Surface-engineered nanomaterials as X-ray absorbing adjuvant agents for Auger-mediated chemo-radiation†

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We report a prototype approach to formulate gold nanoparticle-based X-ray absorbing agents through surface-engineering of a cisplatin pharmacophore with modified polyacrylate. The resulting agents exhibit both chemo-therapeutic potency to cancer cells and Auger-mediated secondary electron emission, showing great potential to improve the therapeutic efficacy of chemo-radiation.

Surface-engineered nanomaterials, and their derivative products, are highly attractive candidates for therapeutic applications. The advantages of using these constructs arise primarily from their nanoscale dimensions and controllable surface chemistry, allowing selective delivery of an incorporated payload to a tumor interstitium via leaky vasculature, while avoiding systemic clearance through renal filtration. The improved therapeutic window and enhanced pharmacokinetic profiles, for example, have demonstrated substantial potential for applications in anticancer drugs and tumor imaging agents.1,2

Among diverse surface-engineered nanomaterials attempted to address nanostructure–biological activity relationships, functionalized gold nanoparticles (AuNPs) are considered one of the most important,3,4 primarily due to their intrinsically non-toxic nature, good redox stability, specific surface plasmon resonance (SPR) bands, and facile surface chemistry. As a result, AuNPs have been widely exploited for a variety of biomedical applications including delivery of small-interfering RNA,5,6 pharmaceuticals,7–9 imaging agents,10,11 facilitation of computed tomography,12 surface-enhanced Raman scattering imaging, and photothermal therapy.13–15

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Using secondary electron emission from AuNPs,16,17 recent developments suggest an exciting potential for using AuNPs to enhance the X-ray dosage at the target site in radiation therapy. Based on this concept, the Auger-type electron emission should have the capacity to deliver sufficient doses of highly localized energy to the cellular organelles (e.g., DNA in nuclear chromosomes) and to induce substantial damage to the targeted cells.18–20 Furthermore, because of their rapid decay in nanoscale volumes, the linear-energy-transfer properties of Auger electrons may prevent damage to the surrounding healthy tissue.21 Moreover, the overall therapeutic performance can be further improved conceptually by incorporating intelligent and complementary design principles.

Herein, we demonstrate an approach to develop and combine chemo- and radiation-therapeutic modalities. Our approach involves engineering of a cisplatin pharmacophore (PtII) onto the surface of stable AuNPs. Beyond the known chemotherapeutic potency,22,23 the PtII-based treatment regimens have also been synchronized with external radiation for inducing additional DNA damage and to interfere with post-radiation DNA-repair processes.24,25 The concept of formulating the (PtII + AuNP) drug complex has also spurred development of a PtII-radiosensitizer for concurrent chemo-radiation therapy.25,26 Moreover, the AuNP-based therapeutic agent demonstrated in this study provides a relevant test bed for development and validation of quantitative measurements for biomedically related functionalized nanoscale materials.

Although the notion of this nanoplatform-mediated delivery (PtII + AuNPs) provides a highly promising strategy for cancer treatments, an obvious challenge thus far has been to accurately engineer this nanomaterial platform on demand to achieve the desired biological functionality. Therefore, understanding the surface chemistry of nanomaterials is of crucial importance in order to optimize the material performance for biomedical applications.

To fabricate surface-tailored PtII–AuNPs, poly(acrylic acid) (PAA) was first adhered to the Au surface via lipoic acid anchor groups.27 Subsequently PtII pharmacophores were bound to the
PAA through metal–carboxylate coordination,28,29 (Scheme 1). Such a formulation allows for a high loading capacity with respect to PtII pharmacophores (more than 1300 PtII ions per particle, see ESI†) without significant aggregation of the AuNPs. More importantly, a moderate amount of PtII-mediated multidentate binding can cross-link polymer networks on the surface of individual AuNPs. This cross-linking should increase the robustness of PAA chains on the AuNP surface and thereby provide improved colloidal and conjugate stability.28 This would be particularly useful to facilitate the PtII pharmacophore release to solid tumor tissue under acidic conditions.31 To this end, our work begins from the foundation of multiple complementary characterization approaches in order to understand the chemical formulation of the drug-tethered nanoplatform, prior to the study of Auger-mediated radiotherapy and in vitro chemotherapeutic efficacy.

