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New Nanomedicine Approaches Using Gold-thioguanine Nanoconjugates as Metallo-ligands

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Abstract

Gold-thioguanine nanoconjugates (AuNP-TG) of size 3–4 nm were synthesized and the ratio between gold and 6-Thioguanine (TG) was estimated as ~1:1.5 using a cyanide digestion method and confirmed by flame atomic absorption spectroscopic analysis. AuNP-TG constructs showed high *in vitro* stability under different pH conditions and biologically relevant solutions for a period of 24 hours. Reaction of AuNP-TG with europium or platinum salts resulted in the formation of organized self-assembled metallo-networks.

1. Introduction

A realistic pathway towards reducing the cancer associated mortality will involve discovery of new molecular approaches that allow selective delivery of therapeutic agents with optimal payloads at the tumor sites with minimal/no collateral damage to healthy tissue/organs.^{1–3} Indeed, this unmet clinical need for the development of target specific cancer therapeutic agents have been emphasized by oncologists. Therefore, it is of paramount importance to develop novel drug delivery systems which provide superior capabilities for achieving optimal bioavailability of cytotoxic drugs at tumor sites while decreasing the systemic toxicity in tandem.^{4–6}

Functionalized gold nanoparticles (AuNPs) continue to play vital roles in the design and development of nanoscale drug delivery systems.^{5, 7} The high reactivity of AuNPs juxtaposed with their biocompatibility has spawned a major interest in the utility of AuNPs for *in vivo* delivery of either therapeutic antibodies or chemotherapeutic drugs.^{5, 7–9} Recently, Phase-I human clinical trials have been completed for AuNPs conjugated with antibody TNF- α (Aurimmune).^{10, 11} The clinical data show that this drug was very well tolerated, with 60–85% cancer response rates.^{10, 11} Likewise, AuNPs conjugated with other chemotherapeutic drugs have shown extraordinary efficacy in treating tumors in small animal models.^{5, 12, 13} Mukherjee and coworkers have recently shown that targeted AuNP-gemcitabine conjugates are effective in treating pancreatic tumors.¹⁴ Similarly, Kotov and coworkers showed that AuNP-mercaptopurine conjugates have increased antiproliferative

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properties in K-562 leukemia cell lines when compared with that of native mercaptopurine.¹⁵ These examples provide unequivocal evidence that AuNPs can carry optimal concentrations of small drug molecules on the nanoparticulate surface.¹⁵ In this context, our approach has encompassed the development of gold nanoconjugates with high loading capacity of 6-thioguanine for potential tumor therapy applications. We have hypothesized that AuNPs can carry high payloads of cytotoxic drug 6-thioguanine on nanoparticulate surface and consequently deliver appropriate therapeutic payloads at tumor sites.

The general structure of AuNP-thioguanine (AuNP-TG) conjugate is shown in Figure 1. Thiopurines, 6-thioguanine (TG) and 6-mercaptopurine are a class of FDA approved chemotherapeutic drugs being currently used for the treatment of leukemia and various cancers.^{16–18} Recent literature reports confirm that TG drugs can be used for other diseases and clinical conditions including immunosuppressants and HIV replication.^{19–22}

As part of our ongoing research efforts focused on new nanomedicine approaches for molecular imaging and therapy of cancer and other diseases,^{7, 8, 23–32} we have carried out a systematic investigation on the design and development of high capacity 6-thioguanine loaded AuNP conjugates. In this study, we also investigated the ability of the AuNP-TG conjugates to serve as metallo-ligands to form complexes with europium and platinum salts. The rationale for developing these types of bimetallic nanoparticles is based on the fact that these conjugates can be effectively used in dual clinical roles serving as diagnostic (using photophysical characteristic of Eu^{3+}) and therapeutic (cytotoxicity of both TG and Pt^{2+}) agents for treating cancers. The results reported in this paper include: (i) synthesis and characterization of gold nanoparticle–thioguanine (AuNP-TG) conjugates, (ii) *in vitro* stability studies of AuNP-TG conjugates toward various biomolecules, and (iii) evaluation of the effectiveness of AuNP-TG to serve as metallo-ligands in the presence of europium and platinum salts.

