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COMMUNICATION

Fluorescent Supramolecular Metal Assemblies as ‘No Quenching’ Probe for Detection of Threonine in Nanomolar Range

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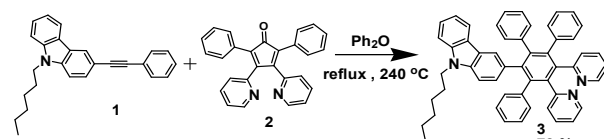
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A highly sensitive detection of threonine is realized using supramolecular ensemble of aggregates of hetero-oligophenylene derivative **3** and Zn^{2+} ions. On the basis of fluorogenic response of aggregates of **3** toward Zn^{2+} ions and threonine, we have constructed a 2-input, 3-output sequential logic circuit at molecular level.

Threonine (**Thr**) is an essential amino acid for human beings and animals as it can promote physical recovery, growth and development^{1a} and is highly effective in preventing stress-induced ulcers.^{1b} **Thr** is also used to treat various nervous system disorders including spinal spasticity,^{1c} multiple sclerosis,^{1d} familial spastic paraparesis,^{1e} and amyotrophic lateral sclerosis^{1f}. However, excess of **Thr** in the body may lead to several side effects such as impairment of intestinal function, headache, nausea and skin rashes.^{1g} Due to the important effects of **Thr** on human health, development of sensitive and fast approaches for its detection is very important. Although, great attention has been paid for the development of new approaches for detection of thiol-containing amino acids² yet development of a method for fast, sensitive and selective detection of **Thr** is still a challenge. Recently, ‘chemosensing ensembles’ have been used as ‘turn on’ sensors for the detection of thiol containing amino acids.^{2b} However, the main disadvantage of this approach is that all the emission changes are centred on same wavelength. However, the chemosensors which exhibit greatly changed emission intensity as well as a shift in the emission band are ideal for sensing.

Our research work involves the development of fluorescent assemblies for selective detection of different types of analytes such as nitroderivatives^{3a,3b} and anions^{3c}. In continuation of this work, we were now interested in development of supramolecular assemblies for detection of amino acids. For this purpose, we designed and synthesized hetero-oligophenylene derivative **3** bearing 2-pyridyl groups and carbazole moiety. Oligophenylene scaffold was chosen because of its self assembling properties. Pyridine is well known for supramolecular constructions⁴ and carbazole moiety was incorporated due to its electroluminescence properties⁵. Interestingly, hetero-oligophenylene derivative **3** formed fluorescent aggregates in aqueous media which exhibited sensitive ratiometric response towards zinc ions which is better in comparison to other Zn^{2+} ion chemosensors reported in literature.⁶ Further, supramolecular ensemble of aggregates of **3** and Zn^{2+} ions exhibit emission modulation at two different wavelengths in the presence of **Thr** and showed selective ‘no quenching’ response towards **Thr**. To the best of our knowledge, this is the first report where supramolecular aggregates of **3**- Zn^{2+} ensemble have used for

Scheme 1. Synthetic scheme of hetero-oligophenylene derivative **3**.

selective and sensitive ‘ON-ON’ detection of threonine. Further, on the basis of fluorogenic response of aggregates of **3** towards Zn^{2+} ions and **Thr**, we have constructed a 2 input, 3-output sequential logic circuit using Zn^{2+} ions and **Thr** as chemical inputs. The design of such signalling devices is of great significance as they can perform molecular level logic operations analogous to those executed by their macroscopic analogues.

The target molecule **3** was synthesized by Diels-Alder reaction of 9-hexyl-3-(2-phenylethynyl)-9H-carbazole **1**⁷ with 2,5-diphenyl-3,4-di(pyridin-2-yl)cyclopenta-2,4-dienone **2**⁸ in diphenylether at 240 °C in 72% yield (Scheme 1, see ESI,† PS4). The structure of compound **3** was confirmed from its spectroscopic and analytical data (see ESI,† PS27–S29).

