Supplementary Information

Enhanced accumulation of microalgal pigments using metal nanoparticle solutions as light filtering devices

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S1. Microalgal growth

Wild type *Chlorella vulgaris* microalgal cultures were initially obtained from the Australian National Algae Culture Collection at CSIRO, Tasmania. The cells were grown in a defined algal media (MLA media) containing several components including nitrates, phosphates, carbonates, several trace metals and vitamins.[1] The culture flasks were grown in a plant-growth room with a controlled temperature around 25 °C, upon orbital shaking at a rotational speed of 75 rpm (Thermoline Scientific) under diurnal illumination of 16 h light/8 h dark cycles (Figure S1e). Light intensity of cool-white fluorescent illumination just at the top-surface of the algal flasks during the illumination periods was measured to be ~75 $\mu mol.m^{-2}.s^{-1}$. Erlenmeyer flasks with a total volume of 30 mL were used as culture reactors, after filling them with 10 mL microalgal solution (Fig. S1a). Fifty milliliters of nanoparticle solutions were placed inside a glass beaker (250 mL) for surrounding the Erlenmeyer flasks containing microalgal cells (Fig. S1b). Both sides and the bottom of the glass beaker shown in Fig. S1b were wrapped with black paper to prevent full spectrum light from reaching the algae flask from the sides (Fig. S1c). Wrapping the mouth of the culture flasks with an aluminum foil was also found to be necessary for avoiding any microbial contamination from air. In order to keep the level of the liquid constant, 1 mL of the growing culture was removed from each flask every 2 days and replaced with an identical volume of fresh growth media (Bolch and Blackburn, 1996) at the time of pigment measurements.

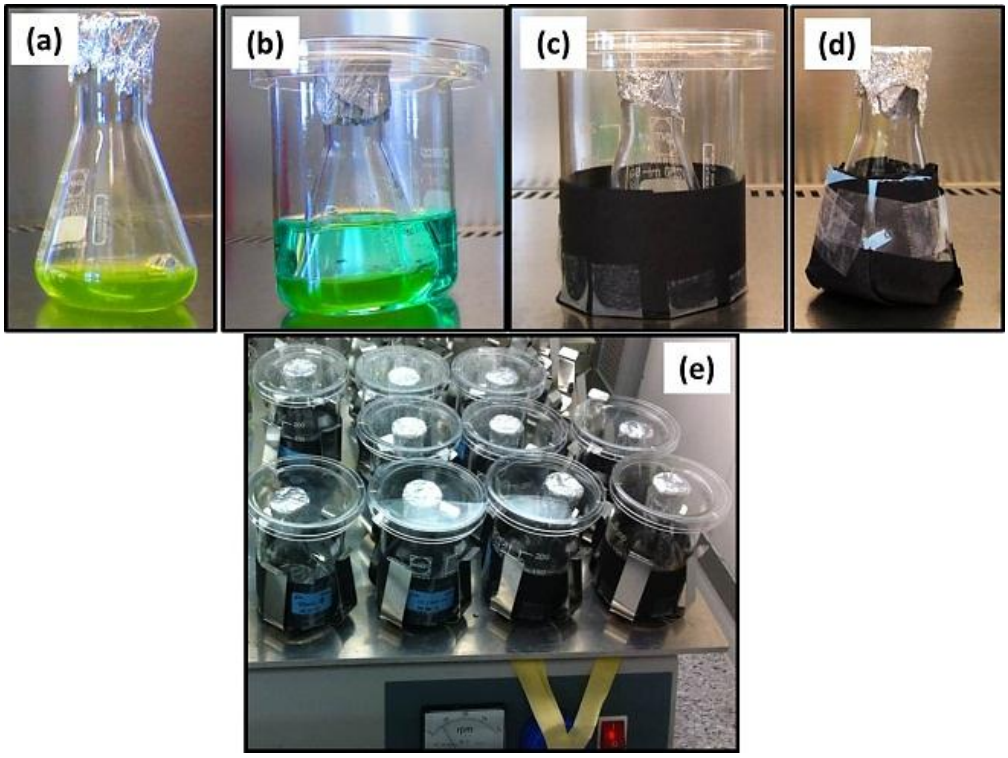

Figure S1. Schematic illustration of the overall process, involving the preparation of nanoparticle solutions **(a-c)**, and negative control **(d)**. Prepared systems were located inside a growth-room working at a constant temperature (25 °C), with flasks shaken at a rotational speed of 75 rpm under diurnal illumination of 16/8 (h/h) light to dark cycles **(e)**.

