Ultrasensitive Detection of 2,4-D at Surface of a Hybrid Silica Nanoparticle Based on Photoinduced-Electron Transfer Enhanced Fluorescence†

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This paper reports the ultrasensitive detection 2,4-D at the surface of hybrid silica nanoparticles through the enhanced-fluorescence intensity based on photoinduced-electon transfer mechanism. Nitrobenzoxadiazole (NBD) fluorophore and organic amine were covalently modified onto the surface of silica nanoparticles to form a hybrid monolayer of dye fluorophores and amine ligands. Photoinduced electron transfer (PET) between nitrobenzoxadiazole and free amine at the spatial approach occurred at the surface of hybrid silica nanoparticles and the fluorescence intensity showed a suitable decrease by comparison of the original intensity of ligands APTS-NBD formed between amine and nitrobenzoxadiazole. The presence of 2,4-D leads to fluorescence enhancement due to inhibition PET between amine and fluorophores. In addition, these nanoparticles can be developed into chips by assembling on arrays onto silicon wafer for detection of proton.

Key Words: Photoinduced-energy transfer, Silica nanoparticles, Ultratrace detection; 2,4-D.

INTRODUCTION

Fluorescent systems, which are capable of sensing various chemically, environmentally, biologically significant species and potential application as molecular switches or devices in information processing, are of great current interest[1-13]. Amine, as one of the simplest receptors, has been widely used in photoinduced electron transfer system[1]. A nitrogen lone pair electron in amino group quenches a nearby fluorophore through nitrogen atom-to fluorophore electron transfer in absence of proton/metal ion. Upon protonation of the amines or coordination with metals, a dramatic increase of the fluorescence intensity results as PET quenching is deactivated. We naturally follow this idea that whether PET system can be used to detect 2,4-D by fluorescence enhancement while show fluorescence enhancement to proton. Normally, PET system has shown low fluorescence[14], which is hard to observe its fluorescence enhancement to 2,4-D. The PET process occurs more efficiently as the spacer connecting the donor and the acceptor becomes approach, which means that fluorescence intensity can be controlled by distance between donor and acceptor. In order to obtain suitable fluorescence intensity, we control the distance between nitrobenzoxadiazole and APTS by modifying them at the surface of silica. Fluorescent dye

molecules together with primary amine were covalently anchored onto the surface of silica nanoparticles, allowing the formation of a hybrid organic monolayer [dye-(NH_2)-silica]. Such sensors based on silica nanoparticles offers access to assemble on arrays and allows real-time visualization in micron-scale fields of importance in detection.

Herein we present the novel fluorescent silica nanoparticles, which possess both free amine as a receptor module responsible for the binding of the analyte and nitrobenzoxadiazole as a signaling unit involving in light absorption and emission. Remarkably, NBD-(NH_2)-silica nanoparticles shows a controllable fluorescent behaivour as a result of competitive coordination of proton. Proton will increase the fluorescence intensity when coordinated to nitrogen of free amine as this coordination will stop the photo induced electron transfer from N atom to nitrobenzoxadiazole molecule.

EXPERIMENTAL

Synthesis of different ratio dye-(NH_2)-silica: Uniform silica nanoparticles with a size of 150 nm were synthesized by the hydrolysis of TEOS with aqueous ammonia. To prepare the silica nanoparticles with a hybrid monolayer of dye and amine ligand [dye-(NH_2)-silica], the dye fluorophores with coupling groups were first conjugated with APTS by an addi-

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tion reaction of amine group with amine-reactive NBD-Cl. The reaction was carried out in dark for at least 12 h by slowly stirring the solution containing the dye and different ratio of APTS in anhydrous ethanol. After the product was separated by centrifugation and washing with ethanol, NBD-(NH₂)-silica nanoparticles with a hybrid monolayer of nitrobenzoxadiazole dye and free amine ligands at surface were finally obtained and then redispersed in ethanol for further use.