Lipoic PAA \( \left( M_n = 2.4 \text{ kDa} \right) \) was prepared via amide coupling of lipoic acid with poly(tert-butyl acrylate) followed by acidolysis (details in the ESI†). Then, a 4-fold excess of lipoic PAA (compared to the available surface atoms of AuNPs) was reacted with tannic acid-stabilized (referred to here as ‘native’) AuNPs (zeta potential, \( \zeta = -24.4 \text{ mV} \pm 4.3 \text{ mV} \), hydrodynamic diameter, \( D_H = 10.4 \text{ nm} \pm 1.9 \text{ nm} \)) and stirred overnight to yield PAA-modified AuNPs. (All reported uncertainties represent one standard deviation calculated from at least three replicate measurements.) This was followed by dialysis to remove unreacted species (PAA–AuNPs, \( \zeta = -64.7 \text{ mV} \pm 5.4 \text{ mV} \), \( D_H = 25.1 \text{ nm} \pm 3.9 \text{ nm} \) in Fig. 1A). PtII conjugation was achieved by the incubation of PAA–AuNP suspension with the PtII pharmacophore, \( \text{cis-[Pt(NH}_3)_2(H_2O)_2]^{2+} \) in different PtII–to-AuNP ratios (i.e., the number of PtII atoms per AuNP, on average, defined as \( \Omega \)) at 25 °C and purified by a centrifugal filter. \(^1\)H NMR spectra exhibited the initial binding of PtII ions to PAA with downshifted proton peaks from the PAA backbone on PtII coordination (Fig. 2A, \( \Omega = 1300 \) as representative). The apparent \( \zeta \) of PtII–AuNPs was increasingly neutralized as the degree of PtII–conjugation increased, yielding values of \((-54.3 \pm 4.3) \text{ mV}\) and \((-50.5 \pm 4.8) \text{ mV}\) for \( \Omega \) of 700 and 1300, respectively, while \( D_H \) decreased to \(21.2 \pm 4.4) \text{ nm}\) and \(18.9 \pm 3.3) \text{ nm}\), respectively (Fig. 1A). The electrophoretic mobility of PAA–AuNPs and PtII–AuNPs in agarose gel on PtII–conjugation (Fig. 1B) was not differentiable, confirming a compensation of decreasing size with charge neutralization (as observed in Fig. 1A).33 The size contraction can be attributed to the PtII–mediated cross-linking of surface-bound PAA chains (vide infra).

Next we evaluated the colloidal stability of the PtII–AuNP vector. Using the concept of Derjaguin, Landau, Verwey and Overbeek theory, the presence of PtII in aqueous solution can significantly reduce the colloidal stability of unprotected AuNPs by shielding the charge repulsion between the particles.34 Indeed, during the PtII–conjugation reaction, only PAA–AuNPs exhibited the characteristic SPR peak wavelength at 520 nm without any spectral changes. Both native AuNPs and lipoic-conjugated AuNPs exhibited a significant red-shift in their SPR spectra (Fig. 2B) due to the ion-mediated particle aggregation. The results confirm that the surface-bound PAA chains provide essential strong electrostatic repulsion and steric hindrance to ensure the AuNPs stability on the addition of PtII ions. Furthermore, the colloidal stability of PtII–AuNPs was maintained even under high ionic strength in cell culture media (Eagle’s Minimum Essential Medium), with no change in the SPR band (Fig. 2B). Formation of nanoparticle aggregates is known to significantly impact the cellular uptake properties.35 Such an enhanced colloidal stability of the PtII–AuNP formulation (i.e., containing lipoic PAA) is critical for subsequent in vitro studies. Note that massive aggregation of AuNPs was observed when \( \Omega > 2500 \), due to interparticle cross-linking2 mediated by excessive PtII ions (Table S2 and Fig. S2B in the ESI†).

Given the apparent stable formation of PtII–AuNPs, as indicated by the characteristic SPR band, and with a desire to further elucidate the binding mechanism of PtII on lipoic–PAA-functionalized AuNPs, we utilized attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy and X-ray

![Image](64x79 to 270x197)

**Scheme 1** Preparation of cisplatin pharmacophore–tethered gold nanoparticles (PtII–AuNPs).

**Fig. 1** (A) Zeta potential and average hydrodynamic diameter (\( D_H \)) of native AuNPs, lipoic acid-modified AuNPs (lipoic AuNPs), PAA–AuNPs, and PtII–AuNPs with 0.7k and 1.3k PtII/AuNP ratios (\( \Omega \)). (B) The corresponding electrophoretic migration of modified AuNPs in 3% agarose gel (150 V, 60 min).

**Fig. 2** (A) \(^1\)H NMR spectra of PAA–AuNPs and PtII–AuNPs. The circled peaks are due to the trace amount of ethanol, added to D_2O as a reference. (B) SPR spectra of AuNPs measured by UV-vis absorption.

