

# Zwitterionic phosphorylcholine as a better ligand for stabilizing large biocompatible gold nanoparticles†

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**Zwitterionic phosphorylcholine showed better stabilization than oligo(ethylene glycol) in protecting big gold nanoparticles.**

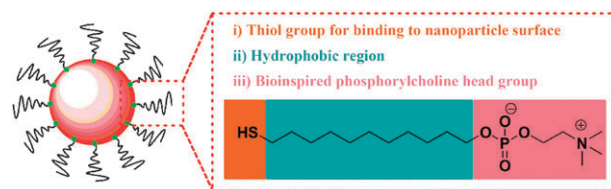
Nanomaterials are currently receiving considerable attention because of their potential applications in biology and medicine. Gold nanoparticles (Au-NPs), for example, have been used in highly sensitive diagnostic assays, thermal ablation and radiotherapy enhancement, as well as drug and gene delivery.<sup>1,2</sup> To further the application of Au-NPs in disease diagnosis and therapy it is important that the nanoparticle systems are not only colloid stable but also biocompatible in physiological environments. Several studies have employed water soluble polymers as stabilizing agents to achieve biocompatible Au-NPs.<sup>3</sup> The use of small molecules as capping agents for biocompatible monolayer protected Au-NPs prevails over polymer-based capping agents since monolayer protected gold nanoparticles are capable of further functionalisation *via* ligand exchange reactions, which is efficient for surface tailoring the nanoparticles with specific target properties. However, only a few examples have demonstrated the application of small molecules to obtain biocompatible monolayer protected Au-NPs.<sup>4</sup> In recent years, because of the protein resistance property, oligo(ethylene glycol) (OEG) self-assembled monolayers (SAMs) have attracted much attention in biotechnology and medical devices performed on either flat or curved surfaces.<sup>5</sup> The introduction of thioalkylated OEG onto Au-NPs resulted in water soluble and biocompatible monolayer protected Au-NPs with sizes up to 5–8 nm.<sup>4a,b</sup> However, the addition of the thioalkylated OEG to bigger 16 nm citrate-stabilized Au-NPs, disappointingly induced the change of the color from red to slightly darker, indicating the aggregation of the Au-NPs.<sup>4c</sup> The discovery of a novel ligand to stabilize Au-NPs with bigger sizes is strongly desired for its potential application relying on optical detection since the molar absorption coefficient increases exponentially with the diameter of the Au-NPs.<sup>6</sup>

Whitesides G. M. *et al.*<sup>7</sup> and Jiang S. Y. *et al.*<sup>8</sup> have shown that the strong resistance of zwitterionic SAMs to protein adsorption on flat surfaces is due to their strong hydration capacity *via* electrostatic interactions. The principle of stabilizing small nanoparticles of less than 10 nm by zwitterionic ligands has been proved by ourselves<sup>9</sup> and others.<sup>10</sup> Herein, we prove that

the thioalkylated zwitterionic phosphorylcholine (PC) showed a much better stabilizing ability than the neutral OEG. For the first time in our knowledge, the Au-NPs with a size of up to 16–50 nm could be stabilized by small molecular ligands. These zwitterionic PC protected nanoparticles are not only stabilized under a wide variety of conditions, including plasma, but are also amenable to functionalisation *via* Murray's ligand exchange route. Using gel electrophoresis we also demonstrated that PC protected Au-NPs have complete resistance to nonspecific protein interactions.

Thioalkylated phosphorylcholine 11-mercaptoundecyl-phosphorylcholine (HS-PC), having a thiol group on one terminus and phosphorylcholine group on the other, was synthesized and purified according to the literature.<sup>8</sup> The novel feature of this ligand is that it combines the stability of strong Au–S linkage with the excellent water solubility of zwitterionic phosphorylcholine, as schematically illustrated in Scheme 1.

