Supporting Information for

Supramolecular Approach to Enzyme Sensing on Paper Discs Using Lanthanide Photoluminescence

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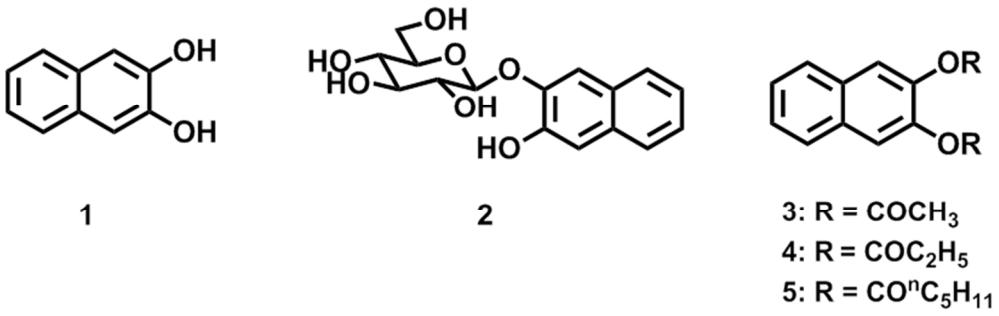


Chart S 1 Structures of sensitizer 2, 3-dihydroxy naphthalene (DHN, **1**), pro-sensitizers (**2-5**) used for β-glucosidase and lipase detection.

1. SEM images of the gel coated paper

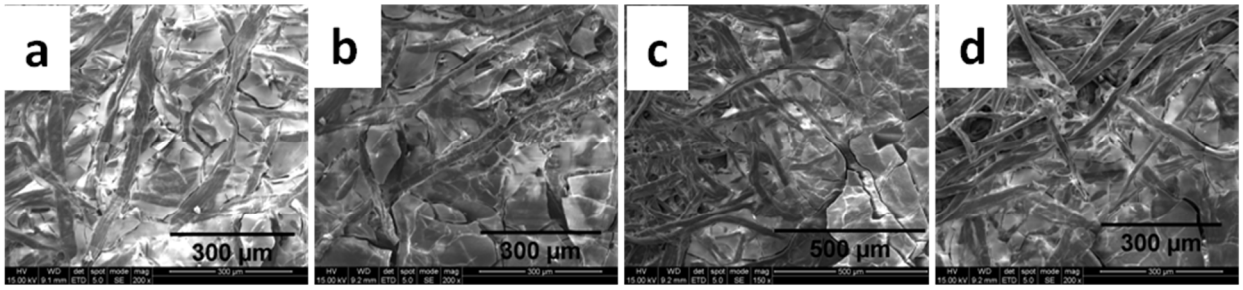


Figure S 1: SEM images of the gel coated paper, **image a** and **image b** are taken at the centre of the gel coated paper, **Image c** and **d** are taken at the edge of gel coated paper

2. (a) Comparison of coated discs prepared by different techniques

In this control experiment, the coated discs were prepared by three methods described below.

- **Method a**: **Disc 1** was treated first with **2** (220 μM) doped Na-cholate solution (20 μL), dried in the air for 15 min, then Tb-acetate solution (20 μL) was added, and the disc was dried again.
- **Method b**: **Disc 2** was treated first with Tb-acetate solution (20 μL), dried in the air for 15 min, then **2** (220 μM) doped Na-cholate solution (20 μL) was added, and finally the disc was dried.
- **Method c**: 40 μL of **2** (108 μM) doped Tb-cholate gel was spread uniformly on **Disc 3** by a micropipette (in 20 μL portions) and air dried for 15 min.

Finally, β-glucosidase (10 μL of 2 mg/mL) was added on each disc, and these were viewed under UV lamp at 15 min intervals (Figure S 2).

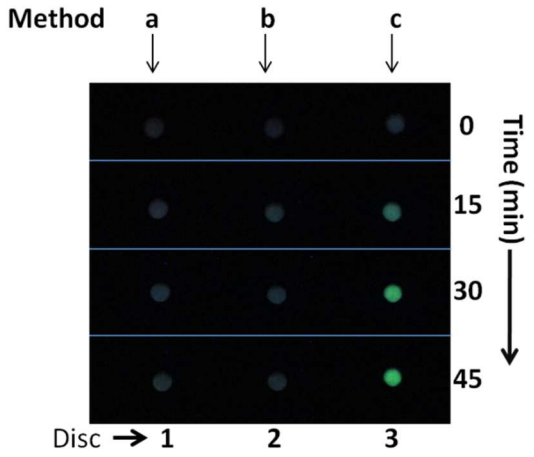


Figure S 2: Images captured under 365 nm UV lamp for discs prepared by methods a, b, c as a function of time.

