Light Activated Metal Coordinated Supramolecular Complexes with Charge-Directed Self-Assembly

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ABSTRACT: Metal coordinated materials are attractive for many applications including catalysis, sensing, and controlled release. Adenine and its derivatives have been widely used to generate many coordination complexes, polymers, and nanoparticles. However, few of these materials display fluorescence. Herein, we report fluorescent gold complexes and nanoclusters formed with adenosine, deoxyadenosine, AMP and ATP, where the former two produced micrometer-sized particles and the latter two produced molecular clusters. Only weak fluorescence was produced with adenine, while no emission was observed with uridine, cytidine or guanosine. We found that adding citrate and light exposure are two key factors to generate fluorescence and their mechanistic roles have been explored. In all the products, the ratio between gold and adenine was determined to be 1:1 using UV-vis spectroscopy. Mass spectrometry showed clusters containing 2, 4, and 6 gold atoms in the gas phase. The fluorescence peak is around 470 nm for the AMP and ATP complex and 480 nm for the (deoxy)adenosine complexes. This work has provided a systematic approach to obtain fluorescent metal coordinated polymers and materials with tunable sizes, which will find applications in analytical chemistry, drug delivery and imaging. The fundamental physical chemistry of these materials has been systematically explored.

Keywords: adenosine, nanoclusters, fluorescence, DNA, gold

Introduction

Supramolecular chemistry and in particular biomolecule-coordinated compounds and materials have attracted a lot of research interest in the past a few decades because of their potential applications in biosensor development, catalysis, tissue engineering, drug delivery and imaging.[1-5] At the same time, such research is also important for understanding metal toxicity and fundamental inorganic and materials chemistry. Out of the numerous biomolecules that can interact with metal ions, DNA and nucleotides have been extensively studied since they can bind to various metal ions via both the hard phosphate ligand and the more soft DNA bases.[6-12] In particular, adenine and its derivatives are very attractive ligands as they contain multiple high affinity metal binding sites.[13-15] For example, Wei *et al.* prepared adenine/Au(III) supramolecularly assembled nanoparticles with a diameter of ~300 nm. These particles were formed via a hierarchical assembled process with a Au:adenine ratio of 4:1.[16] Purohit and Verma managed to obtain the single crystal structure of 9-allyladenine coordinated with Ag and this material showed emission peaks at ~400 nm. This emission was attributed to metal induced nucleotide fluorescence by the authors.[17] Garcia-Teran *et al.* reported a complex formed by adenine and Cu.[18] Rosi and co-workers synthesized a metal-organic framework using Zn(II) and adenine, where Zn was coordinated via oxygen and controlled drug release was demonstrated.[19] Kimizuka and co-workers prepared supramolecular networks of adenosine monophosphate (AMP) with various lanthanides, where both oxygen and nitrogen ligands are proposed to participate in metal binding.[20] This structure was non-crystalline and various guest molecules could be trapped during the synthesis. In addition to experimental studies, a number of theoretical works have also been reported.[13,21-24]

In all the above cited examples, large three-dimensional structures were obtained since adenine has four metal binding sites and each metal ion can also bind multiple adenines. The challenge is thus to control the growth of these materials to form nanoparticles and molecular complexes. Fluorescence was reported only in one case with silver and so far no fluorescent gold complexes were reported with adenine ligands. It is highly desirable to develop more fluorescent materials that could be potentially

used for imaging and biosensing applications. We noticed that all the previous synthesis involved high concentrations of $HAuCl_4$ and adenine containing compounds, making it difficult to control the assembly process. In addition, no reducing agent was added.

In this work, we report a series of fluorescent gold complexes prepared with various adenine containing ligands. Citrate and light exposure or heating are identified to be the key factors to produce fluorescence. The gold complex may extend to large networks of three dimensional structures or may stay in the oligomer state, depending on the amount of charge on the ligand. The smallest complex may contain just a few gold ions, which can be considered as the smallest gold nanoclusters (AuNC) with blue fluorescence.[25-36] The availability of both nanoparticles and molecular species allows us to employ a diverse range of techniques to gain a more complete understanding. While fluorescent gold nanoclusters templated by DNA have been reported,[28,36] nucleotide-templated synthesis is more challenging since DNA could be designed to position a few gold ions at close proximity prior to their reduction. For nucleotides, gold needs to be made close to each other by multivalent coordination.

Materials and Methods

Preparation of Adenosine-Au complex. The complex formation was started by adding 2 μL adenosine (50 mM) to 38 μL water (final concentration: 2mM). To this mixture, 5 μL $HAuCl_4$ (50 mM) was added (final concentration: 0.5 mM) followed by 5 μL citrate-citric acid buffer (500 mM pH 6) (final concentration: 50 mM) to make up a final volume of 50 μL. Several of these parameters (e.g. $HAuCl_4$ and adenosine concentration) were varied before the optimized values above were obtained. For Au-ATP, Au-AMP, Au-deoxyadenosine, and Au-adenine complexes, the procedure was similar. The only difference being that these compounds were used in place of adenosine. For large volume synthesis, the reagents were scaled up accordingly and the incubation time was extended to three days in an open beaker with magnetic stirring.

Optical spectroscopy measurement. Fluorescence was observed using a UV lamp at 245nm in a dark room and recorded using a digital camera (Canon PowerShot SD1200 IS). The samples were also observed under ambient light and recorded. Quantification of fluorescence was normally done by diluting the sample 30 times in water to a final volume of 600 μL and measuring the spectrum on an Eclipse fluorometer (Varian) at an excitation wavelength of 300 nm. UV-Vis absorption spectrums were also obtained from an Agilent 8453A spectrometer after diluting by an appropriate amount. To avoid the interference from the free nucleotides, the samples were centrifuged at 15,000 rpm (for particular samples) or ultracentrifuged at 120,000 rpm (AMP, ATP samples) and washed with water twice before measurement. Dynamic Light Scattering was also performed using a Zetasizer Nano (Malvern) instrument to determine particle size, as well as zeta potential, where the samples were dispersed in the buffer they were synthesized.

XRD, SEM and fluorescence microscopy. Two large scale (500 mL) synthesized samples were prepared and one was stored in dark with the cap tightly sealed and the other exposed to ambient light. The samples were allowed to naturally settle down after three days and concentrated to 3 mL for repeated washing in water. Finally the samples were dried in a vacuum centrifuge. The XRD spectra were collected on a Bruker instrument by drying the sample on a piece of silicon wafer. SEM was measured on a Zeiss instrument. Fluorescence micrographs were obtained on an inverted Nikon fluorescence microscope with the DAPI cube. 2 μL of sample dispersed in water was placed between microscope slides and imaged with either 20× or 100× oil immersion objectives.