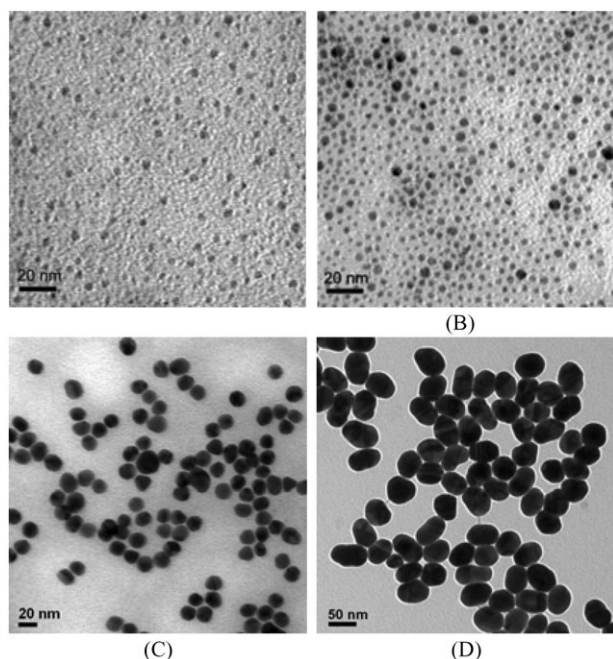
Surface modification with zwitterionic phosphorylcholine appears particularly appealing in nanomedicine as the bioinspired phosphorylcholine has been shown to prevent protein adsorption and blood clotting both on the macroscopic and the microscopic materials.<sup>9–12</sup> Gold nanoparticles are highly polarizable metals with a large Hamaker constant. Gold nanoparticles are prone to aggregation in high ionic strength solutions in which the van der Waals attraction outweighs the electrostatic and/or steric repulsion provided by surface-bound ligands (*e.g.*, citrate). The unique zwitterionic structure of our PC protected Au-NPs is advantageous to attain high biostability and biocompatibility. Four different Au-NPs with sizes of 2–3 nm, 4–6 nm, 16 and 50 nm were prepared using HS-PC as a capping agent (Fig. 1). It is of interest to find, for the first time as far as we know, that the “big” Au-NPs with size ranges of 16–50 nm can also be stabilized by small molecules of thioalkylated zwitterions. The 16 nm Au-NPs, which exhibit a strong plasmon absorption band at around 520 nm in the UV-vis spectrum, was used to check the efficiency of the zwitterionic ligand for improving the stability of nanoparticle systems. The zwitterionic phosphorylcholine monolayer protected Au-NPs were found to be very stable in physiological phosphate buffer solution (PBS) and plasma (see



**Scheme 1** Schematic representation of HS-PC monolayer protected Au-NPs (not to scale).

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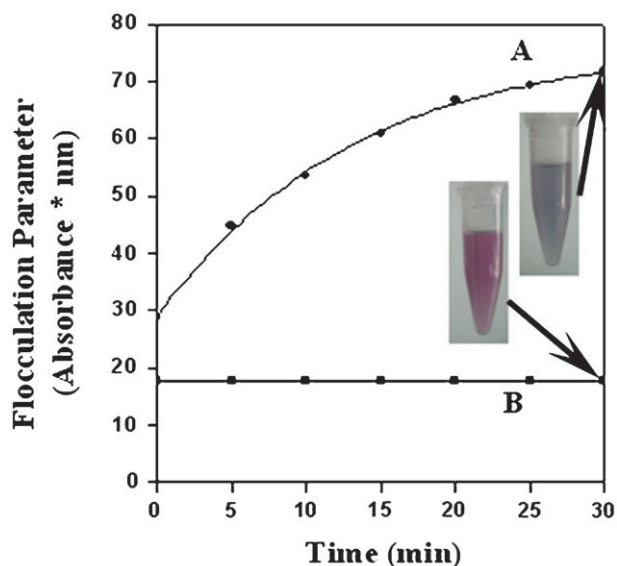


**Fig. 1** TEM images of HS-PC protected Au-NPs with the size of  $3.3 \pm 0.5$  nm (A),  $4.7 \pm 1.5$  nm (B),  $16 \pm 2$  nm (C), and  $50 \pm 4$  nm (D), respectively.

