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Luminescent Gold Nanoparticles with pH-Dependent Membrane Adsorption

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Supporting Information

ABSTRACT: pH regulates many cellular processes and is also an indicator of disease progression. Therefore, pH-responsive materials often serve as either tools in the fundamental understanding of cell biology or medicine for disease diagnosis and therapy. While gold nanoparticles have broad biomedical applications, very few of them exhibit pH-dependent interactions with live cells in a native biological environment due to nonspecific serum protein adsorption. Herein, we report that by coating luminescent gold nanoparticles with a natural peptide, glutathione, and the simplest stable aminothiol, cysteamine, we enabled the nanoparticles to exhibit not only high resistance to serum protein adsorption but also pH-dependent adsorption onto live cell membranes in the presence of serum proteins. Incorporating this pH-dependent membrane adsorption behavior into gold nanoparticles could potentially catalyze new biomedical applications of metal nanoparticles in the fundamental understanding of biological processes as well as disease diagnosis and therapy, where pH changes are involved.

pH is a key parameter for many biological processes[1] and also an important indicator for disease progression.[2] For example, endocytosis, a process wherein substances are engulfed by cells, is involved with a pH change from neutral to acidic (pH 4.5−6.2)[1] Due to the Warburg effect,[3] the acidic pH is also characteristic of solid tumors (extracellular pH 6.0−7.0).[4] Therefore, pH-responsive materials such as organic dye-based indicators, cationic polymers, and some peptides often serve as either tools in the fundamental understanding of cell biology or medicine for disease diagnosis and therapy.[5] Nanoparticles (NPs) often show broad and tunable optical,[6] magnetic,[7] electrical, photothermal properties,[8] and a large surface-to-volume ratio, which allows the integration of different functional groups into one single entity.[9] Therefore, nanoparticles showing pH-dependent interactions with live cells will provide new multifunctional tools for disease diagnosis and therapy.

While metal NPs hold great promise in bioimaging,[10] drug/gene delivery,[11] and phototherapy,[12] their interactions with the cell membrane are generally insensitive to extracellular pH changes in a native biological environment because serum proteins are often adsorbed onto the metal NPs and form a protein "corona".[13] This additional protein corona, rather than surface ligands, governs interactions between NPs and the cell membrane.[13,14] Over 3700 proteins in the serum/plasma and their dynamic adsorption/desorption with the particles create huge uncertainties in rational manipulation of the NP−cell membrane interactions at different pHs. To address this challenge, we took advantage of a natural peptide, reduced glutathione, and the simplest stable aminothiol, cysteamine as surface ligands to create a class of ∼3 nm luminescent gold NPs (AuNPs), which have little interaction with serum proteins but exhibit pH-dependent adsorption onto live cell membranes in a biological pH range from mildly acidic to neutral (5.3−7.4). This simple surface chemistry, where pH-dependent membrane adsorption is enabled is expected to catalyze new biomedical applications of metal NPs in the fundamental understanding of biological processes as well as disease diagnosis and therapy.

In this work, to utilize fluorescence microscopy to probe the interactions between NPs and live cells, we employed luminescent AuNPs, rather than conventional nonluminescent AuNPs, as a model system to investigate how the changes in the surface chemistry of the NPs and the local pH environment influence the NP−cell membrane interaction. Luminescent metal NPs are a class of new metal nanostructures that give intrinsic emission without conjugation with fluorescent dyes.[15] Therefore, potential interferences from organic dyes in the studies of surface chemistry effects on the NP−cell interactions can be circumvented.[16] Glutathione, an abundant triamino acid peptide in nature, was chosen as one surface ligand due to its low affinity to serum proteins and the capability of minimizing nonspecific protein adsorption on the NPs.[11a,17] However, the glutathione-coated luminescent AuNPs (**G-AuNPs**) have very weak interaction with cells within a pH range from 5.3 to 7.4 (Figure S1 Supporting Information [SI]). To make the NP−cell membrane interaction pH dependent, in addition to glutathione, cationic cysteamine was also introduced into the luminescent AuNPs as the second surface ligand because protonated cysteamine can drive NPs to bind nonspecifically to the cell membrane through electrostatic attraction,[18] which was also confirmed by our studies (Figure S2 [SI]).

