Published in final edited form as:

Chem Phys Lett. 2013 March 13; 561-562: 74–76. doi:10.1016/j.cplett.2013.01.028.

Two Photon Induced Luminescence of BSA Protected Gold Clusters

Sangram L. Raut^{†,*}, Dmytro Shumilov[§], Rahul Chib[†], Ryan Rich[†], Zygmunt Gryczynski^{†,§}, and Ignacy Gryczynski^{†,±,*}

[†]Center for Commercialization of Fluorescence Technologies, University of North Texas Health Science Center, 3500 Camp Bowie Blvd. Fort Worth, Texas, USA, 76107

[§]Department of Physics. Texas Christian University, 2800 S. University Dr., Fort Worth, Texas, USA, 76129

[±]Department of Cell Biology and Anatomy, University of North Texas Health Science Center. 3500 Camp Bowie Blvd. Fort Worth, Texas, USA, 76107

Abstract

In this short letter, we have synthesized the BSA protected Au₂₅ nanoclusters and studied their two photon luminescence behavior. We demonstrate that BSA Au₂₅ nanoclusters can be used as a probe with two photon excitation capability. Our results show a quadratic relation between excitation power and emission intensity whereas with one photon excitation shows a linear dependence. The emission spectrum of BSA Au₂₅ nanoclusters with one photon and two photon excitation shows no appreciable change. Due to its long wavelength emission (650 nm) and two photon excitation, BSA Au₂₅ can be potentially used as a probe for deep tissue imaging.

Keywords

Gold clusters; One and two photon excitation; Luminescence; BSA

1. INTRODUCTION

The problems associated with high sensitivity cellular imaging through tissue, have stimulated efforts in synthesizing and using red and near-infrared (NIR) fluorophores that can be excited within the optical window from 630–1100 nm [1–4]. Commercial manufacturers like Molecular Probes, Pierce, and SETA Biomedicals have synthesized a newer class of IR dyes that have several fold higher fluorescence signal to autofluorescence noise ratio compared to traditional IR dyes like Cy 5 and Indocyanine green [5–7].

*Corresponding Authors: Sangram Raut (sraut@live.unthsc.edu), Ignacy Gryczynski (Ignacy.gryczynski@unthsc.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Two photon excitation (2PE) of fluorescence offers high spatial resolution due to its quadratic intensity dependence. Increased use of 2PE induced fluorescence in microscopy and imaging can be well justified by its ability to localize the excitation to a focal point and only excite fluorophores present there [8–12]. Out of focus fluorophores are not excited and hence they are not photo-bleached. Moreover, long wavelengths (> 1000 nm) excitations results in less interference from the auto-fluorescence signal owing to less infrared (IR) absorption, scattering and fluorescence from tissue [13–15].

Currently, considerable research efforts are directed towards synthesizing metal nanoclusters (Au, Ag and Cu) due to their unique photo-physical properties and possible application in cellular and tissue imaging [16–21]. Protein protected, especially bovine serum albumin (BSA), Au clusters has been extensively characterized for one photon spectroscopic properties (red emission, about 6–7% quantum efficiency and photo-stable), metal ion sensing and cellular imaging [22–25]. Despite these reports, little is known about 2PE luminescence of these BSA Au₂₅ clusters. Previously, Patel *et al* have reported 2PE luminescence properties of oligonucleotide protected Ag nanoclusters having a large two photon absorption cross section [26]. In another report, Ramkrishna *et al* show that the 2PE luminescence of alkyl thiolate protected gold clusters dependent on cluster size [27]. In this letter we aim to show that BSA Au₂₅ clusters also exhibit an efficient two photon absorption followed by red to near-infrared luminescence. We think that information regarding spectral properties of BSA protected Au clusters with 2PE is of interest for its application to biophysics and time resolved fluorescence.

2. EXPERIMENTAL SECTION

2.1 Synthesis of BSA Au₂₅ nanoclusters

The Au₂₅ NCs used in this study were synthesized using an approach developed by Xie *et al* [23]. Typically, 5 mL of 10 mM HAuCl₄ was mixed with 5 mL of 50 mg/mL BSA with 0.5mL of 1M NaOH and kept at 37 °C overnight in the incubator. The light brown solution of clusters was further dialyzed (2000 MWCO membrane) against de-ionized water for at least 12 hr with periodic change of water to remove any small impurities. Dialyzed cluster solution was filtered using 0.02 µm syringe filter and used for subsequent measurements.