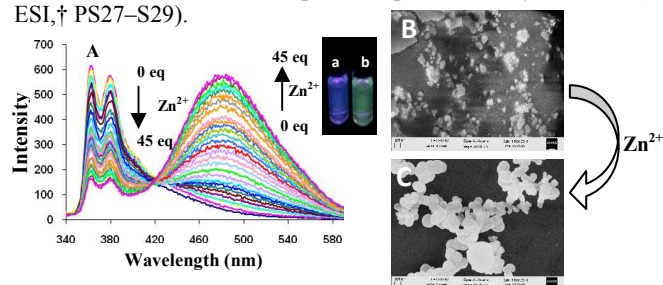


Fig. 1 (A) Change in fluorescence spectra of **3** (1 μ M) with the addition of Zn^{2+} ions (45 equiv.) in $H_2O:THF$ (8:2, v/v) buffered with HEPES, pH = 7.2; λ_{ex} = 300 nm. Inset showing the normalized fluorescence intensity (a) before and (b) after the addition of Zn^{2+} ions. Photographs of SEM images (B) before and (C) after the addition of 45 equiv. of Zn^{2+} ions to the solution of derivative **3** in $H_2O:THF$ (8:2, v/v) solution.

The binding behaviour of aggregates of derivative **3** was studied toward different metal ions (Cu^{2+} , Fe^{2+} , Fe^{3+} , Hg^{2+} , Co^{2+} , Pb^{2+} , Zn^{2+} , Ni^{2+} , Cd^{2+} , Ag^+ , Ba^{2+} , Mg^{2+} , K^+ , Na^+ , and Li^+) as their perchlorates, by UV-vis and fluorescence spectroscopy. The UV-vis spectrum of compound **3** in $H_2O:THF$ (8:2) buffered with HEPES, pH = 7.2, exhibits absorption bands at 225 and 300 nm and level off tail in the visible region (see ESI,† S1). Upon gradual addition of Zn^{2+} ions (0–45 μ M) to the solution of aggregates of derivative **3**, the absorption band at 225 nm is slightly red shifted to 229 nm along with the increase in the intensity of level-off tail (see ESI,† S2). In the

