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*Brief Communication*

# Pick your carats: nanoparticles of gold–silver–copper alloy produced in vivo

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## Abstract

Engineered gold nanoparticle catalysts containing a mixture of metals enable enhanced specificity and reactivity. We report the synthesis by plants of mixed metal nanoparticles suggesting the possibility of using plants to produce catalysts of specific composition, perhaps even those difficult to synthesise by traditional methods. The nanoparticles contain Au, Ag and Cu as an alloy. The structure and composition are confirmed by scanning transmission electron microscopy (STEM) and energy dispersive X-ray analysis (EDX).

## Introduction

The synthesis of nanoparticles of specific composition and size is a burgeoning area of materials science research. The properties of these particles in applications as diverse as catalysis, sensors and medicine depend critically on the size and composition of the nanoparticles. New routes to the manufacture of these materials extend the choice of properties that can be obtained.

One material that has received increasing attention is gold. Nanoparticles of gold are catalytically active for reactions such as the water gas shift reaction and selective oxidation of CO (Andreeva, 2002; Grisel et al., 2002; Hutchings & Haruta, 2005). Gold nanoparticles also have other applications in the field of sensors (Yanez-Sedeno & Pingarron, 2005; Puckett et al., 2005; Liu & Lu, 2004) and in medicine (Paciotti et al., 2004).

Intracellular and extracellular synthesis of metallic nanoparticles, including both gold and silver, by bacteria and fungi have been demonstrated previously (Gericke & Pinches, 2006; Klaus et al., 1999; Mukherjee et al., 2002). Plants have also previously been shown to produce metal nanoparticles, including gold, with plant cells (Reeves & Baker, 2000; Gardea-Torresdey et al., 2002, 2005). The concentration of gold from ores by plants, or phytomining, has also been proposed as an environmentally friendly and economic method of recovering gold (Anderson et al., 1999; Gardea-Torresdey et al., 2005; Lamb et al., 2001a).

To induce accumulation of gold into *Brassica juncea* application of ammonium thiocyanate has been reported, increasing the gold concentration to 57 $\mu g\ g^{-1}$ (Anderson et al., 1998). Other solubilising agents such as cyanide, iodide, bromide, and thiosulfate have been examined in gold accumulation studies (Lamb et al., 2001b).

Increasingly gold catalysts are being specifically tailored to contain a mixture of metals enabling

enhanced specificity and reactivity (Toshima & Yonezawa, 1998). We report the production of such mixed metal nanoparticles by plants opening up the possibility of using plants to produce nanoparticles of specific composition, perhaps even those difficult to synthesise by traditional methods. The material we report contains Au, Ag and Cu as an alloy, the combination of which is likely to be useful catalytically (Liu et al., 2005; Dimitratos et al., 2005; Kariuki et al., 2004; Comotti et al., 2006; Bianchi et al., 2005). While, to our knowledge, this is the first alloy produced by plants to be reported we believe it should be possible to produce a large range of alloys from many metals by a similar route and to have considerable control over the composition of these alloys. The plant based route could be considered to be an environmentally favoured or green chemistry method of production.

## Experimental details

Metal rich soil was produced by applying 100 ml 5 g Au $l^{-1}$ gold chloride solution, silver nitrate and copper chloride solutions to 5 kg of sieved and dried agricultural soil. The soil was dried at 100°C overnight then diluted with Au-free soil to give 48 mg Au $kg^{-1}$, 44 mg Cu $kg^{-1}$ and 31 mg Ag $kg^{-1}$ in the final soil. The soil was distributed into 0.5 l greenhouse pots, and watered daily to dampness but not release for two weeks to allow equilibration of the metals and the soil. Seeds of *Brassica juncea* were then added to the pots. The seedlings were thinned to leave five healthy plants in each pot. After 9 weeks of growth, 100 ml of 4 g $l^{-1}$ potassium cyanide solution was irrigated onto each pot. Fourteen days later the above-ground biomass was harvested and dried at 110°C then ground and sieved to less than 180 μm.

