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Wavelength-dependent metal-enhanced fluorescence using synchronous spectral analysis

Anatoliy I. Dragan, Buddha Mali, Chris D. Geddes *

Institute of Fluorescence and Department of Chemistry and Biochemistry, UMBC, 701 East Pratt Street, Baltimore, MD 21202, USA

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ABSTRACT

The fluorescence spectrum of Au-clusters (8- and 25-atom), which covers the spectral range 350–900 nm, is dramatically enhanced in the presence of plasmon supporting plate-well deposited nanoparticles. The wavelength-dependent metal-enhanced fluorescence (MEF spectrum) correlates well with the plasmon specific scattering spectrum, i.e. the synchronous scatter spectrum of the silver surface of plate wells. Our findings suggest that the synchronous scatter spectra of plasmon enhancing substrates is a good indicator of both the magnitude and the wavelength-dependence of MEF.

1. Introduction

Fluorescence spectroscopy is a powerful tool for the detection of biomolecules (proteins and nucleic acids), studying their interaction with partners and the visualization of macromolecular complexes and cell organelles [1,2]. At the base of these applications is a vast quantity of different fluorescent chromophores, which can be used in different spectral regions, from the UV to NIR. Despite a vast amount of available fluorophores, there is still great demand for probes which are extremely bright and photostable and, subsequently, one's which are able to significantly increase the sensitivity of various detection approaches, including methods such as single-molecule detection and fluorescence correlation spectroscopy [1,3,4]. Modern chromophores, have been specifically synthesized for these purposes, e.g. the Alexa dyes for labeling protein and nucleic acids have large brightness, >100 000 M^{-1} cm^{-1}, and enhanced photostability.

In recent years the metal-enhanced fluorescence (MEF) phenomenon, which dramatically enhances the brightness of chromophores and, at the same time, increases their photostability, has remarkably improved and broadened fluorescence-based applications in the lifesciences [5–8]. While the MEF phenomenon has been well-studied over the last decade, there are still numerous questions unanswered. Our laboratory has contributed significantly to developing the MEF approach and understanding the mechanisms of metal-enhanced fluorescence [5,6,9,10]. At the heart of the MEF phenomenon lies the interaction between the electronic states of chromophores and the near-field, generated around nanoparticles (NPs), and the effective coupling between them occurring at a short (<50 nm) dye-NP distance. Originally, the origin of dye-NP coupling and enhancement of fluorescence was explained by a change in the radiative decay rate of a chromophore, which affords for higher fluorophore quantum yields and a reduced fluorophore lifetime (decay time) [1,11,12]. Subsequently one was previously able to make several conclusions: (1) MEF depends on the free-space quantum yield of a chromophore, i.e. MEF is larger for dyes with a low quantum yield; (2) MEF does not depend on wavelength, as the dye alone radiates the light and the wavelength distribution of the energy is determined solely by inner properties of the chromophore electronic system, and its interactions with the solvent. However, recent experimental works have shown that the interactions in dye-NP systems are significantly more complex than a simple radiatiative rate mechanism. In particular, it has recently been shown that the far-field fluorophore quantum yield, Q_o, has little relationship to fluorescence enhancement factors in MEF [10]. In addition, a wavelength dependence has been shown for Prodan in different solvents [13].

In recent years Geddes and colleagues have shown that the mechanisms of metal enhancement can be considered as due to at least two complementary effects: an enhanced absorption and an enhanced emission component [5,10]. According to this interpretation the enhanced absorption in MEF is facilitated by the electric field generated by NPs, its intensity and spectral distribution. Nanoparticle free oscillating electrons (plasmons) have specific absorption and scattering bands [5,6]. The plasmon scattering component is sensitive to the size, shape and density of nanoparticles, and typically increases and broadens (red shift) with NP size. For silver NP films we have found that the plasmon scattering spectra can be directly measured using the synchronous mode of spectral collection. Knowledge of the plasmon scattering characteristics

Abbreviations: NC, nanocluster; NP, nanoparticle; BSA, bovine serum albumin; ANS, 1-anilino-8-naphthalene sulfonate; Au(8), 8-atom gold cluster; Au(25), 25-atom gold cluster; MEF, metal-enhanced fluorescence.

* Corresponding author. Fax: +1 410 576 5722.
E-mail addresses: geddes@umbc.edu, iof@umbc.edu (C.D. Geddes).

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of silver films gives an opportunity to address some key questions regarding the mechanisms of MEF, for example: does MEF wavelength dependence follow the plasmon scattering spectra? Is there a dependence of the magnitude of MEF upon the incident light wavelength?

To subsequently address these questions we have used fluorescent Au-clusters, an emerging new class of the chromophores, since their spectral properties, specifically their broad emission and absorption spectrum, can be used to study MEF over a broad wavelength range.

2. Materials and methods

Chloroauric acid ($HAuCl_4$), ascorbic acid, fluorescein, 1-anilino-8-naphtalensulphonic acid ammonium salt (ANS) and bovine serum albumin (BSA) were purchased from Sigma (USA) and have been used without further purification.