2.2 Spectroscopic measurements

UV-Vis absorption and luminescence spectra (one photon) were obtained using a Cary 50 bio UV–visible spectrophotometer (Varian Inc.) and Cary Eclipse spectrofluorometer (Varian Inc.) respectively. All the measurements were done in 1cm × 0.4cm cuvettes at room temperature. A solution of rhodamine B (RhB) in water was used as a reference for comparison. Two photon excitation was achieved with Origami-10, 1038 nm pulsed laser (40 MHz repetition rate, 180 mW average power and 140 fs pulse duration) and 10× objective, mounted on a horizontal positioner. Collected emission was analyzed with a FT300 (Picoquant GmbH, Germany) fluorescence lifetime spectrofluorometer.

The hydrodynamic particle size of BSA Au₂₅ clusters was measured using a Nanotracs system (Mircotrac, Inc., Montgomeryville, PA, USA) in water at room temperature.

3. RESULTS AND DISCUSSION

Figure 1 show the cartoon of molecular structure of the BSA protected Au₂₅ clusters and measured hydrodynamic size. Although narrow, there is a size distribution with an average size of 8 nm. This observation points towards the presence of multiple BSA molecules protecting each Au cluster. In Figure 2, the red line shows the corrected absorption spectrum of the BSA Au₂₅ clusters. The measured extinction spectrum of the clusters consists of absorption and Rayleigh scattering ($1/\lambda^4$) components. The scattering component was later subtracted from the total extinction spectrum in order to obtain corrected absorption of clusters. The blue line shows the absorption of RhB in water. These two solutions were used for subsequent one and two photon experiments.

Figure 3, upper panel, shows a photograph in which the cuvette on the left demonstrates one photon excitation of the BSA Au₂₅ solution, excited using the second harmonic of a 1038nm laser. The cuvette on the right shows two photon excitation of the BSA Au₂₅ solution using a 1038nm laser with a focusing lens in the light path. A red filter (560 nm) was used in the excitation light path to avoid transmitting green light. It is evident from the photograph that we can achieve selective and strong excitation at the focal point using invisible near-infrared 1038nm light. Furthermore, we looked at the luminescence spectra of BSA Au₂₅ clusters in one photon and two photon excitation conditions and found that there is no appreciable difference between them (Figure 3 lower panel). The luminescence emission intensity response to the excitation power is different for one and two photon excitations. The relation between excitation power and emission intensity for one photon excitation is presented in Figure 4A which shows linear relationship between excitation power and emission intensity. However, Figure 4B shows a quadratic dependence between excitation power and emission intensity.

Figure 5 shows the 2PE induced luminescence spectra of BSA Au₂₅ clusters and Rh B fluorescence in water. Similar instrument settings were used for clusters and Rh B. The solutions that are used in Figure 1 were used for these measurements. It is difficult to calculate the two photon absorption cross section due to the difficulty in calculating the precise concentration of clusters in the solution. BSA Au₂₅ clusters are composed of more than one molecule of BSA and 25 gold atoms which makes it complex to calculate the exact mass of the complex and hence the concentration. Finding the exact number of BSA molecules in the cluster complex needs further studies. Nonetheless, one can get a qualitative estimation of the two photon absorption cross section of BSA Au₂₅ clusters from the comparison with RhB absorption and fluorescence.

4. CONCLUSIONS

In this report we have synthesized BSA Au₂₅ clusters using the established protocol, and we have confirmed their absorption and luminescence properties. The major observation of our study is the 2PE ability of BSA Au₂₅ clusters. The presence of 2PE properties among these clusters opens up a door to many exciting applications in microscopy and time resolved fluorescence. Moreover, being in the NIR region together with 2PE capability will make them ideal imaging candidate.