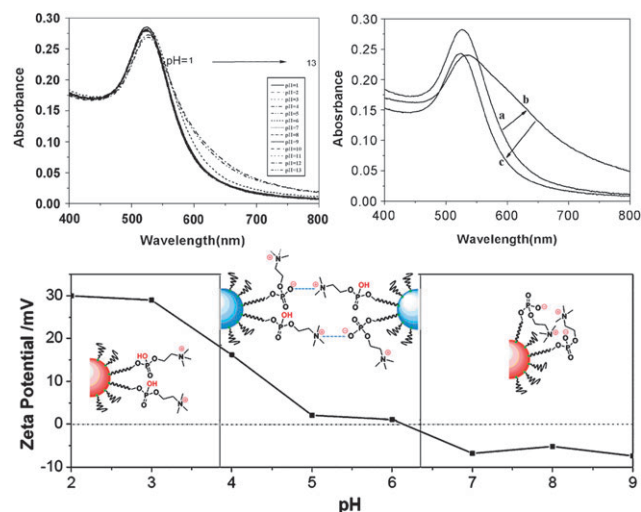
ESI<sup>+</sup>). At the same time, they could withstand high salt concentrations up to 2 M NaCl (see ESI<sup>+</sup>). After 6 months of storage in aqueous solution, no agglomeration was seen.

The “floculation parameter”, which was first defined as the integrated area between 600 and 800 nm in the optical absorption by Whitesides *et al.*,<sup>13</sup> was used here to quantitatively measure the extent of floculation of the PC protected and OEG protected Au-NPs. While the “floculation parameter” of the thioalkylated tetra(ethylene glycol) (HS-EG<sub>4</sub>) stabilized Au-NPs increased rapidly with time, that of the phosphorylcholine monolayer protected Au-NPs were found to be very stable, Fig. 2. The zwitterionic phosphorylcholine showed a much better stabilizing ability than the neutral EG<sub>4</sub>. Also, the nanoparticle systems are readily dissolved in methanol and ethanol. While appearing to be completely inert under these testing conditions, the nanoparticles readily underwent ligand exchange reactions with 1-dodecanethiol (See ESI<sup>+</sup>). Our zwitterion stabilized nanoparticles systems are not only stabilized under wide and stringent conditions but are also amenable to be functionalized *via* Murray’s ligand exchange route.

In order to get more insight into the stabilizing ability of the zwitterionic ligand, the pH effect onto the stabilization was investigated. The nanoparticle systems remained stable when the pH was either higher than 6 or lower than 4, and formed agglomerates between pH 6 and 4 Fig. 3(A). The dispersion-agglomeration states can be reversibly tuned by the pH values, Fig. 3(B). The possible reason for the critical pH value was further investigated by the zeta-potential of the nanoparticles systems (Fig. 3(C)). The PC stabilized Au-NPs presented a distinct positive charge when the pH was lower than 4, which might be ascribed to the protonation of the phosphate groups of the phosphorylcholine<sup>14</sup> and resulting in a net positively charged quaternary ammonium. The PC protected Au-NPs



**Fig. 2** Floculation parameter of 50 nm gold nanoparticles capped by (A) thioalkylated tetra(ethylene glycol), and (B) HS-PC at different time intervals.



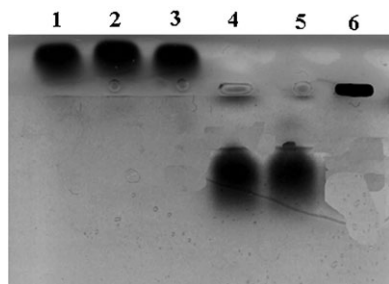
**Fig. 3** (A) UV-Vis spectra of 16 nm HS-PC capped Au-NPs at pH values of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13. (B) UV-Vis spectra evolution of 16 nm HS-PC capped Au-NPs immediately after adjusting the pH to 5 (a), after 30 min incubation at pH = 5 (b), and upon adjusting the pH to 7 (c). (C) Zeta potential vs. pH curves obtained for HS-PC capped Au-NPs. Insets show the schematic evolution of phosphorylcholine on Au-NPs surfaces when pH < 4 (Left), pH = 4–6 (Middle), and pH > 6 (Right).