Results and Discussion

Fluorescent complexes. Most previous work on nucleobase and metal complexes was carried out by simply mixing them at high concentrations, where supramolecular structures were formed immediately. To better control the reaction kinetics, we decreased the concentration of the reagents by more than 10-fold (e.g. ~0.25-0.5 mM $HAuCl_4$) and also introduced citrate as a reducing agent. For the initial test, 1

mM adenosine, uridine, cytidine, or guanosine was respectively mixed with 0.25 mM $HAuCl_4$ and citrate buffer (pH 3 or 6) was added. Citrate acts both as a reducing agent and a buffer. We chose these two pH values to cover the possible protonation effect of adenosine (pk_a=3.5) and cytosine (pk_a=4.2).[37,38] After overnight incubation under ambient light, the samples were observed under 245 nm excitation in a dark room or under ambient light (Figure 1A). Adenosine at pH 6 gave strong blue emission while the samples containing the other nucleosides were dark. Protonation of adenosine at pH 3 also inhibited the synthesis and therefore the N_7 position might be involved in binding to gold. It is interesting to note that large AuNPs were produced with uridine at both pH values since red or blue color was observed under ambient light. Cytosine and guanosine failed to produce either AuNPs or fluorescent species. The fluorescence spectrum of the adenosine/Au complex shows an emission peak at ~480 nm (Ex = 300 nm, Figure 1C, black trace), and the fluorescence excitation spectrum shows a peak at ~300 nm (red trace).

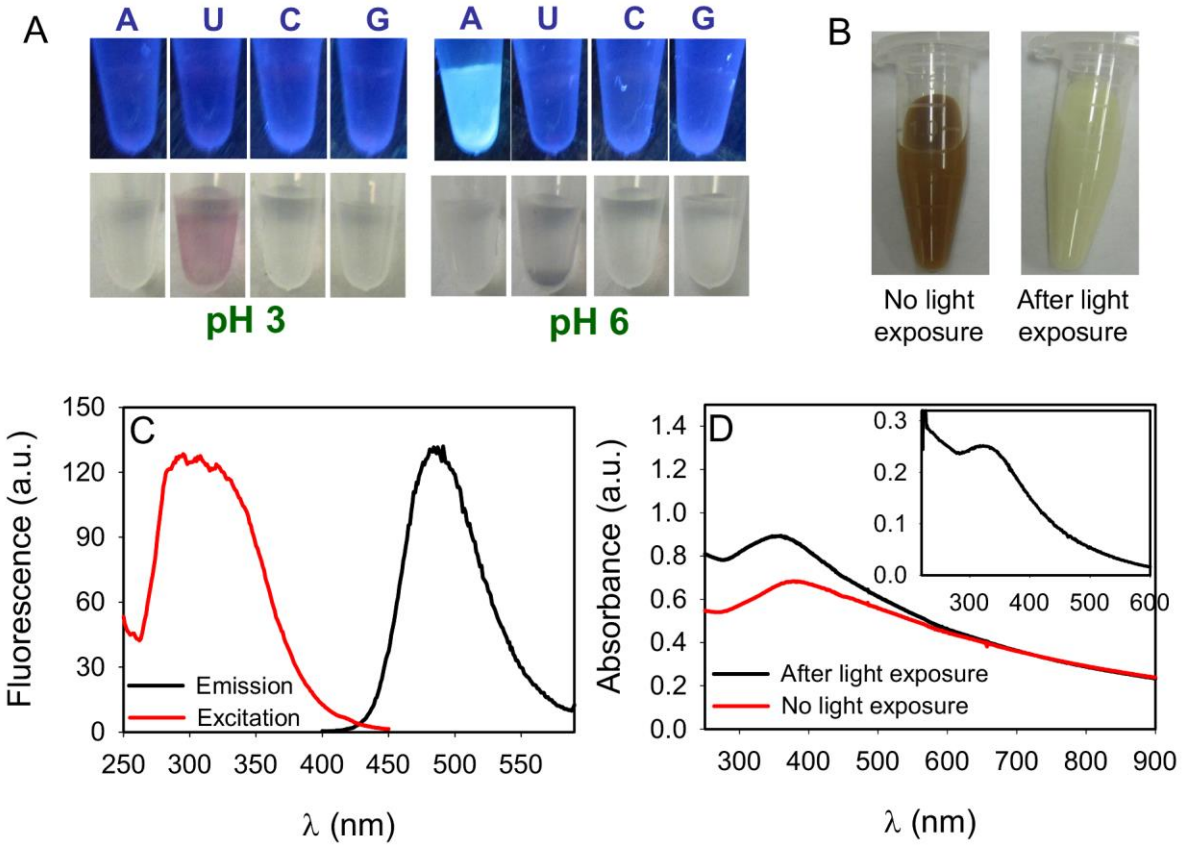

Figure 1. (A) Fluorescence (excited at 245 nm in a dark room) and optical photographs of various ribonucleoside/$HAuCl_4$ mixture with pH 3 or 6 citrate buffer after overnight incubation. Only adenosine at pH 6 produced emission. (B) Photographs of adenosine/$HAuCl_4$/citrate mixture incubated in dark or after light exposure. The samples were concentrated by ~150 times (Au concentration =75 mM) in the pictures. (C) Fluorescence excitation and emission spectra of adenosine/Au complex. (D) Absorption spectra of adenosine/$HAuCl_4$/citrate mixture before and after light exposure. Inset: The difference spectrum obtained by subtracting the spectra in D. Au concentration = 0.2 mM. The samples have been purified by centrifugation and washing in water to remove free adenosine. Since the samples are micrometered particles, they also show light scattering effect to lift up the baseline of the spectra.

Light activated fluorescence. The above samples were prepared by exposing them overnight to ambient fluorescent tube light. Interestingly, no fluorescence was observed when the samples were stored in dark. On the other hand, fluorescence was quickly developed after a short time exposure to UV light, suggesting that light exposure is a required step to produce fluorescence. To systematically understand this, a large volume of sample was prepared by mixing $HAuCl_4$, adenosine and citrate in dark. After overnight incubation, the sample was aliquoted into microcentrifuge tubes in dark. The fluorescence of these samples was measured after exposing each tube for a designated time under the 245 nm UV lamp. As shown in Figure 2A, the maximal emission was achieved after UV exposure for ~5 min. Ambient fluorescent tube lamp could also induce fluorescence but it takes longer time. Next, we tested whether UV exposure alone is sufficient for fluorescence generation. For this purpose, a sample was exposed to UV light immediately after mixing all the reagents; however this sample failed to show fluorescence. Therefore, a certain incubation time was needed for complex formation. As shown in Figure 2B, the maximal emission was achieved ~5 hr after mixing the reagents. Note that all the samples in Figure 2B were exposed to UV light for 5 min before fluorescence measurement. Furthermore, if citrate was not added, no fluorescence was generated either even with light exposure.

Taking together, citrate, an incubation step (e.g. 5 hr) and light exposure were all required to produce fluorescence.

To obtain a large volume of sample, using a handheld UV lamp for light exposure is not practical. Instead, we employed ambient fluorescent tube lamp but with a longer exposure time. The time takes to reach maximal fluorescence is also a function of sample volume. For example, it takes ~3 days when 500 mL sample was prepared. Light exposure has changed the electronic structure of the samples. Before light exposure, the samples were brown (Figure 1B) with a broad absorption feature peaked at ~380 nm (Figure 1D). After light exposure, the sample became greenish but the main absorption peak only slightly blue shifted. The difference spectrum after and before light exposure showed an absorption maximum at ~300 nm (inset of Figure 1D), which agrees with the fluorescence excitation spectrum of the sample (Figure 1C, red curve). In addition to light absorption, the samples also showed light scattering features with a high background, which is the main reason for us to perform the spectrum subtraction step. The light scattering contribution also suggests that the samples are relatively large particles.

