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# Fabrication of a new fluorescent polymeric nanoparticle containing naphthalimide and investigation on its interaction with bovine serum albumin

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ABSTRACT

A new fluorescent nanoparticle was synthesized by chemical crosslinking of polyacrylic acid using 4-aminoethanol-N-hydroxyethanyl-1,8-naphthalimide as the crosslinker. The particle possesses excellent characteristics, including low cytotoxicity, water solubility and good fluorescence properties. The interaction of the particle with bovine serum albumin was investigated by absorbance, fluorescence and circular dichroism spectroscopic methods. The interaction mechanisms, binding model and reciprocal effects on structure and fluorescence between nanoparticle and protein are discussed. The spectral data indicated that the nanoparticle could spontaneously form a reversible complex with bovine serum albumin in solution used mainly by hydrogen bonds and van der Waals forces. Fluorescence resonance energy transfer from tryptophan to naphthalimide in the complex was demonstrated and employed to determine their relative separation distance. During the interaction process, unfolding of polypeptide chains in the protein occurred but no adjustments of local polarities around the tryptophan and tyrosine residues were observed. In addition, it was found that the presence of protein induced a notable enhancement in nanoparticle fluorescence and a blue-shift in the emission maximum. These results will be useful for further applications of the developed nanoparticle in biomedical areas.



## 1. Introduction

Fluorescent polymeric nanoparticles (FPNPs) have attracted great interest in biomedical fields for diagnostics, bioimaging and drug delivery [1–6]. To date, extensive research has been carried out in preparing FPNPs from hydrophobic polymers such as poly(lactic-co-glycolic acid), poly(ε-caprolactone) and poly(methyl methacrylate) derivatives [5]. However, applications of these nanoparticles (NPs) have been limited because their lipophilic surfaces made them easily opsonizable by the mononuclear phagocyte system, which results in difficulty delivering the particles to the target site of interest [5,6]. An improvement in NP hydrophilicity could contribute to addressing this drawback [7–9]. Thus, the development of hydrophilic FPNPs is significant to extend the realistic applications of FPNPs.

Protein–NP interactions have proven to play an important role in biological applications of NPs [10,11]. Advances in this area may help to understand NP transport processes in biological systems and reciprocal effects on structures and properties between NPs and proteins during the interaction process, which are beneficial to develop safe bionanomaterials or to validate in vivo bioimaging results with fluorescent NPs. Spectroscopic methods involving fluorescence, absorbance and circular dichroism (CD) techniques are powerful tools to investigate protein–NP interactions. In the past, these methods were frequently applied to probe the binding of proteins with various NPs, including gold [12], ZnO [13], cholesterol-grafted O-carboxymethyl chitosan [14], magnetic [15] and CdS NPs [16].

In our work, a water-soluble FPNP known as ANPAA-NP was created using polyacrylic acid (PAA) and a naphthalimide compound as precursors. The adoption of PAA was based on its good water solubility, biocompatibility and abundant carboxyl groups ready for modification [17,18]. Naphthalimide fluorophores have good fluorescence properties such as photostability, large Stokes shifts and high fluorescence quantum yields. They have been employed to develop several fluorescent probes for bioimaging [19,20]. Therefore, a dual-hydroxyl naphthalimide derivative, 4-aminoethanol-N-hydroxyethanyl-1,8-naphthalimide (AHN), was used as the fluorescent labeling reagent. In addition, AHN also acted as the crosslinker for chemical crosslinking of PAA to simultaneously achieve nanoparticle formation and fluorescence labeling.

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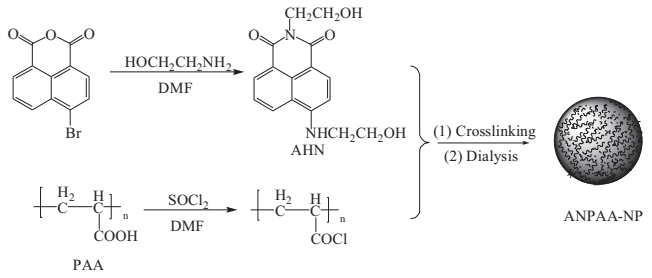


**Fig. 1.** The procedure to prepare ANPAA-NP.

The structure, morphology, fluorescence properties and cytotoxicity of ANPAA-NP were explored. Moreover, the interaction between ANPAA-NP and protein was investigated using spectroscopic methods. In the experiments, bovine serum albumin (BSA) was selected as the protein model due to its medical importance, low cost and homology with human serum albumin. The binding information, BSA conformational change and effect of BSA on ANPAA-NP during the interaction process are discussed. These results will be useful for further applications of ANPAA-NPs in biomedical areas.