were then stabilized by a positive electrostatic interaction at a pH lower than 4. However, all PC stabilized nanoparticles were found to be nearly neutral when the pH was larger than 6. The stabilization of the zwitterionic PC protected nanoparticle might contribute to their strong hydration layer *via* electrostatic interactions. Jiang *et al.*<sup>8</sup> recently reported that a balanced charge and an antiparallel orientation for dipole minimization were essential for zwitterionic PC non-specific resistant properties on a flat surface: it might be the same for zwitterionic PC protected nanoparticles. When the zwitterionic PC have the

balanced charge at high pH ( $\text{pH} > 6$ ), the dipole of the PC may fit themselves as an energy favorable antiparallel orientation with strong non-specific resistance to other colloid particles Fig. 3(C) (right). However, the phosphate groups of the phosphorylcholine became partially protonated at  $\text{pH} 4\text{--}6$ <sup>14</sup> and the unbalanced charge might destroy the antiparallel orientation. The NPs then aggregated, probably due to the ion-pair interactions between the net positively charged quaternary ammonium with the phosphate group on the other nanoparticles, Fig. 3(C) (Middle).

Many biological reactions arise from protein adsorption and nonspecific protein binding could be a serious issue in nanomedicine. Even though a lot of articles have been published dealing with the interactions between nanoparticles and proteins, the issue of nonspecific protein binding with nanoparticles has generally not been addressed.<sup>15</sup> In addition to improving the stability of Au-NPs in high ionic strength media, one potential advantage of zwitterionic phosphorylcholine monolayers is the prevention of nonspecific protein adsorption. Protein binding to Au-NPs can be conveniently monitored by gel electrophoresis, since protein–nanoparticle complexes are expected to migrate differently than the free Au-NPs. Lysozyme and bovine serum albumin (BSA), representative of positively and negatively charged proteins at neutral pH as well as having different hydrophobicities, were chosen for the protein binding test. For comparison, tiopronin protected Au-NPs (Au-Tp), a water soluble nanoparticle, was synthesized and tested for protein binding. Gel electrophoresis results are shown in Fig. 4. When Au-Tp nanoparticles were cultured with lysozyme and BSA, different band-shifts were observed, indicating different degrees of nonspecific adsorption. Incredibly, the Au-Tp nanoparticle solution became turbid on addition of lysozyme, indicating that Au-NPs form aggregates. This is presumably because the negative charged Au-Tp nanoparticles were cross-linked by positive charged lysozyme molecules (lane 6 in Fig. 4). When the same experiment was done with HS-PC protected Au-NPs, no change in the nanoparticle migration was observed (lane 1, 2, 3 in Fig. 4). The zwitterionic phosphorylcholine monolayers protected Au-NPs showed strong resistance to protein adsorption.

In conclusion, we have proposed zwitterionic PC as a novel zwitterionic ligand for preparation of biocompatible monolayer protected Au-NPs. These nanoparticles showed outstanding stability in physiological PBS solution and plasma.



**Fig. 4** Gel image of protein adsorption assay. Lane 1: 10  $\mu\text{L}$  of HS-PC protected Au-NPs at  $\sim 0.1$  mM. Lanes 2 and 3 are the same amount of Au-NPs mixed with 1  $\mu\text{L}$  of BSA and lysozyme, respectively. Protein concentrations are all 10  $\text{mg mL}^{-1}$ . Lanes 4–6 are similar to lanes 1–3, except that Au-Tp nanoparticles were used.

The zwitterionic PC showed much better stabilizing ability than the neutral EG<sub>4</sub>. The fact that these nanoparticles systems with zwitterionic surfaces have excellent biostability, biocompatibility and ligand exchange ability indicate that they can be potentially served as a versatile nanoplatform for the biomedical applications including sensitive diagnostic assays, drug and gene delivery.

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