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Synthesis, chemosensing properties and logic behaviour of a novel ratiometric 1,8- naphthalimide probe based on ICT and PET

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ABSTRACT

A novel fluorescence sensing 1,8-naphthalimide designed on the “*receptor₁-fluorophore-spacer-receptor₂*” model is synthesized and investigated. The novel probe comprising aminotriazole and hydroxyphenyl substituents is capable to operate simultaneously via ratiometric ICT and PET signalling mechanism. The synthesized compound shows excellent signalling properties towards protons, hydroxyl anions and Cu^{2+} ions. Due to the remarkable fluorescence changes in the presence of these analytes the system is able to act as a two output combinatorial logic circuit with three chemical inputs. Input₃-Disabled XNOR, INHIBIT and IMPLICATION logic gates were obtained. Because of the parallel action of disabled-XNOR and disabled-INHIBIT gates a magnitude digital comparator with disable capability is achievable.

Keywords: 1,8-Naphthalimide; Photoinduced electron transfer (PET); Internal charge transfer (ICT); Ratiometric fluorescence probe; Three input disabled-XNOR, disabled-INHIBIT and disabled-IMPLICATION logic gates; Two output combinatorial logic circuit.

GRAPHICAL ABSTRACT

1. Introduction

Over the past years, design and development of molecular systems, which are able to detect specific guest species at low concentration and to provide significant changes in their electrical, electronic, magnetic or optical signals, have been the subject of great interest in research [1-8]. Among them, fluorescent probes have received much attention due to their advantageous properties such as high sensitivity, high selectivity, rapid response time and cheap instrumentation. Also they are excellent diagnostic tools in the medicine and biology since they enable us to spy on events in living cells and organism with high spatial resolution and minimal perturbation to living systems [9-14].

Two common principles for fluorescence molecular switches are used: photoinduced electron transfer (PET) and internal charge transfer (ICT) [15-18]. PET regularly occurs when certain organic systems interact with light, and the format of “fluorophore-spacer-receptor” is the most commonly exploited approach for the design of fluorescent sensors and switches [19-22]. The fluorophore is covalently linked to a receptor with a non- π -electron-conjugating spacer group (usually alkyl group with two carbons). Typically, the receptor contains an amino group, the electrons of which can bind electron poor analytes. In this model the components are chosen so that PET from the receptor to the fluorophore excited state quenches the fluorescence of the system prior to recognition. Upon recognition of the analyte, which binds to the receptor, engaging its lone-pair electrons, the PET process is no longer possible and the fluorescence of the system is recovered [23-26]. In contrast with PET systems, in the ICT chemosensors the receptor is directly attached to the electron-donating/withdrawing unit that is conjugated to the fluorophore electron-withdrawing/electron-donating unit [27-29]. During excitation of the system the fluorophore undergoes donor-acceptor intramolecular charge transfer which strongly depends on the microenvironment of the fluorophore. Thus, recognition of a guest affects the ICT efficiency

that changes the energy between ground and excited state and results in shifting of the fluorophore electronic spectra [30-32]. The attractiveness of the ICT sensors lies on their two-channel output signals - colour change and fluorescence variation allowing the possibility to design a ratiometric fluorescent probes [33-36]. According to the ratiometric method, analyte concentration can be quantified by using the ratio of intensities of fluorescence peaks at two different wavelengths for the analyte free and analyte bound probe. Such self-calibration using two emission bands can eliminate the influence of probe concentration, environmental conditions and instrumental efficiency [37-40]. This method allows precise and quantitative analysis and imaging even in complicated systems reducing the problems of autofluorescence, scattering and stability under illumination during fluorescent sensing within many biological and industrial matrices [41-43].

The simultaneous action of PET and ICT sensing signal outputs could provide new and interesting properties. The amalgamation of PET and ICT pathways has provided examples of multilevel logic systems with multiple logic functions, including arithmetic operations by modulation of the fluorescence output [44-48]. The molecules capable of carrying out a variety of sensing functions simultaneously, and to compute a composite result autonomously, have great potential for real-life applications such as object coding and imaging, intelligent materials, drug delivery and activation, diagnostics or actuation [49-59].