- It is clear from the data shown in Figure S2 that only the disc coated with the *pro*-sensitizer doped gel (**disc 3**) showed luminescence enhancement upon enzyme addition. ***Clearly, the coatings prepared by the non-gel route were ineffective***.

(b) Plate reader data for comparison of discs coated by methods a, b, c

- **Method a**: **Disc 1** was treated first with **2** (200 μM) doped Na-cholate solution (20 μL), dried in the air for 15 min, then Tb-acetate solution (20 μL) was added, and the disc was dried again.
- **Method b**: **Disc 2** was treated first with Tb-acetate solution (20 μL), dried in air for 15 min, then **2** (200 μM) doped Na-cholate solution (20 μL) was added, and finally the disc was dried.
- **Method c**: 40 μL of **2** (100 μM) doped Tb-cholate gel was spread uniformly on **Disc 3** by a micropipette (in 20 μL portions) and air dried for 15 min.

The discs were placed in the wells of a 96 well plate and β-glucosidase (5 μL of 0.5 mg/mL) was added on each disc before plate reader measurement.

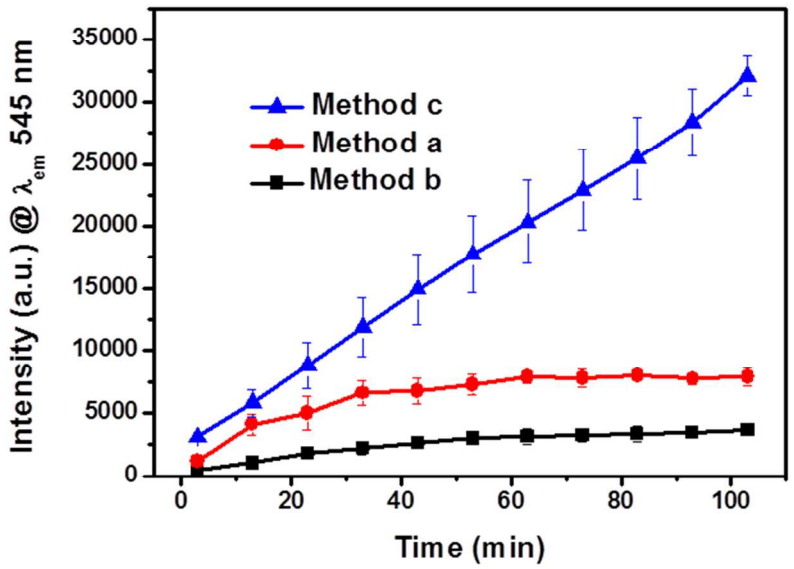


Figure S 3: Emission intensity at 545 nm recorded as a function of time for discs prepared by methods a, b and c (error bars reflect the standard deviations of three sets of measurements)

- The data in Figure S3 clearly support the visual observation from Figure S2, i.e., the gel coating is significantly more effective for enzyme detection.

3. Reproducibility of manual coating of gel on filter paper disc

Nine *identical* coated discs were prepared from DHN (**1**, 150 μM) doped Tb-cholate gel and images of the green emitting discs were captured under a 365 nm UV lamp. Visually the spots appeared to have uniform emission with comparable intensities (Figure S 4). To quantify, the discs were placed in wells of a 96 well plate and emission intensities at 545 nm were recorded in a plate reader (Table S1, Method a). Green color intensity was quantified from each disc using ImageJ and summarized in Table S1, Method b.

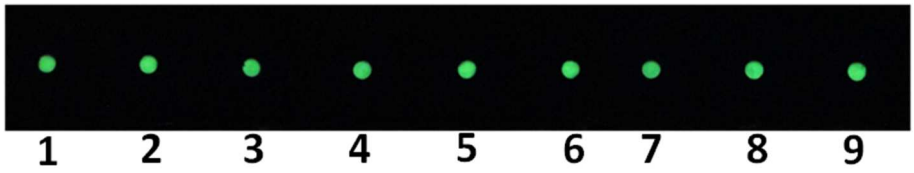


Figure S 4: Image captured under the 365 nm UV lamp for nine identical DHN (1, 150 μM) doped Tb-cholate gel coated discs to check the reproducibility of gel coating

Table S 1: Plate reader data for emission intensity at 545 nm recorded for nine identical DHN (**1**, 150 μM) doped Tb-cholate gel coated discs (row 1) and green color intensity data for nine identical discs (row 2).

