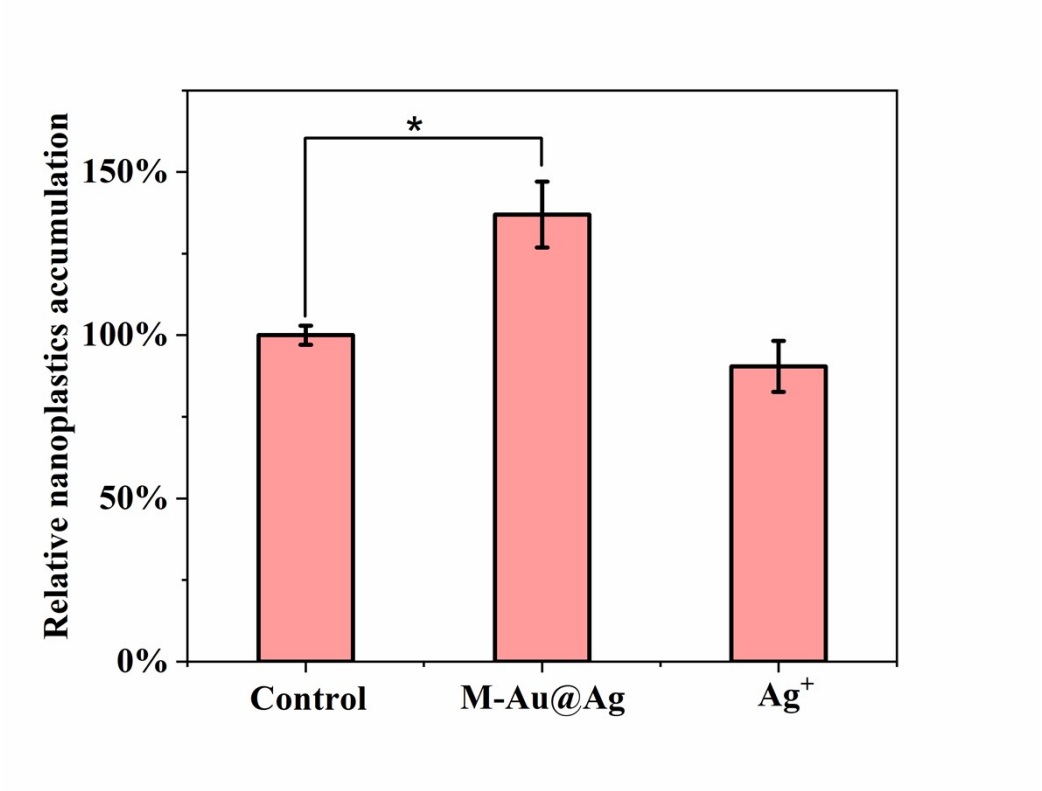

Figure S8 The influence of preincubation with M-Au@Ag or Ag ions on the bioaccumulation of nanoplastic. The preincubation of NPs significantly increases the accumulation of nanoplastic.