Recently we have prepared an ICT and PET based 1,8-naphthalimide bichromophore as a multileveled molecular logic system. However this system didn't exhibit fluorescent ratiometric response which restricts the functionality of the prepared logic system in fluorescent mode. Therefore, in this work we pay attention to the synthesis and fluorescence sensing properties of a novel 1,8-naphthalimide based fluorescent ratiometric probe **2** (Scheme 1) which operates simultaneously via ratiometric ICT and PET signalling

mechanism. The optical chemosensing behaviour of the synthesized compound was studied and sequential logic circuits on molecular level were designed.

Insert here Scheme 1

2. Experimental

2.1. Materials

Commercially available 4-chloro-1,8-naphthalic anhydride, *p*-aminophenol and 3-amino-1,2,4-triazole (Aldrich) were used without purification. All solvents (Aldrich, Fisher Chemical) were pure or of spectroscopy grade. $\text{Zn}(\text{NO}_3)_2$, $\text{Cu}(\text{NO}_3)_2$, $\text{Ni}(\text{NO}_3)_2$, $\text{Co}(\text{NO}_3)_2$, $\text{Pb}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$, $\text{Hg}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$ salts were the sources for metal cations (all Aldrich salts at p.a. grade).

2.2. Methods

FT-IR spectra were recorded on a Varian Scimitar 1000 spectrophotometer. The ^1H NMR spectra (chemical shifts are given as δ in ppm) were recorded on a Bruker Avance II+ 600 instrument operating at 600 MHz. TLC was performed on silica gel, Fluka F60 254, 20×20, 0.2 mm. The melting points were determined by means of a Kofler melting point microscope. The UV-VIS absorption spectra were recorded on a spectrophotometer Hewlett Packard 8452A. The fluorescence spectra were taken on a Scinco FS-2 spectrofluorimeter. The fluorescence quantum yields (Φ_F) were measured relatively to Coumarin 6 ($\Phi_F = 0.78$ in ethanol [60]) as a standard. All the experiments were performed at room temperature (25.0°C). A 1×1 cm quartz cuvette was used for all spectroscopic analysis. To adjust the pH, very small volumes of hydrochloric acid and sodium hydroxide were used. The effect of the metal cations upon the fluorescence intensity was examined by adding portions of the metal cations stock solution to a known volume of the fluorophore solution (10 mL). The addition

was limited up to 100 μ L so that dilution remains insignificant.

2.3. Synthesis of probe (2)

A solution of 4-chloro-1,8-naphthalic anhydride (1.00 g, 0.0043 mol) and *p*-aminophenol (4.7 g, 0.0043 mol) in glacial acetic acid (25 mL) was stirred under reflux for 10 h. After cooling the precipitate was filtered off, washed with water and dried to give 1.32 g of pure 4-chloro-*N*-(4-hydroxyphenyl)-1,8-naphthalimide **1** as brown crystals (1.32 g, 94 %, m.p. > 250°C). Then to a solution of 3-amino-1,2,4-triazole (0.69 g, 8.2 mmol) and KOH (0.9 g, 16 mmol) in DMF (15 mL), a solution of 4-chloro-*N*-(4-hydroxyphenyl)-1,8-naphthalimide **1** (1.32 g, 4.1 mmol) in DMF (5 mL) was added dropwise. The resulting mixture was stirred under reflux for 12 h. After cooling to room temperature, the mixture was poured into water (20 mL) and pH was adjusted to pH 4. The precipitated solids were filtered off and dried to give probe **2** as a yellow solid (0.74 g, 49 %, m.p. > 250°C).

FT-IR (KBr) cm^{-1} : 3309 (ν N-H); 1704 (ν^s C=O); 1648 (ν^{as} C=O). ^1H NMR (DMSO- d_6 , 600 MHz) δ ppm: 9.62 (s, 1H, N-H); 8.48 (d, 1H, J = 7.2 Hz, Naphthalimide H-7); 8.42 (d, 1H, J = 8.1 Hz, Naphthalimide H-2); 8.28 (s, 1H, -N=CH-N-); 8.10 (d, 1H, J = 7.1 Hz, Naphthalimide H-6); 7.49 (d, 1H, J = 8.1 Hz, Naphthalimide H-3); 7.06 (m, 2H, ArH); 6.84 (m, 2H, ArH); 5.87 (s, 1H, Ar-OH). Elemental analysis: Calculated for $\text{C}_{20}\text{H}_{11}\text{N}_5\text{O}_3$ (MW 369.3) C 65.04, H 3.00, N 18.96 %; Found C 65.33, H 3.12, N 18.67 %.

