Size- and distance-dependent excitation energy transfer in fluorophore conjugated block copolymer – gold nanoparticle systems

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ABSTRACT

In the present work, we have demonstrated size- and distance-dependent excitation energy transfer (EET) phenomenon from polymer-bound fluorophores to gold nanoparticles (GNPs) conjugated to the same polymer. Anthracene labeled two block copolymers of controlled block length were synthesized by reversible-addition fragmentation chain transfer (RAFT) polymerization technique using two-arm dithioester chain transfer agent. The block copolymers were grafted onto the gold nanoparticle (GNP) surfaces by taking advantage of the high affinity of the dithioester end-groups located at the two ends of the polymer chains for the gold surface. GNPs of two different sizes, 20 nm and 55 nm, were used for the present study. The bare and polymer-bound GNPs were characterized by UV–vis spectroscopy, transmission electron microscopy and dynamic light scattering measurements. The EET process was monitored through steady-state and time-resolved fluorescence spectroscopic study. Thus we have successfully synthesized fluorophore labeled well-defined triblock copolymers with tailored architectures and subsequently anchored them with GNPs of different sized that enabled us to control the energy transfer process between GNPs and fluorescent polymer.

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1. Introduction

There has been a growing research interest in gold nanoparticles (GNPs) owing to their unique properties and potential applications in several areas like biomedical materials, optics, and electronics [1]. Thiol chemistry has widely been exploited to modify the surface of AuNPs with synthetic polymers [2–4] and biomacromolecules [5–7]. The affinity of the diothioester end-groups for the gold surfaces have been utilized to link well-defined polymers (synthesized via RAFT polymerization method [8]) to gold surfaces [9–13], as demonstrated by McCormick and T. P. Davis et al. Eeisenberg et al. incorporated GNPs into the central of vesicle wall and core of micelle derived from block copolymers of PS-*b*-PAA [14,15].

Excitation energy transfer (EET) from a donor molecule to an acceptor molecule is a very well-known natural process, the most common example being the highly efficient light harvesting system involved in photosynthesis. The EET process involves a non-radiative energy transfer from an electronically excited state of a donor molecule (D*) to the ground state of an acceptor molecule (A) [16]. During the EET process, the total energy of the donor (D) – acceptor (A) system is conserved, and hence, often referred as resonance energy transfer (RET). Experimentally, RET is mostly detected either via decrease/increase in the fluorescence intensity of the donor/acceptor, depending on whichever is fluorescent. Thus, this technique is also popularly known as fluorescence resonance energy transfer (FRET) [16].

In 1948, Förster [17,18] proposed an elegant theory for the rate of EET, that predicted that the rate of EET followed a distance dependence of $1/R^6$ – type, where R is the center-to-center distance between D and A. Förster theory also predicts that the efficiency of ET strongly depends on R and the relative orientation of the participating D and A molecules. This dependence of FRET rate on the distance between the donor and the acceptor has been utilized to develop sensors for various biologically relevant analytes like CO_2 [19], glucose [20,21], and metal ions [22,23]. Moreover, on attaching a suitable D–A pair (generally dye molecules) to a macromolecule, valuable information on the configuration and conformations of the macromolecule can be obtained from the rate of FRET [16]. Förster theory works better when small dye molecules are used in a D–A pair, except for small separation distances. However, use of small dye molecules both as donors and acceptors,

limits the range of distances that can be probed by EET to less than 10 nm [16], which is indeed small when conformations and dynamics of long and complex polymer molecules are being investigated.

In recent years, surface energy transfer (SET) between dye molecule and metal nanoparticles has gained interest because this technique double the range of separations than FRET which would help to understand the large scale conformational dynamics of complex biomolecules in macroscopic detail [24–29]. Persson-Lang [30], Silbey [31], and Gersten-Nitzen [32] have already demonstrated the mechanism of dye quenching at a metal (e.g., gold, silver) surface and the separation of donor and acceptor is d^{-4} dependence. Therefore, development of new polymer-GNP systems

would not only open up possibilities of new applications, also provide newer systems to study as well as controlling the EET process.

In this work, new polymer-GNP systems were designed and prepared. We studied the EET process using polymer-bound anthracene as donor (D) and polymer-bound GNPs of different sizes as acceptor (A). Size and distance-dependent EET process from anthracene to GNPs was demonstrated by the help of steady state and time resolved fluorescence spectroscopic studies.

2. Experimental section

2.1. Materials

t-Butyl acrylate, styrene, 9-anthracene methanol, 4-dimethylaminopyridine (DMAP), dicyclohexylcarbodiimide (DCC), and gold(III) chloride trihydrate (HAuCl$_4$·3H$_2$O) were purchased from Sigma−Aldrich and used without further purification. α,α'-Xylyl-bis(dithiobenzoate) (XBDTB) was synthesized according to the literature reported procedure [33,34]. Trisodium salt of citrate, and trifluoroacetic acid (TFA) were purchased from Sisco Research Laboratories Pvt. Ltd, India, and used as received. 2,2′- Azobis-isobutyronitrile (AIBN, Sigma−Aldrich) was recrystallized twice from methanol before use. MilliQ water was used in all the experiments.

2.2. Synthesis of anthracene conjugated polystyrene-block-Poly(acrylic acid)-block-polystyrene (**P1**, Scheme 1a)

Synthesis of **P1** was consists of the following steps.

