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ORIGINAL PAPER

# Highly Sensitive Quantitation of Human Serum Albumin in Clinical Samples for *Hypoproteinemia* using Metal-Enhanced Fluorescence

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**Abstract** In this paper, we have explored metal-enhanced fluorescence (MEF) of the Human serum albumin indicators: Albumin Blue 580, Merocyanine 540 and Bromophenol Blue in close proximity to silver nano-particles, SiFs, from both buffered and clinical samples. The photostability of the Albumin Blue 580 is shown to be much more prolonged from the SiFs as compared to glass (a control sample), potentially allowing for longer detection times to further improve assay statistics. Our findings suggest the widespread use of nanoparticulate SiFs surfaces for the enhanced detection of HSA, particularly for *Hypoproteinemia*, where an enhanced assay performance at low protein abundance is required.

**Keywords** Metal-enhanced fluorescence · Radiative decay engineering · Plasmon-enhanced fluorescence, Surface enhanced fluorescence · Near-field fluorescence · Hypoproteinemia

## Introduction

Human serum albumin (HSA) is the most abundant protein in the circulatory system and plays critical biological functions in the human body such as regulating water balance between blood and tissues and serves as a physiological carrier for various endogenous and exogenous substances [1, 2]. Variations in the concentration of Human Serum Albumins in body fluids are associated positively with a number of diseases, such as renal disease, diabetes, and liver disease [3]. A low level of albumin in the blood serum, known as hypoproteinemia, may indicate liver failure, cirrhosis, and chronic hepatitis. A high amount of HSA in urine is associated with inflammation in the kidneys. In this regard, the precise quantitative determination of albumins in biological fluids is of importance in diagnosis and in many biomedical sciences. However, it is difficult to precisely determine the concentration of albumins in biological fluids, mostly due to complicated matrices and sometimes low amounts. Usually colorimetric methods, such as Brandford and Lowry assays, have traditionally been used for protein quantitation in solutions. However, these methods are less than ideal because of several drawbacks such as; poor sensitivity, accuracy, and the time consuming nature of the procedure. Currently, biosensors utilizing classical far-field fluorescence have attracted much attention due to their sensitivity, selectivity and rapidity. Albumin Blue 580, Merocyanine 540 and Bromphenol Blue, are today among the most widely used as Human serum albumin (HSA) indicators because of their longer wavelength excitation, which invariably alleviates biological autofluorescence [2, 4]. However, the detection limit in these biological settings is still limited by the quantum yield of the fluorophore, the autofluorescence of the sample and the photostability of the fluorophores, classical constraints of fluorophores, when used in the far-field condition.

In this regard, metallic nanostructures have been used to favorably modify the spectral properties of fluorophores and to alleviate some of there more classical photophysical constraints, such as increasing quantum yields and decreased lifetimes, which invariably lends to an increased system photostability. The use of fluorophore-metal interactions has been termed Metal-Enhanced Fluorescence (MEF) by Geddes [5], whereby large fluorescence enhancements can be obtained from fluorphores that are in the near-field vicinity (less than one wavelength of light) of metal nanoparticles. Owing to these advantages, there has been a

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significant interest in the uses of metal enhanced fluorescence. Many applications of metal-enhanced fluorescence (MEF) have been demonstrated [6–8], which have included improved DNA [9–11] and protein detection [12], the enhanced detection of calcium [13], enhanced wavelength-ratiometric sensing [14], ultra fast and ultra sensitive target analyte detection [15], and metal-enhanced bioluminescence [16], to name but just a few.

In this paper, we have studied the metal-enhanced fluorescence of three HSA indicators: Albumin Blue 580, Merocyanine 540 and Bromphenol Blue in close proximity to silver nano-particles. We observed that Albumin Blue 580, Merocyanine 540 and Bromphenol Blue in the presence of silver nanoparticles, show enhanced far-field fluorescence and become dramatically more photostable. The enhancement factor (i.e. the benefit of using the plasmonic SiFs substrate), was determined as the ratio of the fluorescence intensity from the silver island films as compared to a glass control surface, where no near-field Plasmon enhancement is observed.

