Facile *in situ* preparation of biologically active multivalent glyconanoparticles†

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Biologically active multivalent glyconanoparticles are prepared in an extremely simple method by reduction of well-defined glycopolymers, prepared by RAFT polymerisation, in an aqueous solution of $HAuCl_4$.

In recent years, the understanding of carbohydrate function *in vivo* has increased greatly,[1] revealing that sugars perform highly specialised roles involved in, for example, cell–cell interactions[1] and protein folding.[2] These functions are governed by the specific interaction between carbohydrate moieties present on the surface of cells (typically as glycoproteins or glycolipids), and carbohydrate-binding proteins known as lectins.[3,4] The strength of this interaction is highly dependent on the presence of multiple carbohydrates of the correct type and orientation, a phenomenon known as the 'cluster glycoside effect'.[5] Recently, gold nanoparticles (AuNPs) stabilised with carbohydrate moieties have been prepared and used as model systems[6] and sensors[7] in glycobiology, and also as anti-adhesion agents in potential tumour treatments.[8] Their high affinity has been attributed to the increased carbohydrate density present on the particle surface. However, in almost all published work to date,[9] the carbohydrate ligands themselves are *monovalent* and the synthesis of derivatives suitable for immobilisation on AuNP surfaces requires some non-trivial organic chemistry. Herein we present a simple method for the production of gold nanoparticles stabilised with polymeric glycoconjugates.

Glycopolymers were synthesised by reversible addition–fragmentation chain transfer (RAFT) polymerisation, a versatile technique that utilises thiocarbonylthio compounds, such as dithioesters, as mediating agents.[10] RAFT was chosen for two reasons: i) it has been used previously to synthesise well-defined glycopolymers from unprotected monomers;[11,12] and ii) the dithioester moieties at the chain ends of the polymers can easily be converted to thiols, allowing the polymers to be anchored to gold surfaces.[13] We chose the monomer 2-(β-D-galactosyloxy)ethyl methacrylate (GalEMA, **1**), the polymers of which[14] have previously been shown to bind strongly to the β-galactosyl specific lectin peanut agglutinin (PNA).[15]

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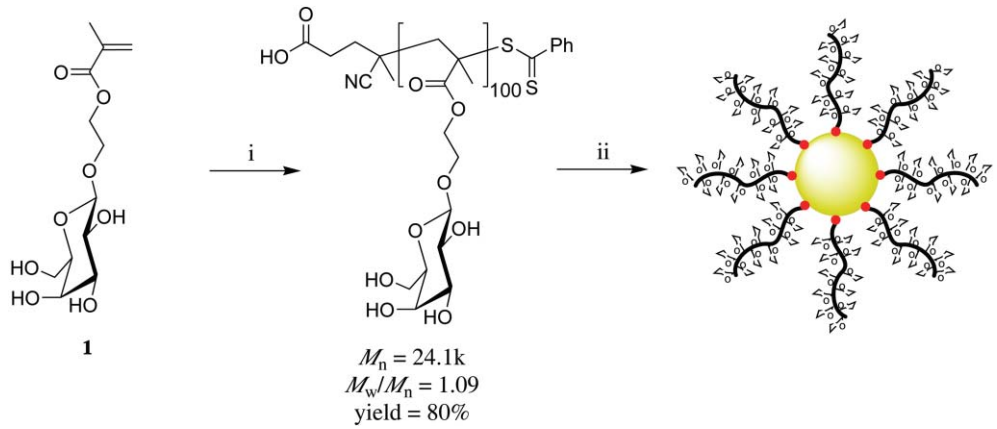
The strategy for the preparation of glycopolymer-stabilised gold nanoparticles is illustrated in Scheme 1. The glycomonomer **1** was synthesised as described previously[14] and was polymerised in aqueous ethanol solution using (4-cyanopentanoic acid)-4-dithiobenzoate (CPADB, **2**) and 4,4′-azobis(4-cyanopentanoic acid) (ACPA, **3**). On completion of polymerisation, the solution was dialysed (molecular weight cut off, MWCO = 3.5 kDa) and subsequently lyophilised to give a pink polymer product (poly[2-(β-D-galactosyloxy)ethyl methacrylate], poly(GalEMA)) in 80% yield. The polymerisation displayed pseudo first order kinetics, following a short induction period (^{1}H NMR in D_2O–EtOH, 9 : 1, see ESI†). As is commonly observed for RAFT polymerisations, the number average molecular weight (M_n) by size exclusion chromatography (SEC; aqueous eluent) was found to agree with that calculated from the initial monomer : RAFT agent ratio, and the polydispersity index was low (M_w/M_n = 1.09).

Glycopolymer-stabilised gold nanoparticles were synthesised directly by addition of $NaBH_4$ to an aqueous solution of poly(GalEMA) and $HAuCl_4$ (2 : 1 [end groups] : [Au]), as demonstrated by Lowe *et al.* and Tenhu *et al.*[13] On addition of the reducing agent, the solution immediately changed from pale orange-yellow to golden brown, with no obvious precipitation (Fig. 1). Commonly, gold nanoparticle dispersions display a size-dependent intense colouration (red to violet) due to surface plasmon resonance. The weak colour here suggests the presence of gold nanoparticles with a diameter smaller than *ca.* 3.5 nm.[16]

Analysis of the nanoparticles by dynamic light scattering gave a peak diameter of 11.5 nm (Fig. 2, **A**), whereas the diameter of the gold core was determined by analysis of TEM images as 1–3 nm (Fig. 2, **B**). This remarkably small diameter was observed recently by Brust *et al.* for particles stabilised with sulfide-terminated poly(acrylic acid)s, prepared by a similar method.[17] Dynamic light scattering produces a larger mean particle diameter since it also detects the polymer corona, whereas in TEM only the gold core is visible.