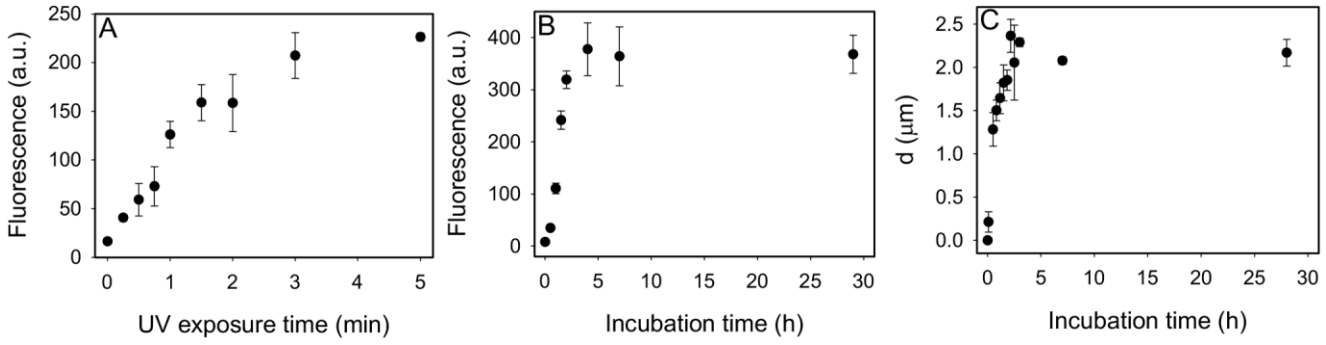

Figure 2. (A) Adenosine/Au fluorescence evolution as a function of UV exposure time. All the samples were incubated overnight in dark before UV exposure. In this study, the UV activation of fluorescence was probed. (B) Fluorescence evolution as a function of incubation time (in dark with $HAuCl_4$, adenosine and citrate). All the samples were exposured to UV for 5 min before measurement. In this

study, the reaction time before UV exposure was probed. (C) Evolution of adenosine/Au particle hydrodynamic diameter as a function of incubation time.

Stoichiometry. To optimize the ratio between adenosine and $HAuCl_4$ during the synthesis, $HAuCl_4$ was fixed at 0.25 mM and higher adenosine concentration appeared to produce stronger fluorescence until 2 mM adenosine was reached (Figure 3A-C). A slight drop was observed with 5 mM adenosine. While the fluorescence spectra differed quite a bit in intensity, the spectral shape and peak maximal remained relatively consistent (Figure 3B), suggesting that the fluorescent species was the same for all the samples. Next, the concentration of adenosine was fixed at 2 mM and the concentration of $HAuCl_4$ was varied. The fluorescence was stronger with higher $HAuCl_4$ concentration until 0.5 mM $HAuCl_4$ was reached (Figure 3D-F). Further increase of gold resulted in decreased fluorescence and the color of the tube also turned slightly brown, suggesting the formation of other types of non-fluorescent species. Therefore, the optimal condition for the synthesis appears to be 2 mM adenosine and 0.5 mM $HAuCl_4$.

To determine the stoichiometry between adenosine and $HAuCl_4$ in the complex, we used an excess amount of adenosine. After centrifugation the absorbance at 260 nm of the supernatant was measured, from which free adenosine concentration was calculated. Based on this, the adenosine:Au ratio in the particle was determined to be 1:1.04, suggesting that the ratio of these two components to be 1:1. Therefore, the requirement of higher concentration of adenosine to reach high fluorescence (e.g. adenosine:Au = 4:1) is likely to avoid the initial formation of large AuNPs.

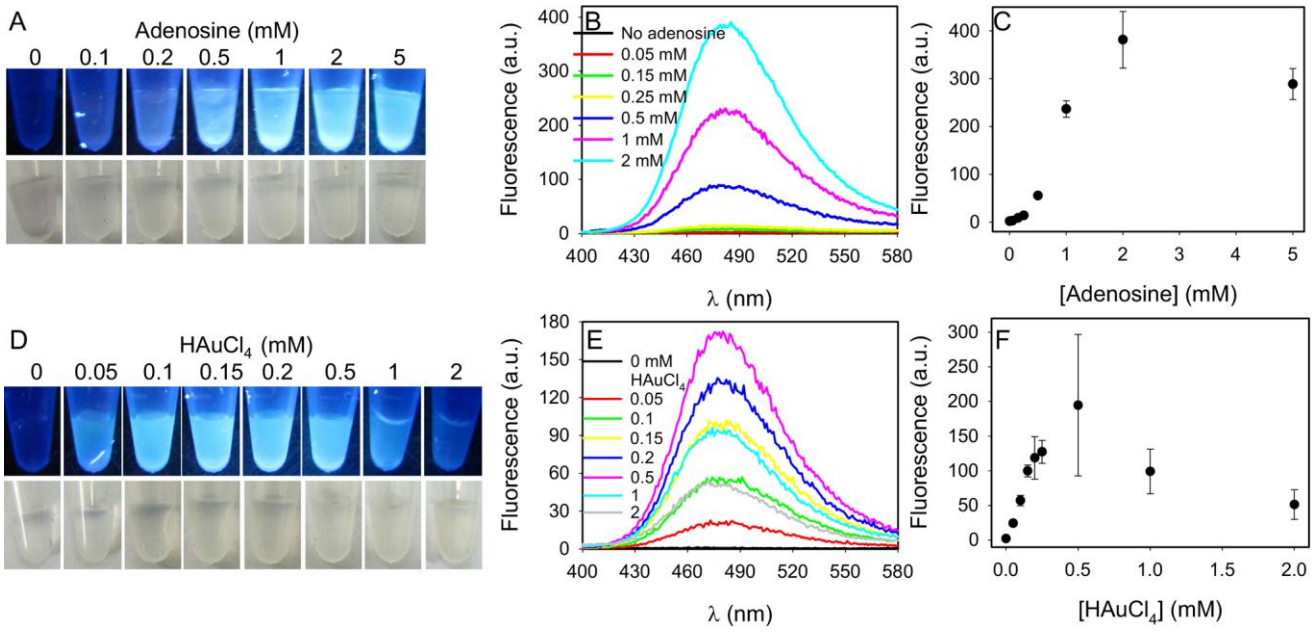

Figure 3. Photographs of AuNCs prepared with adenosine as a function of adenosine concentration (A) or $HAuCl_4$ concentration (D). Fluorescence spectra of AuNPs as a function of adenosine concentration (B) or $HAuCl_4$ concentration (E). Plot of emission peak intensity as a function of adenosine concentration (C) or $HAuCl_4$ concentration (F).

Fluorescent supramolecular particles. We noticed that the samples gradually became cloudy within a few hours after the addition of citrate while the sample without citrate remained clear (Figure 4A). The average size of the particles was measured to be ~2.2 μm using dynamic light scattering (DLS) and the size remained the same after light exposure. Therefore, light exposure does not change the aggregation state of the complex. After centrifugation, the supernatant becomes clear and non-fluorescent while the precipitant is highly fluorescent (Figure 4C), indicating that the fluorescence of the adenosine/gold complex is from colloidal particles instead of from a simply molecular species. We followed the kinetics of particle size change using DLS (Figure 2D); it takes ~5 hr for the particles to reach a stable size distribution. This time scale agrees well with the incubation time required for achieving strong

fluorescence (Figure 2B). Therefore, the formation of the complex between adenosine and gold appears to be a prerequisite for fluorescence generation.