2.1. Production of fluorescent Au-clusters

Condensation of gold atoms into Au-clusters in the presence of protein (albumin) was undertaken according to [14] but using a few changes. In essence, an aqueous solution of chloroauric acid was added to the BSA protein solution in water, followed by the dropwise addition of a reducing agent, ascorbic acid, to trigger the formation of Au-clusters within the protein surface. The pH of the reaction was 11.7. In the original protocol [14] the incubation time for obtaining fluorescent Au-clusters/protein was 6 h at 37 °C. In our modified protocol we employed microwave irradiation of the reactive solution for <30 s in a microwave cavity (GE Compact Microwave Model: JES735BF, frequency 2.45 GHz, power 700 W). The microwave irradiation power was reduced to 20%, which corresponded to 140 W over the entire cavity. Microwave irradiation effectively accelerates the formation of Au-clusters within the protein structure.

2.2. Preparation of 'Fire in the Hole' (FIH) silver-coated plates

Silver coating of Perkin Elmer plate wells was undertaken using a protocol described previously [15]. In short, to prepare the silvering solution, 200 µl of sodium hydroxide solution (0.5% w/v) is added to 60 ml of $AgNO_3$ (0.83% w/v), the solution becomes brown and cloudy, after which 2 ml of ammonium hydroxide (30% solution) is added, or until the solution becomes clear. The solution is then cooled down on ice to 10 °C and, while stirring, 15 ml of fresh D-glucose solution (4.8% w/v) is added.

The silvering solution is then loaded into preheated (40 °C) plate wells for 2 min followed by cooling on ice for several minutes. The solution within the wells is changed several times, followed by continuous heating of the wells for several minutes. Finally, the plate is then washed several times with deionized water and dried in a stream of nitrogen gas.

2.3. Preparation of fluorescent solution of organic dyes (fluorescein and ANS)

To prepare highly fluorescent ANS/BSA complex in solution, concentrated solution of ANS was titrated into the solution of bovine serum albumin (BSA) in PBS at pH 7.4. The final concentration of BSA was 1 mg/ml (1.5×10^{-5} M), the concentration of ANS was 3×10^{-5} M. In this condition most of ANS dye molecules are bound to the protein [16]. The BSA concentration was calculated using molar extinction coefficient $\varepsilon_{280} = 43\,600\ M^{-1}\ cm^{-1}$ [16].

The concentration of fluorescein in TE buffer, pH 7.6 was 1×10^{-5} M. The molar extinction coefficient $\varepsilon_{490} = 80\,000\ M^{-1}\ cm^{-1}$ was used for calculation the fluorescein concentration.

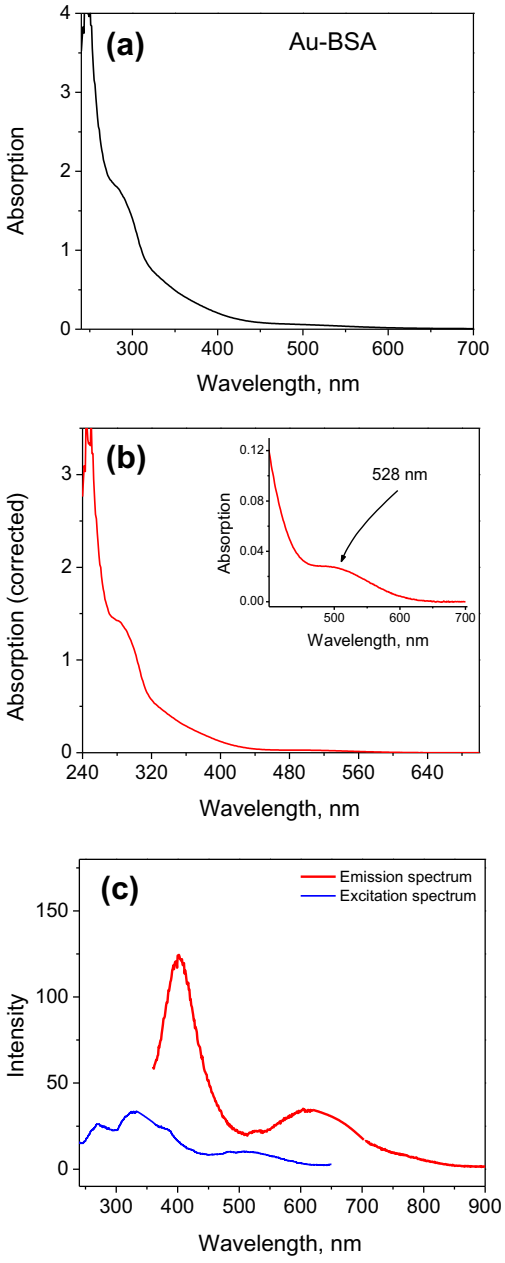

Figure 1. (a) Absorption spectra of Au–albumin solution. Sample was diluted 10-fold with PBS. (b) Corrected for scattering, the absorption spectrum of Au–albumin solution. Insert: enlarged absorption spectrum of Au-clusters shows specific absorption band at 528 nm. (c) Fluorescence and excitation spectra of Au-clusters (8- and 25-atoms). Fluorescence excitation spectrum was recorded using the 652 nm emission wavelength, i.e. maximum of Au–albumin fluorescence. Fluorescence spectra was recorded using the excitation wavelength at 340 nm.

2.4. Fluorescence measurements

Measurements of fluorescence excitation and emission spectra of the Au-protein samples were undertaken using a FluoroMax-4 spectrofluorometer (Horiba, USA).

2.5. Synchronous spectra measurements

Synchronous spectra of silver nanoparticle coated wells, i.e. FIH plates, were measured using a Varian spectrofluorometer plate reader. In synchronous mode the instrument measures the intensity of light from wells at different wavelengths where the wavelength of excitation and emission are equal, i.e. $\lambda_{Ex} = \lambda_{Em}$.