3. Results and discussion

3.1. Design and synthesis

The novel probe **2** was configured on the “*receptor₁-fluorophore-spacer-receptor₂*” model (Scheme 2). The aminotriazole 1,8-naphthalimide part in **2** represents the “*fluorophore₁-receptor₁*” architecture with ICT chemosensing properties, where the 4-amino-

1,8-naphthalimide is the fluorophore and the amino group possessing labile hydrogen is the receptor moiety. 1,8-Naphthalimide derivatives are chosen because of their desirable properties, such as excellent photostability, high luminescence efficiency, large Stokes' shift and easy modification of the molecular structure [61-65]. However, the fluorescent ratiometric response is unusual for the naphthalimide probes. The easiest way to obtain 1,8-naphthalimide architecture with fluorescent ratiometric response is to introduce 3-amino-1,2,4-triazole in the *peri*-position to the carbonyl groups [66,67]. Also, the aminotriazole naphthalimides show high water solubility, low cytotoxicity, clear cellular location and membrane permeability in living cells. That is why in present work the aminotriazole fragment was involved in the examined probe **2**.

Insert here Scheme 2

It is well known that absorption and fluorescence characteristics of the 1,8-naphthalimides depend on the nature of the substituent at C-4 position of the 1,8-naphthalimide ring [68,69]. The 4-amino-1,8-naphthalimide is a "push-pull" electron system in which the light absorption generates a charge transfer interaction between C-4 amine donating substituent and the both *peri*-positioned carbonyl acceptors. When an anion, such as OH⁻, interacts with the amino group in 4-amino-1,8-naphthalimide fluorophore, the amine electron-donating ability is increased due to the deprotonation which generates a strong electron density around the amino nitrogen. As a result the ICT efficiency in the 1,8-naphthalimide fluorophore strongly increases and red shifting of the absorption and emission spectra are expected [66,70]. The *N*-(4-hydroxyphenyl)-1,8-naphthalimide comprises the "fluorophore-spacer-receptor₂" in which the 1,8-naphthalimide moiety is the fluorophore and the phenolic group is the anion receptor. After interaction of the "receptor₂" with anions, such

as OH⁻, the electron density around the phenolic oxygen increases and the PET process quenches the 1,8-naphthalimide fluorophore excited state [71].

The synthesis of the target probe **2** is shown in Scheme 3. First, the intermediate 4-chloro-*N*-(4-hydroxyphenyl)-1,8-naphthalimide **1** was prepared by analogy with previously reported work after condensation of 4-chloro-1,8-naphthalic anhydride and *p*-aminophenol [72]. Then the intermediate **1** was converted into the desired probe **2** by reaction with 3-amino-1,2,4-triazole similarly to the previously reported procedure [66].

Insert here Scheme 3

The structure and purity of the synthesized compound was characterized and confirmed by conventional techniques - elemental analysis data, UV-Vis, fluorescence, FT-IR and ¹H NMR spectroscopy.

3.2 Influence of pH on the absorption and fluorescence properties of probe **2**

The novel compound **2** was designed as a molecular fluorescence probe for determination of pH changes. This was the reason to investigate its photophysical behaviour in water/DMF (4:1, v/v) solution at different pH values.

In acid media at pH = 3-5 the novel probe **2** is present in a neutral form and shows an absorption band in the range between 390-500 nm with well pronounced maximum at 448 nm (Figure 1A), due to the internal charge transfer process in the 1,8-naphthalimide chromophore. Also, under these conditions compound **2** exhibits a blue fluorescence in a spectral range of 450-575 nm with maximum at 483 nm (Figure 1B).

Insert here Figure 1A and Figure 1B

The calculated by Eq. (1) Stokes' shift of 1617 cm^{-1} is unusually low for the 1,8-naphthalimide derivatives and can be attributed to the more rigid structure of the aminotriazole naphthalimide architecture in **2**.

Insert here Equation (1)

The quantum yield of fluorescence $\Phi_F = 0.46$ was calculated using Coumarin 6 ($\Phi_F = 0.78$ in ethanol) [60] as a standard according to Eq. (2), where A_{ref} , S_{ref} , n_{ref} and A_{sample} , S_{sample} , n_{sample} represent the absorbance at the excited wavelength, the integrated emission band area and the solvent refractive index of the standard and the sample, respectively.