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photoelectron spectroscopy (XPS). Compared to the conventional FTIR method, the evanescent waves present in the ATR element allows for much greater sensitivity to surface-bound species \( \text{PtII} \) ions through (Fig. 3A, left inset). As shown in Fig. 3A, PAA–AuNPs exhibit the characteristic C=O stretching peak at 1703 cm\(^{-1} \) associated with the carbonyl group in PAA, while negligible absorbance was observed from COO\(^{-} \) anti-symmetric stretching at 1552 cm\(^{-1} \). On the addition of PtII ions, the peak intensities for both anti-symmetric (1552 cm\(^{-1} \)) and symmetric (1409 cm\(^{-1} \)) COO\(^{-} \) stretching increased while the C=O peak intensity decreased; these effects can be attributed to PtII coordination with the COO\(^{-} \) ligands.\(^{37} \) Given that the PtII–AuNP suspension was kept slightly acidic to prevent the agglomeration of COO\(^{-} \)PAA. Also the relative ratio of peak intensities (Fig. 3A, right inset), leading to size contraction of the PtII–AuNP ratios of 0.7k (low PtII) and 1.3k (high PtII). The intensity ratio (anti/\( \text{PtII} \)) was determined by comparing to the NIST XPS Database.\(^{40} \) Note that the ratio of PtII to AuNP measured by XPS is about six times greater than the \( \Omega \) value in the actual formulation. As the penetration depth of the X-rays was only \( \approx 10 \) nm, there was greater sensitivity to the PtII ions located on the top layer than the Au surface beneath. Details of the XPS analysis are described in the ESI.\(^{\dagger} \)

Auger-mediated secondary electrons figure prominently for radiation therapy, as discussed previously. To evaluate the performance of our PtII–AuNPs as an Auger-emitting adjuvant agent, we monitored the secondary electron emission using non-monochromated Bremsstrahlung radiation, a virtual X-ray source for external radiation. As shown in Fig. 4, Auger electron peaks from both Au and PtII are indeed observed in the kinetic energy spectrum. The results clearly correspond closely to the Auger lines of each element, and are consistent with their Auger parameters\(^{46} \) (Table S1 in the ESI).\(^{\dagger} \) Although the peak intensities are not sufficiently great due to the low relative sensitivity factor of Auger emission, a single photoelectron emission event can trigger the emission of numerous secondary electrons from the outer shells in high-z elements such as Au and PtII. Our results provide a proof of concept for the potential as an adjuvant agent for radiation therapy.

In addition to radiation-mediated Auger lines, the \textit{in vitro} chemotherapeutic potency of PtII–AuNPs was also evaluated with MCF-7 breast cancer and SKOV-3 ovarian cancer cell lines, both of which were known to be partially resistant to cisplatin (Fig. 5). After 48 h and 72 h treatments using both cell lines,
significant cytotoxicity was observed for PtII–AuNPs, which shows comparable potency to that of the cisplatin parent drug (i.e., 20 and 70 μM of PtII–AuNPs for less than 30% of cell viability for MCF-7 and SKOV-3, respectively, after 72 h). Given the acid-triggered release of the PtII pharmacophore observed with PtII–AuNPs (Fig. S6 in the ESI†), such a chemotherapeutic potency can be attributed to the endocytosis-mediated cellular uptake process of nanomaterials as previously reported,9,10 subsequently leading to the triggered drug-release in acidic endosomal environments.42 In contrast, PAA–AuNPs without the PtII-pharmacophore exhibit negligible cytotoxicity after 3 days (Fig. S7 in the ESI†).

In conclusion, PtII pharmacophore–tethered AuNPs can be synthesized via lipoic acid-terminated multidentate PAA chains. Through careful examination by multiple and complementary characterization methods, therapeutic responses of PtII–AuNPs can be correlated with their surface properties accurately. Both the drug loading and colloidal stability can be enhanced significantly by bidentate bridging of PAA to PtII on AuNPs. In vitro chemotherapeutic assays confirm the potency of PtII–AuNPs with MCF-7 breast cancer and SKOV-3 ovarian cancer cell lines. In addition, Auger electron emissions triggered by external ionizing radiation demonstrate the therapeutic potential of PtII–AuNP as an X-ray absorbing adjuvant agent for chemo-radiation cancer therapy. As such, the PtII–AuNP-mediated therapeutic synergy in concurrent chemo-radiation will be evaluated in due course.

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Notes and references


41 For comparison, Pt (4f7/2) peaks range at 75–77 eV for PtIV compounds and 70–71 eV for PtVI compounds. Please see ESI†.