**Measurements of fluorescence response to analytes:** The concentration of stock solution of dye-(NH₂)-silica nanoparticles for the measurements of fluorescence quenching was 17.1 mg/mL in ethanol. Typically, 30 µL of the stock solution was added to a spectrophotometer quartz cuvette. Subsequently, 3 mL of the known concentration of analyte in ethanol was injected into the above cuvette. The final amount of nanoparticles was about 0.17 mg of particles in 3 mL of solvent. Fluorescence spectra were recorded immediately after fully mixing the analyte with dye-(NH₂)-silica nanoparticles in solution. Meanwhile, the fluorescence responses of dye-silica nanoparticles and pure dyes to 2,4-D were also measured by the identical procedure.

RESULTS AND DISCUSSION

Synthesis of amine and nitrobenzoxadiazole hybrid monolayer at the surface of silica nanoparticles: The silica nanopartilces with a hybrid monolayer of nitrobenzoxadiazole dye and amine ligands were prepared by the procedure described in the experimental section. **Scheme-I** illustrates the synthetic procedure and chemical structure of NBD-(NH₂)-silica. PET happened between the nitrogen atom of free amino and nitrobenzoxadiazole fluorophore, which is similar to normally PET process. The quenching efficiency by the PET

process can be controlled by modifying the distance between acceptor and donor. Due to the distance between primary amine and nitrobenzoxadiazole, PET form nitrogen atom of amine to nitrobenzoxadiazole is not efficient, which results in the silica nanoparticles shows mediate fluorescence intensity.