Insert here Equation (2)

Upon addition of NaOH to the solution of probe **2** from pH 5 to pH 9 a significant decrease of the absorption band at 448 nm and an increase in a new longer-wavelength absorption band at 486 nm were observed. This phenomenon was probably due to the deprotonation of amino (N-H) nitrogen in probe **2** (Scheme 4) that enhanced the "push-pull" character of the ICT transition, causing a considerable bathochromic shift (42 nm) of the absorption band. On the basis of the absorption changes of **2** a ratiometric titration curve as a function of pH was obtained using the ratio between 480 nm and 440 nm (Inset of Figure 1A). The analysis of the titration plot according to Eq. (3) gives the pK_a value of 6.20 ± 0.04 [73], which is similar to the other derivatives of a same nature [66].

Insert here Equation (3)

Insert here Scheme 4

Similar to the changes in absorption spectra, after addition of NaOH from pH 5 to pH 9 the fluorescence spectra of probe **2** are red shifted from 483 nm to 511 nm (Figure 1B), as quantum yield of fluorescence remains approximately constant. The calculated quantum yield for probe **2** at pH 9 was $\Phi_F = 0.44$. Using the ratio between 480 and 516 nm as a function of pH the fluorescence ratiometric titration curve of **2** was obtained (Inset of Figure 1B). According to the Eq. (4) [74], the analysis of the fluorescence changes in pH window 5-9 gives pK_a value 6.11 ± 0.04 .

Insert here Equation (4)

Furthermore, the absorption spectrum of **2** does not show significant pH-dependent changes in a pH window 9-12 as the 1,8-naphthalimide fluorophores do not affect their ICT excited states (Figure 2A). In contrast to that the fluorescence spectra are changed remarkably. It was found that the pH increase from pH 9 to pH 12 results in a fluorescence quenching (Figure 2B). This phenomenon was attributed to the deprotonation of the phenolic group which allows a PET quenching process from the generated phenolate anion to the 1,8-naphthalimide excited state (Scheme 4) [71]. The fluorescence quenching FQ = 8.1 have been used as a qualitative parameter. The $FQ = I_0/I$ is determined as the ratio between the maximum fluorescence intensity I_0 at pH 9 and the minimum fluorescence intensity I at pH 12. The analysis of the fluorescence changes at 511 nm in pH window 9-12 (Inset of Figure 2B) according to Eq. (4) gives the pK_a value of 10.51 ± 0.04 for deprotonation of the phenolic group in probe **2**.

Insert here Figure 2A and Figure 2B

3.3 *Influence of metal cations on the absorption and fluorescence properties of probe 2*

The signalling fluorescent properties of compound **2** in the presence of transition metal cations (Co^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Pb^{2+} , Cd^{2+} , Zn^{2+} , and Hg^{2+}) was investigated. To maintain a constant pH values, the experiments were performed in water/DMF (4:1, v/v) solutions in the presence of ammonia/ammonium chloride buffer (pH 9), acetate buffer (pH 4.5) or HEPES buffer (pH 7.3). The ammonia/ammonium chloride (pH = 9) and acetate (pH = 4.5) buffers were used because at these pH values the probe **2** is in its yellow-green or blue emitting form respectively. Furthermore the HEPES buffer was chosen, because it ensures pH in the physiological range (pH = 7.3) which is assumption for applications in living organisms. The effect of the metal cations was observed using two methods. The first method was carried out by adding of the metal stock solutions to different buffered solutions of the examined probe **2**. The second method was performed by addition of buffer solutions to solutions of probe **2** in the presence of metal cations. In both cases a similar effect was observed.

The effect of the metal cations ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) on the fluorescence of **2** ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) in water/DMF (4:1, v/v) solutions is presented in Figure 3. It was found that the addition of metal ion solution to the solution of probe **2** ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) does not cause any photophysical changes in the presence of 10 μM ammonia/ammonium chloride buffer (pH = 9), suggesting that the synthesized compound **2** is not a suitable probe for detection of a metal cation in alkaline media. Probably at this pH the examined metal ions rapidly form hydroxides and do not interact with the probe **2** by any way.

