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COMMUNICATION

Hard X-ray-induced optical luminescence via biomolecule-directed metal clusters

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Here, we demonstrate that bio-molecule directed metal clusters are applicable in the study of hard X-ray excited optical luminescence, promising new direction in the development of novel X-ray-activated imaging probes.

X-rays are widely used in imaging applications such as diffraction imaging of crystals and medical imaging. In particular, X-ray computed tomography (CT) is a critical tool for clinical and disease diagnostics.^{1,2} The principal of conventional CT is based on X-ray attenuation caused by photoelectric absorption and scattering.^{3,4} In addition to conventional CT, a number of novel methodologies are presently under development, including state-of-the-art instrument technologies and chemical probes to fulfill diagnosis criteria.⁵ To advance these new approaches, X-ray fluorescence and X-ray excited optical luminescence (XEOL) are being proposed as potential photo-physical mechanisms to exploit for imaging.⁶ Among these novel methodologies, we have utilized *hard* X-ray induced optical luminescence (*h*XEOL) as a new methodology called X-ray luminescence CT (XLCT).⁷ In previous papers by Anker, Sham, and us, a radio-luminescent probe is employed to specifically target a biological signature and produce optical contrast when irradiated with *hard* X-ray.⁷⁻¹⁰ Despite excellent *h*XEOL properties of inorganic nanoparticles, there are still several drawbacks for medical and biological imaging, such as poor biocompatibility and stability *in vivo*.¹⁰ To overcome these issues, we have reported that iridium complex doped biocompatible polymer dots (P-dots) to induce optical luminescence upon *hard* X-ray irradiation. *In vivo* study of this new class of probes is currently ongoing.¹¹ In addition, the basis of *h*XEOL has not yet been reported in detail except in few studies.^{8,9} Therefore,

to understand the nature of *h*XEOL, there is still a need to explore and discover compounds and materials that emit light upon *hard* X-ray irradiation.

Luminescent metal clusters upon ultraviolet (UV) or visible light irradiation have recently been developed as imaging probes. In particular, biomolecule-directed metal clusters have emerged as a promising approach for applications such as *in vitro* and *in vivo* bio-sensing and fluorescence imaging.¹²⁻¹⁶ These biomolecule-directed metal clusters emit in the near infrared region, upon ultraviolet UV light or blue light irradiation.^{12, 17} Furthermore, a few studies on protein-directed metal clusters suggested their possible use as the X-ray CT absorption contrast agents.^{18, 19} Prompted by these studies, we hypothesized that bio-molecule directed metal clusters will produce luminescence upon *hard* X-ray excitation. Herein, for the first time, we explore the possibility of *h*XEOL via biomolecule-directed metal clusters and propose it as a potential platform for new X-ray imaging. Figure 1 shows the strategy of this study. We hypothesized that XEOL

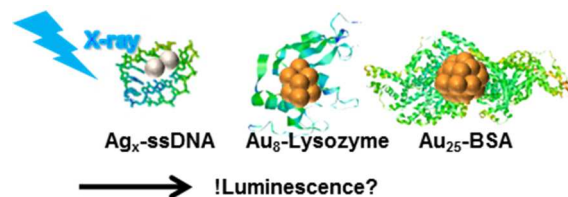


Figure 1. Overview of the study. X-ray excited optical luminescence was characterized to investigate the hypothesis that hard X-ray induced optical luminescence would be induced via ssDNA-, Lyz-, and BSA-directed metal clusters.

would be induced from biomolecule-directed metal clusters and that obtaining a distinctive luminescence image from these metal clusters upon X-ray irradiation is possible. In the present study, we choose previously described bio-molecule-directed metal clusters, the single strand DNA (ssDNA) directed Ag clusters,¹³ lysozyme VI (Lyz)¹⁶ or bovine serum albumin (BSA)¹⁵ directed Au clusters as proof of concept. These metal clusters have been demonstrated by several groups for *in vitro* as well as *in vivo* imaging studies with UV or visible light irradiation. In the present study, their X-ray activity is studied further to investigate whether these probes could function for XLCT. These metal clusters were synthesized as previously described by Dickson *et al.*,²⁰ Ying *et al.*,¹⁵ and Tseng *et al* with slightly modified methods.¹⁶ The detailed procedures were described in the experimental section.