Method	Disc#	1	2	3	4	5	6	7	8	9	Mean with SD
a	Intensity (a.u.) by Plate reader	12045	12896	12932	13400	13900	13888	13739	13700	14109	13400 ± 660 (< 5%)
b	Intensity by ImageJ software	205	219	200	219	222	198	215	210	205	210 ± 8.83 (<5%)

4. Almond β-glucosidase detection

Almond β-glucosidase detection was carried out in three different sets. Discs were prepared by coating with **2** doped Tb Cholate gel. Addition of water and denatured almond extract showed no luminescence enhancement from disc 1 & 2, respectively. But the fresh almond extract addition led to green emission enhancement from the discs 3 for all the sets.

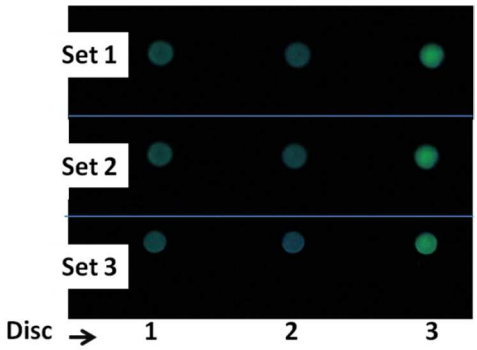


Figure S 5 Images captured under 365 nm UV lamp after 60 min of addition of 10 μL portions of Water (disc 1), Heat treated almond extract (disc 2), Almond extract (disc 3).

5. Plate reader measurement for almond β-glucosidase detection

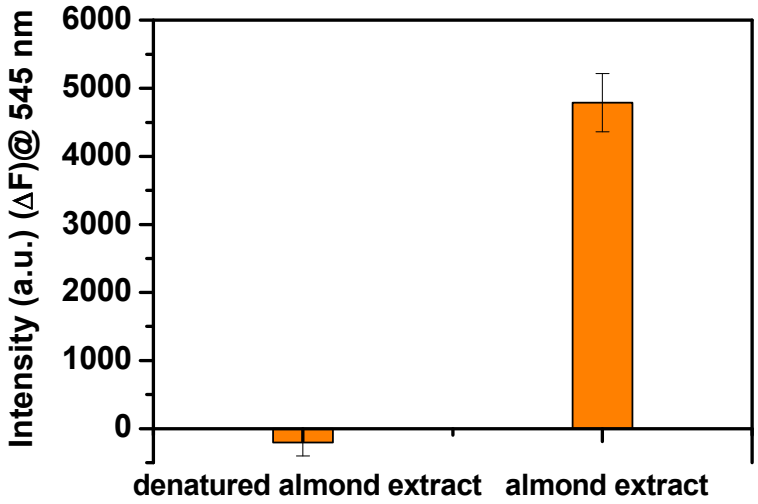


Figure S 6 Plate reader data (λ_{em} 545 nm) of the gel coated discs 90 min after the addition of heat treated almond extract (disc 2), almond extract (disc 3). ($\Delta F = F_{sample} - F_{blank}$)

6. Lipase detection with sensor disc coated with DHN-diacetate (3) doped Tb-cholate gel

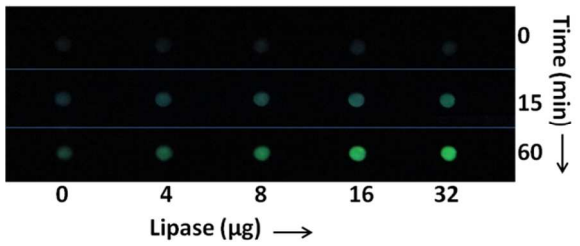


Figure S 7: Image captured under 365 nm UV lamp after addition of variable amounts of lipase on **3** (78 µM) doped Tb-cholate gel coated paper discs.

- Compound **3** was not stable on paper disc. It underwent slow decomposition on paper.