fluorescence spectrum, the aggregates of derivative **3** in H₂O:THF (8:2) solution buffered with HEPES pH = 7.2, exhibit an emission band at 363 nm ($\phi = 0.34$)⁹ when excited at 300 nm (Fig. 1A). Upon addition of Zn²⁺ ions (0–45 μ M) to the solution of derivative **3** in H₂O:THF (8:2) a gradual decrease in intensity of the emission band at 363 nm is observed along with the appearance of a new band at 490 nm (Fig. 1A). These spectral changes are accompanied by the color change of the solution from blue to green with the addition of Zn²⁺ ions (365 nm UV light, Inset Figure 1A). As a function of the addition of Zn²⁺ ions, the intensity of emission band at 363 nm is decreased and intensity of emission band at 490 nm ($\phi = 0.89$) increased in a ratiometric manner (see the ESI,† S3). The ratio of the two emission bands at 363 and 490 nm, respectively, established that aggregates of derivative **3** served as dual-emission ratiometric probe for Zn²⁺ ions. We believe that coordination of Zn²⁺ ions with nitrogen atoms of pyridyl groups enhances the planar rigidity¹⁰ of the system which results in excited state intramolecular charge transfer¹¹ from carbazole to pyridyl groups leading to fluorescence enhancement at 490 nm. This type of ratiometric fluorescence behavior was not observed with the addition of any other metal ions (see ESI,† S4). The presence of different metal ions such as Fe²⁺, Fe³⁺, Hg²⁺, Co²⁺, Pb²⁺, Ni²⁺, Cd²⁺, Ag⁺, Ba²⁺, Mg²⁺, K⁺, Na⁺, and Li⁺ caused no interference, whereas Cu²⁺ ions (0–30 μ M) quenched the fluorescence emission such that intensity ratio I_{490}/I_{363} could not be measured (see ESI,† S5). Thus, aggregates of derivative **3** work as efficient ratiometric fluorescence probe for Zn²⁺ ions. The detection limit for Zn²⁺ was found to be 110 nM which is sufficiently low in comparison to detection limit for Zinc reported in literature (see ESI,† S6 and PS31 Table S3). We also carried out time resolved fluorescence studies to determine the radiative (K_r) and non-radiative decay constants (K_{nr}) of derivative **3** in the aggregated state and in the presence of zinc ions (see ESI,† PS9).¹² The fluorescence radiative rate constant (k_f) of derivative **3** increases in the presence of zinc ions, however, the non radiative decay constant (k_{nr}) of aggregates of derivative **3** ($0.18 \times 10^9 \text{ s}^{-1}$) was found to be much higher than that in the presence of zinc ions ($0.03 \times 10^9 \text{ s}^{-1}$) (see ESI,† pS9, Table 1). These studies clearly show that presence of zinc ions accelerate the decrease in non emissive rate constant, thus, suggesting rigidity as an important cause of observed enhancement in the fluorescence emission intensity.¹³ To get insight into the binding interactions between aggregates of derivative **3** and Zn²⁺ ions, we carried out ¹H NMR studies in THF-d₈/D₂O (1:1). In the ¹H NMR spectrum, the pyridyl protons of derivative **3** undergo downfield shift in presence of Zn²⁺ ions, thus, proving that Zn²⁺ ion is interacting with aggregates of **3** through nitrogen atom of the pyridyl groups (see ESI,† S8 and PS11, Scheme 2). Further, the ESI/MS spectrum of the **3-Zn**²⁺ ensemble shows a molecular ion peak at m/e 872.22 corresponding to [M + Zn²⁺ + ClO₄⁻] complex (see ESI,† S9). We also carried out fluorescence titration of derivative **3** with Zn²⁺ ions in THF and the detection limit was found to be 190 nM (see ESI,† S10). Thus, from these studies we may conclude that aggregates give a better detection limit towards zinc ions. We believe that ordered molecular organization in case of aggregates of derivative **3** is responsible for the amplified sensing response.¹⁴ The dynamic light scattering (DLS) studies of derivative **3** were carried out in H₂O/THF solvent mixture and it was found that size of the particles decreases upon increase in volume fraction of water in THF (see the ESI,† S11). Further, the scanning electron microscopy (SEM) images of derivative **3** at low water fraction also show large sized sponge like aggregates which get converted into small sized aggregates with increase in volume fraction of water in THF (see the ESI,† S12). Thus, we believe that aggregation behaviour of derivative **3** may be controlled by varying water fraction in THF. The SEM images of **3-Zn**²⁺ ensemble show the

presence of spherical aggregates (Fig. 1C and S13). The DLS studies clearly show that, in the presence of Zn²⁺ ions, the smaller sized aggregates (average diameter of particles = 220 nm) are self-assembled to closely packed spherical aggregates (average diameter of particles = 631.92 nm) (see ESI,†, S14). These results indicates modulation of aggregates of derivative **3** in presence of Zn²⁺ ions. Biological applicability of aggregates of **3** to sense Zn²⁺ ions was checked by carrying out fluorescence titrations of *in situ* prepared Zn²⁺ ensemble and titrating this by varying the concentrations of blood serum as well as bovine serum albumin (BSA), capable of capturing Zn²⁺ ions. No significant change in the fluorescence emission intensity of **3-Zn**²⁺ ensemble at 490 nm was observed either in presence of BSA or in the presence of blood serum milieu (see ESI,† S15).

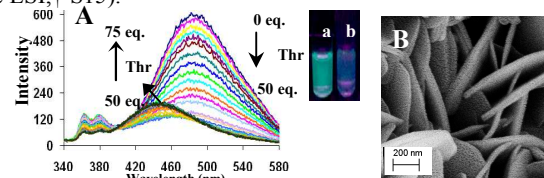


Fig. 2 (A) Fluorescence emission spectra of **3-Zn**²⁺ ensemble (1 μ M) with the addition of 75 eq. of **Thr** in H₂O:THF (8:2, v/v) buffered with HEPES, pH = 7.2. Inset showing the normalized fluorescence intensity (a) before and (b) after the addition of **Thr** ions to **3-Zn**²⁺ ensemble. (B) Photograph of SEM image of **3-Zn**²⁺ ensemble after the addition of 75 μ M of **Thr**.