Insert here Figure 3A, Figure 3B and Figure 3C

In contrast to the above, after the addition of Cu^{2+} cations ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) to water/DMF (4:1, v/v) solutions of the probe **2** ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) buffered with acetate buffer (pH 4.5) or 10 μM HEPES (pH = 7.3) fluorescence quenching was observed. The other cations did not change remarkably the fluorescence of **2** at these pH values. Possibly the quenching effect of Cu^{2+} is due to the better complexation and paramagnetic properties of Cu^{2+} which are resulting in unspecific fluorescence quenching by energy or electron transfer [75,76]. The quenching (FQ) of the fluorescence emission have been used as a qualitative parameter. The $\text{FQ} = I_0/I$ has been determined from the ratio between the maximum fluorescence intensity (I_0 - solution free of metal cations) and the fluorescence intensity in the presence of metal cations (I). The calculated fluorescence quenching was $\text{FQ} = 2.9$ and $\text{FQ} = 2.0$ at pH 7.3 and pH 4.5, respectively. The higher fluorescence quenching at pH 7.3 (Figure 3B) could be attributed to a better coordination of Cu^{2+} with the deprotonated form of **2** which is formed at this pH.

The stoichiometry of the coordination between Cu^{2+} and the probe **2** was determined using the method of continuous variations (Job's method). The Job's plot analysis with respect to the emission of **2** at 510 nm in the presence of Cu^{2+} in different concentration (Figure 4A) showed a maximum near 0.5 which can be attributed to the existence of 1:1 (Probe **2** : Cu^{2+}) complex in the solution (Figure 4B).

Insert here Figure 4A and Figure 4B

Furthermore, it was found that the addition of 1 equivalent of Cu^{2+} to the solution of probe **2** buffered with HEPES (pH = 7.3) induces a hypsochromic shift of the absorption maximum ($\Delta\lambda_A = 10 \text{ nm}$) due to the complexation of Cu^{2+} with probe **2** (Figure 5). That means the triazole amino group is subjected to the coordination of probe **2** with Cu^{2+} cations.

The cation coordination in aminotriazole fragment partially decreases the push-pull character of the ICT state of probe **2** and results in a blue shift.

Insert here Figure 5

3.3 *Logic behaviour of probe 2*

Due to the remarkable fluorescence changes of probe **2** ($c = 10^{-5}$ mol L⁻¹) in the presence of H⁺ (0.01 M HCl), OH⁻ (0.01 M NaOH) and Cu²⁺ ($c = 10^{-5}$ mol L⁻¹) it would be able to act as a two output combinatorial logic circuit with three chemical inputs. Starting from pH = 8, by monitoring the emission output at 482 nm (Output 1) of probe **2** an input₃-disabled-INHIBIT gate at a molecular scale was obtained where the disabling Input 3 is Cu²⁺, and H⁺ and OH⁻ are Input 1 and Input 2, respectively (Table 1).

Insert here Table 1

First, it could be seen that the fluorescence of **2** at 482 nm was high (coded for binary 1) with the input of acid only (Figure 6). This behaviour mimicked the INHIBIT logic gate. At the same time, in the presence of Cu²⁺ the fluorescence of the probe **2** is always quenched and the action of the above INHIBIT gate was prevented. This behaviour is correlated very well with a three-input Disabled INHIBIT gate [52].

Insert here Figure 6

By monitoring the fluorescence emission at 511 nm as output (Table 1, Output 2), as acid-base two input logic gate, probe **2** works as XNOR gate at a molecular scale. As can be

seen from Figure 6, starting with pH = 8 the fluorescence of **2** at 511 nm was low (coded for binary 0) with the input of either acid or base. The simultaneous inputs of acid and base annihilated each other and generated the initial high emission (coded for binary 1) at 511 nm. This behaviour mimicked the XNOR logic function. In the presence of Cu^{2+} as third input the fluorescence of **2** is always quenched that results in low fluorescence output value and disables the action of the XNOR gate. As a whole input₃-disabled-XNOR is achieved [52] where the disabler Input 3 is Cu^{2+} , and H^+ and OH^- are Input 1 and Input 2.

On the basis of the present study and the results obtained the logic behaviour of probe **2** could be summarized with the electronic representation in Scheme 5.