7. Comparative study of sensitivity among three different sensor discs coated with DHN-diester (3, 4, 5) doped Tb-cholate gel for lipase detection

Figure S8

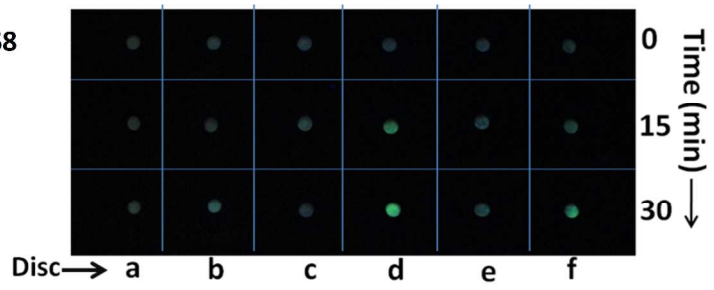
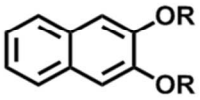


Table S2

	a	b	c	d	e	f
Substrate 3						
Substrate 4						
Substrate 5						
*Lipase (32 µg)						
Water						



3: R = $COCH_3$
4: R = COC_2H_5
5: R = $CO^nC_5H_{11}$

Green colour: The presence of a compound in the coated disc.
Red colour: The absence of a compound in the coated disc.

Figure S 8 with Table S2: Images captured under 365 nm UV lamp for sensor discs ((a, b) DHN-dihexanoate (**5**) doped Tb-cholate gel, (c, d) DHN-dipropanoate (**4**) doped Tb-cholate gel, (e, f) DHN-diacetate (**3**) doped Tb-cholate gel after addition of 16 µL water at a, c, e discs and 32 µg (*16 µL of 2 mg/mL) of lipase at b, d, f discs.) and **Table S 2**: The tabular description of the composition of each disc in Figure S8, and the structures of the pro-sensitizers at the right side of Table S2.

8. Lipase detection in blood serum

Blood serum lipase/esterase detection was carried out on three different sets with the pro-sensitizer **4** doped Tb Cholate gel coated discs. When water (5 µL) and denatured blood serum (5 µL) were added on discs no emission was observed (disc 1 & disc 2, respectively) but with native blood serum (disc 3) intense emission was observed and it reached maximum within 30 min.

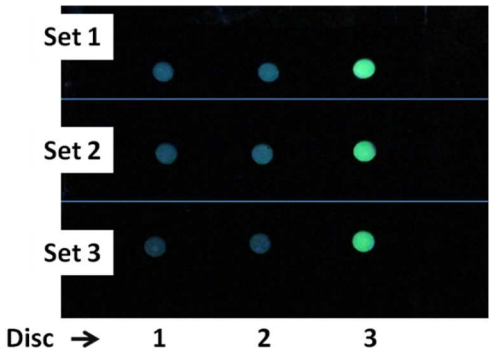


Figure S 9 Images captured under UV lamp for three different sets of **4** doped Tb Cholate gel coated disc 30 minutes after addition of 5 µL H_2O (disc 1), 5 µL denatured blood serum (disc 2), 5 µL blood serum (disc 3), respectively.

9. Sensor disc stability study

To check the stability of sensor disc, paper strip containing sensor discs was prepared as displayed below and stability was checked for the same by keeping in freezer.

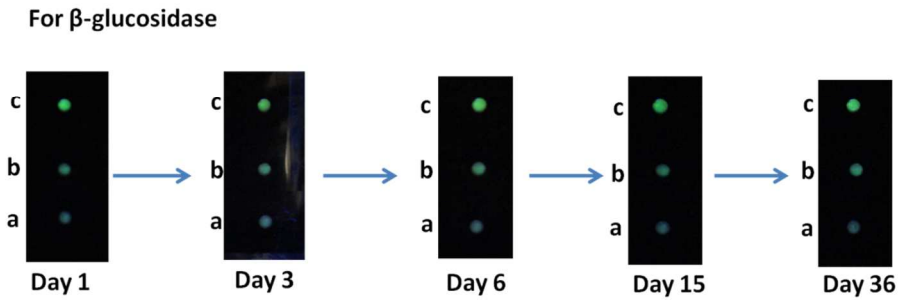


Figure S 10: Image capture under 365 nm UV lamp with time for paper strip with (From bottom to top), **disc a-** Tb-cholate gel (40 µL), **disc b-** 2 (80 µM) doped Tb-cholate gel, **disc c-** 2 (80 µM) doped Tb-cholate gel with 8 µg (16 µL of 0.5 mg/ mL) of β-glucosidase