Since Zn²⁺ ions have strong tendency to form complexes with amino acids, we used the aggregates of **3-Zn**²⁺ ensemble for detection of various amino acids such as Ala, Arg, Val, Gln, Lue, Lys, Gly, Met, Phe, Pro, Ser, **Thr**, His, Tyr, Asn, Glu, Asp and Cys. In the fluorescence emission spectra, with the addition of 75 eq. of **Thr** to the solution of **3-Zn**²⁺ complex, the emission band at 490 nm ($\phi = 0.89$) was quenched and a new band is formed at 440 nm ($\phi = 0.25$) (Fig. 2). These spectral changes are accompanied by the color change of the solution of **3-Zn**²⁺ from green to blue with the addition of **Thr** (365 nm UV light, Inset Figure 2A). We also carried out fluorescence studies of derivative **3** with Zn²⁺ ions and **Thr** in CH₃CN:H₂O (2:8, v/v) mixture, almost same results were obtained as with THF:H₂O (2:8) mixture (see ESI,† S16). Further, the calibration curve for **Thr** showed linearity ($R^2 = 0.9852$) with in the dynamic range of 2.18–35 μ M **Thr** (see ESI,† S17).¹⁵ We believe that interaction between **Thr** and Zn²⁺ ions is responsible for this emission behaviour. Under similar set of conditions as used for **Thr**, with the addition of 45 eq. of **His** to the solution of **3-Zn**²⁺ supramolecular ensemble resulted in restoring the original emission spectra (see ESI,† S18), which may be attributed to the displacement of Zn²⁺ ions by **His** through the formation of an imidazole bridge between the Zn centers.¹⁶ However, in case of other amino acids, no significant change in emission intensity is observed (see ESI,† S19). Thus, supramolecular **3-Zn**²⁺ ensemble can easily differentiate between **Thr** and **His**. The limit of detection (LOD) of **3-Zn**²⁺ ensemble for **Thr** was found to be 120 nM (see ESI,† S20). Biological applicability of supramolecular **3-Zn**²⁺ ensemble to detect **Thr** ions was checked by carrying out fluorescence titrations of *in situ* prepared solution of supramolecular **3-Zn**²⁺ ensemble and **Thr** and titrating this by varying the concentrations of BSA and blood serum milieu. No significant change in the emission behaviour of solution of supramolecular **3-Zn**²⁺ ensemble and **Thr** was observed in presence of BSA and blood serum milieu (see ESI,† S21). We also carried out time resolved emission studies of aggregates of **3-Zn**²⁺ ensemble in presence of **Thr** and calculated the radiative as well as non radiative rate constants (see ESI,† S7 and PS9 Table 1). In the presence of **Thr**, a decrease in radiative rate constant of aggregates of **3-Zn**²⁺ was observed. On the other hand, increase in

k_{nr} value of aggregates of $3-Zn^{2+}$ ensemble was observed. On the basis of these results, we propose that **Thr** interacts with Zn^{2+} ions which results in the weakening of existing $3-Zn^{2+}$ bond. We also carried out 1H NMR studies in THF- d_6/D_2O (1:1) to get insight into the binding interactions between $3-Zn^{2+}$ ensemble and **Thr** (see the ESI,† S22). Upon addition of **Thr** to the solution of $3-Zn^{2+}$ ensemble in THF- d_6/D_2O (1:1), the protons of pyridyl group, assigned as **a**, **b**, **c**, **d** undergo upfield shift of 0.16, 0.1, 0.12, 0.14 ppm and other aromatic protons of derivative **3** undergo average upfield shift of 0.06 ppm (see ESI,† S22). Interestingly, the original spectra of aggregates of derivative **3** is not restored and all the protons are even more upfield shifted as compared to their original position. This result corroborates our assumption that in presence of **Thr**, the interaction between zinc ions and the aggregates of **3** gets weakened and zinc ions are not displaced from the supramolecular ensemble. Further, this upfield shifting of aromatic protons indicate the tendency of the molecule to self-assemble through π - π interactions between neighbouring molecules (see ESI,† PS10, Scheme S2). The morphology of the probe aggregates of $3-Zn^{2+}$ changed from spherical to cross-linked flakes in presence of **Thr** (Fig. 2B). The dynamic light scattering studies show that, in the presence of **Thr**, the size of aggregates of $3-Zn^{2+}$ complex (average diameter of particles = 631.92 nm) is increased (average diameter of particles = 825.13 nm) (see the ESI,† S23). Further, the 1H NMR studies of $3-Zn^{2+}$ complex in presence of **Thr** suggest the presence of π - π stacking between neighbouring molecules (see the ESI,† S22). On the basis of these results, we believe that π - π stacking between neighbouring molecules are responsible for this modulation of self-assembled structure of $3-Zn^{2+}$ complex in aqueous media. The ESI/MS spectrum of the $3-Zn^{2+}$ -**Thr** adduct shows a molecular ion peak at m/e 892.32 corresponding to $[M + Zn^{2+} + Thr]^-$ complex (see ESI,† S24).