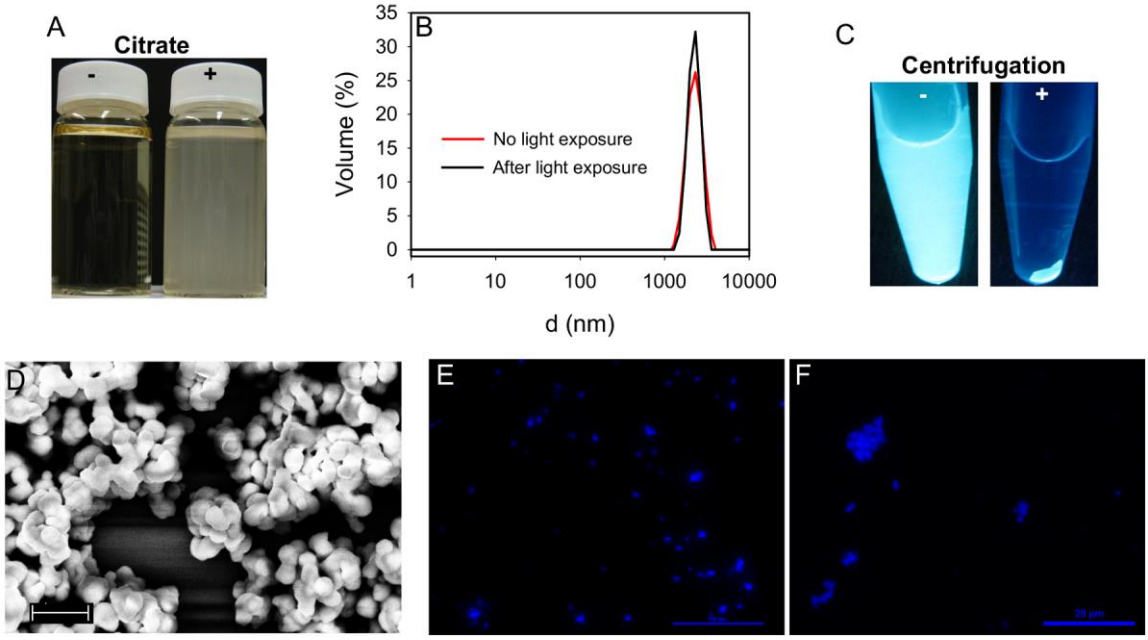

Figure 4. (A) Photographs of samples after a typical synthesis. If no citrate was added, the sample remained clear. (B) Hydrodynamic size distribution of particles after overnight incubation. (C) Fluorescence images of the particles when dispersed in water and after centrifugation. (D) SEM micrograph of dried particles. (E, F) Fluorescence micrographs of the particles dispersed in water. Scale bar = 100 μm in (E), 20 μm in (F) and 1 μm in (D).

To characterize the morphology of the particles, a sample was dried and imaged using scanning electron microscopy (SEM) (Figure 4D). The sample was not conductive and displayed the charging effect during the SEM experiment. The individual particles are spherical with a size of ~300 nm. The 2.2 μm hydrodynamic size is thus a consequence of aggregation. This conclusion is also supported by optical microscopy of the sample dispersed in water, where clustering of small particles were observed (Figure 4E, F). Therefore, the aggregation in SEM was not an artifact of drying. We measured the zeta-potential of the sample to be just -2 mV in 50 mM citrate buffer (pH 6), explaining the tendency to aggregate.

Even after drying, the particles remained fluorescent (see Figure S1). However, powder X-ray diffraction experiments showed that the particles were non-crystalline before or after light exposure (see Figure S2).

Complexes formed with other adenine derivatives. To further understand the interaction between adenosine and gold, we next tested a few adenine derivatives including adenine, deoxyadenosine, AMP and ATP. For each compound, the fluorescence photographs and spectra were measured as a function of the compound concentration. As shown in Figure 5A-C, deoxyadenosine showed a similar trend as adenosine. Therefore, the 2′-OH is not important for fluorescence generation. The product was also particles (particle size = ~2 μm, Figure 5D and zeta-potential = -2 mV). On the other hand, adenine failed to produce strong emission (Figure 5E-G). The best fluorescence was observed at a lower ratio between adenine and gold (e.g. 0.5 mM adenine) and the strongest emission was just ~5% of that of adenosine. The major difference is that the 9′ nitrogen is available for binding to gold in adenine, which appears to have an adverse effect on fluorescence generation. The adenine/Au complex also appeared to be particles of 1.4 μm with its zeta-potential being -14 mV. It is interesting that AMP or ATP produced clear solutions, while blue fluorescence was still observed (Figure 5I-P). The fluorescence spectra were blue shifted by ~10 nm to 470 nm for these two negatively charged compounds. DLS showed that AMP produced an average size of ~10 nm and ATP has an average size of just 2 nm. Therefore, the degree of aggregation is a function of charge associated with the ligands. Zeta-potential measurement showed values of -24 and -28 mV for AMP and ATP complexes, respectively. This experiment shows that producing fluorescence does not require extensive aggregation, while aggregation with adenosine is due to a lack of charge. The fluorescence intensity of samples prepared with (deoxy)adenosine, AMP and ATP was quite comparable but not exactly the same. This might be related to the different particle size and difference in the amount of light exposure. We have used the AMP-Au complex to determine the fluorescence quantum yield to be 1.6% with anthracene as a reference.