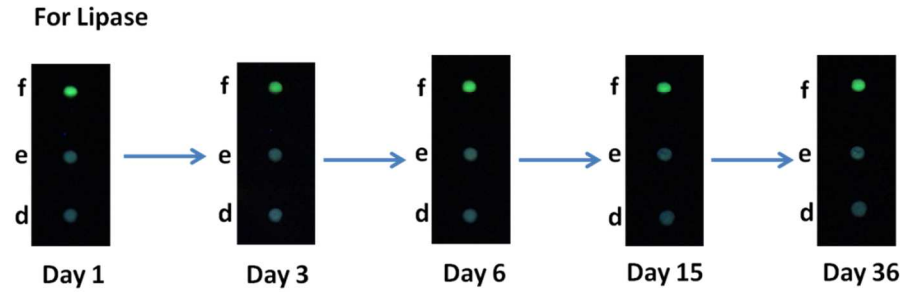


Figure S 11: Image capture under 365 nm UV lamp with time for paper strip with (From bottom to top), **disc d-** Tb-cholate gel (40 µL), **disc e-** 4 (65 µM) doped Tb-cholate gel, **disc f-** 4 (65 µM) doped Tb-cholate gel with 8 µg (16 µL of 0.5 mg/ mL) of lipase.

Table S3: tabular description of Figure S7 and Figure S8

Compound	Fig. S6			Fig. S7		
	Disc a	Disc b	Disc c	Disc d	Disc e	Disc f
Tb-Cholate						
Substrate 2						
Substrate 4						
β-Glucosidase						
Lipase						

Green colour: The presence of a compound in the coated disc.
Red colour: The absence of a compound in the coated disc.

The sensor discs were found to be stable for at least over a month when kept at -18 °C (freezer).

10. HPLC analysis to confirm DHN as the reaction product on the coated disc upon enzyme action

HPLC analysis was carried out for the filter paper discs coated with *pro*-sensitizers (**2**, **4**) doped Tb-cholate gel with and without the addition of enzyme to confirm the formation of DHN. Filter paper discs of 5 mm diameter were coated with Tb-cholate gel (40 µL) doped with **2** (200 µM) for β-glucosidase and **4** (70 µM) for lipase. On one of the disc enzyme solution was added and on the other disc an identical volume of water was added. After 1 h the disc were taken in a small test tubes (5 mL), and MeOH (350 µL), 5 % AcOH/ H_2O (50 µL) and H_2O (100 µL) were added, The mixture was sonicated for 10-12 s to extract the coated material from the disc and filtered through nylon membrane (0.45 micron).The filtrate was analyzed by HPLC (25 cm C18 analytical column with 45:55 H_2O/ MeOH as the mobile phase for **2** doped disc extract and with 35:65 H_2O/ MeOH as the mobile phase for **4** doped disc extract.

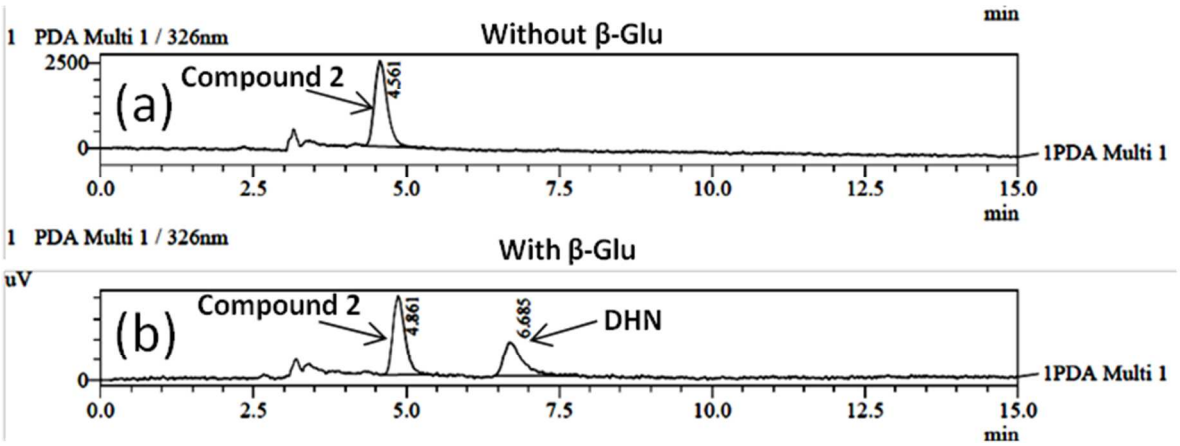


Figure S 12: Chromatogram of (a) **2** doped Tb-cholate gel coated paper disc extract 1h after preparation of sensor disc and (b) **2** doped Tb-cholate gel coated paper disc extract 1 h after addition of 2.5 µg of β-glucosidase (mobile phase 45:55 H_2O/ MeOH).

