Straightforward and robust synthesis of monodisperse surface-functionalized gold nanoclusters

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Abstract
Gold nanoclusters are small (1–3 nm) nanoparticles with a high surface area that are useful for biomedical studies and drug delivery. The synthesis of small, surface-functionalized gold nanoclusters is greatly dependent on the reaction conditions. Here, we describe a straightforward, efficient and robust room temperature one-pot synthesis of 2 nm gold nanoclusters using thioglucose as a reducing and stabilizing agent, which was discovered by serendipity. The resultant monodisperse gold nanoclusters are more stable than those generated using some other common methods. The carboxylic acid contained in the stabilizing agent on the cluster surface serves as anchor for nanocluster functionalization. Alternatively, the addition of thiols serves to functionalize the nanoclusters. The resulting non-cytotoxic nanoclusters are taken up by cells and constitute a tuneable platform for biomedical applications including drug delivery.

Findings
Nanoparticles ranging in size from 1 to 100 nm are ideal tools to study biological processes [1,2]. Many different materials, including gold, have been used to create nanoparticles [3-6]. Gold nanoparticles are an attractive platform because of their biocompatibility, low toxicity, and low immunogenicity [7], their inherent optoelectronic properties [8] and high transmission electron microscopy (TEM) contrast. They are relatively easy to synthesize, functionalize, are biocompatible and have controllable optical properties [3,9-12]. Therefore, gold nanoparticles functionalized with carbohydrates [13], proteins [14], antibodies [15] and DNA [16] are commonly used as multivalent materials for biological studies. Gold nanoparticles have been
used in vivo as radiotracers [15,17], for targeted delivery [18] and, when functionalized with carboxylic acids, inhibit β-amyloid fibril growth related to Alzheimer’s disease [19]. Gold nanoclusters (NCs) are gold nanoparticles ranging in size between 1 and 3 nm, with interesting physicochemical properties and increased surface area for drug delivery applications [20].

There are several methods to synthesize gold nanoparticles. In addition to the reduction of HAuCl₄ with citrate at high temperatures [21], sodium borohydride can act as a reducing agent while an alkanethiol stabilizes the nanoparticles [22]. The latter method was used to prepare glycoconjugates by adding thiol-terminated glycoconjugates [23]. Gold nanoparticles have also been prepared under reflux using 1-thioglucose as reducing and stabilizing agent [24] but the resulting nanoparticles are too unstable to be used as biosensors [25].

In an effort to create monodisperse, stable and surface-functionalized gold nanoclusters, we explored 1-thioglucose as a stabilizing and reducing agent. By serendipity we discovered a novel one-pot method to prepare gold nanoclusters using 1-thioglucose at room temperature. This simple and robust synthesis produces stable, and monodisperse nanoclusters. Oxidation of the carbohydrate results in carboxylic acid as determined by X-ray photoelectron spectroscopy (XPS). Coupling to the carboxylic acids or addition of thiols functionalizes the NCs that are taken up by cells but are less cytotoxic than NCs prepared by other methods.

During experiments exploring different methods for the synthesis of gold tetrapods [26], we found that simply the addition of 1-thioglucose as reducing agent to gold salts resulted in the formation of monodisperse gold NCs (Figure 1A). The reaction produced the same products at any temperature between 0 and 90 °C and thereby stood in stark contrast to all known literature procedures [27-29] that were sensitive to all reaction conditions including the speed of the stirrer. The influence of the gold to 1-thioglucose ratio on the yield and quality of the glucose-stabilized gold nanoclusters (Glc-NCs) was determined (Table S1,