2. Experimental

2.1. Physical measurements and materials

Tetrachloroauric acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), sodium borohydride (NaBH_4), 6-thioguanine (TG), glacial acetic acid (CH_3COOH), sodium hydroxide (NaOH), hydrochloric acid (HCl), methanol (MeOH), diethyl ether (Et_2O), sodium chloride (NaCl), dimethyl sulfoxide (DMSO), histidine, human serum albumin (HSA), bovine serum albumin (BSA), cysteine, sodium cyanide (NaCN), europium trichloride trihydrate ($\text{EuCl}_3 \cdot 3\text{H}_2\text{O}$), cis-diammine dichloroplatinum (II), trisodium citrate were purchased from Aldrich and used as received. For the preparation of an aqueous solution and for the rinsing of gold nanoparticles, only Milli-Q (DI) water ($\rho > 18\text{M}\Omega$) was used.

Transmission electron microscope (TEM) images were obtained on a JEOL 1400 transmission electron microscope (TEM), JEOL Ltd, Tokyo, Japan. TEM samples were prepared by placing 5 μL of gold nanoparticle solution on a 300 mesh carbon-coated copper grid and allowing the solution to stand for five minutes. Excess solution was removed carefully and the grid was allowed to dry for an additional five minutes. The average size and size distribution of the gold nanoparticles synthesized were determined by the processing of the TEM image using image processing software such as Adobe Photoshop (with Fovea plugins). DCS was used to measure size and was measured on a DC 24000, CPS Instruments Inc. USA. The hydrodynamic radius and the zeta potential were obtained on a Zetasizer Nano S90, Malvern Instruments Ltd. USA. The absorption measurements were carried out using Varian Cary 50 UV/Vis spectrophotometers with 1 mL of gold nanoparticle solution in disposable cuvettes with a 10 mm pathlength. Estimation of gold

concentration in AuNP-TG conjugates were performed using furnace Atomic Absorption Spectroscopy (AAS) using a standard curve spanning 0–40 $\mu\text{g L}^{-1}$. The furnace parameters were as specified in the user's manual for the Perkin–Elmer AAnalyst 800 ThGA graphite furnace. Quality-control materials (duplicates, spikes, and instrument-calibration verification) were within appropriate ranges. For elemental analysis, Energy Dispersive Spectroscopy (EDS) was used to determine the concentration of Gold, Europium and Platinum.

2.2. Synthesis of TG stabilized AuNPs

The TG functionalized AuNPs were prepared *via* a modified protocol by Brust et. al.³³ Briefly, 200 mg (0.5mmol) of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was dissolved in 60 ml of methanol. In a separate flask, 100 mg (0.6mmol) of 6-thioguanine was dissolved in 40 ml of 0.01M aqueous NaOH solution. This solution was added to aqueous solution of gold salt to produce a murky orange solution. To this mixture, 4 ml of glacial acetic acid was then added followed by an immediate addition of 190 mg (5mmol) of NaBH_4 dissolved in 14 ml of DI water under vigorous stirring at room temperature. A dark brown to black flocculate was produced followed by total black flocculate. The resultant mixture was allowed to stir for 1 hour at room temperature and then 5 ml of 1M aqueous HCl was added. This black solution of gold nanoparticles was then subjected to centrifugation at 7000 RPM for 20 minutes. The supernatant was removed and the particles were washed twice with 0.01M HCl keeping the centrifugation parameters same as above. The particles were further washed thoroughly and successively with DI water followed by diethyl ether. The resulting black powder of AuNP-TG was dried under vacuum and stored at -20°C . As needed, the particles were readily dispersed in 0.01M NaOH and stable for over a month.

