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Sensing of Hg^{+2} and Ag^{+} through a pH dependent FRET system: Fabrication of molecular logic gates

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ABSTRACT

The fluorescence resonance energy transfer (FRET) from the donor 6,7-dimethoxy-2,4[1*H*,3*H*]-quinazolinedione (**Q**) to the acceptor lumichrome (**L**) exhibits strong pH dependency and in aqueous solution at pH 9 it is used to sense Hg^{+2} or Ag^{+} ions, selectively. **QL** hybrid forms organic nanoparticles through self-assembly at pH 9 as evident from TEM micrographs. The **QL** system can sense Hg^{+2} or Ag^{+} ion in aqueous medium at pH9 at a nanomolar concentration range using FRET pathway via complexation and nanoparticle formation, respectively. In the **QL** system, the ions can be easily distinguished with the help of UV–vis spectroscopic study or through the naked eye. We have successfully utilized the above metal ions for the fabrication of NOR and INHIBIT molecular logic gates using FRET of **QL** system as a signal transducer and Hg^{+2}, Ag^{+} ion and pH as chemical activators.

1. Introduction

Heavy metal ion contamination of the atmosphere is a significant global concern due to their toxicity to the human health. Mercury, which can accumulate in vital organs and tissues, is highly toxic and can have deadly effects on living systems. It originates mainly from the oceanic and volcanic emissions, coal-burning power plants, gold mining, waste combustion and different industrials waste [1]. Methyl mercury, the most toxic form of Hg^{+2}, is produced from the microbial bio-methylation of Hg^{+2} ions that plays a role of an effective neurotoxin agent which passes through the food chain to the tissues of fish and marine mammals [2,3]. Ag^{+} also has toxicity in the living system [4], so, it is highly necessary to develop a sensitive and selective heavy metal ion detection method that can provide simple, realistic, and high-throughput routine determination of them. Many attempts have been devoted to design the sensing systems for metal ions, including sensors based on organic chromophores [5–7] or fluorophores [8–10], gold nanoparticles [11,12], conjugated polymers [13], semiconductor quantum dots [14], proteins [15], etc. In recent times, fluorescence spectroscopy has become a powerful tool for detecting heavy metal ions in ultra trace quantity due to its high label of sensitivity. Most of the sensors developed for heavy metal ions is based on the conventional fluorescence emission technique either by quenching (turn off) or by enhancement (turn on) of the fluorescence intensity but few are based on fluorescence resonance energy transfer (FRET) [16–21]. FRET involves the non-radiative transfer of excitation energy from an excited donor to a proximal ground-state acceptor and its efficiency depends on several factors such as distance between the donor and acceptor chromophore (1–10 nm), spectral overlap etc. [22]. FRET allows a larger wavelength gap between the excitation and emission wavelengths, so it permits the use of lower resolution devices to measure emission without interference from the excitation source but it lacks in the conventional fluorescence [23,24].

There are many examples of FRET systems constructed from donor acceptor fragments connected through covalent bond in one molecule [25–30]. This type of FRET system has several advantages over a simple mixture of donor acceptor molecules. When the donor and acceptor fragments are present in one molecule linked by covalent bonding the distance between donor and acceptor are fixed and also the orientation of their dipoles is highly specific causing an efficient excited state energy transfer in a given direction in the FRET system. As a result the FRET system is responded greatly by the external analyte. But such molecules are difficult to synthesise and also difficult for processing. For this purpose mixture of the commercially available donor and acceptor ingredients is a good choice. But in a solution containing mixture of donor and acceptor molecules the above requirements of efficient FRET system are not fulfilled. So we have chosen such a mixture of donor

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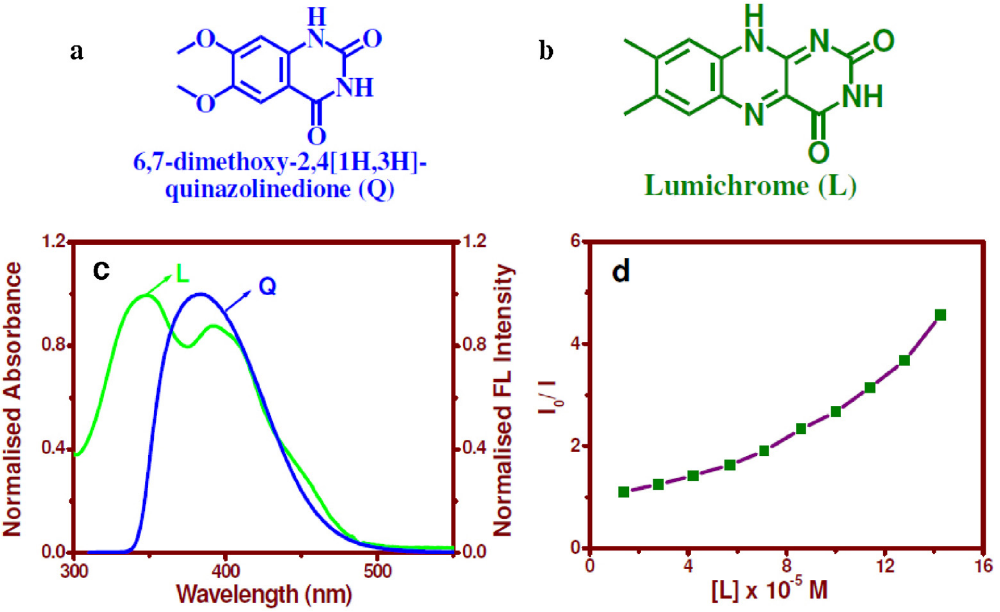

