In situ generation of gold nanoparticles on a protein surface: Fischer carbene complex as reducing agent†

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Suitably designed, hydrophilic Fischer carbene complexes reduce $HAuCl_4$ to produce stable gold nanoparticles localized on proteins in aqueous buffer solution.

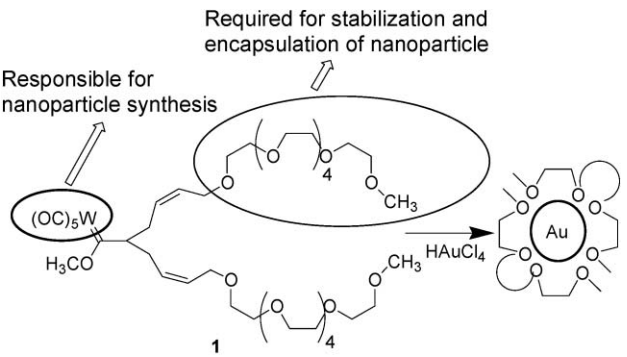
Accurate, robust, and highly selective detection of water-soluble analytes, such as toxins, carbohydrates, ionic species, and various biomolecules including proteins, peptides, and DNA remains a highly sought after scientific goal with implications in healthcare, safety, and defense applications. The past few years have witnessed intense research activities aimed at the development of such molecular diagnostics using nanomaterials that can address the deficiencies of conventional technologies.[1] A number of optical methods such as colorimetric detection, fluorescence resonance energy transfer (FRET)/quenching, surface plasmon resonance analysis, and scattering-based sensing that are based on nanoparticles have been developed towards this objective.[2] The use of gold nanoparticles in immunological staining for electron microscopy is one such application.[3] These particles are available in a range of sizes, making them perfect contrast agents for identification of cellular compartments or determination of protein localization or addressing related biological questions.

The general route to protein–metal colloid conjugates involves a simple solution mixing process of proteins with metal colloidal sols formed prior to the mixing.[4] The thiol or amine functional groups in the protein bind with the metal nanoparticle surface, leading to the formation of the bioconjugate. However, due to the inhomogeneous dispersion between the protein and the metal colloid, the resulting conjugates may show large aggregated structures either in solution or in the dried state, which is not a useful attribute in the context of their potential application.[5] A greater control of the generation and dimensions of nanoparticles can perhaps be achieved if the gold nanoparticle is generated *in situ* on a protein surface by use of a reducing component, either added externally[6] or already present on the surface of a protein.[7]

Although known for decades because of their rich and unique chemistry,[8] Fischer carbene complexes have seldom been used as reducing agents for other metal salts. Hyeon *et al.*[9] have used Fischer carbene complexes as precursors of metal nanoparticles that were generated by thermal decomposition and such synthesis was only limited to chromium and tungsten. Our preliminary experiments revealed that a Fischer carbene complex of tungsten can reduce $HAuCl_4$ to colloidal gold particles in water‡. To achieve efficiency, we designed a carbene complex **1** [$(OC)_5W{=}C(OCH_3)CH\{CH_2CH{=}CHCH_2(OCH_2CH_2)_6OCH_3\}_2$] that contains two hexaethylene glycol (HEG) tethers that were expected to encapsulate the newly generated gold nanoparticle.[10] Incorporation of the HEG tether also makes the carbene complex water soluble and hence adaptable for applications in biology.[11]

As a proof of concept, 3 ml of 1 mM aqueous solution of carbene complex **1** was added dropwise to 2 ml of 1 mM aqueous hydrogen tetrachloroaurate solution with stirring for 1 minute (Scheme 1). The solution instantly turned red with a very characteristic surface plasmon centred at 523 nm in the UV-vis spectrum (Fig. 1A) indicating the formation of gold nanoparticles.[12,13] This plasmon band did not show any red shift even after several days indicating the stability of the nanoparticles against spontaneous agglomeration.[14] This stability is attributed to the spontaneous assembly of the highly flexible HEG moiety forming a "crown" type cage to encapsulate the nanoparticles.§ The multipoint attachment of an ethylene glycol chain on a gold surface was evident from a clear shift of IR peak position (C–O stretch of the ether) from 1108 to 1031 cm^{-1} upon complexation accompanied by broadening of the signal (Fig. 1B).[15] The stabilizing role of the ethylene glycol chain was further established by a simple control experiment. When an acylmetal complex **2**, $(CO)_5W{=}C(CH_3)ONEt_4$[16] devoid of any oligoethylene glycol tethers was used, rapid agglomeration of gold nanoparticles was observed. Agglomeration did not occur if hexaethylene glycol dimethyl ether was present in the medium prior to reduction (Scheme 2).