First, the steady state luminescence under UV light (365 nm) excitation was investigated. Consistent with earlier works,^{15, 16, 20} visible range luminescence from the biomolecule-directed metal clusters was clearly observed upon UV irradiation (Figure S1a). The ssDNA directed Ag cluster exhibits a pink color under UV excitation, although the ssDNA sequence used in this study has been reported to emit blue,²¹ yellow,²⁰ green²² in earlier works. This is possibly because of different synthetic conditions, such as the variety of buffers and their concentrations. Protein-directed Au clusters synthesized in this study emit blue for Lyz and red for BSA under UV light excitation, consistent with earlier works.^{15, 16} To further confirm the formation of luminescent metal clusters, the steady state excitation and luminescence spectra were measured (Figure S1b). The luminescence maximum was at 607 nm for ssDNA-, 421 nm for Lyz- and 667 nm for BSA-directed metal clusters, respectively. For ssDNA clusters, the number of Ag atoms has been estimated to be about 5-11, according to the literature, and determining the number of the silver atoms from steady state photo-luminescence measurement is not easy.²³ Therefore, in this study, we term Ag cluster with ssDNA used in this study, Ag_x-DNA from the following sessions. Consistent with earlier works,^{15, 16} it was also confirmed on the basis of Spherical Jellium model that Lyz- or BSA-directed Au clusters are Au₈ or Au₂₅, respectively. Absolute quantum yields (QYs) for metal clusters were measured to quantify photo-physical properties upon UV or visible light irradiation (Table S1). The measurement of photo-luminescence quantum yields upon UV or visible light irradiation showed that Ag_x-DNA cluster is the brightest among the three samples. These results further confirm that metal clusters directed biomolecules produce luminescence under UV and visible light irradiation.

To test the hypothesis of *h*XEOL via biomolecule-directed metal clusters, *hard* X-ray induced luminescence from these metal clusters was measured in aqueous solution (Figure 2a). A *hard* X-ray source was employed for X-ray irradiation. The X-ray spectrum of the source used in this study is shown in the supporting information (Figure S2). A maximum X-ray excitation was observed around 0.05 nm (25 keV) when the X-ray source was operated at 50 kVp. For *h*XEOL imaging, a sample solution of the biomolecule-directed cluster was placed in a plastic tube and irradiated with *hard* X-rays to induce optical emission. The emission was measured with an EM-CCD camera to analyze the *h*XEOL. The detailed method is written in the experimental section and in previous our works.¹¹ Figure 2a shows a typical image of optical luminescence upon *hard* X-ray irradiation. It is noteworthy that significant luminescence from Ag_x-DNA and Au₈-Lyz clusters was not observed, despite clear luminescence and reasonable QYs upon UV or visible light irradiation. In contrast, distinctive luminescence was observed in the Au₂₅-BSA cluster upon *hard* X-ray irradiation. It is worth noting that Au₂₅-BSA has the lowest absolute QY upon visible light irradiation among the three samples investigated in this study (Table S1). These results suggest that the steady state photo-physical properties do not necessarily

represent the *h*XEOL properties. The relative intensity of luminescence of Au₂₅-BSA against water is greater by a factor of 11 (25 mg/mL of BSA for the synthesis), which is comparable to that of iridium P-dots described previously.¹¹ Metal ions (gold and silver ions), ssDNA, and proteins themselves (Lyz and BSA) did not provide any distinctive luminescent signals upon *hard* X-ray irradiation (Figure S3). These results further suggest that the *hard* X-ray induced luminescence from Au₂₅-BSA is produced by the metal Au clusters in the BSA molecule. We also confirmed comparable luminescence from transferrin and human serum albumin directed gold clusters (data not shown). Furthermore, to confirm whether the luminescence originates from the metal clusters upon *hard* X-ray excitation, the XEOL spectrum for Au₂₅-BSA was measured and is shown in Figure 2c. A luminescence spectrum similar to the steady state luminescence spectrum shown in Figure S1 was observed. This result further supports the hypothesis that the *h*XEOL arises from Au₂₅-BSA clusters.