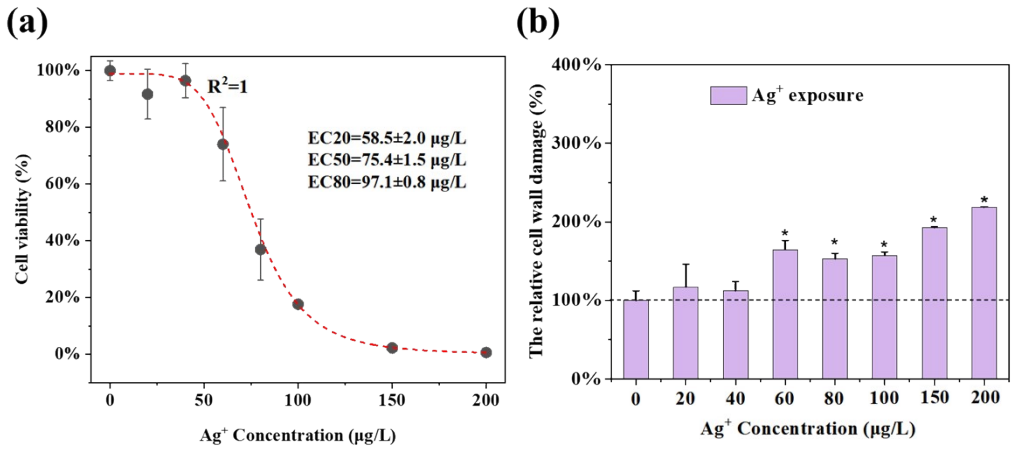

Figure S9 (a) The dose-effect relationship between Ag^+ concentration and mortality. The maximum concentration of Ag ions released by NPs is 16.5 μg/L, which would not induce cell death. (b) The change of cell membrane damage with the increase of Ag^+ concentration. It is indicated that free Ag ions released by NPs in the extracellular medium could not damage the cell wall.

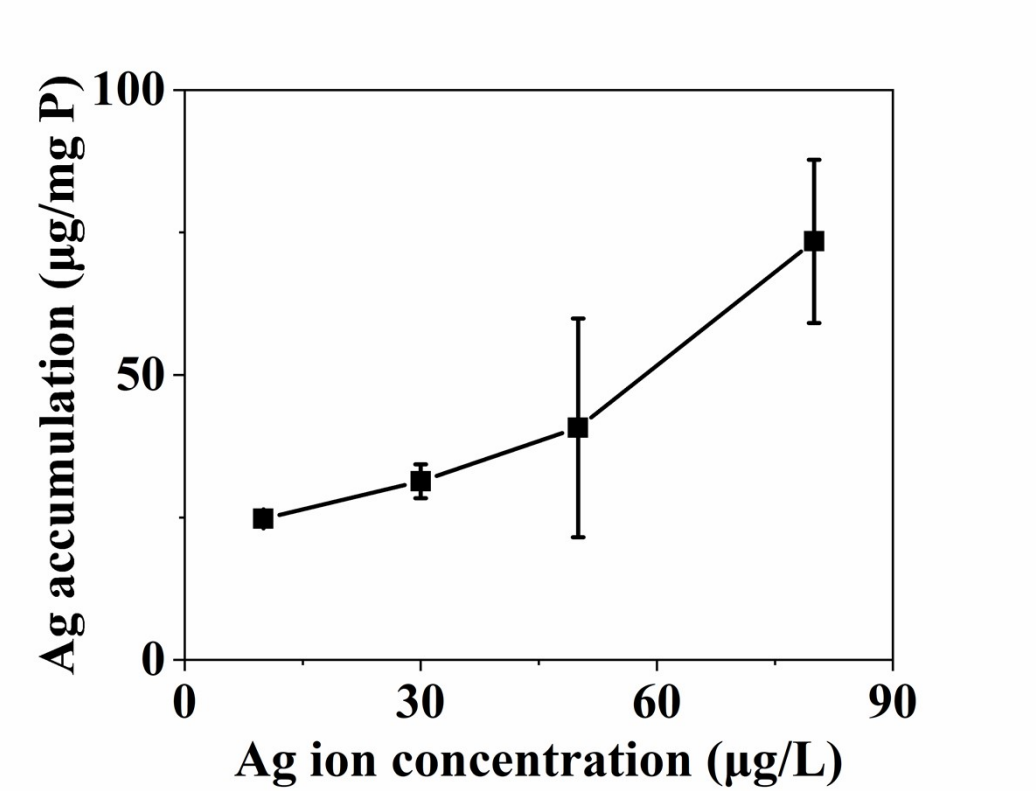

Figure S10 The Ag accumulation in the algae after 24 h exposure of Ag ion.