## Experimental

### Materials

Silver nitrate (99.9 %), sodium hydroxide (99.996 %), ammonium hydroxide (30 %), *D*-glucose and premium quality silane-prep glass slides (75×25 mm), Albumin Blue 580, Merocyanine 540 and Bromphnol Blue were purchased from Sigma-Aldrich. All chemicals were used as received.

### Preparation of Silver Island Films (SiFs)

Silver island films were prepared in accordance with previously established procedures [17]. In this procedure, *D*-glucose is used to reduce silver nitrate to form silver islands on one surface of the glass slide. The thickness of the SiFs and indeed nanoparticle size can be varied depending on the length of time silver is allowed to deposit on the slides [18]. The SiFs used for this study had a deposition time of 2 min.

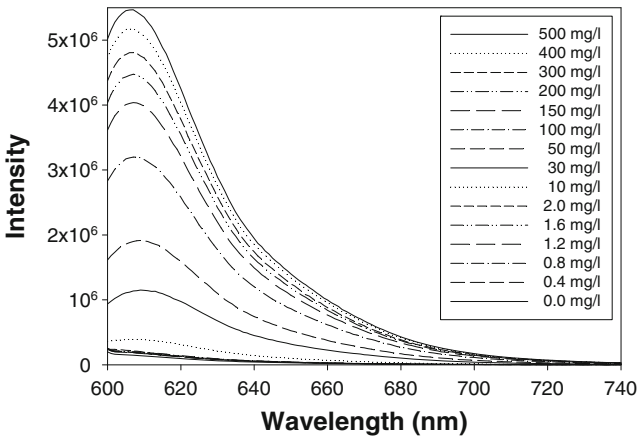


**Fig. 1** Fluorescence spectra of Albumin Blue 580 (1.2 μM) at a constant temperature of 21.2 °C in the presence of Human serum albumin, at various concentration in PBS buffer (from 2 mg/L to 500 mg/L). Ex: 594 nm, slit 5/10 nm

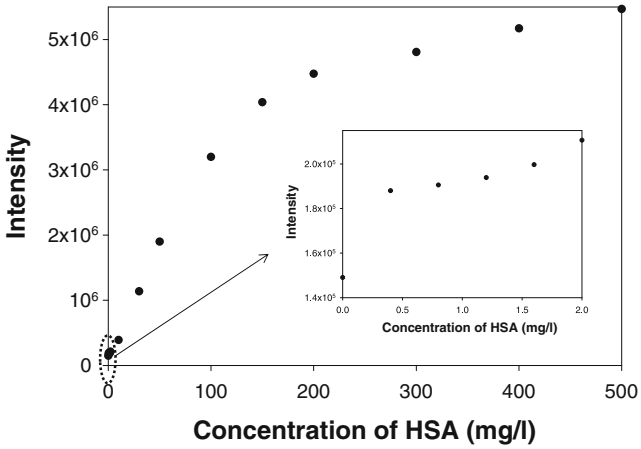


**Fig. 2** Calibration curve for human serum albumin from 0 mg/L to 500 mg/L in a cuvette

### Preparation of the Sandwich Format Sample

Ten μL of Fluorophore (Albumin Blue 580 1.2 uM :2-chloro-3-(2,2-dicyanoethenyl)-2-cyclohexen-1-ylidene)methylpropanedinitrile potassium salt), Merocyanine 540 and Bromphenol Blue was trapped in a sandwich-like format along with Human serum albumin at various concentration (from 0 to 500 mg//L) between the glass slides and the silver island films, respectively.

### Fluorescence Measurements

To measure the emission spectra of Albumin Blue 580, 3 μL of stock solution was added to 1 mL of each of the concentrations of buffers in a quartz cuvette. The final concentration of Albumin Blue 580 was 1.2 μM. A similar procedure was followed to obtain the emission spectra of Merocyanine 540 and Bromphenol Blue. Fluorescence spectra were collected with a Varian Eclipse spectrofluorometer with an excitation of 594 nm, 532 nm and 594 nm, respectively.

