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Charge Dependence of Ligand Release and Monolayer Stability of Gold Nanoparticles by Biogenic Thiols

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The effect of surface charge on the stability of gold nanoparticles (AuNPs) to the biogenic thiols glutathione (GSH), dihydrolipoic acid (DHLA), and cysteine was quantified. It was observed that the rate of release of fluorescein-tagged ligand was determined by the surface charge of the AuNPs, with cationic particles much more labile than anionic analogues. This ability to tune stability is significant for the design of both delivery vehicles and intracellular probes.

The design of functional materials that are capable of controlled release of therapeutic materials is of great importance in biomedical and materials chemistry (1, 2). Numerous drug release strategies have been employed, including those relying on external stimuli (e.g., photochemical (3, 4) or ultrasound (5, 6) or intracellular environment (e.g., enzymes (7, 8) or pH (9, 10)).

One of the most promising intracellular release strategies relies on glutathione (GSH) (11, 12). GSH is the most abundant thiol species inside the cell and plays an essential role in protecting organisms from free radical damage and oxidative stress through balancing the GSH/oxidized glutathione (GSSG) equilibrium (13). The concentration of GSH is significantly lower in the extracellular environment than inside the cell (10 μ M in blood plasma (14) vs 10 mM in liver cells (15)). This 1000-fold difference in concentration makes GSH a promising trigger for intracellular release, a method that has been exploited through disulfide-bond based drug delivery systems (16). Other biogenic thiols are found in cells, including DHLA (17) and cysteine (18).

Recent interest has been focused on drug delivery using gold nanoparticles (AuNPs). The core of AuNPs are essentially inert and nontoxic, and the capability to tune the core size and surface functionalities provide convenient access to explore and optimize delivery parameters (19, 20). These materials also offer controlled release of payload through place-exchange of GSH with thiols on the AuNP surface, thus leading to efficient protein (21), DNA (22), and small molecular drug payload release *in vitro* and inside cells (11). However, it will be very desirable to develop a simple approach to modulate payload release kinetics by systemic alteration of functionalities of AuNPs (23).

Here, we report the use of a series of fluorophore-labeled gold nanoparticles with varied surface charges to investigate the role of particle charge on the stability of gold nanoparticles to thiols. Our hypothesis was that the systemic tuning of the surface charge on AuNP would affect the electrostatic interaction between AuNPs surface and anionic GSH molecule, thus alternating concomitant payload release. In addition, intracellular thiols DHLA and cysteine were systematically examined.

Monolayer-protected AuNPs of 2 nm diameter featuring controlled surface charge were synthesized by the Brust-Schiffrin reduction (24) followed by the Murray place-exchange reaction (25). The charges on these particles range from positive

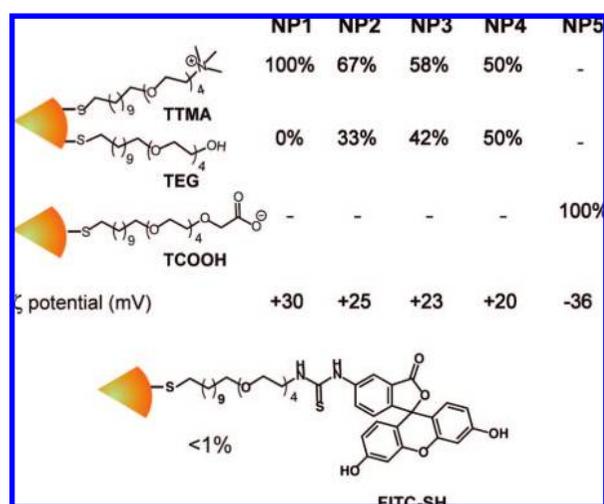


Figure 1. Ligand ratios as determined by NMR for NP1–NP5.

to negative (Figure 1). Briefly, the cationic AuNPs (NP1–NP4) were fabricated with different ratios of tetra(ethylene glycol) (TEG) ligands onto 1-pentanethiol AuNPs, while the anionic AuNP (NP5) was obtained in analogous fashion using a carboxylate-terminated tetra(ethylene glycol) (TCOOH) ligand (see Supporting Information). In placed-exchanged protocol, the fluorophore was also incorporated onto these water-soluble nanoparticles ($\leq 1\%$ loading as determined by NMR) with fluorescein-functionalized thiol FITC-SH. The obtained AuNPs were subjected to dialysis to remove excess fluorophore and displaced ligands. The ligand ratios for the cationic and neutral ligands were determined through NMR endgroup analysis; the fluorescein ligand was at concentrations too low to quantify. As expected, the zeta potential tracked qualitatively with ligand ratio for the particles (Figure 1).