## 2. Experimental

### 2.1. Materials

4-bromo-1,8-naphthalic anhydride (≥98.0%) was obtained from Huifeng Chemicals Co. (Anshan, China), and PAA (Mw = 2.6 kDa) was purchased from Kaite Chemical Co. (Zhengzhou, China). BSA from Sigma was dissolved in water to produce a stock solution at 2.0 mg mL$^{-1}$. The stock solutions of BSA and ANPAA-NP (1.0 mg mL$^{-1}$ in water) were stored at 4 °C and appropriately diluted to obtain the working solutions. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma was dissolved in PBS to a concentration of 5.0 mg mL$^{-1}$ and filtered through a 0.2 μm filter for use. All other reagents were of analytical-reagent grade and were utilized without further purification. Millipore-Q water was used throughout.

### 2.2. Preparation and characteristics of ANPAA-NP

Fig. 1 illustrates the method to prepare ANPAA-NP. 4-bromo-1,8-naphthalic anhydride was treated with aminoethanol to produce AHN. Poly(acryloyl chloride) prepared from PAA was reacted with AHN for 5 h at room temperature. The final reaction mixture was dialyzed against water to purify the product. The purified ANPAA-NP solution was lyophilized and easily re-dissolved in water to a concentration of 1.0 mg mL$^{-1}$. The ANPAA-NPs were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS) and infrared (IR), absorbance and fluorescence spectrometry. The degree of substitution (DS) of the naphthalimide group was measured by an absorbance spectroscopic method with a calibration curve obtained using standard solutions of AHN.

The detailed process is provided in the Supplementary Data (Part S1).

### 2.3. MTT assay

In a 96-well plate, HepG2 cells (40–50% confluence) were incubated in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum and various quantities of ANPAA-NPs at 37 °C (5% $CO_2$) for 48 h. Subsequently, MTT (20 μL/well) was added and the cells were further incubated for 4 h. The medium was replaced with DMSO (150 μL/well), and the cells underwent gentle shaking for 10 min. Then, the optical densities (OD) of the wells were determined at 490 nm using a microplate reader (KHB ST-360, Shanghai Kehua Laboratory System Co., China), and the percentages of viable cells were calculated.

### 2.4. Interaction of ANPAA-NP with BSA

ANPAA-NP solution, 2.0 mL of phosphate buffers (pH 7.4) and appropriate concentrations of BSA solution were added to test tubes and thoroughly mixed. Then, the solutions were diluted to 5.0 mL with water and incubated for 10 min before being measured for fluorescence, absorbance and CD spectra. The slit widths were set to 5.0 nm and 2.5 nm for the fluorescence excitation and emission spectra, respectively.

## 3. Results and discussion

### 3.1. Characteristics of ANPAA-NPs

The absorbance spectra revealed that PAA had no absorbance peak in the visible light range while both ANPAA-NP and AHN had absorption peaks at 444 nm. In the IR spectrum of ANPAA-NP, a peak at 1577 cm$^{-1}$ appeared corresponding to the stretching vibration of the naphthalimide moiety. Because of the prior removal of free AHN by dialysis, these results proved that naphthalimide fluorophores were immobilized within the PAA matrices. DS of naphthalimide in ANPAA-NP was calculated to be 1.13%, which suggests that numerous carboxyl groups still exist in the structure of ANPAA-NP.

ANPAA-NP had a fluorescence emission wavelength ($\lambda_{em}$) of 544 nm with a maximum excitation wavelength ($\lambda_{ex}$) of 444 nm. Having an emission wavelength $\lambda_{em}$ longer than 500 nm is useful to minimize the interference from background fluorescence ($\lambda_{em}$ < 500 nm). In addition, the exceptionally large Stokes shift of 100 nm resulted in a small overlap between the absorption and emission spectra, which efficiently reduces the effect of self-absorption.

The TEM images in Fig. 2(a) demonstrate that ANPAA-NPs had spherical morphology. The size distribution of ANPAA-NPs in water was evaluated by DLS. The result in Fig. 2(b) shows that the hydrodynamic diameters of ANPAA-NPs were mainly in the range of 40–140 nm with an average size of 73 nm.

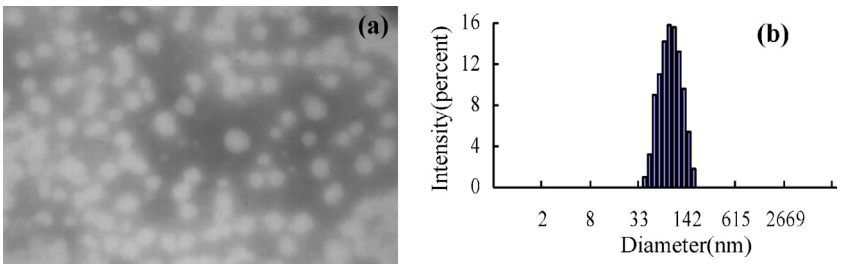


**Fig. 2.** (a) The morphology of ANPAA-NPs measured by TEM and (b) the size distribution of ANPAA-NPs measured by DLS.