### Emission of Fluorescence on SiFs and Glass Slides

A 100 μL solution of AB 580, Merocyanine 540 and Bromphenol Blue and different HSA concentration buffers was sandwiched between two glass slides and then SiFs with a glass slide on top, to measure the fluorescence emission. The same SiFs slide was washed between samples with de-ionized water and then dried

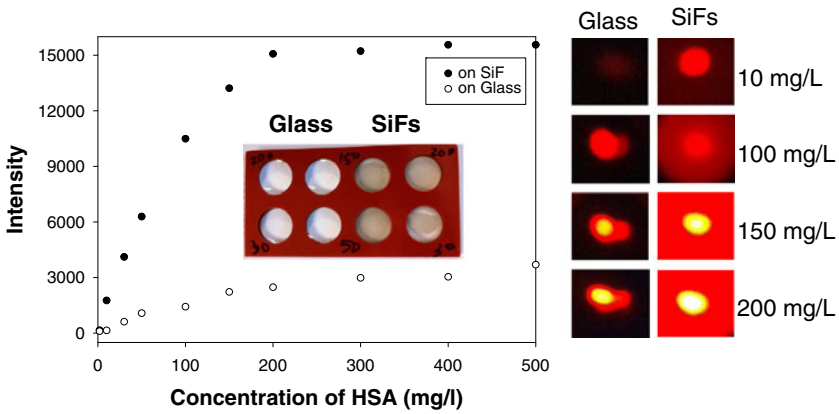


**Fig. 3** Fluorescence intensity of 80 μL Albumin Blue 580 (1.2 μM) in the presence of Human serum albumin at various concentration in PBS buffer from both glass and SiFs. The signal from the SiFs surface is significantly enhanced as compared to the control sample. Ex: 594 nm. **SiFs** – Silver Island Films

with nitrogen gas. Samples were excited using a 473 nm laser, and emission spectra were collected with an Ocean Optics HR2000 Fluorometer and a RazorEdge (TM) 473 nm filter. An integration time of 300 ms was kept constant for all measurements.

Photostability (Intensity Vs Time)

The steady-state fluorescence intensity decay of AB580 was measured using the sandwich format with an Ocean Optics HR2000 Fluorometer and a RazorEdge (TM) 473 nm filter. Glass slides functioned as a control sample by which to compare with SiFs and the glass slide sandwich. The initial intensity on SiFs was adjusted to that observed on glass using a neutral density filter (Edmund Optics) in order to compare fluorescence intensity decays, and to further demonstrate the benefit of the MEF effect.

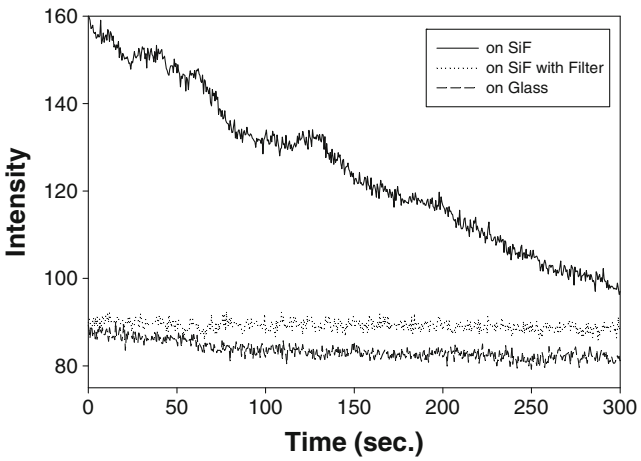


**Fig. 4** Emission intensity vs. time (photostability) of Albumin Blue 580 (1.2 μM) in the presence of 2 mg/L Human serum albumin from glass and SiFs, and also with the laser power adjusted to give the same initial steady-state fluorescence intensity as observed from SiFs

## Results and Discussion

Albumin Blue 580 Indicator for HSA

Albumin Blue 580 is the first derivative of the Albumin Blue series that provides sufficient stability of its stock solution to allow application in detection. Isopropanol was chosen as a solvent because of its stabilizing effect, low toxicity, and lack of effect on the assay. It also is an effective antifreezing agent for dye solutions at -20 °C. Albumin Blue 580 is anionic and binds to albumin in a highly specific way and undergo's strong fluorescence emission. Because the assay was found to depend strongly on temperature, the temperature was maintained at 21.2 °C during all measurements. Emission spectra of AB580 in the presence of human serum albumin at concentrations from 0 to 500 mg/L, when excited at 594 nm, are shown in Fig. 1. It was observed that the fluorescence increases with the [HSA] increasing from 0 to 500 mg/L. The calibration curves are nonlinear and approach saturation at high albumin concentration. From Fig. 2, we readily observe that when Human serum albumin concentration exceeds≈200 mg/L, the maximum fluorescence intensity approaches a plateau.