Glutathione and cysteamine-coated luminescent AuNPs (**GC-AuNPs**) were synthesized using a modified method that we used to create G-AuNPs.[19] Briefly, a fresh aqueous solution (400 μL) containing 12.5 mM glutathione and 12.5 mM cysteamine was added into a 25 mM $HAuCl_4$ aqueous solution (200 μL). Because of the strong Au(I)−S interaction, the two thiolated ligands immediately reacted with gold ions to form Au(I)−glutathione/cysteamine polymers,[19] which dissociated into AuNPs naturally coated by glutathione and cysteamine (Scheme S1 and Figure S3 [SI]). The FTIR analysis revealed

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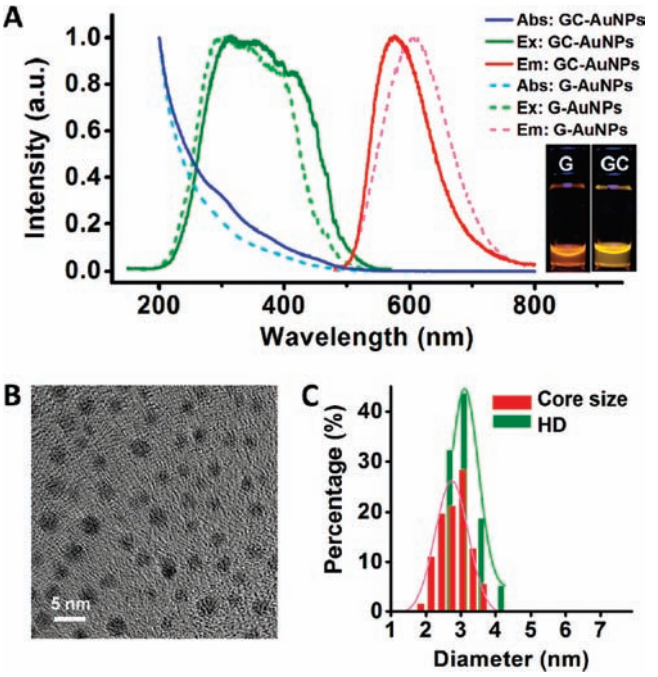

Figure 1. (A) Absorption, excitation, and emission spectra of glutathione-coated luminescent gold nanoparticles (G-AuNPs) and glutathione/cysteamine-coated luminescent gold nanoparticles (GC-AuNPs) in aqueous solution. G-AuNPs: One adsorption shoulder peak at 400 nm, λ^{ex}_{max} = 300 nm, λ^{em}_{max} = 605 nm. GC-AuNPs: three adsorption shoulder peaks at 305, 375, and 450 nm, λ^{ex}_{max} = 315 nm, λ^{em}_{max} = 575 nm. Inset: Pictures taken under 365 nm UV excitation. (B) Typical transmission electron microscopy image of GC-AuNPs showing (C) a core size of 2.7 ± 0.5 nm, and dynamic light scattering analysis showing a hydrodynamic diameter (HD) of 3.1 ± 0.4 nm in aqueous solution.