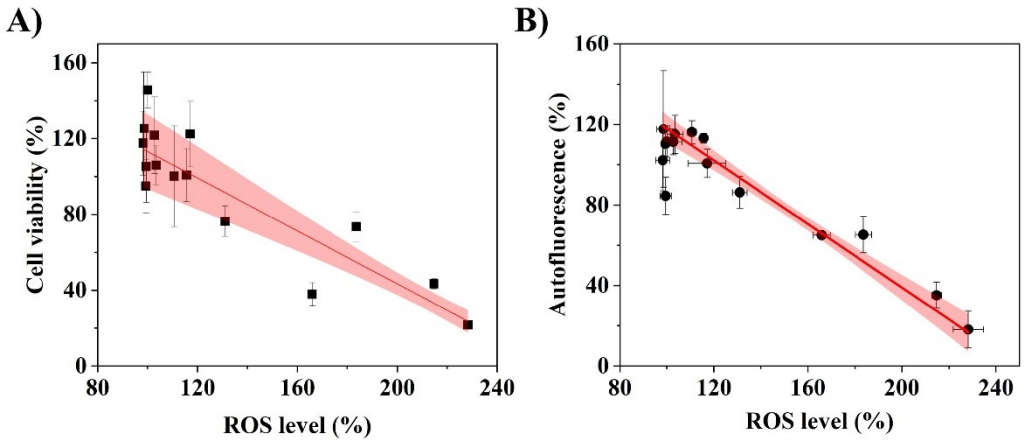

Figure S11 The correlation analysis between ROS level and (A) cell viability/ (B) autofluorescence. These scatters represent all Au@Ag NPs groups.

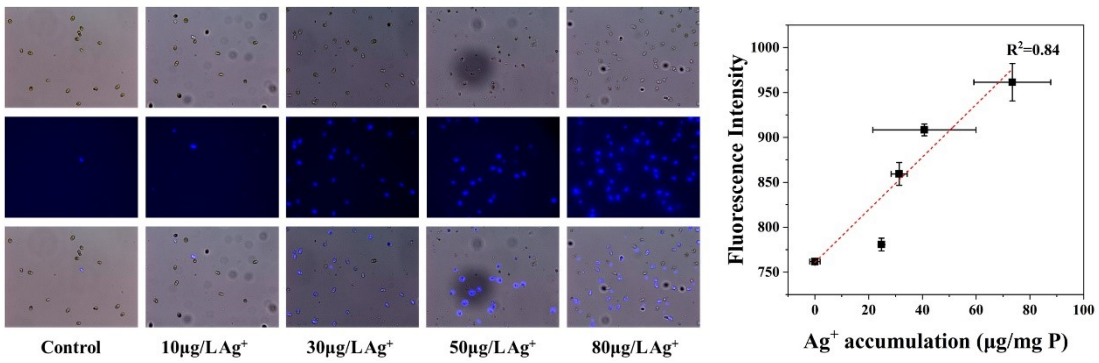

Figure S12 The left panel represents fluorescence images of algae after being exposed with different concentrations of Ag ions. The blue color represents the fluorescence of Ag^+ ion. The right panel plot the linear relationship between fluorescence intensity and Ag accumulation, which is used as standard curve to calculate the free Ag ion concentration in the algae.