X-ray photons above the L3 absorption edge of Au (~12 keV) have sufficient energy to knock out inner-shell electrons (photoelectric absorption) or outer-shell electrons (incoherent Compton scatter) from Au atoms. Additionally, photoelectric absorption is followed by emissions of fluorescent X-rays and Auger electrons as the inner-shell holes get filled with electrons from lower energy states, leading to a cascade of events. Similar processes also occur in organic molecules that surround the gold nanocluster. Energetic electrons produced during these events travel through matter up to tens of micrometers (continuous slowing down approximation), losing their energy through electron-electron inelastic scattering (thermalization). This

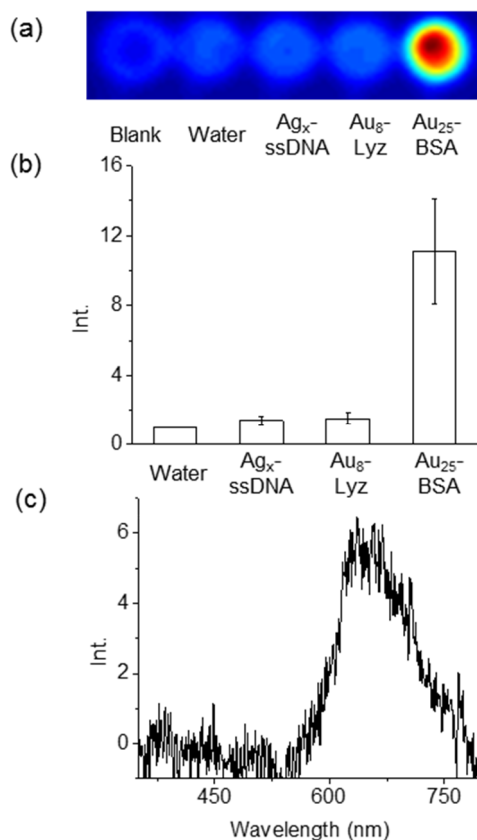


Figure 2. *h*XEOL imaging and spectra in solution. (a) Images upon X-ray irradiation. From left; blank, water, Ag_x-ssDNA, Au₈-Lyz and Au₂₅-BSA cluster. (b) Relative luminescence intensity upon X-ray irradiation. (c) X-ray excited optical luminescence spectra for Au₂₅-BSA clusters.

thermalization process produces excited electrons and holes within the gold nanocluster that can recombine to emit luminescence.²⁴ Although not all of the metal clusters are applicable for the *h*XEOL and the specific mechanisms of the luminescence upon hard X-ray irradiation should be examined in future, these results clearly demonstrate that **BSA**-directed metal clusters produce optical luminescence upon *hard* X-ray irradiation. Further experiments such as the measurement of the XAFS spectrum via resulting photoluminescence yield (PLY) with synchrotron X-ray source by other specialists of X-ray spectroscopy would provide more detailed mechanistic aspects after the publication of this communication paper.^{25, 26} One big issue concerning XEOL imaging or X-ray imaging in general is radiation damage. Fortunately for hard X-rays, the absorption cross section is low for low Z elements. If low brightness source (conventional X-ray) is used, it would further reduce the risk but studies are necessary to establish the protocol.

Conclusions

In this study, the hard X-ray excited optical luminescence of biomolecule-directed metal clusters was investigated. It was found, for the first time, that luminescence is induced from **BSA**-directed metal clusters, whereas **Ly**-directed metal clusters and **ssDNA**-directed silver clusters show less or no significant emission upon X-ray irradiation. To the best of our knowledge, this is the first demonstration of luminescence imaging with biomolecule-directed metal clusters upon hard X-ray irradiation. Although the specific mechanisms of XEOL in metal clusters are still unclear, these results are likely to provide a novel application of these metal clusters and expand the new field in radiation chemistry. Future works will be dedicated to detailed mechanistic studies such as metal cluster size dependency on XEOL and, more importantly, tumour imaging with biomolecule-directed metal clusters for the realization of multimodal imaging with CT and X-ray fluorescence imaging.²⁷ Moreover, these systems may work as useful tools in crystallographic studies to determine the position of crystals during mounting of the samples on the stage.

This work was supported by JST PRESTO program to YO, World Premier International Research Center Initiative (WPI) and Foundation Advanced Technology Institute.

Notes and references

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Electronic Supplementary Information (ESI) available: [FigureS1-3 and Table S1 and experimental information]. See DOI: 10.1039/c000000x/

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