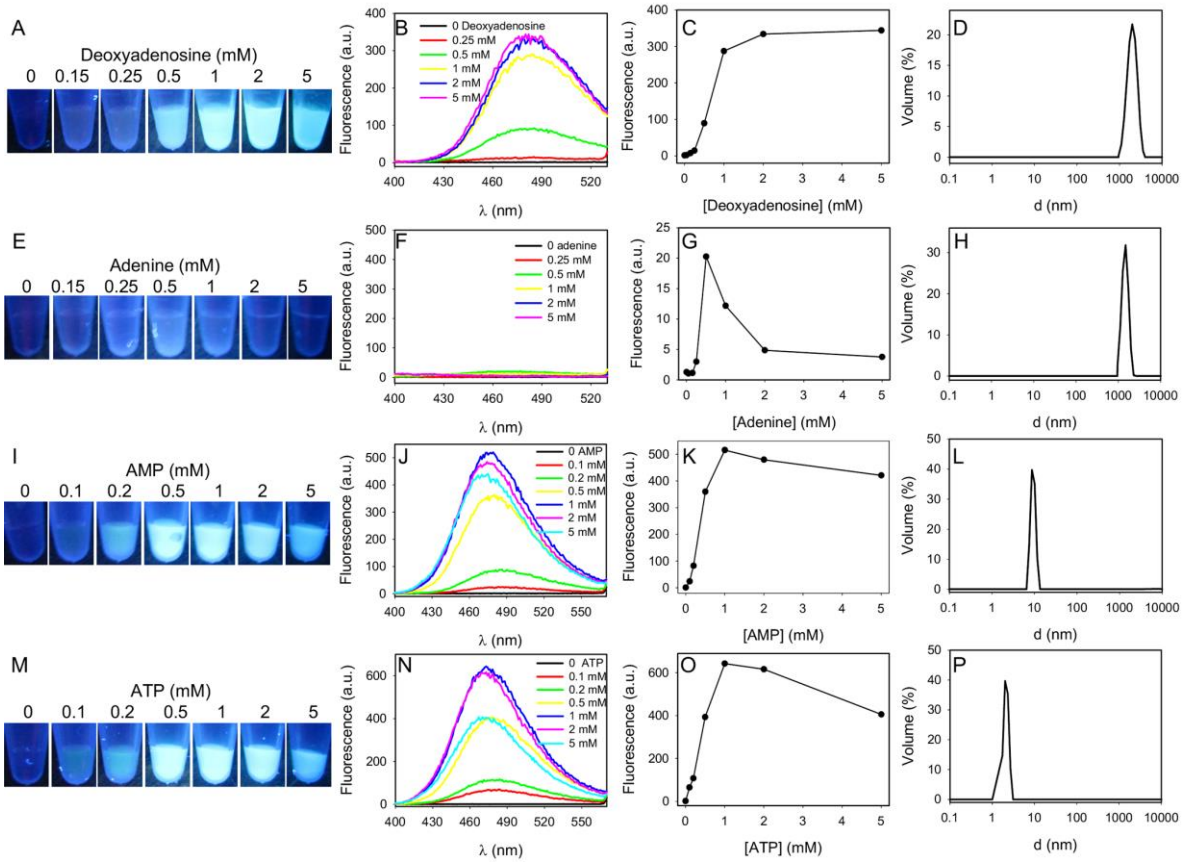

Figure 5. Fluorescence images of the complexes formed by Au and deoxyadenosine (A), adenine (E), AMP (I) and ATP (M). The fluorescence intensity of the samples (B, F, J, N) and peak intensity as a function of the concentration of deoxyadenosine (C), adenine (G), AMP (K) and ATP (O). Hydrodyanmic diameter of the complexes formed by Au and deoxyadenosine (D), adenine (H), AMP (L) and ATP (P). In all the experiment, the $HAuCl_4$ concentration was fixed to be 0.5 mM and citrate pH 6.0 (50 mM).

Since the adenosine sample formed aggregated nanoparticles, it cannot be characterized by mass spectrometry. Fortunately, the emission property of AMP and adenosine samples was very similar and DLS showed that AMP might generate molecular species. We performed ultracentrifugation to concentrate and purify the AMP/Au complex and carried out mass spectrometry studies (Figure 6A). Interestingly, AMP_nAu_n complexes were observed, where n = 2, 3, or 4. This is consistent with our

previous stoichiometry measurement using UV-vis spectroscopy. Essentially no mass corresponding to the AMP_1Au_1 species was found. Therefore, we reason that at least two gold ions are required to form a stable complex and to generate fluorescence, and Au-Au interaction might be crucial for fluorescence.[39-41] It needs to be pointed out that the mass spectrometry only displays species that are stable in the gas phase and it may not completely reflect the situation in the solution phase. The fact that these clusters could be detected in the mass spectrometer conditions indicates that they are very stable. At the same time, the small clusters predicted by the mass spectrometry matches the small sizes measured using DLS.

To understand the origin of fluorescence, we measured the emission lifetime. The absorption spectrum of the AMP/Au complex showed a peak at 300 nm (Figure 6B), which is similar to the difference spectrum we obtained for the adenosine/Au complex before and after light exposure (inset of Figure 1D). The lifetime decay of both AMP and adenosine complexes were measured to see the effect of dispersed versus aggregated molecules (Figure 6C). In both cases, the decay times were on the order of 1 μs and contained multiple decaying components. The decay with adenosine appeared to be even slower. There are literature reports showing fluorescence lifetime of gold nanoclusters to be around a few hundred nanoseconds, which is comparable with our observation.[42-45] The mechanism of such emission is still under investigation but it is believed to be related to the charge transfer from ligand.[39,42] For example, Wu and Jin reported that the $Au_{25}SG_{18}^-$ cluster has decay times of 246 nsec and 1200 nsec, where SG denotes for glutathione.[42] Therefore, our adenosine/Au complex could be a form of such AuNC. When the lifetime and the Stokes shift (~180 nm) are considered, it might be more appropriate to call our emission luminescence instead of fluorescence. In other cases, AuNCs could also display lifetime characteristic of molecular fluorescence. For example, Zheng *et al.* reported the preparation of poly(amidoamine) dendrimer stabilized AuNC whose fluorescence lifetimes are below 10 ns.[25] In that example, it is believed that fluorescence is from the gold cluster itself (not charge transfer from the

ligand) and larger AuNCs emit longer wavelength. In our case, the emission color is almost independent of the cluster size, consistent with the charge transfer mechanism.

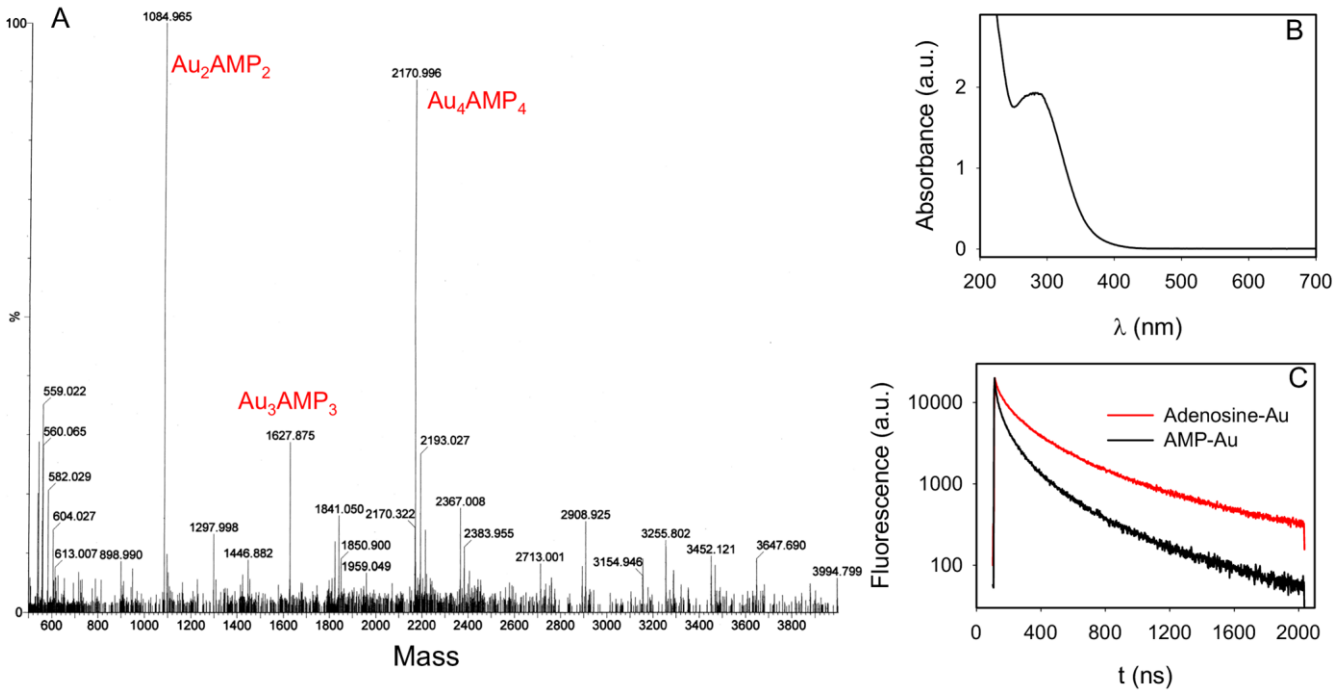

Figure 6. (A) Mass spectrometry with electrospray ionization of the AMP/Au complex. The sample has been purified using ultracentrifugation to remove free AMP. (B) UV-vis spectrum of AMP/Au complex. (C) Fluorescence lifetime decay of the adenosine/Au complex and AMP/Au complex in water.