Analysis of the images (Fig. 1C) from transmission electron microscopy (TEM) showed that the particles have a mean diameter of 10.1 ± 1.1 nm and are generally separated from each other. Typical X-ray diffraction patterns of the Au nanocrystallites



Scheme 1 Synthesis of gold nanoparticles by a Fischer carbene complex.

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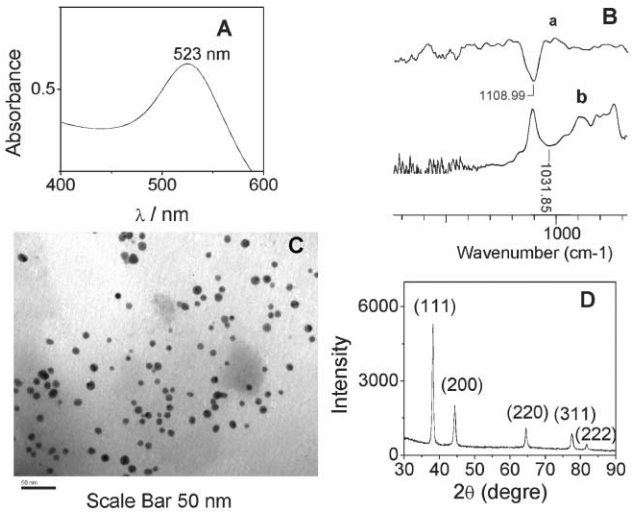
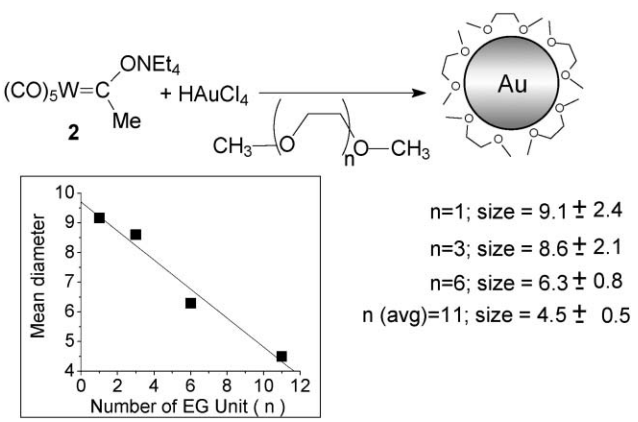


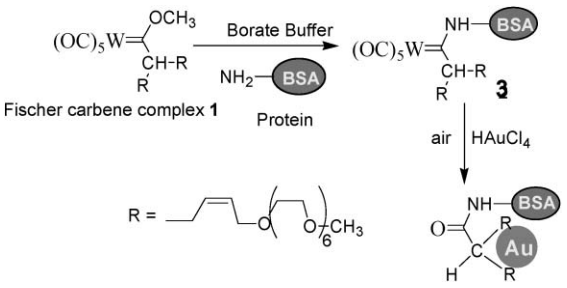
Fig. 1 (A) UV-vis absorbance spectra of Au nanoparticles prepared using carbene complex **1**, (B) IR spectra: (a) Fischer carbene complex **1** displays the ether C–O stretch at 1108.99 cm^{-1}; (b) complex **1** absorbed on a Au nanocrystal shows broadening as well as a shift of the ether C–O absorption band to 1031.85 cm^{-1}. (C) TEM micrograph of the same Au nanoparticles. (D) XRD pattern recorded for the same gold nanoparticles.

of the sample recorded from a nanoparticle film deposited on a glass surface is shown in Fig. 1D. Diffraction features appear at about 38.2, 44.3, 64.8, 77.7 and 81.7°, which correspond to the (111), (200), (220), (311), and (222) planes of the cubic phase Au nanocrystal, respectively. The average size of the gold nanoparticles was also determined from the width of the reflection according to the Scherrer formula, $D = 0.9\lambda/(\beta\cos\theta)$, where β is the full width at half-maximum of the peak, θ is the angle of diffraction, and λ is the wavelength of the X-ray radiation.[17] The value of D calculated from the (111) reflection of the cubic phase is 12 nm, which is in good agreement with the TEM results.

Interestingly, when acylmetal complex, $(CO)_5W{=}C(CH_3)ONEt_4$ (**2**) was used as reducing agent and different oligoethylene glycols of the same concentration were used externally as capping agent,¶ the size of the generated nanoparticles was dependent on the chain



Scheme 2 Synthesis of gold nanoparticles by an acylmetal complex in the presence of oligoethylene glycol ether. Inset picture shows the linear relationship between the size of the particles and the length of the ethylene glycol unit.