It is well-known, that for classical far-field fluorescence based assays, the assay detection limit is underpinned *in part* by the quantum yield of the probe, in this case AB580. Here, metallic nanostructures have been used to favorably modify the spectral properties of AB580 and to alleviate some of their more classical photophysical constraints, such as quantum yield. When a solution of AB580 is in close proximity to silver nanoparticles in the SiFs sandwich geometry, the fluorescence emission intensities of AB580 can be increased dramatically. From Fig. 3, we observe that the fluorescence was significantly enhanced as compared to the control sample from glass/glass, an otherwise identical control sample, but containing no silver nanoparticles. The real-color photograph (Fig. 3 right) also shows that the emission intensity is much more visually detectable from the SiFs/

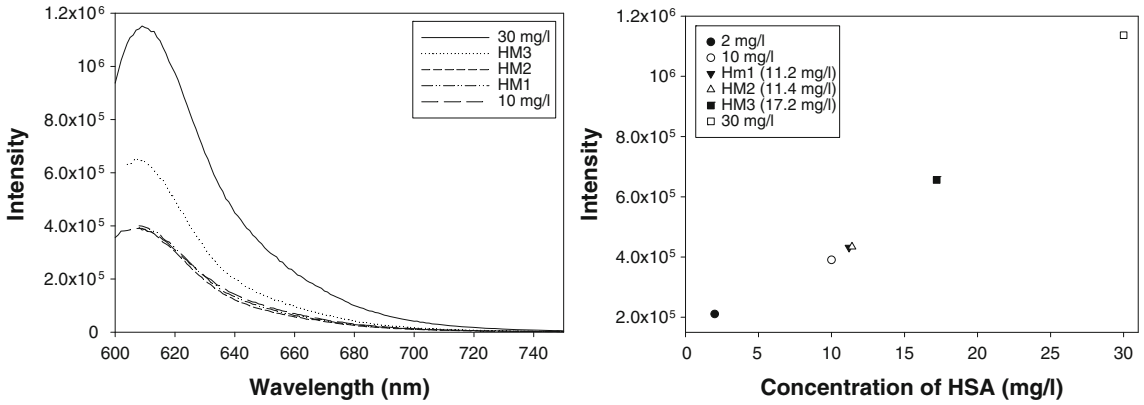


**Fig. 5** Fluorescence spectra of Albumin Blue 580 (1.2 μM) in the 3 Urine samples, HM1⟶3, (Male). From the Calibration curve of human serum albumin, the HSA concentration from within urine was determined to be 11.2, 11.4, and 17.2 mg/L. The calibration points 2, 10 and 30 mg/L are also shown on the right plot

glass as compared to the glass/glass control slide, significantly improving the detectability of the protein. Our laboratories mechanistic interpretation of this observation, i.e. MEF, is underpinned by a model whereby non-radiative energy transfer occurs from excited distal fluorophores to surface plasmons in non-continuous films, in essence a fluorophore induced mirror dipole in the metal. The surface plasmons, *in turn*, radiate the photophysical characteristics of the coupling fluorophores [19, 20]. In essence, the *system radiates* as a whole. As a result, the *system* exhibits modified overall increased radiative rates, in contrast to the fluorophore itself whose radiative rate is thought unchanged. Ultimately, the increased radiative rate for the system lends to enhanced fluorescence signals for fluorophores in close proximity to metallic structures. The important factors which affect the magnitude of the fluorescence enhancement via the localized surface Plasmon resonance (LSPR) are the size and shape of the nanoparticles, the degree of overlap between the LSPR and the emission band of the dye [21], as well as the distance of the fluorophore from the metal [22]. A detailed description of the factors which underpin the MEF effect can be found in a recent report by us [20].

