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A small, steady, rapid and selective TICT based fluorescent HSA sensor for pre-clinical diagnosis

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ABSTRACT

Quantitative detection of HSA in biological fluids is essential for pre-clinical diagnosis. Herein a TICT based HSA sensor NJUP1 was introduced for detection in healthy and hypertension blood samples. It interacted with domain IIA of HSA to generate a turn-on response. The detection mechanism was convinced by HSA destruction, site competition, molecular docking and isothermal titration calorimetry. NJUP1 suggested advantages such as rapid (response time 5 s), steady (more than 24 h), sensitive (LOD 18.1 nM, up to 200-fold enhancement), selective (~6 fold over 10 times BSA in mass), wide detection range (covering blood samples requirements) and low toxicity, among which several were top class. Most importantly, it possessed only ~300 molecular weight and one connecting double bond. It could be applied in urine system and blood samples. Thus, NJUP1 provided a potential approach for further applications.

1. Introduction

Despite measuring small signaling and functional molecules, recent reporters show increasing interests on detecting various proteins in biological systems [1–3]. Among them, Human Serum Albumin (HSA), the most abundant transport protein in circulatory system [4,5], performs as a vital one in regulating oncotic pressure [6–8] and transporting cargo including nutrients [9,10], metabolites [11,12] and drugs [13–15]. Abnormal HSA concentration is associated with coronary heart diseases [16,17], multiple myeloma [18,19], diabetes mellitus [20–22], kidney diseases [23–26], neurometabolic disorders [27,28] and liver cirrhosis [29–32], thus considered as a potential parameter in pre-clinical diagnosis [33,34]. HSA concentration in blood plasma is typically 35–50 mg/mL [35]. Therefore, quantitative detection of HSA in biological fluids including blood serum has become an emergency for preventive medicine and therapy [36,37].

Until now, basis of HSA detedting methods include proteomic techniques [38], radioimmunoassay [39], electrochemistry [40–43] and fluorescence [44–46]. Fluorescence methods show advantages such as free of expensive equipments or complicate preparation, satisfactory sensitivity and capability [47]. Common fluorescence HSA sensors rely on mechanism including Green Fluorescent Protein mimic strategy [48], nanoparticle-based enhancement [49], dye-binding imaging [50] and site-binding interactions [51,52]. Among them, binding certain site of HSA provides superior convenience and selectivity over BSA (Bovine Serum Albumin), owing to a usual TICT (Twisted Intra-molecular Charge Transfer) mechanism. However, they still face some drawbacks such as interferences by polarity [53] or viscosity [54]. Moreover, the probes are usually positive charged salts with ~500 molecular weight and conjugated double bonds [55,56], casuing undesirable biocompatibility and randomicity.

In this work, we provided a small, steady, rapid and selective HSA sensor **NJUP1** based on TICT-related mechanism. Possessing fine sensitivity and selectivity, this sensor reduced the interferences of polarity and viscosity of media. Detecting mechanism was convinced stepwisely through HSA destruction, site competition and molecular docking. Most importantly, **NJUP1** possessed only ~300 molecular weight and one connecting double bond, being a relative amphiphilic molecule instead of positive charged salts. It maintained favorable biocompatibility and detecting steadiness. Being low toxic on cells, **NJUP1** was then applied in urine and blood samples, thus providing a potential approach for pre-clinical applications.

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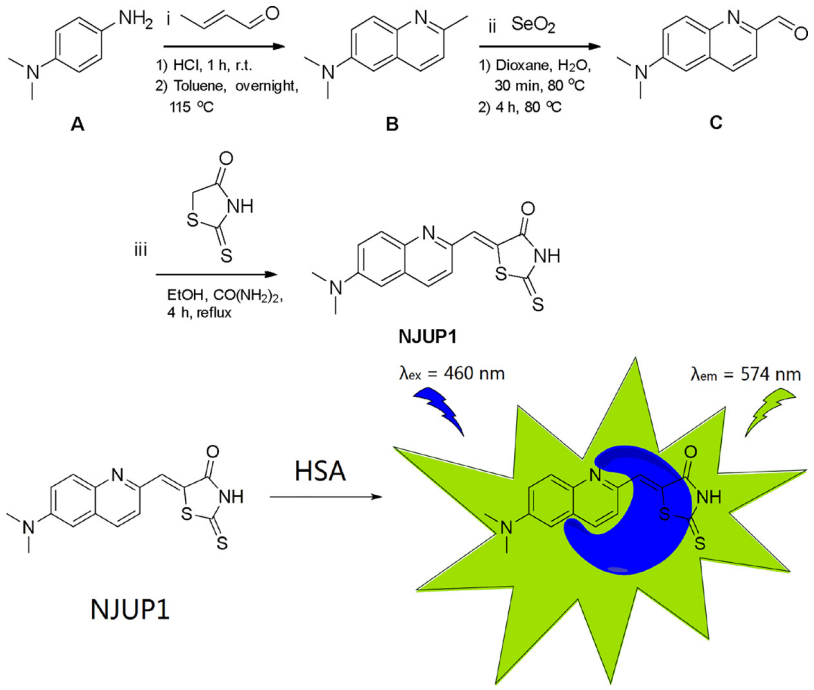

Fig. 1. General synthesis route of **NJUP1** and its detection mechanism into HSA.