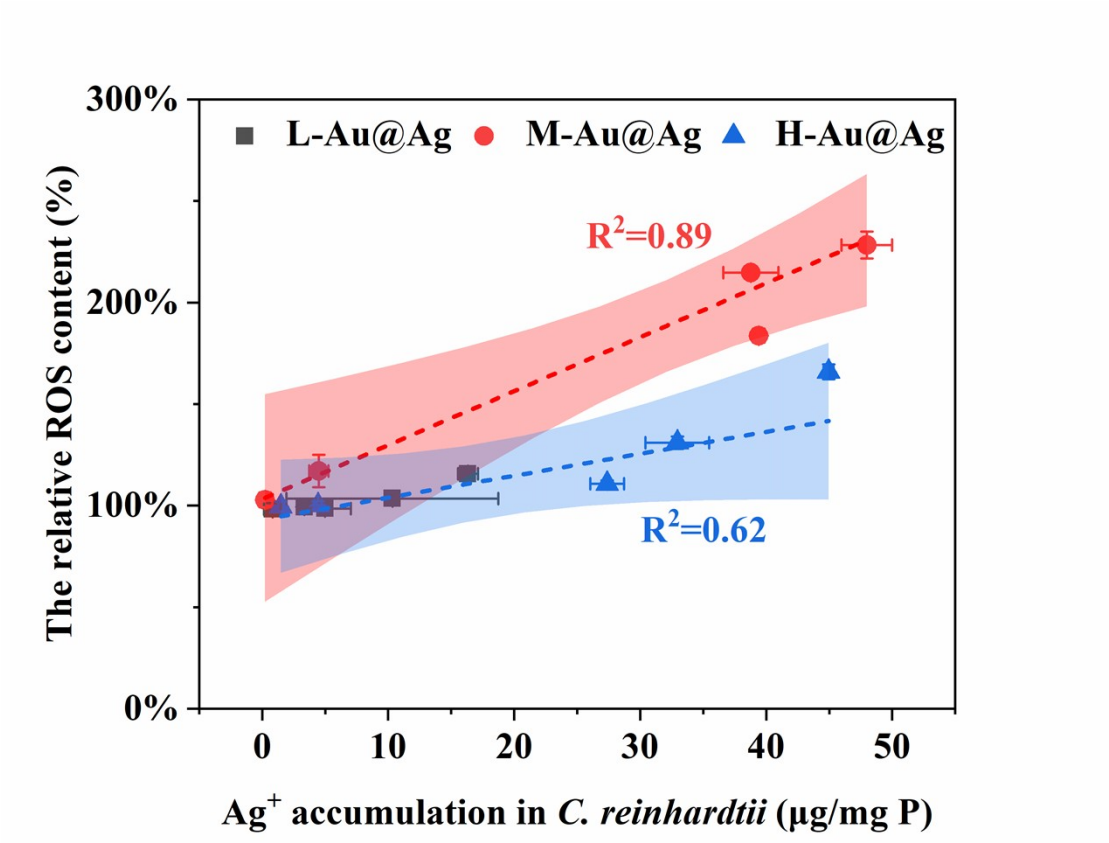

Figure S13 The correlation analysis between ROS level and Ag ion bioaccumulation. As the ROS change for L-Au@Ag group is minor, therefore the correlation analysis is conducted in the M-Au@Ag and H-Au@Ag group.