Virtually all fluorophores are photobleached when exposed to continuous light illumination. Photobleaching is a ubiquitous problem in the application of fluorescence. In this regard, the photostability of AB580 on SiFs and Glass has been studied. Figure 4 shows AB580 emission as a function of time, excited at 594 nm and observed through a long pass filter. The relative intensities of the plots reflect that more detectable photons can be observed per unit time from the SiFs film, as compared to glass (a control sample), where the integrated ar*eas* under the plots is proportional to the photon flux from the respective samples. By additionally adjusting the laser power, to match the same initial steady-state intensities of the samples, the AB580 on SiFs can be seen to be more photostable as compared to that on glass/glass. This finding suggests that the fluorescence lifetime (decay time) on the SiFs is also shorter than on the glass film, the AB580 in essence spending less time on average in

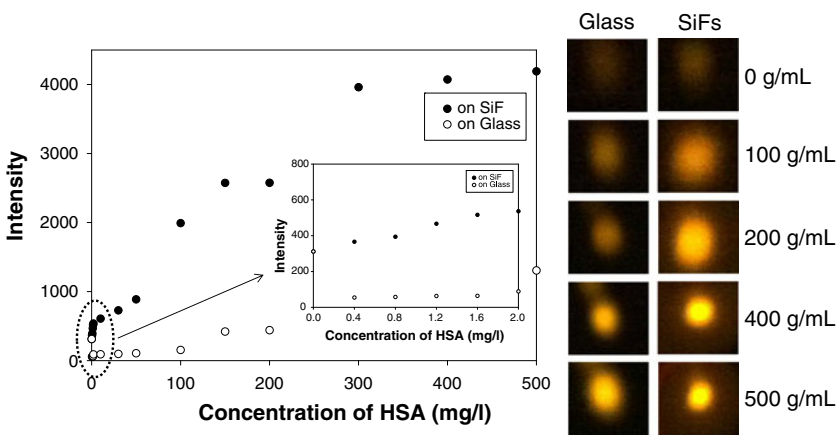


**Fig. 6** Fluorescence intensity of Merocyanine 540 (1.2 μM) in the presence of Human serum albumin at various concentration in TE buffer from both glass and SiFs substrates. Ex: 532 nm

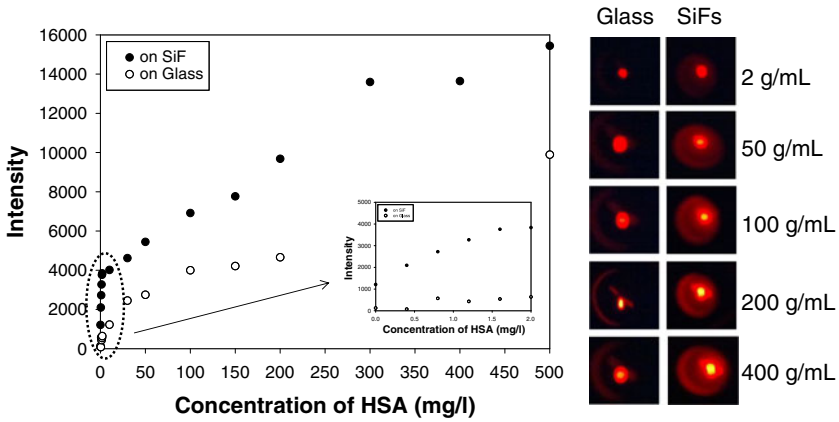


**Fig. 7** Fluorescence intensity of Bromophenol Blue (1.2 μM) in the presence of Human serum albumin at various concentration in PBS buffer from glass and SiFs. Ex: 594 nm

an excited state due to the fast non-radiative energy transfer to the SiFs, and therefore is less prone to photo destruction, i.e. is more photostable.

Albumin Blue 580 Fluorescence-Based Urine Assay

To test the applicability of our MEF assay with clinical samples, we have measured the HSA concentration of three male urine samples. Figure 5 shows the fluorescence spectra of albumin blue 580 in the urine samples. From the calibration curves, the HSA concentration could readily be determined as 11.2, 11.4, and 17.2 mg/L, respectively.

