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Fluorescence enhancement for the complex PAMAM–BSA in the presence of photonic crystal heterostructures

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ABSTRACT

The paper presents the optical characterization of the multilayer film composed of styrene (ST)–poly(ethylene glycol) methyl ether methacrylate (PEGMA 1100)–gold nanoparticles (Au)–poly(amidoamine) PAMAM (G4)–bovine serum albumin (BSA). The addition of the last layer composed of BSA resulted in an unusual optical behaviour, i.e. increase of the fluorescence emission intensity, respectively the intensity of the UV–vis reflection, compared with the ST–PEGMA 1100–Au–G4 film. The explanation could be attributed to the presence of photonic crystal heterostructures. The multilayer film has been characterized by optical microscopy, AFM, UV–vis, and fluorescence.

1. Introduction

Serum albumin is one of the most extensively studied proteins for many years. It is the most abundant protein in blood plasma with a typical concentration of 50 g/L and functions as a transport protein for numerous endogenous and exogenous substances. It also plays an important role in regulating the colloid osmotic pressure of blood. It provides about 80% of the osmotic pressure and is responsible for the pH maintenance in blood [1]. Many researchers have studied the structures, functions and properties of serum albumins to understand their interactions with other molecules and ligands. Serum albumin can interact with dendrimers [2–4], which are relatively new class of globular polymers. They possess a central core, branches expanding from the core and many terminal groups [5,6].

The full generations of PAMAM dendrimers have hydroxyl or amino groups on their surfaces whereas the half generations have carboxyl or sodium carboxyl groups. The abundance of different functional surfaces add dendrimers numerous unique properties, e.g. aqueous solubility and stimuli (temperature, pH, etc.) responsibility [7,8]. These properties make dendrimers favourable in many biomedical and pharmaceutical areas such as bio-imaging, tissue engineering, drug and gene delivery [9–12]. By far (PAMAM) dendrimer is the most intensively investigated [13–19].

The effect of dendrimers on bovine serum albumin (BSA) consists in a decrease/quenching of the fluorescence of the two tryptophan residues contained in BSA [20–24]. The possible reason for the decreasing fluorescence intensity is the electrostatic dendrimer–protein interactions (Scheme 1).

Metal nanoparticles can be used as physical support for BSA–PAMAM complex, whereas gold nanoparticles (Au)–PAMAM nanocomposites are used as biosensors [24,25], quantum dots [26], and drug delivery systems [27]. Au nanoparticles can be used as a quencher to decrease the fluorescence intensity of fluorophores [28–30]. The dynamic quenching process included an instantaneous exciplex formed between excited fluorescent molecules and quenchers [31]. These exciplexes could not emit fluorescence or become different from the original fluorescent molecules, which cause quenching to happen. In the process of resonance energy transfer, the efficiency depends on the overlap degree of emission spectra of the donor and the absorption spectra of the acceptor. The higher the overlap, the higher the efficiency [24]. The prominent overlap between the absorption spectrum of Au and the emission spectrum of PAMAM provides increased probability of energy transfer from the excited dendrimer to Au, and hence the intense quenching of the fluorescence (Scheme 2).

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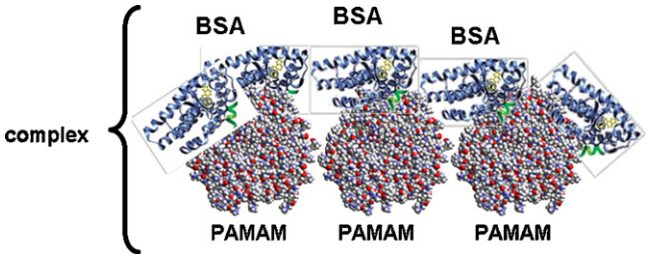


Scheme 1. PAMAM–BSA complex.

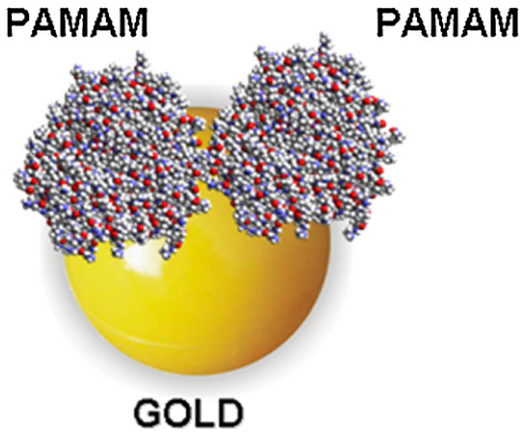
Photonic crystals (PCs) constitute a fascinating class of materials – promising candidates for nanoscale optoelectronic devices for the next generation information technology [32–34]. They are generally characterized by artificial structures with a periodic dielectric arrangement which does not allow propagation of light in all directions for a given frequency range. This phenomenon induces the opening of photonic stop-bands or band gaps due to Bragg diffraction [35,36]. The stop-band of the PCs represents the narrow range of specific wavelengths in which the propagation of light is prohibited. In this context, the synthesis of monodisperse colloidal spheres with sub-micronic diameters has lately attracted a lot of interest from various researchers, due to their self-assembling properties leading to crystalline structures of synthetic opal after the removal of the dispersion medium. Crystalline lattices of inorganic or polymer particles have a highly ordered structure that leads to PCs properties. One of the most promising methods of obtaining monodisperse colloidal polymer particles is the soap-free emulsion polymerization [37–41]. Various methods for obtaining films of colloidal particles, such as gravitational sedimentation, centrifugation, vertical deposition, physical confinement, interfacial or electric field induced self-assembly have been described in the literature [42–48].