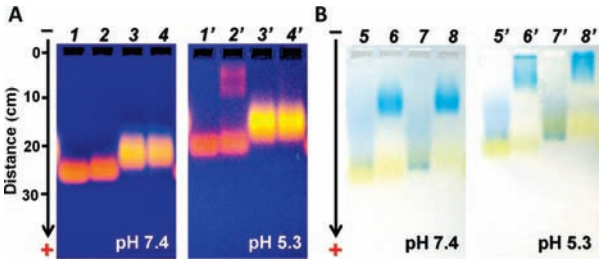

Figure 2. Agarose gel electrophoresis of GC-AuNPs with or without incubation with fetal bovine serum (FBS) at pH 7.4 and pH 5.3, respectively. (A) G-AuNPs (well 1, 2, 1′, 2′) and GC-AuNPs (well 3, 4, 3′, 4′) were incubated at pHs 7.4 or 5.3 in the absence (well 1, 3, 1′, 3′) or presence (well 2, 4, 2′, 4′) of 10% (v/v) FBS at 37 °C for 30 min (pictures taken under 365 nm UV excitation). (B) After G-AuNPs (well 5, 6, 5′, 6′) and GC-AuNPs (7, 8, 7′, 8′) were incubated with or without FBS, serum proteins were stained by CBB250. Blue band in wells 5, 7, 5′, 7′ (without FBS) = free CBB250. Blue band in wells 6, 8, 6′, 8′ (with FBS) = CBB250-stained proteins.

the ratio of cysteamine to glutathione on the GC-AuNPs was approximately 10 to 7 (Figure S4 [SI]). The GC-AuNPs exhibited intensive yellow emission with a maximum at 575 nm (Figure 1A) and a quantum efficiency of about 9% in a pH range of 4.8−8.0 (Figure S5 [SI]), which is two times higher than that of G-AuNPs. The differences in the adsorption, excitation and emission spectra of G-AuNPs and GC-AuNPs indicated that the introduction of cysteamine actually enhances the emission property of luminescent AuNPs (Figure 1A). The mean core size and hydrodynamic diameter of GC-AuNPs were 2.7 ± 0.5 nm and 3.1 ± 0.4 nm, respectively (B and C of Figure 1), which are slightly larger than the ∼2 nm G-AuNPs.

While the positively charged cysteamine ligand in a mildly acidic environment can drive the AuNPs to bind nonspecifically to the cell membrane (Figure S2 [SI]), cationic cysteamine coated-AuNPs became negatively charged in the presence of serum proteins, indicating that serious nonspecific protein adsorption on NPs occurred (Figure S6 [SI]), consistent with a previous report.[20] Therefore, to assess whether GC-AuNPs coated with both glutathione and cysteamine can still resist protein adsorption, the NPs were incubated in the absence or presence of fetal bovine serum at 37 °C for 30 min and then were analyzed by agarose gel electrophoresis. GC-AuNPs displayed no changes in mobility after incubation with fetal bovine serum at pHs 7.4 and 5.3, suggesting that GC-AuNPs did not aggregate in the serum protein-containing medium and that serum proteins were not bound to the particles at neutral and mildly acidic pH values (Figure 2A). In contrast, G-AuNPs started binding to proteins at pH 5.3, revealed by two luminescent bands after fetal bovine serum treatment. After the NPs were incubated with fetal bovine serum, additional protein staining by Coomassie brilliant blue 250 (CBB250) showed the blue protein band was well separated from the yellow band of GC-AuNPs at both pH 7.4 and pH 5.3 (Figure 2B), further confirming that GC-AuNPs have little interaction with serum proteins in the biological pH range from mildly acidic to neutral.

The pH-responsive membrane adsorption of GC-AuNPs in an extracellular pH range from 5.3 to 7.4 was investigated using fluorescence microscopy imaging of live HeLa cells as a model system. HeLa cells were also used in a previous study of pH-dependent insertion of a peptide because their membranes remain integral in the range of pH 5.5−7.4.[5e] As is shown in A−D of Figure 3 and in Figure S7, GC-AuNPs had little interaction with cells at pH 7.4 in phosphate-buffered saline (PBS). Once the extracellular pH was lowered to pH 6.0 and further dropped to 5.3, luminescence intensity of the cells increased dramatically, suggesting the adsorption of the NPs to the cell membrane was significantly enhanced in a slightly acidic environment. Z stack imaging of cells stained by GC-AuNPs at pH 5.3 indicated that the NPs were not internalized into the cells but bound on the cell surface (Figure S8 [SI]), which was further confirmed by the colocalization of GC-AuNPs with DiR, a phospholipid bilayer membrane dye that can embed in the lipid bilayer (Figure 3E and Scheme S2 [SI]). Due to the membrane adsorption, filopodia, the slender cytoplasmic projections for cell migration, were also clearly visualized at pH 5.3 using fluorescence microscopy, similar to membrane staining by DiR (Figures 3D and S9 [SI]). Moreover, real-time imaging of live cells revealed that the accumulation of GC-AuNPs on the cell membrane reached a maximum within 30 s, implying a fast membrane adsorption of the particles (Figure S10 [SI]). These results clearly indicated that the additional surface ligand cysteamine made GC-AuNPs exhibit pH-dependent membrane adsorption in the biological pH range.