Merocyanine 540 and Bromphenol Blue Indicators for HSA

Similar to AB580, Merocyanine 540 and Bromphenol Blue dilution curves in the presence of human serum albumin at concentrations of 0 to 500 mg/L, when excited at 532 and 594 nm respectively, are shown in Figs. 6 and 7. The fluorescence was significantly enhanced as compared to the control sample on glass/glass, increasing the signal-to-noise significantly. The real color photographs (Figs. 6 and 7 right) also show visually that the emission intensity is much more clearly detectable from the SiFs/glass as compared to the glass/glass control slide. While not shown in the data, and similar to AB580, the photostability of both probes was significantly more pronounced from the SiFs surface as compared to the control sample. Given the greater signal from SiFs of all the 3 probes studied, coupled with a much more pronounced photostability, then our MEF approach suggests that significantly more sensitive assays can be realized, where emission intensity can be collected and integrated over a longer time period.

## Conclusions

For Albumin Blue 580, Merocyanine 540 and Bromphenol Blue in close proximity to silver nanoparticles, the fluorescence emission intensities can be increased dramatically. For the urine samples, the fluorescence enhancement factor (MEF) was over 20 fold (sample I) as compared with the control sample on glass/glass. The photostability of the fluorophore (AB580) was also significantly improved on the SiFs film as compared to glass (a control sample), potentially allowing for the longer acquisition of data, which ultimately improves assay statistics. Given the widespread use of fluorescence-based detection of HSA in clinical medicine, then our findings suggest that SiFs surfaces can be potentially employed as a more sensitive alternative detection platform, particularly for *Hypoproteinemia*, where there is a real need for both the detection and quantitation of HSA at abnormally low protein levels in physiological fluids.

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## References

1. Hu Y-J, Liu Y, Xiao X-H (2009) Biomacromolecules 10:517–521
2. Hong Y, Feng C, Yu Y, Liu J, Lam JWY, Luo KQ, Tang BZ (2010) Anal Chem 82:7035–7043
3. Kessler MA, Meinitzer A, Wolfbeis OS (1997) Anal Biochem 248:180–182
4. Grynkiewicz G, Poenie M, Tsien RY (1985) J Biol Chem 260:3440–3450
5. Geddes CD, Lakowicz JR (2002) J Fluoresc 12:121–129
6. Aslan K, Previte MJR, Zhang Y, Gallagher T, Baillie L, Geddes CD (2008) 80, 4125–4132.
7. Guarise C, Pasquato L, De Filippis V, Scrimin P (2006) Proc Natl Acad Sci U S A 103:3978–3982
8. Li CZ, Male KB, Hrapovic S, Luong JH (2005) Chem Commun (Camb) 3924–3926.
9. Aslan K, Malyn SN, Geddes CD (2006) Biochem Biophys Res Commun 348:612–617
10. Tennant SM, Zhang Y, Galen JE, Geddes CD, Levine MM (2011) PLoS One 6(4):e18700
11. Zhang Y, Agreda P, Kelly S, Gaydos C, Geddes CD (2011) IEEE Trans Biomed Eng 58(3):781–784
12. Dragan A, Albrecht MT, Pavlovic R, Keane-Myers AM, Geddes CD (2012) Anal Biochem 425:54–61

13. Bondre N, Zhang Y, Geddes CD (2011) Sensors Actuators B Chem 152:82–87
14. Aslan K, Lakowicz JR, Szmacinski H, Geddes CD (2005) J Fluoresc 15:37–40
15. Aslan K, Geddes CD (2005) Anal Chem 77:8057–8067
16. Eltzov E, Prilutsky D, Kushmano A, Marks RS, Geddes CD (2009) Appl Phys Lett 94:083901
17. Aslan K, Leonenko Z, Lakowicz JR, Geddes CD (2005) J Fluoresc 15:643–654
18. Pribik R, Dragan AI, Zhang Y, Gaydos C, Geddes CD (2009) Chem Phys Lett 478:70–74
19. Dragan AI, Geddes CD (2011) Phys Chem Chem Phys 13:3831–3838
20. Aslan K, Geddes CD (2010) In Geddes CD (ed) Metal-enhanced fluorescence. New Jersey. Wiley, pgs 1–24, ISBN: 978-0-470-22838-8
21. Dragan A, Geddes CD (2012) Appl Phys Lett 100:093115
22. Dragan AI, Bishop ES, Casas-Finet JR, Strouse RJ, McGivney J, Schenerman MA, Geddes CD (2012) Plasmonics - In Press