The rate of ligand release from the nanoparticles was determined using fluorescence spectroscopy, exploiting the efficient quenching of fluorophores by the Au core of the particles. The increase in light emission observed upon GSH-mediated displacement of the fluorophores provides a direct tool for the quantification of release *in vitro* and in the cellular environments (11). The fluorophore-conjugated AuNPs NP1–NP5 were treated with GSH solution (Figure 2). Upon place exchange with GSH, the fluorescent ligands are released from the AuNP surface and the fluorescence recovered. The

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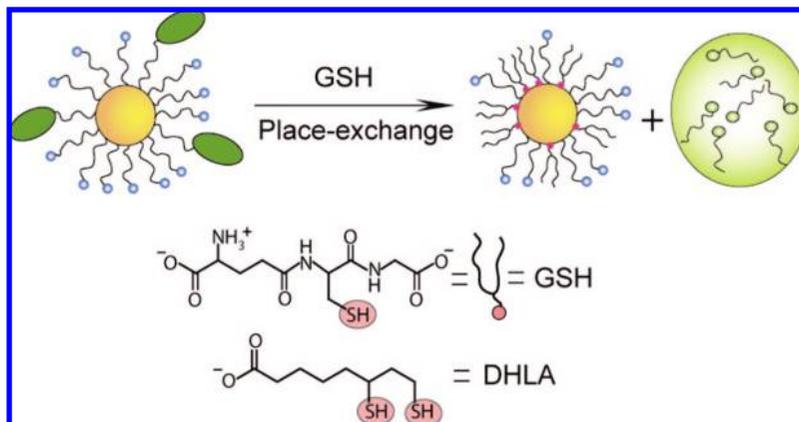


Figure 2. Schematic representation of GSH-mediated ligand release.

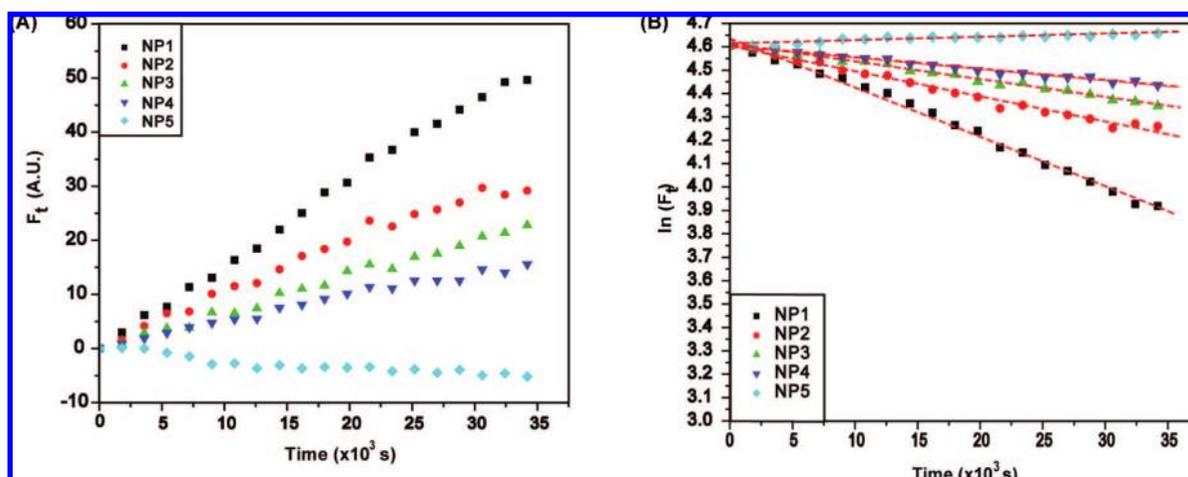


Figure 3. Percentage (A) and rate (B) for GSH-mediated release of fluorophores from different NPs. The fraction of the released FITC-SH ligand (F_t) at a given incubation time point (t). The concentration of AuNPs was $1.5 \mu\text{M}$ in PBS buffer (pH ~ 7.4) at 37°C .