2.3. Alternative Synthesis of TG Capped AuNPs

The citrate stabilized AuNPs were synthesized according to the method developed by Frens et. al.³⁴ Briefly, 10 mg (0.025mmol) of chloroauric acid was added to 75 ml of DI water, and the solution was heated to boiling ($110\text{--}120^\circ\text{C}$). To the solution, 22 mg (0.085mmol) of trisodium citrate dissolved in 1 ml of DI water was added. The solution was refluxed until a color change from dark blue to red was observed. Once cooled to room temperature, the nanoparticles were purified using diafiltration method. Minimate tangential flow filtration system and Minimate TFF capsule membrane (50k) along with a peristaltic pump was used. As synthesized citrate stabilized gold nanoparticles (20 ml) were passed through the Minimate TFF capsule membrane to reduce the content of trisodium citrate and unreacted gold salts and also to reduce the volume to ~ 1 ml which was used for the conjugation with 6-thioguanine. Typically, to 1 ml of purified citrate gold nanoparticles (Au: 0.027 mmol) were added varied concentrations of 6-thioguanine *viz.* 1.5 mg (0.0089 mmol), 2.5 mg (0.015 mmol), 3.5 mg (0.020 mmol), 4.5 mg (0.026 mmol), 5.5 mg (0.033 mmol) and excess amount of 40 mg (3.0 mmol) in 5 ml of 0.01 M NaOH solution. Each reaction mixture was stirred for 24 hrs and the gold nanoparticles were found to change the color from wine red to blue as the concentration of TG increases. The resultant 6-thioguanine bound gold nanoparticles were purified to remove unbound 6-thioguanine from the surface of gold nanoparticles by two-steps centrifugation at 5000 rpm for 10 min. AuNP-TG nanoconjugates obtained from citrate method using 5.5 mg of 6-thioguanine and 1 ml of purified citrate gold nanoparticles and borohydride method were used to compare the amount of 6-thioguanine on the surface of gold nanoparticles.

2.4. Determination of Concentration of TG in AuNP-TG Conjugates

To determine the content of TG on the surface of gold nanoparticles, a previously reported cyanide digestion experiment was performed.¹⁵ Briefly, 10 mg of synthesized AuNP-TG were suspended in 5 ml of 0.01M NaOH. To this solution 24.5 mg of NaCN was added to

make 0.1M as a final concentration. This mixture was stirred for 24 hrs at room temperature and the resultant colorless solution was analyzed by UV-Visible spectroscopy and compared with a standard solution of TG. The same procedure was followed for TG capped AuNPs prepared by citrate method.

2.5. In vitro Stability of AuNP-TG Conjugates

In vitro stability studies were performed by subjecting the AuNP-TG to various pH conditions: 2, 5, 7, 10 and 12 for the period of 24 hrs. The stability behavior of AuNP-TG was also monitored by challenging aqueous solutions of AuNP-TG (0.5 mL) with 0.5 mL each of 0.2M cysteine, 0.2M histidine, 0.2M HSA and 10% saline solutions. The stability was measured by monitoring the UV-visible absorbance at 30 mins and 24 hrs. A negligible change in plasmon band was observed in UV-Vis spectra for AuNP-TG, which confirmed the retention of nanoparticulate composition in all of the mixtures.

2.6. Reaction of AuNP-TG with Europium trichloride

To a solution of AuNP-TG (10.0 mg) in 0.1M NaOH (5 ml) was added europium trichloride trihydrate (29.13 mg, 0.113 mmol) dissolved in 17.5 ml of 0.1 M NaOH. The reaction mixture was stirred for an hour at room temperature. A turbid brown color precipitate due to self assembled network was observed at the end of reaction. The reaction supernatant was aspirated and the residue was washed thoroughly and successively with 0.01M NaOH solution to remove excess of europium salt and dried under vacuo.

2.7. Reaction of AuNP-TG with Cis-diamminedichloroplatinum(II)

To prepare the platinum complex analog, cis-diamminedichloroplatinum(II) was used as the starting material. Typically, 100 mg of platinum precursor dissolved in 5 mL of DMSO was added drop wise to AuNP-TG conjugate (5 mg in 0.01N NaOH) and stirred for one hour at room temperature. The supernatant was then removed *via* aspiration and the precipitate was washed repeatedly with 0.01M NaOH to ensure complete removal of uncomplexed platinum. The resultant conjugate was subjected to detailed analysis by TEM and EDS.

3. Results and Discussion

3.1. Synthesis and Characterization of AuNP-TG

In the present study, AuNP-TG conjugates were synthesized by two different procedures. In first procedure, sodium borohydride was utilized as a reducing agent to generate gold nanoparticles with concomitant conjugation of TG over the surface of the nanoparticle. The second procedure involved a ligand exchange reaction of citrate stabilized AuNPs with thioguanine molecules. Even though both the procedures yielded nanoconjugates, AuNP-TG generated by borohydride reduction showed high *in vitro* stability toward biologically relevant solutions and high loading of TG over the surface of gold nanoparticles (*vide infra*). On the other hand, AuNP-TG conjugates generated using citrate-AuNPs showed less stability with fewer number of TG on the surface. In this paper, AuNP-TG conjugates synthesized *via* borohydride reduction method was used for detailed analysis.