Mechanistic studies. Since citrate and light exposure are the two key components to generate fluorescence, we aim to further study their effects. We first mixed adenosine and $HAuCl_4$ in the absence of citrate. Citrate was then added after waiting for designated time periods and the samples were incubated overnight under ambient light. As shown in the inset of Figure 7A, we found that if citrate was added after 2 hr, the sample appeared yellow. The fluorescence decreased significantly as the waiting time was increased, where essentially no fluorescence was observed at time 2 hr (Figure 7A). Similar observations were obtained for AMP but ~40% fluorescence still remained at 2 hr. Therefore, adenosine and AMP can bind to $HAuCl_4$ in the absence of citrate and form yellow colored complexes. If these complexes are stably formed, adding citrate cannot reduce gold to a fluorescent state. We propose

that citrate needs to be added first to reduce $HAuCl_4$ to a lower oxidation state and that may result in a different coordination geometry favored for fluorescence (e.g. with appropriate Au-Au distance for aurophilic interaction). Adenosine can form a 3D network structure to confine coordinated gold very tightly while AMP forms smaller clusters that can be adjusted more easily. This may explain the difference between these two in Figure 7A.

Next we tested the addition of citrate before adding adenosine. As shown in the inset of Figure 7B, AuNPs were formed and aggregated if adenosine was added at 30 min after mixing $HAuCl_4$ and citrate, where blue colored precipitants were observed. Within 10 min, strong fluorescence was still obtained. Therefore, citrate initially reduced $HAuCl_4$ to a lower oxidation state and if adenosine is present to stabilize it, fluorescent complex can form. If it is not stabilized and the reduction reaction proceeded further, large nanoparticles are produced.

To further understand the mechanism, we followed the reaction by UV-vis spectroscopy. As show in Figure 7C, ATP alone has a peak at 260 nm and addition of $HAuCl_4$ has slightly decreased the 260 nm peak while a small peak at ~300 nm showed up due to the added $HAuCl_4$ (blue line). This spectrum is relatively stable and does not change much in 30 min. If citrate is added, the 260 nm peak is dropped significantly and the 300 nm peak is increased (red line). Overnight incubation results in a slight change with the same trend (red dashed line). If free ATP is removed, no peak at 260 nm is observed and only 300 nm showed the complex peak (pink line). The change of electronic absorption feature also provides evidence for the effect of citrate in assisting coordination of gold by adenosine into a different configuration. Since our materials are non-crystalline, it is difficult to determine the exact coordination geometry of the gold complexes. The only other reported luminescent adenine/metal complex is 9-allyladenine/Ag,[17] where the ratio between the ligand and the metal is also 1:1 and each Ag is bound by three adenines and vise versa.

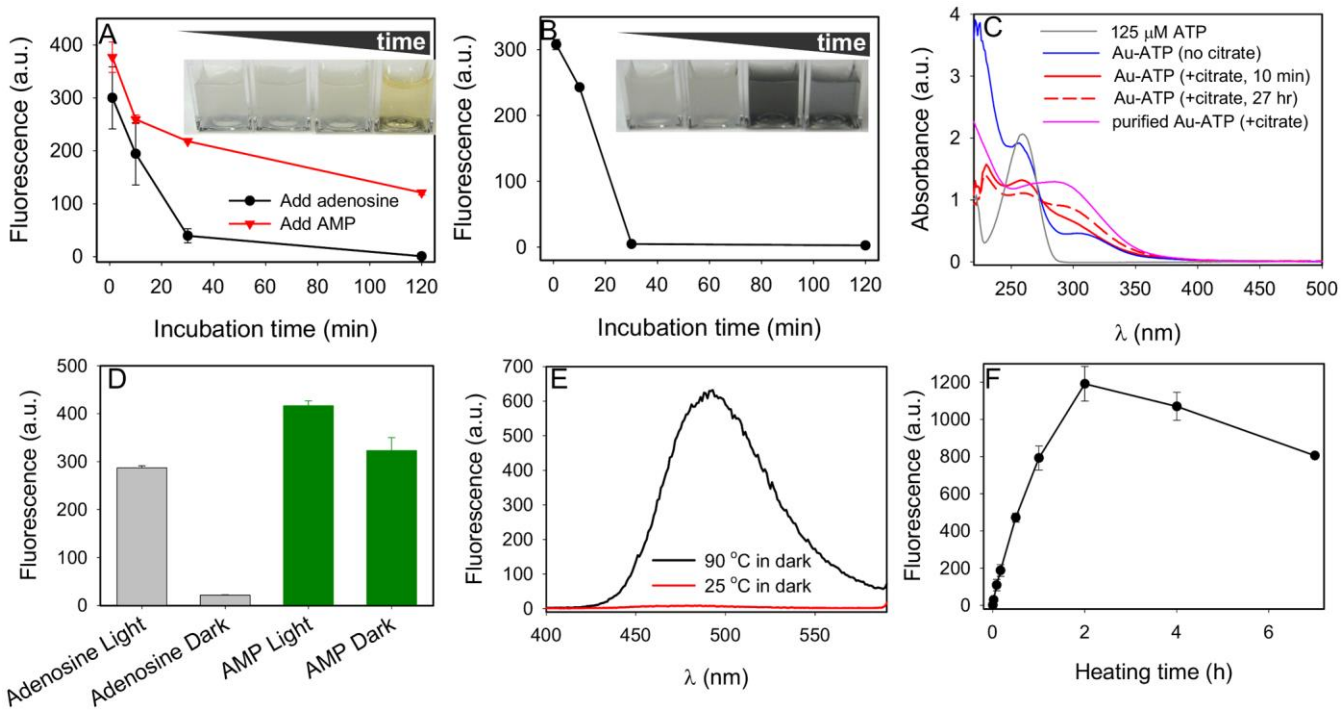

Figure 7. (A) Fluorescence of adenosine/Au or AMP/Au complex when citrate was added at designated time points after mixing adenosine or AMP with $HAuCl_4$. Inset: a photograph of the adenosine/Au samples. (B). Fluorescence of adenosine/Au complex when adenosine was added at designated time points after mixing $HAuCl_4$ with citrate. Inset: a photograph of the adenosine/Au samples. (C) UV-vis spectra of ATP, ATP/$HAuCl_4$ before and after citrate addition. Purified Au-ATP was prepared after ultracentrifugation and removal of free ATP. (D) Fluorescence intensity of adenosine/Au and AMP/Au with or without after light exposure. (E) Fluorescence spectra of adenosine/Au samples stored in dark at 25 °C or 90 °C for 2 hr. (F) Fluorescence of adenosine/Au samples exposed to ambient light and 90 °C water bath as a function of time.