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**Figure 1:** A) One-pot synthesis of Glc-NCs at room temperature; B) UV–vis spectra of freshly synthesized Glc-NCs and after three days are indicative of stable particles; C) high-resolution TEM bright-field image of Glc-NCs showing monodisperse nanoclusters, scale bar 2 nm; D) size distribution of 110 nanoclusters of Glc-NCs yielding diameters of 2.02 ± 0.18 nm.
Figure 2: A) Functionalization of Glc-NCs by coupling trifluoroethanol to the carboxylic acid groups on the surface of the nanocluster to yield Glc-NC@F and via thio-substitution of pentylthiol mannoside on the gold surface giving Glc-NC@Man; B) $^{19}$F NMR of Glc-NC@F after dialysis showing one product peak; C) vials containing (1) Glc-NCs (2) Glc-NC@Man (3) Glc-NCs + ConA (4) Glc-NC@Man + ConA. Aggregation is only observed upon addition of the lectin ConA (4).
stabilizer molecules per nanocluster were measured by comparing the number of trifluoroethanol molecules coupled to Glc-NCs using an internal standard (Figure S10b, Supporting Information File 1). Thus, we demonstrated that Glc-NCs can be functionalized via coupling to carboxylic acid groups.

To illustrate the surface functionalization of Glc-NCs, pentythiol mannoside (Scheme S1, Supporting Information File 1) was incubated with Glc-NCs overnight to yield Glc-NC@Man (Figure 2A). After dialysis, the IR spectrum revealed two new peaks corresponding to the C–H bonds of the linker at 2856 and 2925 cm$^{-1}$. Apparently, all carbohydrates reacted as no S–H peaks were observed (Figure S11, Supporting Information File 1). Functional evidence for the formation of Glc-NC@Man was obtained by aggregating Glc-NC@Man with the addition of the mannose-binding lectin concanavalin A (ConA). Unfunctionalized Glc-NCs fail to aggregate since oxidized thio-glucose is not recognized by ConA (Figure 2C). Glc-NC@Man are monodisperse prior to aggregation by the addition of ConA as judged by TEM (Figure S12, Supporting Information File 1).

Nanocluster cytotoxicity was assessed by incubating the nanoclusters for one day with the mouse cell line L929 for a proof-of-principle study. Cell viability was measured using the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium inner salt] assay [30]. The cytotoxicity of Glc-NCs, CTAB-NCs and THPC-NCs was compared. CTAB-NCs were toxic even at low concentrations (0.2–25 µM), whereas both THPC-NCs (Figure S13, Supporting Information File 1) and Glc-NCs did not show any toxicity at 500 µM (Figure 3A). To test whether the free stabilizers were affecting the cytotoxicity, both Glc-NCs and THPC-NCs were measured after synthesis without dialysis. Glc-NCs were not

![Figure 3](image-url)
cytotoxic at any concentration tested (Figure 3B), whereas THPC-NCs were toxic at 100 μM (Figure S14, Supporting Information File 1), indicating that Glc-NCs are suitable for biological experiments even without purification.

Cellular uptake of the nanoclusters was studied by incubating 12.5 and 25 μM Glc-NCs with the same cell line. After 24 h, the cells were washed with buffer and then treated with aquea regia to transform the gold nuclei into gold ions that were detected by an inductively coupled plasma optical emission spectrometer (ICP-OES). The Glc-NCs are cell permeable as 10⁸–10⁹ gold atoms were delivered per cell and can potentially be used in cellular delivery applications (Figure 3C).

In summary, we developed a straightforward, robust and efficient one-pot method to prepare glucose-stabilized gold nanoclusters (Glc-NCs). The resulting 2 nm nanoclusters are monodisperse and more stable than gold nanoclusters synthesized by other methods. Functionalization of the Glc-NCs is achieved either via coupling to the carboxylic acid of the stabilizing agent or substitution with a thio-functionalized molecule. Glc-NCs are non-toxic, and are taken up by L929 cells. Surface functionalization of Glc-NCs with biomolecules opens opportunities for drug delivery applications.

**Supporting Information**

**Supporting Information File 1**
Additional experimental data. [http://www.beilstein-journals.org/bjnano/content/supplementary/2190-4286-7-118-S1.pdf]

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