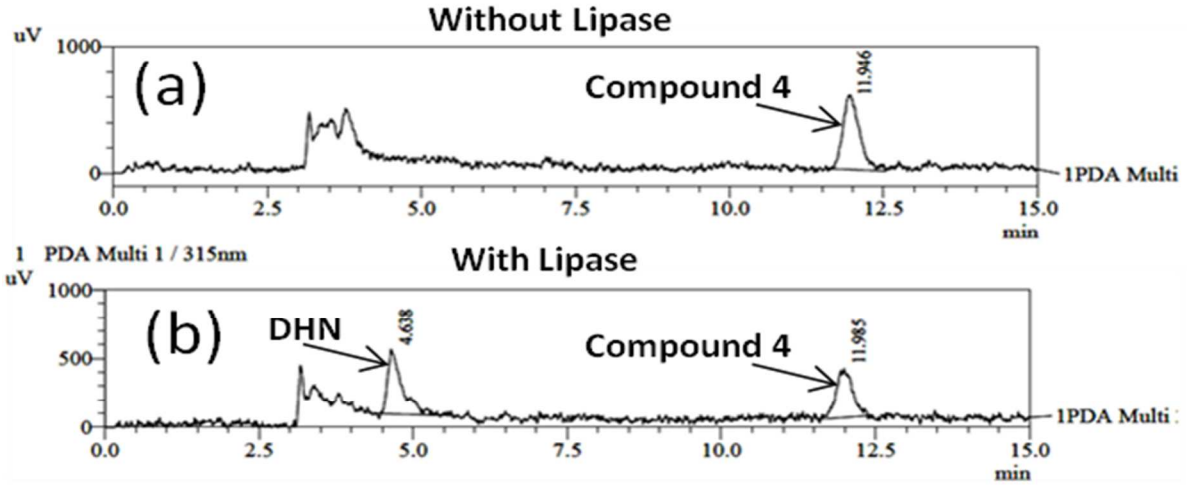


Figure S 13: Chromatogram of (a) **4** doped Tb-cholate gel coated paper disc extract 1h after preparation of sensor disc and (b) **4** doped Tb-cholate gel coated paper disc extract 1h after addition of 2.5 µg of lipase (mobile phase 35:65 H_2O/ MeOH).

- The conclusion from the HPLC analysis is that the sensitizer peak (DHN) was observed only after adding the enzyme solution on the sensor discs.

11. Plate reader data,

a. for β-glucosidase detection

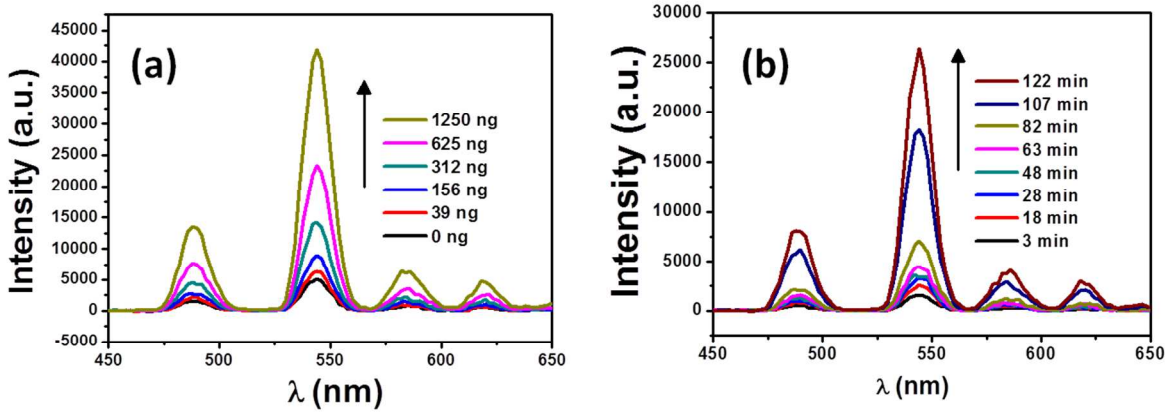


Figure S 14 Evolution of luminescence spectra (a) with increasing amounts of β-glucosidase, (b) as a function of time with 1.2 µg of β-glucosidase.