The SEM image of Fig. 1A shows that the silica nanoparticles are still spherical and monodisperse and the surface of particles is very smooth after the chemical modification. The absorption and fluorescence spectra of NBD-(NH₂)-silica in ethanol are shown in part B of Fig. 1. The emission peak of NBD-(NH₂)-silica nanoparticles are slightly red-shifted with respect to the emission peak of pure APTS-NBD. This bathochromic shift of the fluorescence band maximum of NBD-(NH₂)-silica relative to that of NBD-APTS is suggestive of the photoinduced electron transfer between fluorescne and free amino of APTS.
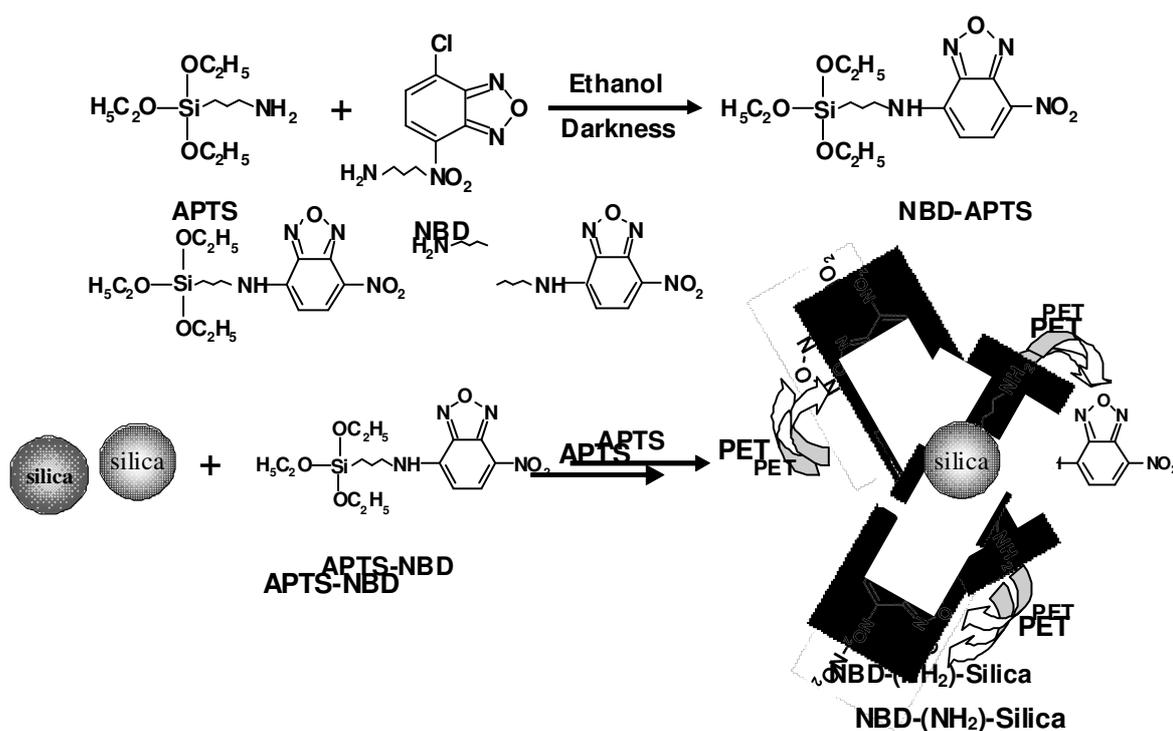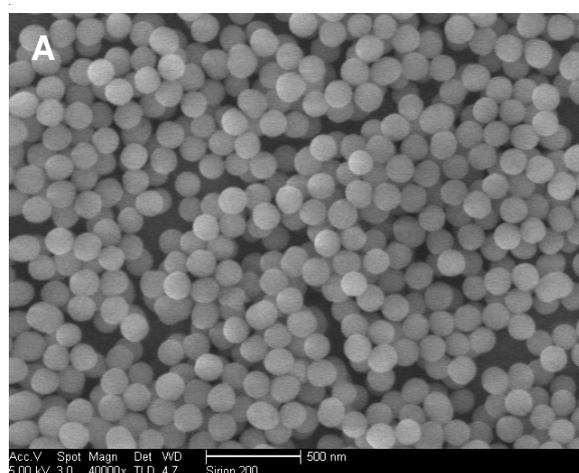
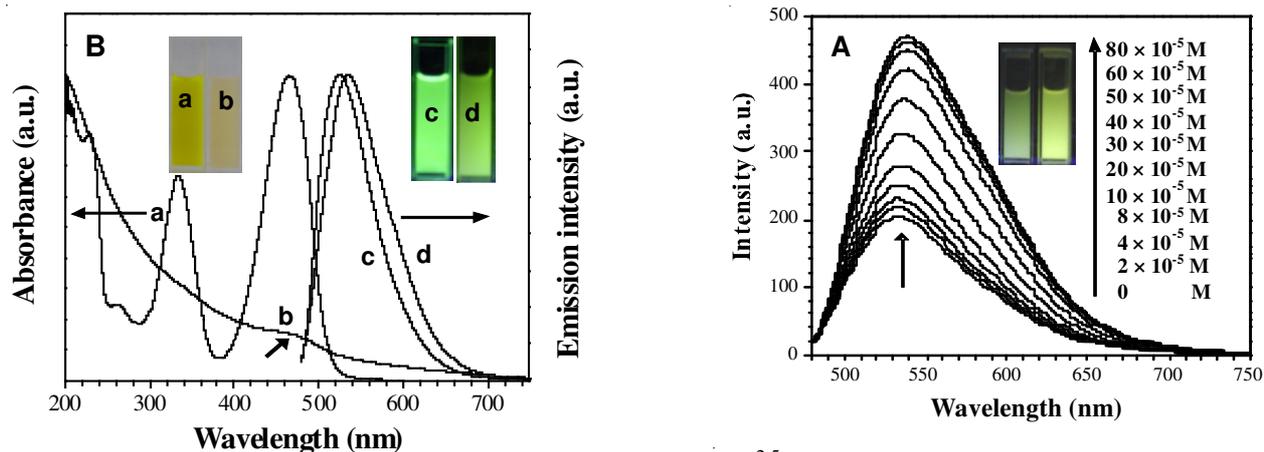**Scheme-I:** Schematic illustration for the synthesis of NBD-APTS and NBD-(NH₂)-silica