Insert here Scheme 5

Moreover due to the parallel action of both disabled-XNOR and disabled-INHIBIT logic gates, a magnitude digital comparator at the molecular level with disable capability is achievable. The operation of the **2**-based digital comparator was illustrated on Table 2. As can be seen the XNOR gate plays the role of a mathematical symbol “equal” because its output is 1 (true) only when both of the inputs have the same value. Furthermore, the role of the INHIBIT gate is to serve as mathematical symbol “greater than” and it is 1 (true) when the first input (H^+) is higher than the second one (OH^-). When the second input (OH^-) is higher than the first one (H^+) both of the outputs are 0 (false). In the presence of Cu^{2+} the XNOR and INHIBIT logic functions of probe **2** are disabled and the action of the above digital comparator was prevented. That is why the probe **2** works as magnitude digital comparator with disable capability.

Insert here Table 2

Also, starting from acid solution, by monitoring the emission output at 482 nm of probe **2** an input₃-disabled-IMPLICATION gate at the molecular scale was obtained (Table 3).

Insert here Table 3

We focused our attention on the IMPLICATION gate realization due to the importance of this logic gate. It is referred to the IF-THEN operation and very recently on the molecular IMPLICATION gate a Tic-Tac-Toe computer game was realized [77]. The starting conditions were prepared after addition of 0.01 M NaOH to a solution of probe **2** at pH 8 in water/DMF (4:1, v/v). First, by monitoring of the fluorescence at 482 nm as output (Figure 7, Output 1), as acid-base two input logic gate probe **2** works as IMPLICATION gate at molecular scale.

Insert here Figure 7

As can be seen from Figure 7, the fluorescence output at 482 nm was low (coded for binary 0) with the input of acid only. For the other three combinations of the two inputs, the fluorescence at 482 nm was high (coded for binary 1). This behaviour correlates very well with an IMPLICATION gate. In the presence of Cu²⁺ as third input the fluorescence of **2** is quenched in all cases and the presented IMPLICATION gate was disabled. In other words, input₃-disabled-IMPLICATION was achieved [52] where the disabler Input 3 is Cu²⁺, and H⁺ and OH⁻ are Input 1 and Input 2, respectively.

4. Conclusions

A novel 1,8-naphthalimide derivative with fluorescence sensing properties based on PET and ICT was synthesized and its photophysical behaviour as a function of pH and in the presence of representative metal ions was studied. The novel probe was designed on the “*receptor₁-fluorophore-spacer-receptor₂*” model comprising C-4 3-amino-1,2,4-triazole (“*receptor₁*”) and *N*-4-hydroxyphenyl (“*receptor₂*”) substituents and as such is able to operate simultaneously via ratiometric ICT and PET signalling mechanism. The synthesized compound shows excellent signalling properties towards protons, hydroxyl anions and Cu²⁺ ions. In an acid media (pH = 3-5) probe **2** emits blue fluorescence, while in an environment near to the neutral is changed to yellow-green due to the deprotonation of N-H in the “*receptor₁*”. In an alkaline media (pH > 9) due to deprotonation of the hydroxyl group (“*receptor₂*”), the fluorescence is quenched as a result of the activation of the photoinduced electron transfer to the fluorophore. In the presence of representative metal ions only Cu²⁺ remarkably quenches the fluorescence of the system, buffered with HEPES (pH = 7.3). Due to the remarkable fluorescence changes in the presence of protons, hydroxyl and Cu²⁺ ions the novel system is able to act as a two output combinatorial logic circuit with three chemical inputs and to execute input₃-disabled-XNOR, input₃-disabled-INHIBIT and input₃-disabled-IMPLICATION logic gates. Furthermore, due to the parallel action of both disabled-XNOR and disabled-INHIBIT logic gates a magnitude digital comparator at a molecular level with disable capability is achievable.

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Tables

Table 1

The truth table for the operation of compound 2.

	Input 1 (H^+)	Input 2 (OH^-)	Input 3 (Cu^{2+})	Output 1 (Fl_{482})	Output 2 (Fl_{511})
A	0	0	0	0	1
B	1	0	0	1	0
C	0	1	0	0	0
D	1	1	0	0	1
E	0	0	1	0	0
F	1	0	1	0	0
G	0	1	1	0	0
H	1	1	1	0	0

Table 2

The truth table for the operation of the 2-based digital comparator.