Next we studied the effect of light by storing adenosine and AMP samples in dark or under ambient light. After overnight reaction, the adenosine sample was fluorescent only if exposed to light. On the other hand, the AMP sample was much less affected by light and light exposure only increased fluorescence by ~20% compared to that stored in dark. We already know that the complexes formed

with AMP are much smaller clusters. It is likely that the adjustment of gold coordination is more easily to take place in such smaller AMP clusters rather than that in large aggregated adenosine complexes. We reason that if the role of light is just to facilitate re-organization of gold binding to adenosine, thermal energy might also be useful for this purpose. To test this, we prepared a sample completely sealed in dark and then exposed to a 90 °C water bath for 2 hr and indeed this sample showed strong fluorescence (Figure 7E). For comparison, the sample without heating (but still in dark) remained non-fluorescent. Therefore, the role of light can be replaced by heating. Finally, we combined light exposure and heating. As shown in Figure 7F, we obtained much stronger fluorescence in 2 hr (e.g. light alone produces ~500 a.u. fluorescence). Further heating leads to fluorescence quenching. Overall, the thermostability of the fluorescence is very high.

We previously reported the preparation of fluorescent AuNCs using DNA such as C_{30} at low pH and A_{30} at neutral pH.[28] In this current work using nucleosides or nucleotides, only adenine derivatives could afford fluorescence. We reason it is easier for a DNA chain to position a few gold ions close to each other (intramolecular binding) so that a cluster can form upon reduction. For single nucleotides, to form a cluster, it is likely that intermolecular multivalent coordination needs to establish. This is probably why only adenine derivatives can produce such nanoclusters since no other nucleosides are known to form network of coordination complexes with gold.

Conclusions.

In summary, we reported the first series of fluorescent gold complexes with adenine derivatives. All the compounds emit blue light with a long lifetime of ~1 μs, which is attributed to charge transfer. In all previous work, the adenine derivatives and gold complexes were non-fluorescent. We found two critical factors for fluorescence generation: citrate and light exposure. Citrate is needed to reduce $HAuCl_4$ to a lower oxidation state, which is likely to be Au(I) based on its luminescence property. In particular, citrate needs to be added soon after mixing $HAuCl_4$ and adenosine. Otherwise, after forming stable complex between these two, no fluorescence was produced even after adding citrate. The ratio of

adenosine and gold in the complex is 1:1. The coordination chemistry of this complex is different from one directly achieved with mixing $HAuCl_4$ and adenosine (no citrate). To achieve strong fluorescence, the 9′-position of the adenine needs to be blocked since adenine itself generated very weak fluorescence. The size of the complex could be tuned by adding charged nucleotides such as AMP or ATP. In the case of adenosine, the complex is non-charged and tends to aggregate. This reductive complexation synthesis method is likely to open many new opportunities to achieve fluorescent gold complexes that could be used for analytical and biomedical applications.

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Supporting Information. Dried sample fluorescence, XRD, additional methods. This information is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

(1) Lehn, J. M. Toward Complex Matter: Supramolecular Chemistry and Self-Organization. *Proc. Natl. Acad. Sci. U.S.A.* **2002,** *99*, 4763-4768.

(2) Liu, Y.; Tang, Z. Nanoscale Biocoordination Polymers: Novel Materials from an Old Topic. *Chem. Eur. J* **2012,** *18*, 1030-1037.

(3) Daniel, M.-C.; Astruc, D. Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology. *Chem. Rev.* **2004,** *104*, 293-346.

(4) James, S. L. Metal-Organic Frameworks. *Chem. Soc. Rev.* **2003,** *32*, 276-288.

(5) Tang, Z. Y.; Kotov, N. A. One-Dimensional Assemblies of Nanoparticles: Preparation, Properties, and Promise. *Adv. Mater.* **2005,** *17*, 951-962.

(6) Martin, R. B. Nucleoside Sites for Transition Metal Ion Binding. *Acc. Chem. Res.* **1985,** *18*, 32-38.

(7) Sigel, H. Interactions of Metal Ions with Nucleotides and Nucleic Acids and Their Constituents. *Chem. Soc. Rev.* **1993,** *22*, 255-267.

(8) Navarro, J. A. R.; Lippert, B. Molecular Architecture with Metal Ions, Nucleobases and Other Heterocycles. *Coord. Chem. Rev.* **1999,** *185-186*, 653-667.

(9) Lu, Y. New Transition Metal-Dependent Dnazymes as Efficient Endonucleases and as Selective Metal Biosensors. *Chem. Eur. J.* **2002,** *8*, 4588-4596.

(10) Miyake, Y.; Togashi, H.; Tashiro, M.; Yamaguchi, H.; Oda, S.; Kudo, M.; Tanaka, Y.; Kondo, Y.; Sawa, R.; Fujimoto, T.; Machinami, T.; Ono, A. MercuryII-Mediated Formation of Thymine-HgII-Thymine Base Pairs in DNA Duplexes. *J. Am. Chem. Soc.* **2006,** *128*, 2172.

(11) Barton, J. K.; Lippard, S. J. Heavy Metal Interactions with Nucleic Acids. *Metal Ions in Biology* **1980,** *1*, 31.

(12) Storhoff, J. J.; Mirkin, C. A. Programmed Materials Synthesis with DNA. *Chem. Rev.* **1999,** *99*, 1849-1862.

(13) Verma, S.; Mishra, A. K.; Kumar, J. The Many Facets of Adenine: Coordination, Crystal Patterns, and Catalysisâ§. *Acc. Chem. Res.* **2009,** *43*, 79-91.

(14) Prizant, L.; Olivier, M. J.; Rivest, R.; Beauchamp, A. L. Metal Binding to Four Different Sites in Adenine Ligands. Crystal Structures of 2:1 Methylmercury Complexes with Adenine and 9-Methyladenine. *J. Am. Chem. Soc.* **1979,** *101*, 2765-2767.

(15) Gillen, K.; Jensen, R.; Davidson, N. Binding of Silver Ion by Adenine and Substituted Adenines. *J. Am. Chem. Soc.* **1964,** *86*, 2792-2796.

(16) Wei, H.; Li, B.; Du, Y.; Dong, S.; Wang, E. Nucleobase-Metal Hybrid Materials: Preparation of Submicrometer-Scale, Spherical Colloidal Particles of Adenine-Gold(III) Via a Supramolecular Hierarchical Self-Assembly Approach. *Chem. Mater.* **2007,** *19*, 2987-2993.

(17) Purohit, C. S.; Verma, S. A Luminescent Silver-Adenine Metallamacrocyclic Quartet. *J. Am. Chem. Soc.* **2005,** *128*, 400-401.

(18) Garcia-Teran, J. P.; Castillo, O.; Luque, A.; Garcia-Couceiro, U.; Roman, P.; Lezama, L. An Unusual 3d Coordination Polymer Based on Bridging Interactions of the Nucleobase Adenine. *Inorg. Chem.* **2004,** *43*, 4549-4551.