Fig. 1. (A) SEM image of dye-(NH$_2$)-silica nanoparticles. (B) The absorption and emission spectra of (a, c) NBD-APTS and (b,d) NBD-(NH$_2$)-silica nanoparticles solution, normalized at their respective peak positions, in ethanol. The inset shows the optical images of NBD-APTS and NBD-(NH$_2$)-silica particles solution under natural light (left) and under 360 nm UV lamp (right), respectively

Fluorescence responses of NBD-(NH$_2$)-silica to proton: Nitrobenzoxadiazole showed decrease fluorescence intensities with increase in solvent polarity. In our experiment, we choose ethanol as solvent ignoring the effect of solvent. Part A of Fig. 2 shows the fluorescence responses of NBD-(NH$_2$)-silica to 2,4-D in ethanol. The fluorescence intensities increase with adding successive aliquots of 2,4-D concentrations in the system, which can be ascribed to the proton coordinate with nitrogen of primary amine at the surface of silica nanoparticles stop the process of PET between nitrogen atom and nitrobenzoxadiazole. When APTS and NBD-APTS were grafted at the surface of silica nanoparticles, fluorescence of nitrobenzoxadiazole was quenched by the nitrogen of free primary in the form of PET. However, the fluorescence would recover when PET process was stopped through proton coordinating with nitrogen atom. Coordination of proton with the nitrogen atom can effectively decrease its electron-donating ability, so the quenching PET process form the nitrogen atoms to the nitrobenzoxadiazole is inhibited, which results in a large increase in fluorescence intensity at *ca.* 535 nm. An enhancement factor (EF, I/I$_0$) of 2.4 at 535 nm was observed in the presence of proton indicated that the PET quenching pathway is efficiently blocked by protonation of free amine at the surface of silica nanoparticles. In order to identify whether this reference was true, we modified with different free amine at the surface of silica. Curves of Fig. 2B shows that the fluorescence increasing percentage of different ratio of amine to nitrobenzoxadiazole at the surface of silica as a function of HCl. Through titration with HCl, the different ratios of amino to nitrobenzoxadiazole show various fluorescence increasing degree. It was clearly detected that the fluorescence increasing of nanoparticles contain more free amine relative to nitrobenzoxadiazole is much larger than that of less free amine. Moreover, pure NBD-APTS only exhibits an extremely slight fluorescence increasing with HCl analyte and the increasing efficiency of pure APTS-NBD dye is the lowest in the four different ratio of amine to nitrobenzoxadiazole systems. These measurements confirm that the PET process happened between the free amine
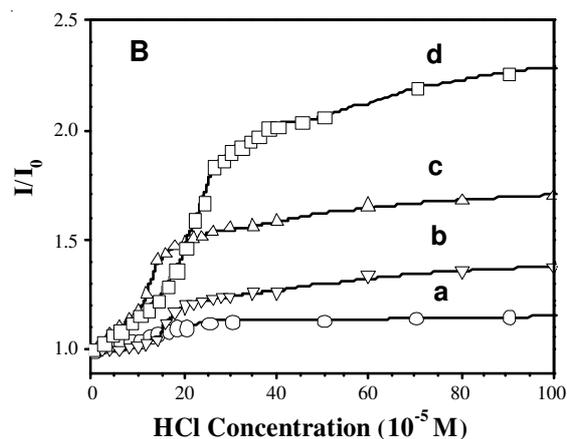

Fig. 2. (A) Evolution of fluorescence spectra of NBD-(NH$_2$)-silica with increasing of 2,4-D concentration. (The insert shows the fluorescence NBD-(NH$_2$)-silica before and after addition of 2,4-D, sample were excited at 360 nm using UV lamp). (B) Fluorescence spectra of NBD-(NH$_2$)-silica with different free amine amounts (a) pure NBD-APTS, (b) NBD:APTS = 3:1, (c) NBD:APTS = 1:10, (d) NBD:APTS = 1:40 upon titration with HCl

and nitrobenzoxadiazole fluorophore at the surface of silica nanoparticles. In the case of NBD-(NH$_2$)-silica with higher ratio of free amine, the chance of electron transfer between the nitrogen atom and nitrobenzoxadiazole will be enlarged, as described in **Scheme-I**. Therefore, NBD-(NH$_2$)-silica nanoparticles with more free amine has more chance to adsorb proton, leading to a higher fluorescence increasing percentage.