AuNP-TG conjugates were synthesized at room temperature by reduction of commercially available HAuCl_4 using NaBH_4 in presence of TG. The UV-visible absorption spectroscopy of these nanoparticles showed an intense peak appeared at 320 nm due to TG ($\pi \rightarrow \pi^*$) and a weak absorption band at ~520 nm. The weak surface plasmon band at 520 nm is characteristic of smaller size gold nanoparticles.

Physicochemical properties, such as size, charge, and morphology of AuNP-TG were determined by three independent analytical techniques *viz.* transmission electron microscopy

(TEM), differential centrifugal sedimentation (DCS), and dynamic light scattering (DLS). TEM and CPS were used to determine the core size of AuNP-TG conjugate, while DLS was used to evaluate the hydrodynamic size. Analysis of TEM image (Figure 2) showed that the nanoparticles are spherical with an average diameter of 3.5 ± 1 nm. Histogram analysis confirmed that these conjugates are uniform in size. DCS measurements confirmed that the core size of AuNP-TG conjugates is ~ 3.5 nm. In sharp contrast to the core size, the hydrodynamic size of AuNP-TG conjugate was determined as 170 nm using DLS measurements. This data revealed that such increased hydrodynamic radius might be attributed toward the formation of a strong self-assembled network by TG molecules. Indeed, literature reports confirm the presence of H-bonding and π -stacking between TG molecules.³⁵ In a similar fashion, we presume that TG molecules present on the surface of AuNPs possesses intramolecular interactions with neighboring AuNP-TG molecules. Zeta potential of the nanoconjugates provide crucial information on the stability of the nanoparticle dispersion as the magnitude of the charge is an indication of the mutual repulsion between particles that can be used to predict the long-term stability of the nanoparticulate dispersion. AuNP-TG conjugates exhibited high negative zeta potential (ζ) value (-50.0 mV) suggesting high stability of these nanoconjugates. The negative value can be attributed to the charged thioguanine molecules present on the surface of nanoconjugates.

In order to estimate the concentration of TG present over the surface of a nanoparticle, cyanide digestion method¹⁵ (Figure 3) was utilized. In this method, cyanide ions interact with gold atoms on the nanoparticle and liberate the bound TG molecules. After 24 hours of digestion, concentration of TG ligand was estimated using UV-Visible absorption band at 320 nm. This data revealed the concentration of TG present on 1 mg of AuNP-TG conjugate was ~ 0.4 mg. To further confirm the above results, we performed fAAS to determine the concentration of gold in AuNP-TG. This data shows that 1 mg of AuNP-TG conjugate contains 0.53 mg of Au and the remaining 0.47mg corresponded to the concentration of TG. These data establish that 1 mg of AuNP-TG contains ~ 0.4 – 0.5 mg of TG molecules. Previously, Kotov and coworkers showed that the $\sim 25\mu\text{g}$ of mercaptopurine was present on 1 mL of AuNP.¹⁵ On comparison, AuNP-TG conjugates synthesized in the present study have high loading of TG molecules on the surface of AuNPs.

3.2. In vitro stability of AuNP-TG nanoconjugates

In vivo biomedical applications require nanoconjugates to exhibit remarkable stability over different pH buffers, saline, and other biologically relevant solutions.^{7, 8, 23–29, 32} In order to understand the stability of AuNP-TG, the conjugate was suspended in different pH buffers and incubated at room temperature for a period of 24 hours (Figure 4(a)). The stability was monitored using the surface plasmon resonance of AuNPs. If the conjugate is unstable, it will either disintegrate or aggregate; both the processes are expected to alter the surface plasmon resonance band of AuNPs. Our studies revealed that AuNP-TG is stable for a period of 24 hours under different pH conditions. Likewise, AuNP-TG showed excellent stability in 10% saline solutions for extended period. We also investigated the stability of AuNP-TG conjugates towards coordinating amino acids such as cysteine and histidine (Figure 4(b)). Even though the conjugate did not show any change in surface plasmon band after treatment with histidine, a marked change is observed on treatment with cysteine. This change in plasmon resonance is presumably due to the interaction of sulfur atoms in cysteine with gold surface and this interaction destabilizes the nanoparticles. In a similar fashion, slight change in surface plasmon band is observed when AuNP-TG is treated with BSA or HSA solutions. From our *in vitro* studies, it is evident that AuNP-TG may be suitable for *in vivo* tumor therapy applications.