S2. Preparation of silver nanoparticles

In flask one, 30 mg $NaBH_4$ and 120 mg sodium dodecyl sulfate (SDS) was added to 50 mL of Milli-Q water and placed in the fridge at 2 degrees for 30 minutes. In flask two, 1 mL of 0.1 M $AgNO_3$ was added to 50 mL of Milli-Q water and placed in a fridge at 2 °C for 30 minutes. Flask one was placed in an iced bath and stirred rapidly the contents of flask two was added to flask one over 10 minutes. The solution was stirred for a further 1 hr. The plasmon maximum was at 404 nm (see, Figure 2).

S3. Preparation of gold seed solution

In flask one 7.6 μL of 0.33 M $HAuCl_4$ was added to 5 mL of Milli-Q water. In flask two 5 mL of Milli-Q water was added to 364 mg of cetyltrimethylammonium bromide (CTAB) and was stirred rapidly while heating until the solution became clear. Flask two was then added to flask one to result in a dark yellow solution. The combined solution was stirred rapidly while 0.8 ml of ice cold 15 mM $NaBH_4$ was added. This resulted in the brown gold seed solution.

S4. Preparation of gold nanorods

In flask one 76 µL of 0.33 M $HAuCl_4$ was added to 50 mL of Milli-Q water. In flask two 50 ml of Milli-Q water was added to 3.64 g of CTAB and was stirred rapidly while heating until the solution became clear. Flask two was then added to flask one to result in a dark yellow solution. To this solution, 22 µL of 0.1 M $AgNO_3$ was added while stirring and followed by 552 µL of 0.1 M ascorbic acid to produce a clear solution. To this solution 120 µL of the gold seed solution was added and the flask was transferred to an oil bath at 30 °C and left for 2 hours without stirring. The plasmon maximum was at 642 nm (Figure 2). By changing the added amount of $AgNO_3$ solution to 24 µL and 28 µL, two other gold nanorod solutions were produced that have plasmon maximum values of 662 and 710 nm (Figure 2), respectively.

For the gold-nanoparticle solutions with plasmon maximum values higher than 600 nm, crystallization problem is most likely to occur at 25 °C due to the presence of CTAB. This difficulty has been solved by removing the crystals that were settled at the bottom of the flasks. Gentle-rotation of the culture flasks at around 75 rpm was also found to be necessary for avoiding any bubble formation on the surface of each solution. In order to reach a plasmon maximum at an exact blue region (around 450 nm), Ag-Au alloys prepared after adding $HAuCl_4$ solution to the aforementioned silver nanoparticle solution were also investigated, which results in a shift in the overall plasmon maximum to 440 nm. However this solution could not be used within our microalgal growth experiments, as it tends to precipitate during the experimental period, requiring improvements for further investigations. During the growth experiments, liquid levels of the nanoparticle solutions surrounding the cultures were controlled on a daily base, in order to avoid any liquid-loss that can be caused by the evaporation of water.

S5. Preparation of Nickel (II) Chloride solution

The nickel(II) chloride solution was prepared by the dissolution of $NiCl_2$(58 g) in a mixed solution of H_2O (250 mL) and concentrated HCl (30 mL).

S6. Characterization

A JEOL 2100-TEM instrument operating at 80 kV was used for determining the size of silver nanoparticles and gold nanorods. Each sample was prepared by inserting the nanoparticle solutions on top of carbon-coated 200 mesh copper grids and letting them to dry.

Spectrophotometric measurements have been carried out using the UV-1800 Shimadzu UV Spectrophotometer.

References

1. C.J.S. Bolch and S.I. Blackburn, *J Appl Phycol.*, 1996, **8**, 5-13.