By quantifying the luminescence intensity of the cell membrane after incubation with GC-AuNPs at different pHs in PBS, we found the adsorption of GC-AuNPs onto live cell membranes exponentially increase with the increase of H^+ concentration in the pH range of 4.8−8.0 (Figure S11 [SI]). Luminescence intensity of the cell membrane increased about 28 times once the

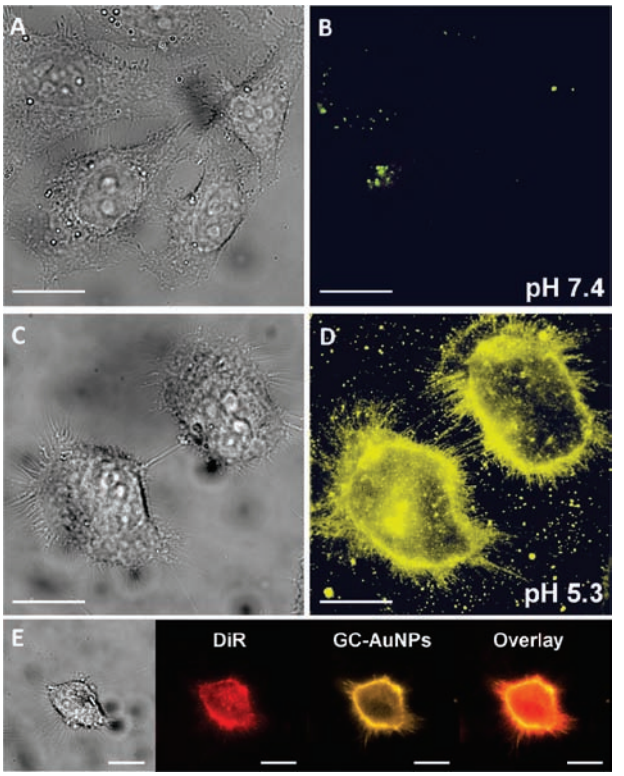

Figure 3. pH-dependent adsorption of GC-AuNPs on live HeLa cell membrane. Brightfield (A, C) and fluorescence (B, D) images of live HeLa cells incubated with 0.2 mg/mL GC-AuNPs at pH 7.4 (A, B) and pH 5.3 (C, D) in PBS at 25 °C for 10 min, respectively (scalar bar, 20 μm). (E) Co-localization of GC-AuNPs with DiR (a phospholipid bilayer membrane dye) on live cell membrane (scalar bar, 20 μm).

pH dropped from 7.4 to 5.3 (Figure 4A). A similar pH-dependent membrane adsorption of GC-AuNPs was also observed in serum protein-containing cell culture medium (Figures S12, S13 [SI]). The pH thresholds for the membrane adsorption of GC-AuNPs in the absence or presence of serum proteins are 6.5 and 6.4, respectively (Figure 4A), indicating that serum proteins did not interfere with the interaction of GC-AuNPs with the cells. This pH-dependent membrane adsorption of GC-AuNPs in the presence of serum proteins is attributed to a collaborative effect of glutathione and cysteamine ligands: glutathione prevents nonspecific protein adsorption, whereas the positively charged cysteamine enables particles to nonspecifically bind to the negatively charged cell membrane through electrostatic interactions.