b. for lipase detection

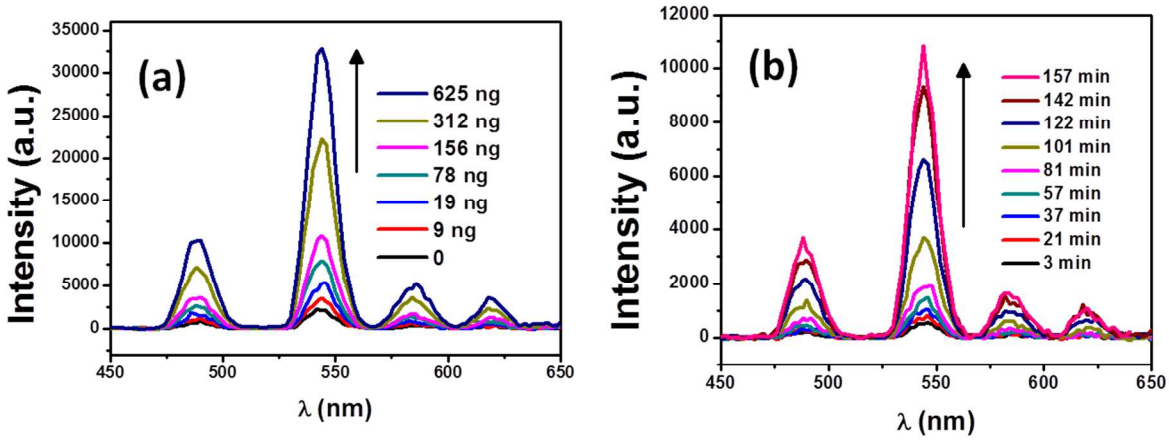


Figure S 15 Evolution of luminescence spectra (a) with increasing amounts of Lipase, (b) as a function of time with 156 ng of lipase.

12. Checking activity of the β-glucosidase doped Tb Cholate gel coated disc

When enzyme was the analyte of interest, *pro*-sensitizer was integrated on paper by using Tb-Cholate hydrogel and enzyme was added externally. If we can integrate the enzyme on paper surface and still it shows its activity, than it will be useful for detection of enzyme responsive analytes. So enzyme doped Tb-Cholate gel was prepared, paper disc was coated with this gel and the enzyme activity was checked by adding *pro*-sensitizer solution externally. It was quite interesting to see the enzyme showed very quick luminescence response (Figure S16-a, Disc 2) when integrated on paper so the immobilized enzyme on paper can be useful for detecting other biological and environmentally important analytes.

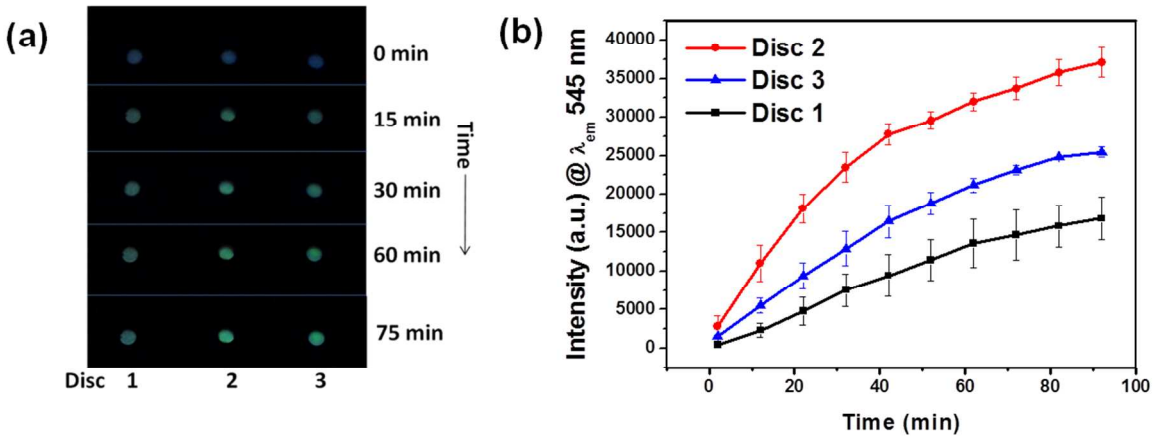


Figure S 16: (a) **Disc 1** was coated with 40 µL Tb-cholate gel, 20 µL DHN-glucoside and 20 µL β-glucosidase (10 µg, 0.5 mg/ mL) were added sequentially. **Disc 2** was coated with 40 µL β-glucosidase (10 µg) doped Tb-cholate gel and 20 µL aq. DHN-glucoside (400 µM) was added. **Disc 3** was coated with 40 µL DHN-glucoside (200 µM) doped Tb-cholate gel and 20 µL β-glucosidase(0.5 mg/ mL, 10 µg) was added. (b) Corresponding plate reader data. (error bars reflect the standard deviations of three sets of measurements)