Nanoparticles-assembled chips for detection of 2,4-D: Meanwhile, we develop the inexpensive solid-state chips for detection of 2,4-D by assembling the NBD-(NH$_2$)-silica nanoparticles into the etched microwell array of silicon wafer. When the ethanol suspension of NBD-(NH$_2$)-silica nanoparticles were cast onto the silicon chip with etched microwells of 5 μm in side length and 2 μm in depth and dried under ambient condition, the microwells were spontaneously filled up with the silica particles. Fig. 3 shows the colourful fluorescence images under laser excitation on a confocal microscope. The fluorescent dot array is highly regular and extremely bright and all microwells are completely filled with the fluorescent silica particles. The evolution of the brightness of fluorescent dots are clearly observed with dropping only 10 μL of 2,4-D solution of different concentrations onto the 1 cm × 1 cm sized chip. As is shown in Fig. 3, the brightness of fluorescent dots become larger and larger and ultimately super bright with
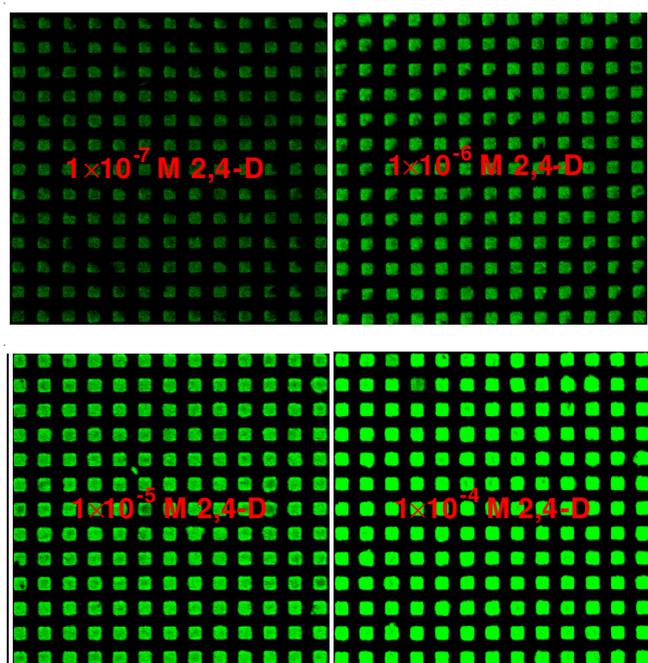
Fig. 3. Regular array assembly of NBD-(NH_2)-silica nanoparticles on the silicon wafer with etched microwells. Confocal fluorescence images show the evolution of the brightness and size of fluorescent dots with dropping 10 µL of analyte solution of different concentrations

increasing 2,4-D concentration from 1×10^{-7}-10^{-4} M. Furthermore, one of the main advantages of the detection chips is that less amount of sample is needed for detection, due to the collective effect of particle assembly in the microwells. Therefore, the microchips can be used as a convenient indicator of 2,4-D residues.

Conclusion

This paper demonstrates that a hybrid monolayer of nitrobenzoxadiazole fluorophores and free amine ligands at the surface of silica nanoparticles can be used as dual fluorescence switch showing the enhanced fluorescence intensity to proton. The primary amines quench nitrobenzoxadiazole fluorescence due to suitable photoinduced electron transfer. "OFF-ON" was observed due to proton block PET process when proton was added to suspension of nanoparticles. To the best of our knowledge, this is the first reported example of a single sensor with one receptor and one fluorophore capable of showing dual fluorescence switch. Meanwhile, the dual fluorescence switch can be repeated used by washed with proper ligands. The principle may also be extended to other receptors to improve their selectivity and could potentially lead to a new generation of optical sensors.

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