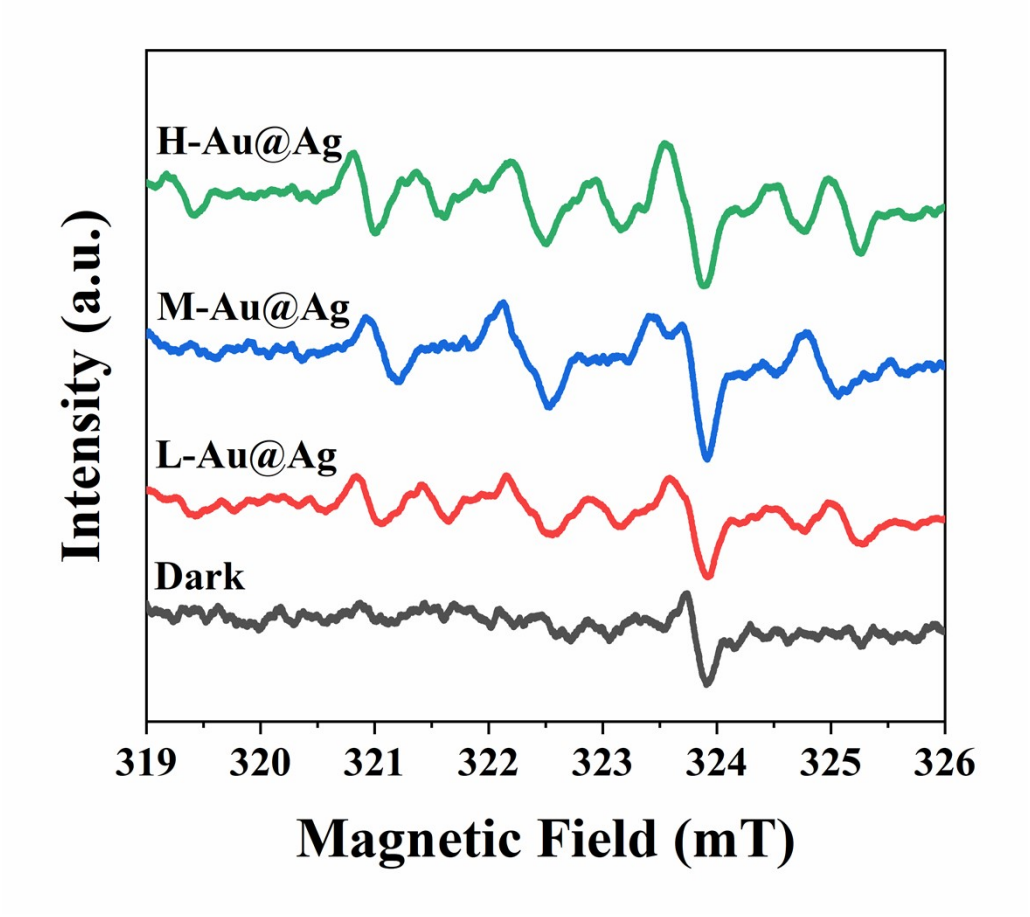

Figure S14 The ESR spectrum for all Au@Ag NPs.

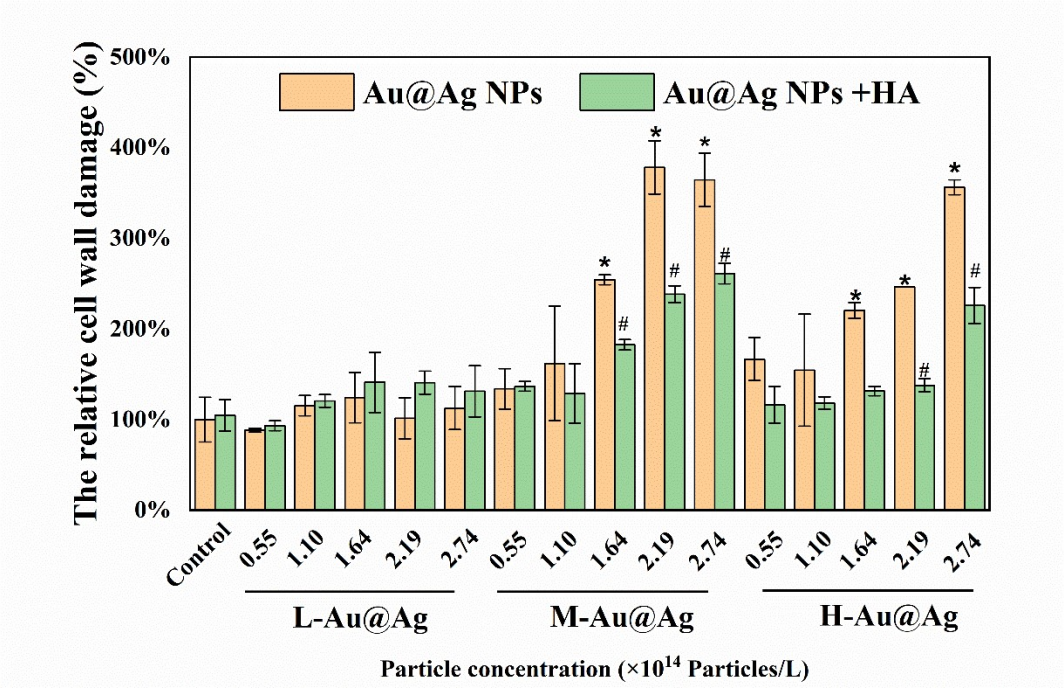

Figure S15 The influence of HA on the cell membrane damage induced by Au@Ag NPs exposure.