The concentration of gold in the plant material was measured using AA spectroscopy (GBL Avanta Σ, $\lambda = 242.8$ nm). The biomass was ashed at 550°C for 4 h in silica crucibles and digested in aqua regia in sealed glass tubes at 150°C for 2 h. The samples were then diluted with 2 mol $l^{-1}$ HCl to a Au concentration of 2–10 ppm. A gold standard solution (1,000 ppm Au, Spectrasol) was used to prepare standard solutions ranging from 2 to 20 ppm Au in 2 mol $l^{-1}$ HCl.

For TEM imaging the plant material was embedded in Spurr's resin. Sections of about 60 nm thickness were prepared using a Leica EM-UC6 ultramicrotome, collected on a water surface and transferred to a slightly etched (Ar plasma) Ni grid and dried. The thin sections were then covered with a thin layer of carbon to prevent charging during analysis. A JEOL JEM-2010F-HR TEM was used, with a 200 kV electron beam (field emission gun), equipped with a scanning unit (STEM) and a Thermo Noran energy dispersive X-ray (EDX) and the sample held in a Be holder. We recorded some images with High Angle Annular Dark Field mode (HAADF) using a dark field detector where only electrons that are significantly scattered by the sample material are detected, which leads to heavier elements in the sample appearing bright. EDX spectra and maps were recorded under analytical probe (1.0 nm) conditions. A particle diameter of more than 5 nm is required for it to be visible in the STEM-EDX maps under our measurement conditions.

## Results and discussion

The dried plants contained an average of 760 μg Au $g^{-1}$, 300 μg $g^{-1}$ Cu, and 730 μg $g^{-1}$ Ag. Typically in plants containing such a high level of gold about half of the gold is in the form of metal and half in the form of a soluble $Au^{+}$ salt. The metallic portion of the gold is present in the form of nanoparticles which appear to range in size from 5–50 nm (Figure 1) although there may be some particles smaller that were more difficult to observe. We confirmed that these were particles of high atomic mass initially by HAADF imaging. A STEM-EDX map for Au, Ag and Cu was also recorded from which it was apparent that all three elements are present in all particles (Figure 2).

The elemental compositions of 30 particles were determined by spot analysis. Complete solid solution is possible between Au, Ag and Cu and this forms the basis of the common gold alloys for jewellery. The gold content of the larger nanoparticles varied between 20 and 55 atomic % with corresponding 80–44 atomic % Ag and up to 1 atomic % Cu. The average analysis for the larger nanoparticles (for which we were able to get a more accurate analysis than for the smaller particles, and we have averaged about half the spot

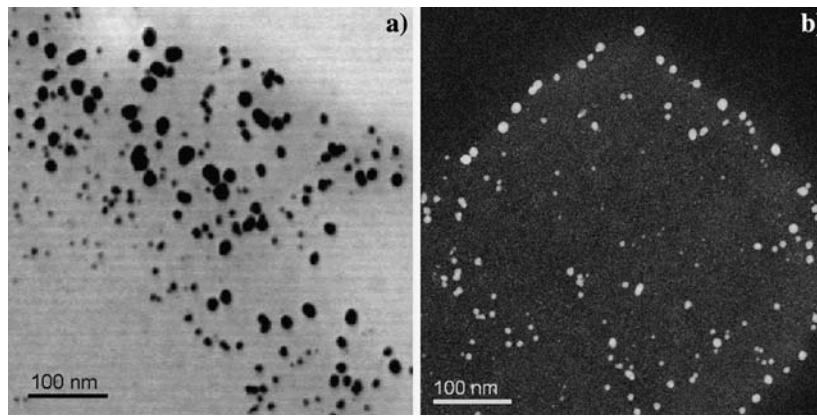


*Figure 1.* (a) STEM micrograph of metallic nanoparticles from *Brassica juncea*; (b) STEM-HAADF image of nanoparticles in plant material (bright areas indicate high atomic mass).