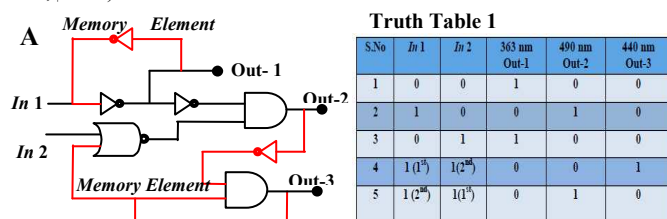


Fig. 3 (A) Sequential logic circuit displaying memory units with two inputs (**In 1** and **In 2**) and three outputs; Table 1 is the truth table for sequential logic circuits A.

Nowadays, the development of sequential logic devices has emerged as an active research area of unconventional computing which involves the conversion of chemically encoded information into fluorescent signals.¹⁷ Sequential circuits are essential for the recognition of memory devices capable of storing information and operating through the feedback loop where one of the outputs of the device functions as the input and is memorized as “memory element”. Thus, depending upon the different chemical inputs (Zn^{2+} and **Thr**) and fluorescent signals as outputs, sequential logic circuits are constructed. The two chemical inputs Zn^{2+} and **Thr** are designated as **In 1** and **In 2**, respectively. The threshold values of fluorescence intensities specified at Out-1 (363 nm), Out-2 (490 nm) and Out-3 (440 nm) are 350, 350 and 100, respectively (see ESI,† S25). Fluorescence intensities higher than the threshold values are assigned as “1” and intensities lower than the threshold values are assigned as “0”, corresponding to the “On” and “Off” states of the readout signals, respectively. On the basis of this, we designed sequential logic circuit with two inputs (**In 1** and **In 2**) and three outputs at 363 nm (Out-1), 490 nm (Out-2) and 440 nm (Out-3) (Fig. 3A). The combination of different inputs as shown in the truth table

(Table 1) permits the execution of a sequential logic circuit in which Out-2 and Out-3 representing the set/reset element that corresponds to the memory device (Fig. 3A). As shown in sequential logic circuit 4B, the ‘0’ or ‘1’ state of ‘Out-2’ is defined by ‘Out-3’. Similarly, the ‘0’ or ‘1’ state of ‘Out-3’ is defined by ‘Out-2’ which means it is operating through the feedback loop where one of the outputs of the device functions as the input and is memorized as “memory element”. The ‘0’ or ‘1’ state of ‘Out-1’ depends on ‘Out-1’ which defines the presence or absence of **In 1**. The development of such sequential circuits is important to store encoded information and execute it when required.

In conclusion, we designed and synthesized hetero-oligophenylene derivative **3** which formed fluorescent aggregates in aqueous media and these aggregates exhibited selective ratiometric response towards zinc ions. Further, supramolecular zinc ensemble of derivative **3** exhibit ‘ON-ON’ response towards **Thr** and can detect **Thr** in nanomolar range. On the basis of fluorogenic response of aggregates of **3** toward Zn^{2+} ions and **Thr**, we have constructed a 2-input, 3-output sequential logic circuit at molecular level.

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† Electronic Supplementary Information (ESI) available: Synthesis of compound **3**, characterization data, UV-vis and fluorescence studies. See DOI: 10.1039/b000000x/

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