Fig. 1. (a) and (b) Chemical structures of **Q** (donor) and **L** (acceptor). (c) Spectral overlap of the absorption (green) of **L** and emission spectra (blue) of **Q**. (d) Intensity ratio (I_0/I) vs. **L** concentration for emission peak of **Q**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Q) and acceptor (L) system where noncovalent interactions can cause quite a similar order required for efficient FRET emission. Here we report the energy transfer from the donor 6,7-dimethoxy-2,4[1*H*,3*H*]-quinazolinedione (**Q**) to the acceptor lumichrome (**L**) (Fig. 1a and b) in aqueous solution where the noncovalent H-bonding interaction may occur between Q and L. At pH 9 using the FRET property of the mixture we have made a sensing analysis of Hg^{+2} and Ag^{+} at a nanomolar level. With the addition of these two metal ions fluorescence intensity of **L** decreases i.e. FRET is perturbed and it is highly dependent on pH. So, using metal ions and pH as inputs and FRET emission as output we have fabricated NOR and INHIBIT logic gates.

2. Experimental

6,7-Dimethoxy-2, 4[1*H*,3*H*]-quinazolinedione (**Q**) Lumichrome (**L**) and MCl_2 (M = K^+, Na^+, Mg^{+2}, Ca^{+2}, Cu^{+2}, Fe^{+2}, Mn^{+2}, Cd^{+2} Ni^{+2}, Co^{+2} Pb^{+2}, Zn^{+2} Hg^{+2} Ag^{+}) were purchased from Aldrich, USA and were used as received. Ammonium hydroxide was purchased from International Chemical India (Kolkata), and hydrochloric acid (HCl) from Merck Specialities Private Limited (Mumbai). In the preparation of aqueous solution of **Q** a few drops of DMSO were added to increase the solubility of **Q** and for **L** we made the aqueous solution at pH9.

Transmission electron microscopy (TEM) of **QL**-Ag nanoparticale was performed by dropping a small volume of **QL**-Ag nanoparticale solution on a carbon coated copper grid (300 mesh) and was dried in air at room temperature., It was then observed through a TEM instrument (JEOL, model 2010EX) directly under a voltage of 200 kV.

The UV–vis spectra of the samples were recorded with a Hewlett-Packard UV–vis spectrophotometer (model 8453) using a cuvette of 0.1 cm path length. Fluorescence study of all the samples in a sealed cuvette were carried out in a Horiba Jobin Yvon Fluoromax 3 instrument. Each sample in a quartz cell of 1 cm path length was excited at 297 nm wavelength and the emission scans were recorded from 320 nm to 700 nm using excitation slit width of 2 nm and emission slit width of 5 nm with an increment in wavelength of 1 nm having an integration time of 0.1 s. Fluorescence lifetimes were measured by using a time-correlated single photon counting fluoremeter (Fluorecule, Horiba Jobin Yvon). The system was excited with 295 nm radiation (nano LED of Horiba Jobin Yvon having λ_{max} at 368 nm) with pulse duration <200 ps. Average fluorescence lifetimes ($\langle \tau_f \rangle$) were calculated for exponential iterative fitting from the decay times (τ_i) and the relative amplitudes (a_i) using the following relation

$$\left\langle \tau_f \right\rangle = a_1\tau_1 + a_2\tau_2 + a_3\tau_3 \qquad (1)$$

where a_1, a_2, a_3 are relative amplitudes and τ_1, τ_2, τ_3 are the respective lifetimes.

3. Result and discussion

Lumichrome (**L**), a class of nitrogen heterocycles, related to lumazines, is a decomposition product of biologically important flavins and has excellent photophysical properties [31–33]. The absorbance spectrum of pure **L** (pH 9.0) has two peaks at 348 nm and 392 nm (Fig. 1c) corresponding to π–π^* transition and π–π^* transition coupled with n–π^* transition [31], respectively. **Q** has absorption peaks at 258 nm and 321 nm for the π–π^* and n–π^* transitions (Fig. S1) and on excitation at 297 nm it emits blue fluorescence at 384 nm. The emission spectra of **Q** and absorption spectra of **L** strongly overlap (Fig. 1c) and the calculated overlap integral ($J(\lambda)$) is $8.6 \times 10^{14}\ M^{-1}\ cm^{-1}\ nm^4$ (SI). So, an efficient energy transfer from **Q** to **L** may occur.

A gradual decrease of fluorescence intensity of **Q** is observed due to the addition of **L** to the aqueous solution of **Q** (3.3×10^{-4} M) excited at 297 nm indicating FRET from **Q** (donor) to **L** (acceptor) (Fig. S2). To identify whether the quenching takes place only for energy transfer or for ground state complex formation, Stern–Volmer plot (Fig. 1d) is made. It exhibits a curvilinear increase of I_0/I with increase in **L** concentration where I_0 and I are the fluorescence intensity of donor in the absence and presence of a quencher. It can be explained from the Stern–Volmer equations as follows:

$$I_0/I = 1 + k_a[q] \qquad (2)$$

$$I_0/I = 1 + \tau_0 k_q[q] \qquad (3)$$