#### 2.2.1. Synthesis of Poly(t-Butyl Acrylate) Macro-CTA
α,α'-Xylyl-bis(dithiobenzoate) (XBDTB) (0.16 g, 0.39 mmol) and AIBN (0.012 g, 0.073 mmol) were added to a 50 mL round bottomed flask. t-Butyl acrylate (5.0 g, 39.06 mmol) was then added to it, followed by addition of 4.0 mL of dry DMF. The reaction vessel was then degassed, purged with nitrogen and then placed in an oil bath at 70 °C. The polymerization was continued for 6 h, and the product was recovered by precipitation from ice cold MeOH/H$_2$O (2:1). M$_n$ (^{1}H NMR) = 5600, M$_n$ (GPC) = 7100, PDI = 1.3.

2.2.2. Synthesis of polystyrene-block-Poly(t-butyl acrylate)-block-polystyrene
Poly(t-butyl acrylate) macro-CTA (1.0 g, 0.180 mmol) and AIBN (7.3 mg, 0.047 mmol) were added to a 30 ml tube. Styrene (3.7 g, 36.0 mmol) was then added followed by addition of 3.0 ml of dry DMF. The reaction vessel was then degassed thrice, purged with nitrogen and allowed to warm to room temperature. The reaction mixture was then placed in an oil bath at 70 °C with vigorous stirring for 16 h. Thereafter, the polymerization was quenched, product diluted with minimal amount of THF and precipitated from ice-cold hexane. The polymer was isolated by filtration and washed several times with hexane and then dried under high vacuum. Poly(t-butyl acrylate)-*block*-polystyrene was obtained as pale pink solid (54% yield). Mn (^{1}H NMR) = 9800, Mn (GPC) = 11,800, PDI = 1.45.

2.2.3. Hydrolysis of t-butylacrylate groups of polystyrene-b-Poly(t-butylacrylate)-b-polystyrene
Polystyrene-b-poly(t-butyl acrylate)-b-polystyrene (0.5 g, 0.048 mmol with respect to t-butyl acrylate) was dissolved in 5 mL dry DCM, stirred for 15 min followed by addition of trifluoroacetic acid (TFA, 1.5 mL). The reaction mixture was stirred for 16 h at room temperature. Then the solvent was removed in rotary evaporator and TFA was completely removed by azeotropic distillation using
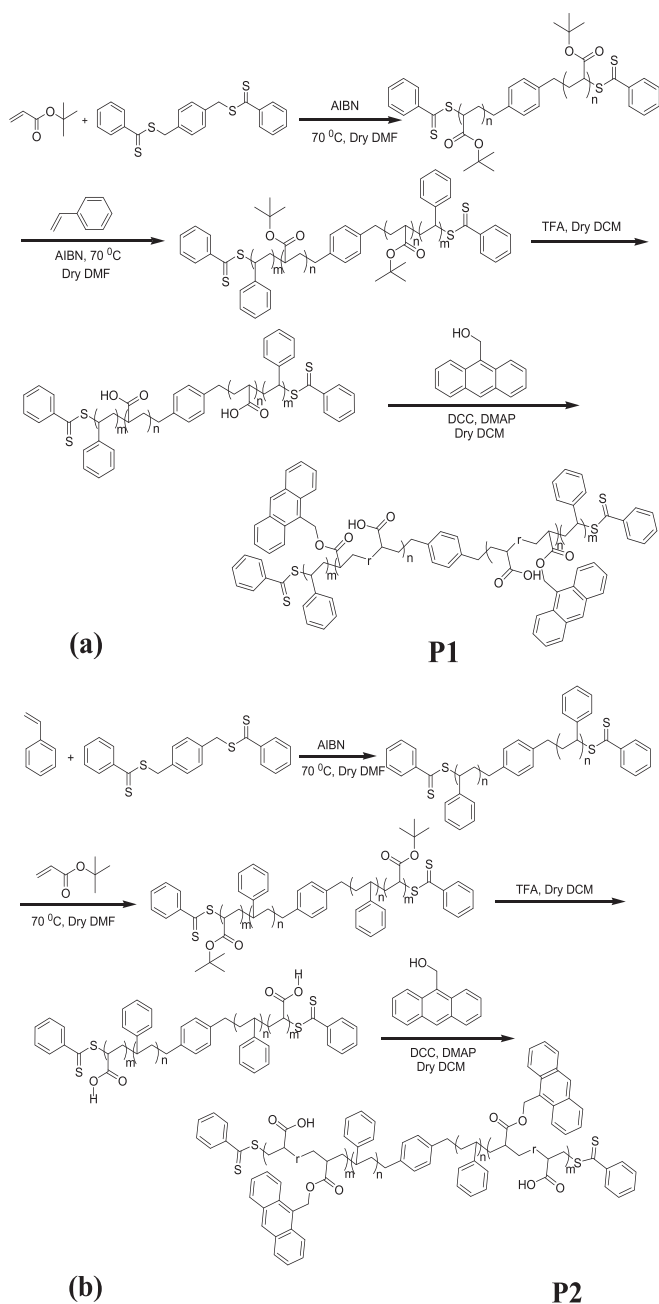
Scheme 1. Synthetic strategy of (a) anthracene containing polystyrene-*block*-poly(-acrylic acid)-*block*-polystyrene (**P1**) − and (b) anthracene containing poly(acrylic acid)-*block*-polystyrene-*block*- poly(acrylic acid) (**P2**).