Acknowledgments

This work was supported by the NIH GRANT R01EB12003

References

1. Pham W, Medarova Z, Moore A. *Bioconjug Chem.* 2005; 16:735. [PubMed: 15898745]
2. Shibata Y, Kruskal JB, Palmer MR. *J Neurosci Methods.* 2005; 147:82. [PubMed: 15885798]
3. El-Deiry WS, Sigman CC, Kelloff GJ. *J Clin Oncol.* 2006; 24:3261. [PubMed: 16829650]
4. Bai M, Achilefu S. *Bioorg Med Chem Lett.* 2011; 21:280. [PubMed: 21106373]
5. Patsenker L, Tatarets A, Kolosova O, Obukhova O, Povrozin Y, Fedyunyayeva I, Yermolenko I, Terpetschnig E. *Ann N Y Acad Sci.* 2008; 1130:179. [PubMed: 18596347]
6. Steinberg T, Nyhus J, Aggeler B, Greenfield L, Leong L. 2006
7. [Accessed on 3rd Dec 2012] WWW.Piercenet.com
8. Periasamy A, Skoglund P, Noakes C, Keller R. *Microsc Res Tech.* 1999; 47:172. [PubMed: 10544332]
9. Vogel SS, Thaler C, Blank PS, Koushik SV. *FLIM Microscopy in Biology and Medicine.* 2009; 1:245.
10. Xu C, Webb WW. *JOSA B.* 1996; 13:481.
11. Piston DW. *Trends Cell Biol.* 1999; 9:66. [PubMed: 10087621]
12. Schneider M, Barozzi S, Testa I, Faretta M, Diaspro A. *Biophys J.* 2005; 89:1346. [PubMed: 15908572]
13. Qian J, Wang D, Cai FH, Xi W, Peng L, Zhu ZF, He H, Hu ML, He S. *Angew Chem Int Ed Engl.* 2012; 51:10570. [PubMed: 23002009]
14. Geng J, Li K, Ding D, Zhang X, Qin W, Liu J, Tang BZ, Liu B. *Small.* 2012
15. Lakowicz, J., editor. *Topics in fluorescence spectroscopy.* Plenum Press; New York: 1997.
16. Raut S, Rich R, Akopova I, Thyraug E, Shtoyko T, Shumilov D, Gryczynski Z, Gryczynski I. *Chemical Physics Letters.* 2012; 549:72. [PubMed: 23772092]
17. Qian H, Zhu M, Wu Z, Jin R. *Acc Chem Res.* 2012; 45:1470. [PubMed: 22720781]
18. Xu H, Suslick KS. *Adv Mater.* 2010; 22:1078. [PubMed: 20401932]
19. Lu Y, Chen W. *Chem Soc Rev.* 2012; 41:3594. [PubMed: 22441327]
20. Retnakumari A, Setua S, Menon D, Ravindran P, Muhammed H, Pradeep T, Nair S, Koyakutty M. *Nanotechnology.* 2010; 21:055103. [PubMed: 20023317]
21. Durgadas CV, Sharma CP, Sreenivasan K. *Analyst.* 2011; 136:933. [PubMed: 21152627]
22. Wang XX, Wu Q, Shan Z, Huang QM. *Biosens Bioelectron.* 2011; 26:3614. [PubMed: 21382705]
23. Xie J, Zheng Y, Ying JY. *J Am Chem Soc.* 2009; 131:888. [PubMed: 19123810]
24. Hu L, Han S, Parveen S, Yuan Y, Zhang L, Xu G. *Biosens Bioelectron.* 2012; 32:297. [PubMed: 22209331]
25. Das T, Ghosh P, Shanavas MS, Maity A, Mondal S, Purkayastha P. *Nanoscale.* 2012; 4:6018. [PubMed: 22915187]
26. Patel SA, Richards CI, Hsiang JC, Dickson RM. *J Am Chem Soc.* 2008; 130:11602. [PubMed: 18686957]
27. Ramakrishna G, Varnavski O, Kim J, Lee D, Goodson T. *J Am Chem Soc.* 2008; 130:5032. [PubMed: 18357982]

Research Highlights

BSA Au₂₅ nanoclusters of about 8nm

NIR (650 nm) fluorescence emission

Excitation power and emission intensity quadratic dependence

Two photon excitation capability

Similar emission spectra for one and two photon excitation

Figure 1.
Cartoon of BSA protected Au₂₅ clusters and hydrodynamic particle size of the BSA Au₂₅.

Figure 2. Absorption spectra of BSA Au₂₅ (red line) and rhodamine B (blue line). These solutions were used for 2 photon experiments.

Figure 3.

Upper panel: left cuvette shows the one photon excitation of BSA Au₂₅ solution excited using second harmonic of 1038 nm laser and right cuvette shows two photon excitation of BSA Au₂₅ solution using 1038nm laser. Lower panel one photon and two photon emission spectra of BSA Au₂₅ clusters in water at room temperature using 519 nm and 1038nm laser respectively.

Figure 4.

(A) One photon emission intensity dependence on excitation power of BSA Au₂₅ clusters showing linear relation. (B) Two photon emission intensity dependence on excitation power of BSA Au₂₅ clusters showing quadratic relation.

Figure 5. Two photon emission spectra of RhB (blue line) and BSA Au₂₅ clusters in water at room temperature using 1038nm laser.