Interestingly, both gel electrophoresis and zeta potential measurements have confirmed that GC-AuNPs are still negatively charged at pHs 7.4 and 5.3 (zeta potential: -29.76 ± 1.81 mV and -15.71 ± 1.71 mV, respectively, Figure 4B). However, the zeta potentials of GC-AuNPs are much lower than those of G-AuNPs at the same pHs (-48.03 ± 2.26 mV and -35.54 ± 2.77 mV at pHs 7.4 and 5.3, respectively), suggesting that the additional cysteamine ligand in the GC-AuNPs indeed the significantly decreases negative charge of the NPs. Since the cell membrane remains negatively charged at a pH above 5,[21] the mildly acidic pH-triggered membrane binding of anionic GC-AuNPs implies that such pH-dependent adsorption behavior fundamentally arises from the decrease of global charge repulsion between GC-AuNPs and the cell membrane and an increase of

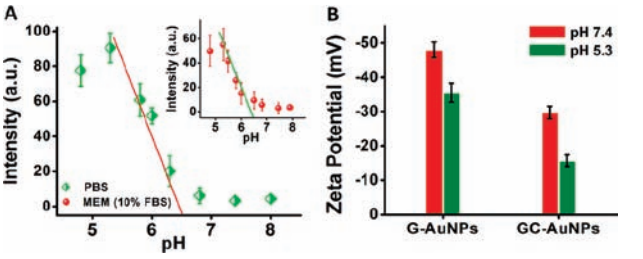

Figure 4. Luminescence intensities of the cell membrane and zeta potentials of GC-AuNPs at different pHs. (A) Luminescence intensity of the cell membrane incubated with GC-AuNPs at different pHs in PBS or (Inset) in minimum essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum (FBS). (B) Zeta potentials of G-AuNPs and GC-AuNPs at pH 7.4 and pH 5.3, respectively. Results presented as mean $\pm$ SD ($n = 6$).

attraction between protonated cysteamine ligand and the cell membrane. At pH 7.4, GC-AuNPs are still highly negatively charged; therefore, strong repulsion between the NPs and the cell membrane prevents the membrane adsorption. When the pH reaches a critical point, ~pH 6.5, the threshold for adsorption, the local attraction between cysteamine and the membrane can overcome global electrostatic repulsion, and thus membrane adsorption of anionic GC-AuNPs onto negatively charged membranes starts being observed.

The observation that negatively charged GC-AuNPs were able to be bound to the negatively charged membrane seems against intuition but is consistent with recent molecular dynamic simulation on the nonspecific binding of anionic ~2 nm AuNPs to a negatively charged lipid membrane in a serum-free environment[22] and the experimental observation that anionic AuNPs with an ordered hydrophobic and hydrophilic surface structure can overcome the global repulsion between the NPs and the cell membrane and can cross negatively charged lipid membrane.[23] We currently cannot determine whether glutathione and cysteamine formed an ordered surface structure on the NPs; however, since pure cysteamine-coated AuNPs can nonspecifically bind to the cell membrane (Figure S2 [SI]),[24] the driving force for the binding of GC-AuNPs to the cell membrane mainly arises from cysteamine ligand. Using a trypan blue assay,[25] we found that the cell membrane remains intact after fusion with the NPs (Figure S14 [SI]), revealing that adsorption of negatively charged NPs to the cell membrane in acidic conditions does not induce the membrane disruption. This observation is distinct from interactions between cationic NPs and the cell membrane, which induce nanopore formation on lipid bilayers, but is consistent with previously reported cell-permeable negatively charged AuNPs which also do not destroy membrane integrity.[23]

In conclusion, by using a natural peptide, glutathione, and a simple aminothiol, cysteamine, as surface ligands, we facilely synthesized a negatively charged luminescent AuNP, which exhibits not only high resistance to nonspecific protein adsorption but also strong pH-dependent adsorption live cell membranes within a biological pH range (5.3−7.4). This simple surface chemistry strategy offers us a new way to manipulate NP−cell membrane interactions in a native biological environment. Considering that pH plays a key role in many cellular processes and is an indicator for a pathological environment, metal NPs with pH-dependent membrane adsorption might find new applications in tumor diagnosis and therapy.

■ ASSOCIATED CONTENT

Supporting Information. Materials and equipment, synthesis and characterization of luminescent gold nanoparticles, cell culture and microscopy imaging and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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