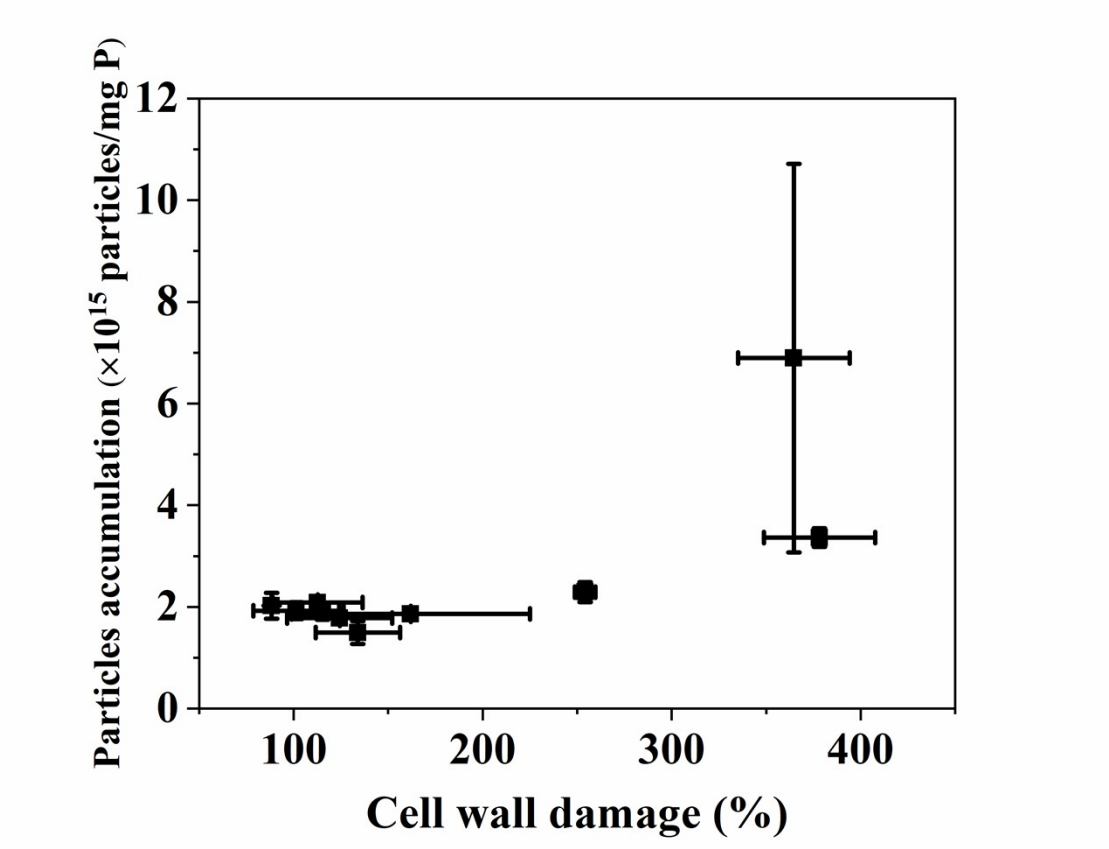

Figure S16 The relationship between cell membrane damage and accumulation of particles. It is indicated that particles could only penetrate into the cell wall after cell wall damage is beyond 150%.