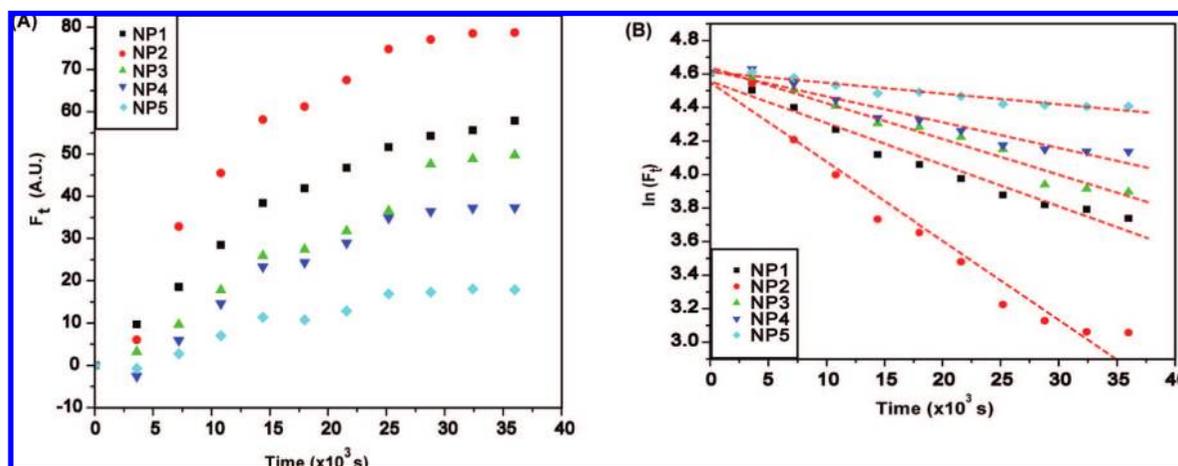


Figure 4. Percentage (A) and rate (B) for DHLA-mediated release of fluorophore. The fraction of the released FITC-SH ligand (F_t) at a given incubation time point (t). The concentration of AuNPs was $1.5 \mu\text{M}$ in PBS buffer (pH ~ 7.4), at 37°C .

fluorescence intensity thus corresponds to the amount of fluorophore that is released from the NP surface (Figure 3).

From Figure 3, it is evident that fluorophore release from the AuNPs surface is time- and charge-dependent. Moreover, the kinetic analysis of the parameter of $\ln F_t$ (logarithm of the normalized percentage of fluorophore release) as a function of time shows a linear dependency with respect to time, indicating pseudo first-order kinetics (see Supporting Information).

In addition to GSH, the AuNPs were investigated in the presence of other biologically relevant free thiols, i.e., DHLA and cysteine. In comparison with GSH, dihydrolipoic acid (DHLA) features two thiols and a higher degree of hydrophobicity and has been used to functionalize nanoparticles, including quantum dots. The results from DHLA-mediated release of FITC from AuNP surface are shown in Figure 4. Cysteine, a zwitterionic thiol, was likewise tested (Figure 5). In both cases, pseudo first-order kinetics were observed.

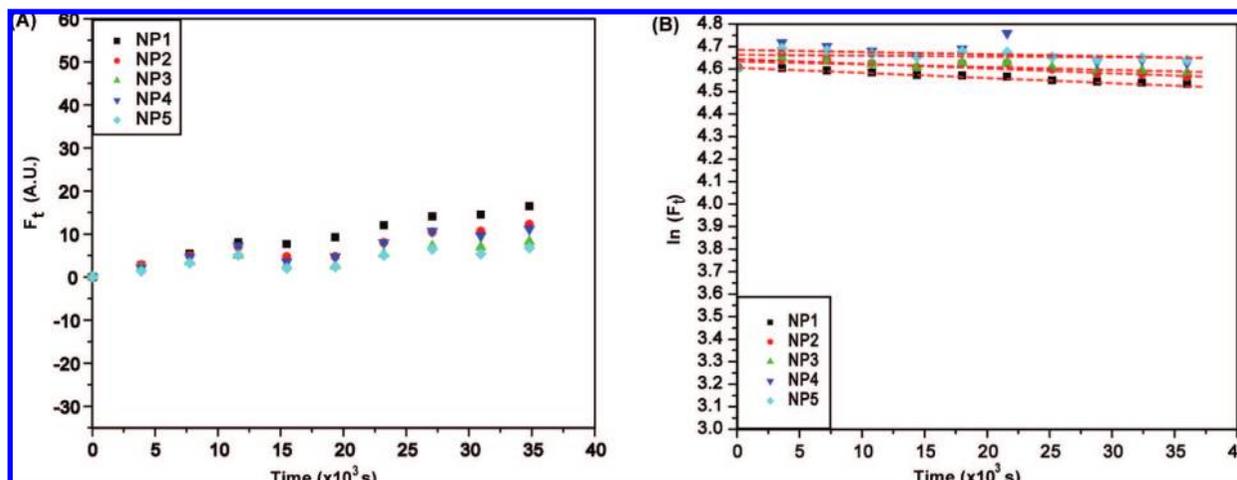


Figure 5. Percentage (A) and rate (B) for cysteine-mediated release of fluorophores from different NPs. The fraction of the released FITC-SH ligand (F_t) at a given incubation time point (t). The concentration of AuNPs was 1.5 μM in PBS buffer (pH \sim 7.4), at 37 $^\circ\text{C}$.