(19) An, J.; Geib, S. J.; Rosi, N. L. Cation-Triggered Drug Release from a Porous Zinc-Adeninate Metal-Organic Framework. *J. Am. Chem. Soc.* **2009,** *131*, 8376-8377.

(20) Nishiyabu, R.; Hashimoto, N.; Cho, T.; Watanabe, K.; Yasunaga, T.; Endo, A.; Kaneko, K.; Niidome, T.; Murata, M.; Adachi, C.; Katayama, Y.; Hashizume, M.; Kimizuka, N. Nanoparticles of Adaptive Supramolecular Networks Self-Assembled from Nucleotides and Lanthanide Ions. *J. Am. Chem. Soc.* **2009,** *131*, 2151-2158.

(21) Jena, N. K.; Chandrakumar, K. R. S.; Ghosh, S. K. DNA Base-Gold Nanocluster Complex as a Potential Catalyzing Agent: An Attractive Route for Co Oxidation Process. *J. Phys. Chem. C* **2012,** *116*, 17063-17069.

(22) Vyas, N.; Ojha, A. K. Interaction of Gold Nanoclusters of Different Size with Adenine: A Density Functional Theory Study of Neutral, Anionic and Cationic Forms of [Adenine + (Au)(N=3,6,9,12)] Complexes. *Comput. Theor. Chem.* **2012,** *984*, 93-101.

(23) Cao, G. J.; Xu, H. G.; Li, R. Z.; Zheng, W. J. Hydrogen Bonds in the Nucleobase-Gold Complexes: Photoelectron Spectroscopy and Density Functional Calculations. *J. Chem. Phys.* **2012,** *136*.

(24) Lv, G.; Wei, F. D.; Li, Q. N.; Shen, Q.; Jiang, H.; Zhou, Y. Y.; Wang, X. M. Dft Study on the Interactions between Au(N) (N=2 ... 4) and Adenine. *J. Nanosci. Nanotechnol.* **2010,** *10*, 809-818.

(25) Zheng, J.; Zhang, C.; Dickson, R. M. Highly Fluorescent, Water-Soluble, Size-Tunable Gold Quantum Dots. *Phys. Rev. Lett.* **2004,** *93*, 077402.

(26) Jin, R. C. Quantum Sized, Thiolate-Protected Gold Nanoclusters. *Nanoscale* **2010,** *2*, 343-362.

(27) Zheng, J.; Nicovich, P. R.; Dickson, R. M. Highly Fluorescent Noble-Metal Quantum Dots. *Annu. Rev. Phys. Chem.* **2007,** *58*, 409-431.

(28) Kennedy, T. A. C.; MacLean, J. L.; Liu, J. Blue Emitting Gold Nanoclusters Templated by Poly-Cytosine DNA at Low pH and Poly-Adenine DNA at Neutral pH. *Chem. Comm.* **2012,** *48*, 6845-6847.

(29) Qian, H.; Zhu, M.; Wu, Z.; Jin, R. Quantum Sized Gold Nanoclusters with Atomic Precision. *Acc. Chem. Res.* **2012,** *45*, 1470-1479.

(30) Saitoh, M.; Balch, A. L.; Yuasa, J.; Tada, K.; Onoda, M.; Nakashima, T.; Kawai, T. Highly Photoluminescent Nanocrystals Based on a Gold(I) Complex and Their Electrophoretic Patterning. *Langmuir* **2011,** *27*, 10947-10952.

(31) Luo, Z.; Yuan, X.; Yu, Y.; Zhang, Q.; Leong, D. T.; Lee, J. Y.; Xie, J. From Aggregation-Induced Emission of Au(I)-Thiolate Complexes to Ultrabright Au(0)@Au(I)-Thiolate Core-Shell Nanoclusters. *J. Am. Chem. Soc.* **2012,** *134*, 16662-16670.

(32) Zheng, J.; Zhou, C.; Yu, M.; Liu, J. Different Sized Luminescent Gold Nanoparticles. *Nanoscale* **2012,** *4*, 4073-4083.

(33) Xie, J. P.; Zheng, Y. G.; Ying, J. Y. Protein-Directed Synthesis of Highly Fluorescent Gold Nanoclusters. *J. Am. Chem. Soc.* **2009,** *131*, 888-889.

(34) Zhou, C.; Sun, C.; Yu, M. X.; Qin, Y. P.; Wang, J. G.; Kim, M.; Zheng, J. Luminescent Gold Nanoparticles with Mixed Valence States Generated from Dissociation of Polymeric Au(I) Thiolates. *J. Phys. Chem. C* **2010,** *114*, 7727-7732.

(35) Yang, X.; Gan, L.; Han, L.; Wang, E.; Wang, J. High-Yield Synthesis of Silver Nanoclusters Protected by DNA Monomers and DFT Prediction of Their Photoluminescence Properties. *Angew. Chem., Int. Ed.* **2013**, DOI: 10.1002/anie.201205929.

(36) Liu, G. Y.; Shao, Y.; Ma, K.; Cui, Q. H.; Wu, F.; Xu, S. J. Synthesis of DNA-Templated Fluorescent Gold Nanoclusters. *Gold Bulletin* **2012,** *45*, 69-74.

(37) Zhang, X.; Servos, M. R.; Liu, J. Instantaneous and Quantitative Functionalization of Gold Nanoparticles with Thiolated DNA Using a pH-Assisted and Surfactant-Free Route. *J. Am. Chem. Soc.* **2012,** *134*, 7266–7269.

(38) Zhang, X.; Servos, M. R.; Liu, J. Surface Science of DNA Adsorption onto Citrate-Capped Gold Nanoparticles. *Langmuir* **2012,** *28*, 3896-3902.

(39) Wing-Wah Yam, V.; Kam-Wing Lo, K. Luminescent Polynuclear d10 Metal Complexes. *Chem. Soc. Rev.* **1999,** *28*, 323-334.

(40) Pyykko, P. Strong Closed-Shell Interactions in Inorganic Chemistry. *Chem. Rev.* **1997,** *97*, 597-636.

(41) Schmidbaur, H.; Schier, A. A Briefing on Aurophilicity. *Chem. Soc. Rev.* **2008,** *37*, 1931-1951.

(42) Wu, Z.; Jin, R. On the Ligand's Role in the Fluorescence of Gold Nanoclusters. *Nano Lett.* **2010,** *10*, 2568-2573.

(43) Shang, L.; Azadfar, N.; Stockmar, F.; Send, W.; Trouillet, V.; Bruns, M.; Gerthsen, D.; Nienhaus, G. U. One-Pot Synthesis of near-Infrared Fluorescent Gold Clusters for Cellular Fluorescence Lifetime Imaging. *Small* **2011,** *7*, 2614-2620.

(44) Allendorf, M. D.; Bauer, C. A.; Bhakta, R. K.; Houk, R. J. T. Luminescent Metal-Organic Frameworks. *Chem. Soc. Rev.* **2009,** *38*, 1330-1352.

(45) Rodriguez, L.; Ferrer, M.; Crehuet, R.; Anglada, J.; Lima, J. C. Correlation between Photophysical Parameters and Gold-Gold Distances in Gold(I) (4-Pyridyl)Ethynyl Complexes. *Inorg. Chem.* **2012,** *51*, 7636-7641.