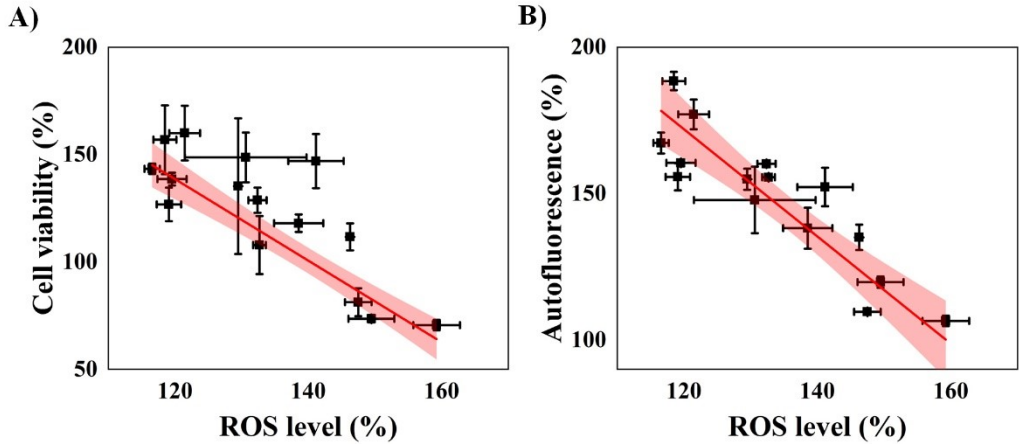

Figure S17 The correlation analysis between ROS level and (A) cell viability/ (B) autofluorescence. These scatters represent all Au@Ag NPs groups in the existence of humic acid.

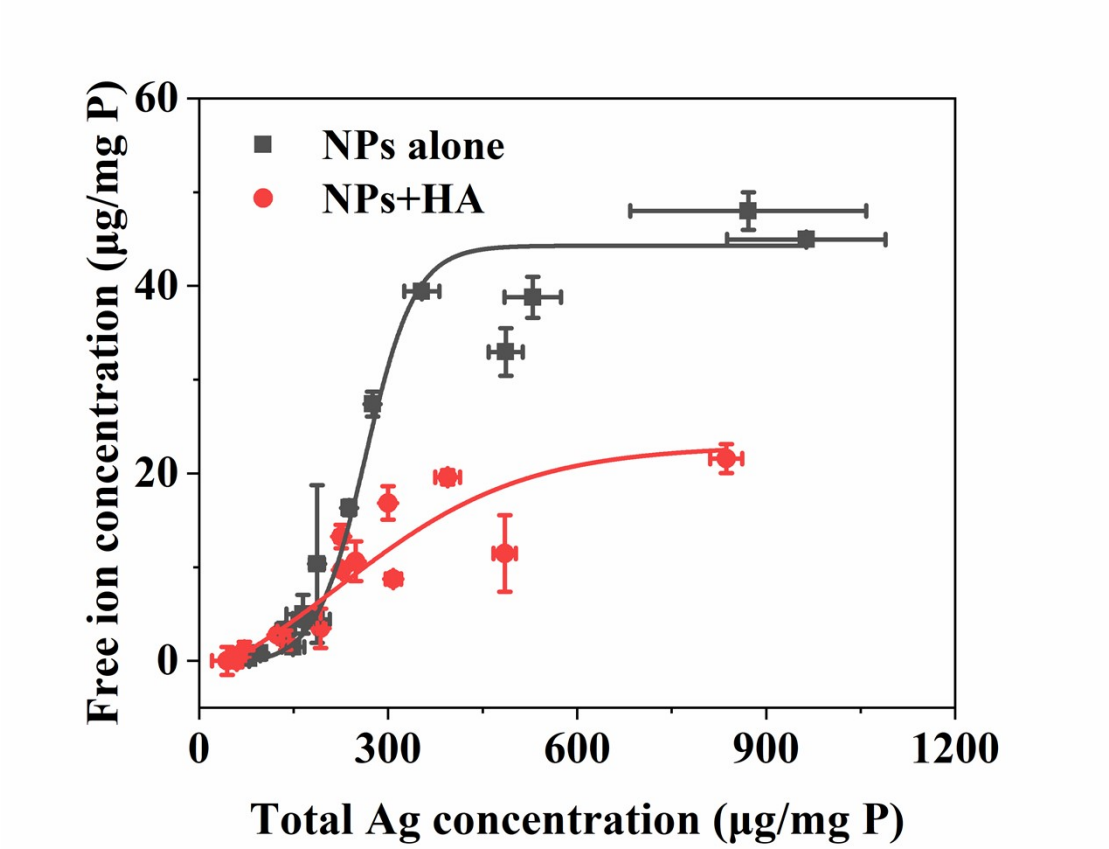

Figure S18 The relationship between total Ag concentration and free Ag ion concentration in the algae. The addition of HA significantly reduces the free Ag ion concentration at the equivalent total Ag concentration, which would reduce the algal toxicity.