3.3. Reaction of AuNP-TG with EuCl_3

To investigate the ability of AuNP-TG to function as metallo ligands, we treated purified AuNP-TG, dissolved in 0.1 M NaOH, EuCl_3 . After 1 hour of stirring, a brown color precipitate was formed, and it was purified by washing with NaOH solution and dried under vacuo for further investigations. Each TG molecule contains 1 sulfur atom and 5 nitrogen atoms, the sulfur atom forms a strong bond with gold atoms on the surface. However, the nitrogen atoms present in purine base will be available for conjugation with other metal ions. On the other hand, each Eu^{3+} has a coordination number which varies from 6 to 11.³⁶ After the addition of Eu^{3+} to AuNP-TG, nitrogen atoms of conjugated TG interact with europium to form an organized self-assembly of metallo-networks. In this self assembly, Eu^{3+} can interact either with TG present in neighboring gold nanoparticle or with TG present in the same AuNP. Due to the geometric constraint imposed by coordination of TG from the same AuNP, Eu^{3+} will prefer to coordinate with TG from neighboring AuNP. Presence of two AuNPs close together will generate steric repulsion from future coordination of Eu^{3+} with other nanoparticles. From the TEM image (Figure 5), it is evident that each nanoparticle interacts with other AuNP and assembles around a central nanoparticle. Together, it forms a hexagonal type of assembled network (Figure 5). Additionally, metalla-hexagons could interact with additional Eu^{3+} to fuse with other hexagon or it may branch to form a network of overlapping hexagons (Figure 5). Our studies revealed that both geometric and steric repulsion between AuNPs play vital roles in forming a branched non-hexagonal shape metallo-networks. However, it is evident from the TEM image that nanoparticles forms an organized clustered self-assembly of metalla-network with both hexagonal and branched network. In order to further confirm the presence of Europium in the clustered network, EDS spectrum was recorded. As shown in Figure 6(a), the presence of europium is unequivocally confirmed by the spectral peaks corresponding to europium salts. Our additional studies involved understanding the luminescence characteristics of AuNP-TG-Eu self-assembled network. The luminescence studies were accomplished by exciting the EuCl_3 , TG, AuNP-TG and AuNP-TG-Eu conjugates at 300 nm and scanning the visible region for emission peaks associated with Eu^{3+} ions and TG ligand (Figure 6). It is important to note that the excitation wavelength of ~320 nm corresponds to the maximum absorption due to π - π^* transition of the TG ligand in the nanoconjugates. Luminescence property of Eu^{3+} is a result of ground-state electronic configuration of $[\text{Xe}]4f^6$ with six unpaired electrons, which leads a 7F_J ground state with spin-orbit coupling $J = 0, 1, 2, 3, 4, 5, 6$ corresponding to seven possible transitions.³⁶ Our studies were initially focused on understanding the fluorescence property of EuCl_3 in NaOH solution, as this data will serve as a control. Our data shows an intense- sharp peak at 600 nm ($^5D_0 \rightarrow ^7F_1$) and three weak-broad peaks at 407 nm, 668 nm and 694 nm ($^5D_0 \rightarrow ^7F_J$, $J = 0, 2, 3$ respectively). (Figure 6(b)). The peak at 600 nm indicates long life time (τ) for the excited states of Eu^{3+} . As a second step, we recorded the fluorescence spectrum of AuNP-TG-Eu conjugate. In this conjugate, the initial Eu^{3+} luminescence intensity at 600 nm decreased significantly in presence of AuNP-TG and a new broad emission peak appeared at 411 nm due to the TG. It is well known that, TG can emit at 410 nm when excited at 330 nm.³⁷ This phenomenon clearly suggests that the Eu^{3+} is attached to TG through nitrogen centers of base. One possible explanation for quenching Eu^{3+} emission in presence of AuNP-TG is that there is an energy transfer from the long-lived excited state of Eu^{3+} (donor) to the short-lived excited state of TG (acceptor). It is also important to note that the self-assembled network forms a dark brown precipitate, which may also result in decreased fluorescence intensity. The energy transfer from the excited states of the donor to the excited states of the acceptor is a competitive process with the relaxation to the ground state of the donor. If the luminescence lifetime of the donor is long and that of the acceptor is short, energy transfer can be very efficient at the excited levels, which causes the present quenching of luminescence.³⁸ This energy transfer is also evident in the emission profile of TG at 411 nm. Due to efficient