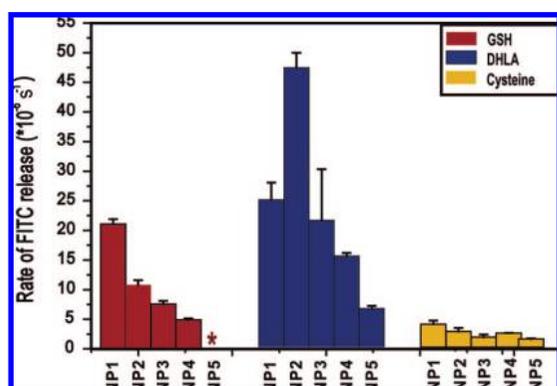


Figure 6. Summary of release rates at 20 mM thiol concentration.

The pseudo first-order rate constants of NP1–NP5 with GSH, DHLA, and cysteine are shown in Table 1 and summarized in Figure 6. Of the particles, NP1 (100% positively charged) shows the highest release rate with GSH, presumably due to the strong electrostatic interaction between the positive charge on the particle surface and the negative charge on the thiol molecule (Figure 7). The rate of release with the other positively charged NPs show that increasing positive charge results in more rapid fluorophore release. In the case of negatively charged AuNPs, no measurable change in fluorescence was observed, indicating complete nanoparticle stability. This low reactivity presumably arises due to repulsion between the negatively charged NP surface and negative charge on the GSH (Figure 7).

DHLA-mediated release was qualitatively similar to that observed with GSH. The one major difference is that NP2 shows

Table 1. Summary Rate of FITC Release Mediated by Different Thiol Species at 20 mM Concentration

AuNPs	rate of release ($\times 10^{-6} \text{ s}^{-1}$)		
	GSH	DHLA	cysteine
NP1	21.10	24.90	3.90
NP2	10.80	47.20	2.69
NP3	7.59	21.40	1.72
NP4	4.91	15.40	2.42
NP5	-	6.55	1.41

the highest release rate. This behavior suggests that the hydrophobic properties and electrostatic interaction of NP2 interact in a cooperative fashion. As expected, release by DHLA is more rapid than with GSH, as DHLA is both more hydrophobic and contains a dithiol in the molecule facilitating interactions with the AuNP core. Cysteine, while zwitterionic, will be partially anionic (pI = 5.07) under the conditions studied. The rate of release with cysteine was very slow, with the most rapid release occurring with NP1, as expected based on electrostatic arguments presumably the slow rate of exchange arises from the lack of hydrophobicity of the highly charged amino acid.

In summary, we found that the stability of gold nanoparticles toward thiols is governed by the surface charge of AuNPs. The ability to control the rate of release of ligands via place exchange provides a potentially potent tool for drug delivery applications. Likewise, these studies provide a strategy for enhancing the stability of nanoparticles in cells, and important consideration for the design of imaging agents.

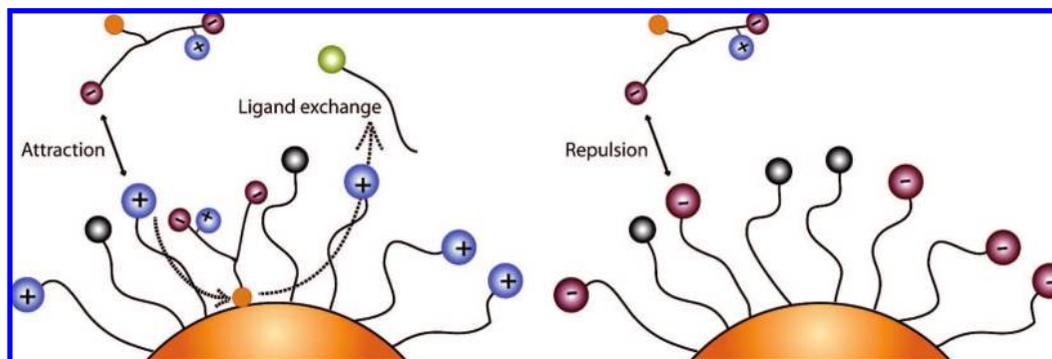


Figure 7. Proposed mechanism for GSH-mediated FITC release from cationic and anionic AuNPs surface.

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Supporting Information Available: Synthesis of ligands and particles, data analysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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