In this work, a multilayer film composed of PCs, Au, PAMAM and BSA was obtained (Scheme 3). The optical influence of PCs substrate has been investigated, given that Au decreases the fluorescence of PAMAM and PAMAM also decreases the BSA fluorescence.

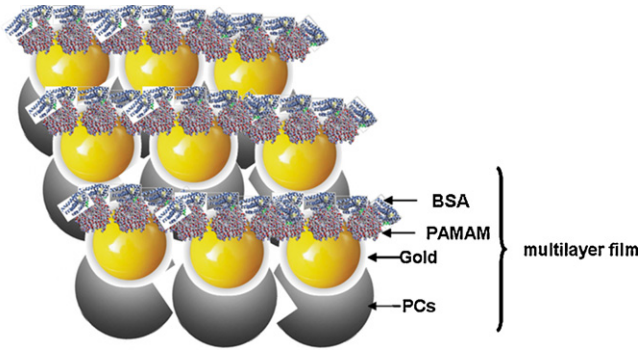
2. Materials and methods

2.1. Materials

Styrene (ST) (Merck) was purified through vacuum distillation. Poly(ethylene glycol) methyl ether methacrylate (PEGMA 1100) (Aldrich) was used without purification. Potassium persulphate (KPS) (Merck) was recrystallised from an ethanol/water mixture and then vacuum dried. Tetrachloroauric acid trihydrate 99.5% ($HAuCl_4{\cdot}3H_2O$) (Merck), trisodium citrate dihydrate ($Na_3C_6O_7{\cdot}2H_2O$) (Fluka), PAMAM (G4) (Aldrich), BSA (Fluka) were used as received.



Scheme 2. PAMAM–Au complex.



Scheme 3. PCs–Au–PAMAM–BSA complex, multilayer film.

2.2. Soap-free emulsion polymerization

6.5 ml ST and 0.25 ml PEGMA 1100 were added in 100 ml distilled water together with 0.0625 g KPS. The reaction mixture was nitrogen purged and then maintained for 8 h at 75° C under continuous stirring. The final dispersion was dialyzed in distilled water for 7 days, using cellulose dialysis membranes (molecular weight cut-off: 12,000–14,000), in order to remove the unreacted monomer and initiator.

2.3. Preparation of gold (Au) nanoparticles

Au colloids were prepared by $Na_3C_6O_7{\cdot}2H_2O$ reduction of $HAuCl_4$. 90 ml $HAuCl_4$ 3×10^{-4} M aqueous solution was allowed to boil and then 3.6 ml $Na_3C_6O_7$ 6.8×10^{-2} M aqueous solution was added dropwise under stirring. Following the addition of citrate, the solution began to darken and turn bluish-gray or purple. After approximately 10 min, the reaction was completed and the final colour of solution was a deep wine red. The solution was cooled to room temperature under continuous stirring.

2.4. Synthesis of PCs heterostructured film

The ST–PEGMA 1100 colloidal dispersion was mixed with Au colloidal solution (1:5 volume ratios). The hybrid film (ST–PEGMA 1100–Au) was obtained by gravitational sedimentation of the colloidal mixture and kept at 60 °C for 20 min.

2.5. Surface treatments of PCs heterostructured film

The hybrid film was previously immersed in G4 methanol solution (10%) and kept for 24 h at room temperature. After the treatment, the G4 modified film (ST–PEGMA 1100–Au–G4) was dried at 30 °C for 6 h. In the next step, BSA solution was dropwise added on the surface of the film. The resulting film (ST–PEGMA 1100–Au–G4–BSA) was kept at 24 °C for 24 h.

2.6. Characterization

The morphologies of polymer particles were investigated through scanning electron microscopy (SEM) using a Philips XL-30-ESEM TMP microscope. The samples were sputtered with a thin layer of gold prior to imaging. The particles size measurement through dynamic light scattering (DLS) and the *Z* potential were obtained with a Nani ZS device (red badge). Transmission