Scheme 3 Synthesis of gold nanoparticles with the reducing agent "fixed" to the protein.

length (Scheme 2). This is in accordance with other observations[18] where the size selective synthesis of gold nanoparticles is possible in the presence of different chain length surfactants. Significantly, in the present case a linear relationship between the size of the nanoparticles and the chain length of the oligoethylene glycol was observed when all other conditions were the same (Scheme 2 inset picture).‖ The longer the ethylene glycol chain, the narrower the particle size distribution (for TEM pictures, see ESI†).

It was recognized that Fischer carbene complexes can be anchored on the protein surface by reacting with a pendent amino group of the protein. For the generation of gold nanoparticles *in situ* in the presence of a protein, the Fischer carbene complex **1** was initially grafted onto the surface of the protein BSA (Scheme 3) using the method described by Jaouen and others.[19] A characteristic shift of the wavenumber of the two prominent ν_{CO} bands was observed in the IR spectrum upon conversion of the methoxycarbene into the protein carbene conjugate (see ESI†). No change was observed in the CD spectrum of BSA before or after the grafting; revealing that there was no structural change of the protein consequent to metalation (see ESI†).

Dropwise addition of a solution of the Fischer carbene complex grafted onto protein to 2 ml of 1 mM aqueous hydrogen tetrachloroaurate solution (Scheme 3) followed by incubation at 25 °C gave a solution of red color with a characteristic surface plasmon band centered at 530 nm (Fig. 2B curve a).

A TEM image (Fig. 2A) demonstrated that the diameter of the nanoparticles synthesized *in situ* is 4.1 ± 1.1 nm, which is half of the size of the nanoparticles described above. The smaller particle size and their stability in the absence of oligoethylene glycol molecules, except those grafted onto the protein, strongly suggest that the gold particles are localized on the protein surface where HEG tentacles are present.

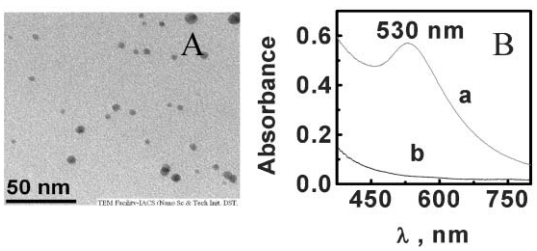


Fig. 2 (A) TEM micrograph of gold nanoparticles produced by the BSA–carbene conjugate. (B) UV-vis spectra of: (a) gold nanoparticles generated by BSA–carbene conjugate **3**; (b) control experiment with BSA – no gold nanoparticles formed.

A control experiment with pure protein BSA and hexaethylene glycol ether instead of the BSA–Fischer carbene conjugate produced neither a red solution nor a surface plasmon band (Fig. 2B, curve b).

In summary, the results of preliminary experiments described in this paper provide: a) the first convincing example that a Fischer carbene complex can behave as a reducing agent (a water soluble metal(0) reductant) and reduce Au(III) to gold nanoparticles; b) a convenient way to generate gold nanoparticles *in situ* for grafting onto proteins to afford protein–metal nanoconjugates; and, c) a clear evidence that oligo- or poly-ethylene glycol is an efficient complexing agent that can stabilize and control the size of gold nanoparticles in water. Further studies pertaining to the application of such nanobioconjugates to biological problems are in progress.

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Notes and references

‡ Exploratory experiments show that $AgNO_3$, K_2PtCl_4 or K_2PdCl_4 are reduced by Fischer carbene complex **1** to nanosized silver, platinum or palladium metal particles respectively under mild conditions in water. An account of their preparation and characterization will be reported in due course.

§ The nanoparticles thus stabilized can be extracted into organic solvents using long chain alkanethiols.

¶ 0.1 ml of 1 mM aqueous suspension of carbene complex **2** was added dropwise to 1 ml of 0.01 mM aqueous hydrogen tetrachloroaurate solution with stirring during 1 minute in the presence of 100 μL of 1 mM different oligoethylene glycol ethers. The solution turned red within five minutes indicating the formation of gold nanoparticles.

‖ Size was determined from TEM micrographs (see ESI†). A gradual shift of UV maxima towards higher wavelength with increasing chain length of oligoethylene glycol ether was also observed (see ESI Fig. 3†).

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13 If the carbene complex **1** is oxidized with 4-methylmorpholine *N*-oxide, the resulting ester can no longer produce gold nanoparticles under identical conditions. Hence, the group $(CO)_5W{=}C$ of **1** is essential for the reduction of Au(III) to gold nanoparticles.

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