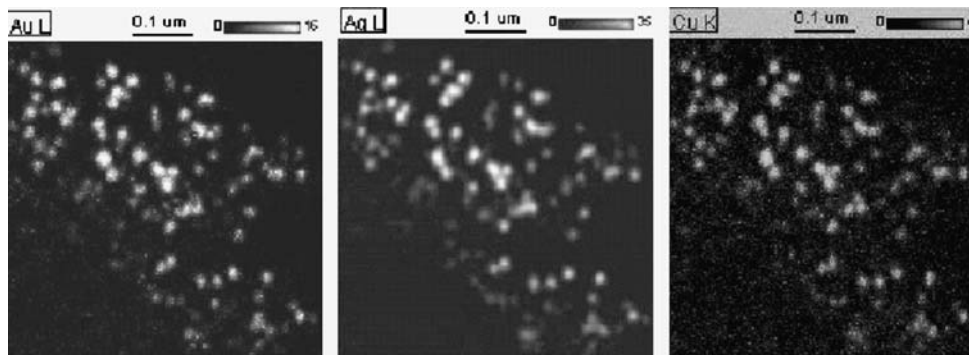


*Figure 2.* STEM-EDX maps of Au, Ag and Cu.

analyses) was 43 atomic % Au, 57 atomic % Ag with less than 1% Cu. There were variable minor amounts of Mg, Si, P, S Cl and Ca which are likely to be from covering or surrounding material and these analyses we report have excluded these elements when calculating the Au, Ag and Cu. The atomic ratios of Au/Ag varied from 0.25–1.2 (with an average of 0.80) compared with the ratio for the bulk plant material of 0.57.

We found a lattice spacing of 0.22–0.23 nm from a Fourier transform (Figure 3a inset) and also 0.22–0.23 nm from a measurement of lattice spacing directly off the images (Figure 3). This is commensurate with a mixture of Au (111) which has a spacing of 0.235 nm (Akita et al., 2005), Ag (111) of 0.235 nm and Cu (111) of 0.209 nm (Somorjai, 1994) although the accuracy of the measurement is such that we can not make a definitive statement about composition from the spacing alone. This combined with the EDX analysis indicates that the nanoparticles consist of an alloy and not a mixed phase of separate elements (Frenkel et al., 2000).

## Conclusion

In summary, we have used *Brassica juncea* to produce nanoparticles of an alloy. We are not aware of any other instances of metallic alloys being produced by plants. This preliminary work points the way to a new biosynthetic method for the production of nanoparticles of metal alloys for a range of nanotechnology applications. We propose that these nanoparticle alloys can be tailor made using plants by adjustment of the metal

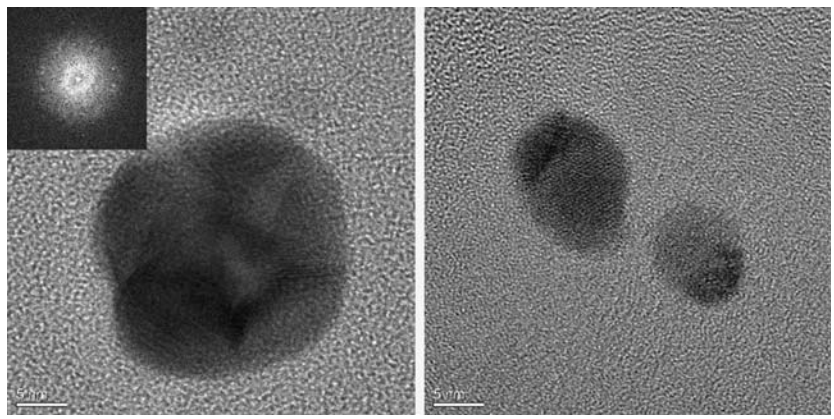

*Figure 3*. STEM images of nanoparticles (inset – Fourier transform).

components in the growth medium. Specifically we have demonstrated the synthesis of the Au–Ag–Cu class of alloy.

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