energy transfer from the excited state of Eu^{3+} to the excited state of TG, the peak intensity of TG in AuNP-TG-Eu has been increased remarkably compare to TG in AuNP-TG. The TG concentration was same in both AuNP-TG and AuNP-TG-Eu nanoconjugates for quantitative comparison. To confirm this property, we performed additional experiment of mixing TG with EuCl_3 ; the data shows that quenching of $\text{EuCl}_3 \cdot 3\text{H}_2\text{O}$ is also seen when it has been separately treated with TG. (Figure 6(b), inset) We consider that the quenching effect of AuNPs in AuNP-TG-Eu is minimal due to very weak absorption of AuNPs in the visible region. Notably, the broad emission peak of TG at 411 nm has not been even quenched by gold nanoparticles in AuNP-TG nanoconjugate.

3.3. Reaction of AuNP-TG with cis-Platin

It is well known that in cells, *cis*-Platin interacts preferentially with N-7 of guanine and not with sulfur atom of thioguanine.³⁹ It would be of interest to understand the nature of AuNP-TG with *cis*-platin, as it would shed light about the possibility of using AuNPs as carriers for *cis*-platin. With this objective, we treated AuNP-TG with *cis*-platin in DMSO solvent for 30 minutes. Reaction was performed in DMSO solution to maximize the solubility of cisplatin. TEM image was shown in Figure 7 and it shows the formation of self-assembled linear network. As explained in detail above in section 3.2, nitrogen atoms present in AuNP-TG is available for interaction with metal atoms. After the interaction of nitrogen atoms, AuNP-TG forms a complex with platinum. Next, Pt has an opportunity to interact with TG present in the same conjugate or TG from the neighboring AuNP. If the interaction arises from the neighboring AuNP, then two AuNPs will be closely aligned with each other. Further, the coordination number of platinum is non-extendable and severely limited both geometric and steric constraints. Consequently, AuNPs will be linked with other nanoparticle by platinum atoms, which serve as a linker between the nanoparticles (Figure 7). It is also evident from the TEM image that a linear network of AuNP-TG is formed. In order to verify the presence of platinum atoms in the linear network, EDS spectrum of the resultant conjugate was performed (Figure 7). The EDS spectrum confirmed the presence of platinum in the conjugate.

4. Conclusions

In summary, AuNP-TG nanoconjugates of average size 3.5 nm, were synthesized by reducing Au salts with borohydride in presence of 6-thioguanine. Our results show that high loading of thioguanine on AuNPs were achieved. Additionally, the data suggest that AuNP-TG conjugates are highly stable in various *in vitro* biologically relevant conditions. Our results convincingly demonstrate that AuNP-TG conjugates can serve as metallo-ligands by coordinating with europium or platinum salts. With europium, a novel hexagonal type metallo-network was formed and with platinum AuNP-TG forms a linear self-assembly of nanoparticles. Future studies will involve studying the cytotoxicity and cellular internalization of AuNP-TG nanoconjugates and metallo-networks. The utilization of Au-Eu nanoconjugates as dual CT-fluorescence imaging will also be studied in future.

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Figure 1.
General structure of AuNP-TG nanoconjugate

Figure 2.
(a) TEM image and (b) Histogram analysis of AuNP-TG conjugates

Figure 3. Cyanide digestion of AuNP-TG and determination of concentration of TG conjugated with AuNPs (10mcg/ml).

Figure 4.

(a) UV-Visible spectrum of AuNP-TG under different pH conditions after 24 hours; (b) UV-visible spectrum of AuNP-TG after 24 hours of treatment with NaCl, different aminoacids, HSA and BSA

Figure 5.
TEM images of self-assembly of AuNP-TG after treatment with EuCl_3

Figure 6.

(a) EDS spectrum of AuNP-TG-Eu metallo-network; (b) Fluorescence spectra of EuCl₃ (Red), AuNP-TG (Blue), and AuNP-TG-Eu metallo-network (Green); The inset shows quenching of EuCl₃·3H₂O in presence of TG.

Figure 7. TEM images of self-assembly of AuNP-TG after treatment with cis-platin, EDS spectrum confirms the presence of both Pt and Au.