	Input 1 (H^+)	Input 2 (OH^-)	Output 1 (Fl_{511})	Output 2 (Fl_{482})
			XNOR (equal)	INHIBIT (greater than)
$\text{H}^+ = \text{OH}^-$	0	0	1	0
$\text{H}^+ > \text{OH}^-$	1	0	0	1
$\text{H}^+ < \text{OH}^-$	0	1	0	0
$\text{H}^+ = \text{OH}^-$	1	1	1	0

Table 3

The truth table for the operation of compound **2** as input₃-disabled-IMPLICATION gate.

	Input 1 (H ⁺)	Input 2 (OH ⁻)	Input 3 (Cu ²⁺)	Output 1 (Fl ₄₈₂)
A	0	0	0	1
B	1	0	0	1
C	0	1	0	0
D	1	1	0	1
E	0	0	1	0
F	1	0	1	0
G	0	1	1	0
H	1	1	1	0

Scheme Captions

Scheme 1. 1,8-Naphthalimide based probe **2**.

Scheme 2. Design of probe **2** on the “*receptor₁-fluorophore-spacer-receptor₂*” model.

Scheme 3. Synthesis of 1,8-naphthalimide based probe **2**.

Scheme 4. Photophysical behaviour of probe **2** as a function of pH.

Scheme 5. Combinatorial logic circuit on the basis of probe **2**.

Figure legends

Fig. 1. Absorption (A) and fluorescent (B) spectra of probe **2** in water/DMF (4:1, v/v) solution at different pHs in pH window 3-10.

Fig. 2. Absorption (A) and fluorescent (B) spectra of probe **2** in water/DMF (4:1, v/v) solution at different pHs in pH window 9-12.

Fig. 3. Effect of metal cations ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) on the fluorescence of **2** ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) in water/DMF (4:1, v/v) solutions buffered with (A) 10 μM acetate buffer (pH 4.5), (B) 10 μM HEPES (pH 7.3) and (C) 10 μM ammonia/ammonium chloride buffer (pH 9).

Fig. 4. Fluorescence changes (A) and Job's plot (B) of probe **2** ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) in water/DMF (4:1, v/v) solution (buffered with 10 μM HEPES) after addition of Cu^{2+} .

Fig. 5. Absorption spectra of probe **2** ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$) in water/DMF (4:1, v/v) solution (buffered with 10 μM HEPES) before and after addition of Cu^{2+} ($c = 1 \times 10^{-5} \text{ mol L}^{-1}$).

Fig. 6. Fluorescence changes of probe **2** ($c = 10^{-5} \text{ mol L}^{-1}$) in the presence of HCl ($c = 10^{-2} \text{ mol L}^{-1}$), NaOH ($c = 10^{-2} \text{ mol L}^{-1}$) and Cu^{2+} ($c = 10^{-5} \text{ mol L}^{-1}$) as chemical inputs.

Fig. 7. Fluorescence spectra of probe **2** ($c = 10^{-5} \text{ mol L}^{-1}$) based input₃-disabled-IMPLICATION gate using HCl ($c = 10^{-2} \text{ mol L}^{-1}$), NaOH ($c = 10^{-2} \text{ mol L}^{-1}$) and Cu^{2+} ($c = 10^{-5} \text{ mol L}^{-1}$) as chemical inputs.

Equations

$$(\nu_{\text{A}} - \nu_{\text{F}}) = \left(\frac{1}{\lambda_{\text{A}}} - \frac{1}{\lambda_{\text{F}}} \right) \times 10^7 \quad (1)$$

$$\Phi_{\text{F}} = \Phi_{\text{ref}} \left(\frac{S_{\text{sample}}}{S_{\text{ref}}} \right) \left(\frac{A_{\text{ref}}}{A_{\text{sample}}} \right) \left(\frac{n_{\text{sample}}^2}{n_{\text{ref}}^2} \right) \quad (2)$$

$$\log \left[(A_{\text{max}} - A) / (A - A_{\text{min}}) \right] = pH - pK_a \quad (3)$$

$$\log \left[(I_{F \text{ max}} - I_F) / (I_F - I_{F \text{ min}}) \right] = pH - pK_a \quad (4)$$

2

Scheme 3

Figure 1A-R1

Figure 1B-R1

A)

Figure 3A

Figure 3B

Figure 3C

Figure 4A

Figure 4B

B)

Figure 5-R1

Figure 6

Figure 7