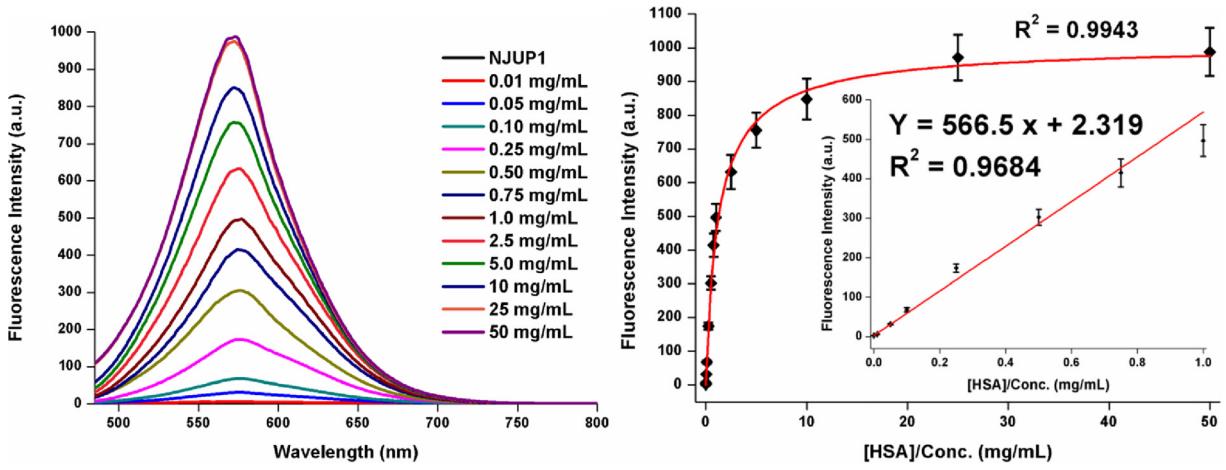

Fig. 2. (Left) Fluorescence emission spectra of **NJUP1** upon addition of increasing concentration of HSA (0–50 mg/mL). The fluorescence intensity enhanced along with the increase of HSA concentration; (Right) The logistic fitting in the range of 0–50 mg/mL; (Right-inner) The linear correlation in the range of 0–1.0 mg/mL.

2. Experimental

2.1. Materials and methods

All commercially available chemicals were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-600 spectrometer. Mass spectra were from Agilent 6540 UHD Accurate Mass Q-TOF LC/MS. PHS-25 pH-meter was used for pH measurement. UV–vis spectra were recorded on Shimadzu UV-2550 spectrometer. Fluorescence measurements were obtained on Hitachi Fluorescence Spectrophotometer F-7000. Cell viability were performed using HeLa (human cancer cell line) and LO_2 (human embryonic liver cell line) by MTT method. The absorbance was measured at 490 nm on an ELISA microplate reader.

The chloride salt dissolved in distilled water for metal ions (1 mM) except Pb^{2+} (from $Pb(OAc)_2$) and Ag^{+} (from $AgNO_3$). Anions were prepared from their sodium or potassium salt except H_2O_2. Other concentrations were obtained by dilution. **NJUP1** was dissolved in DMSO to get the solution of 1.0 mM. The final concentration of **NJUP1** was settled at 10 μM with 5% DMSO in PBS buffer (10 mM, pH 7.4). In selectivity experiments, HSA was settled at 0.5 mg/mL, other proteins and saccharides were at 5 mg/mL. Hemocyte resuspension was at physiological concentration. The tested amino acids, anions and metallic ions were settled at 0.5 mM. The excitation wavelength was 460 nm. Both excitation and emission slit widths were 5 nm. The photomultiplier voltage was 500 V. After joining substrate for 30 min, we started measuring. The emission spectrum was scanned from 480 nm to 800 nm at 1200 nm/min.

2.2. Determination of the fluorescence quantum yield

Participation ratio method was recruited with formula: $\Phi_u = [(A_sF_un^2)/(A_uF_sn_0^2)]\Phi_s$. Herein, $\Phi_s = 0.69$, $A_s = 0.003$, $F_s = 10.197$, $n_0 = 0.914$; $A_u = 0.015$, $F_u = 7.025$, $n = 1.480$; $\Phi_u = 0.27$.