The biological activity of the particles was demonstrated using an extremely facile visual method involving the agglomeration of peanut agglutinin (PNA)-coated agarose beads (PNA loading 2–4 mg ml^{-1}). A 20 μl aliquot of the agarose suspension was pipetted onto a microscope slide, a 40 μl aliquot of the poly(GalEMA) functionalised particles was then added and the interaction monitored by optical microscopy. Prior to nanoparticle addition, the beads were seen to be well dispersed (Fig. 2, **C**). Following addition of the poly(GalEMA) nanoparticles, the beads were observed to agglomerate, with near complete agglomeration after 30 min (Fig. 2, **D** and **E**). The agglomeration is most likely caused by poly(GalEMA) nanoparticles binding to PNA lectin



Scheme 1 Preparation of glycopolymer-stabilized gold nanoparticles. Reagents and conditions: i (4-cyanopentanoic acid)-4-dithiobenzoate (0.01 eq.), 4,4′-azobis(4-cyanopentanoic acid) (0.005 eq.), 70 °C, 3 h, H_2O–EtOH (9 : 1); ii $HAuCl_4$ (0.5 eq.), $NaBH_4$ (12.5 eq.), H_2O, r.t.

Fig. 1 A stable dispersion of glycopolymer-stabilized nanoparticles in aqueous solution.

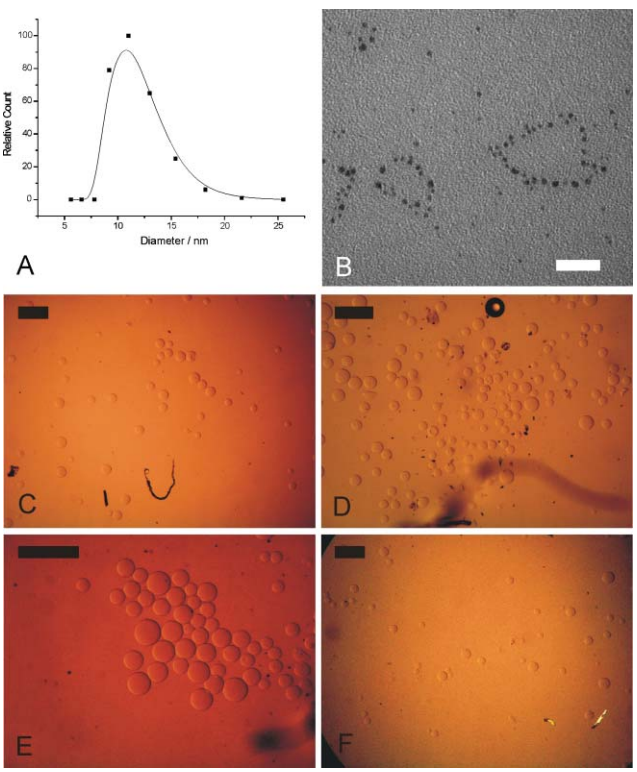


Fig. 2 Characterisation of glycopolymer-stabilised gold nanoparticles and *in vitro* assay of their biological activity. (**A**) Dynamic light-scattering data and (**B**) TEM micrograph (scale bar represents 20 nm) of poly(GalEMA)-functionalised gold nanoparticles. (**C–F**) Optical micrographs of peanut agglutinin (PNA)-coated agarose beads: (**C**) before, (**D**) 5 min after and (**E**) 30 min after addition of poly(GalEMA)-functionalised gold nanoparticles; (**F**) after addition of methyl β-D-galactopyranoside to suspension shown in (**E**). Scale bars in (**C–F**) represent 500 μm.

molecules adsorbed on the surface of agarose beads. Thus, this assay is similar to the well-known hemagglutination assay for plant lectins.[4]

To probe the specificity of the glyconanoparticle–bead interaction, two further experiments were conducted. Firstly, a solution of either methyl β-D-glucopyranoside or methyl β-D-galactopyranoside was added to a portion of agglomerated beads. On addition of the glucoside, no change in the agglomerated status of the beads was apparent; conversely, on addition of the galactoside, the beads were seen to redisperse rapidly (Fig. 2, **F**). The free galactoside will displace the polymer bound moieties from the lectin binding sites, breaking the crosslinking and allowing redispersion. In the case of the glucoside, there is no interaction with the lectin and subsequently the crosslinking is unaffected. Secondly, the agglomeration experiment was repeated using nanoparticles functionalised with poly(methyl 6-*O*-methacryloyl-α-D-glucoside) prepared by RAFT;[12] this time no agglomeration occurred, confirming that binding between the nanoparticles and lectin is carbohydrate-specific.

To conclude, we have described an extremely simple and versatile method for the production of very small glyconanoparticles stabilised with multivalent neoglycopolymers and have demonstrated their biological activity with a simple agglomeration assay involving lectin-coated agarose beads. Due to the increased carbohydrate density on the particle surface, we expect enhanced affinities with lectins compared to particles stabilized with individual carbohydrates, leading to improved performance in applications such as sensors for toxins and viruses.

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