13. Material cost for coated disc preparation:

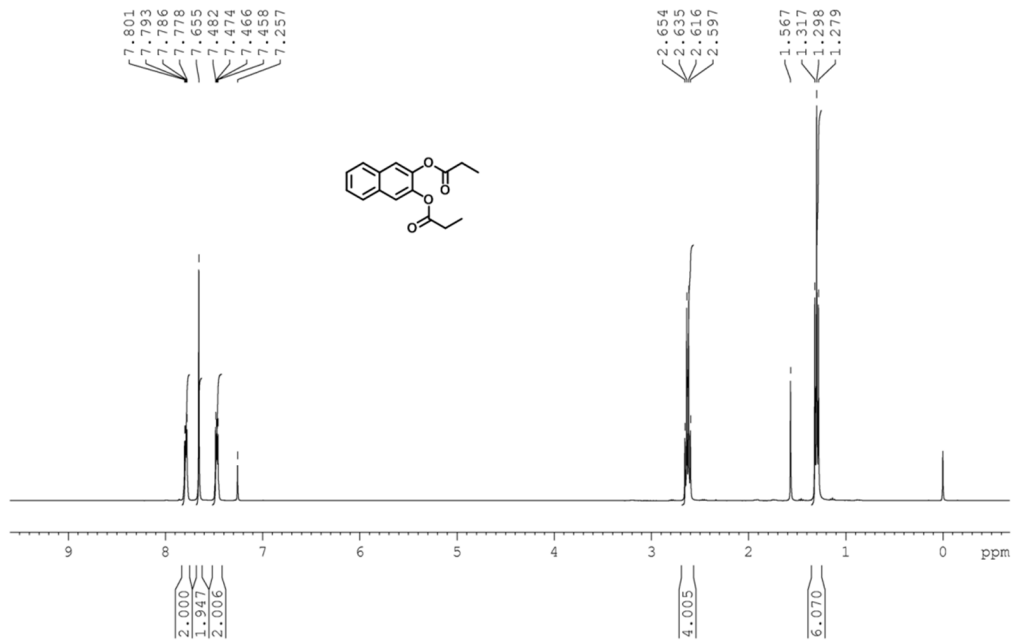
Table S 4: Material cost (based on Aldrich prices) for coated disc preparation for lipase detection

Material	Cost for a single disc (INR)
Whatman paper grade 3 (38 mm^2)	0.03
Na Cholate (0.258 mg)	0.0164
Tb Acetate (0.067 mg)	0.140
Pro-sensitizer (0.001 mg)*	0.001
Total	INR 0.187/- or $ 0.0028/-

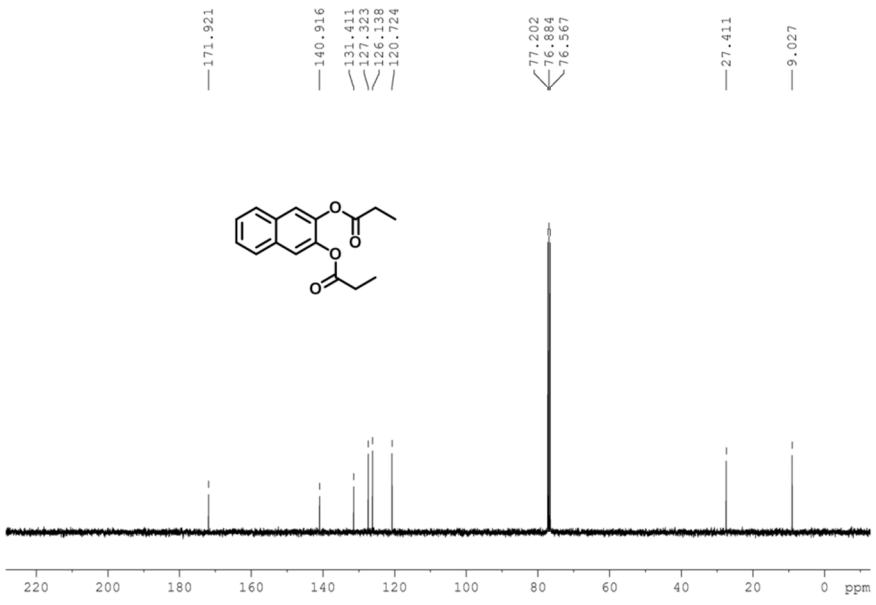
***Calculated based on the synthesis of pro-sensitizer 4 (include cost of starting materials, reagents, silica gel and solvent used for column purification, and the time spent by TG converted into cost based on her current fellowship).**

14. NMR spectra:

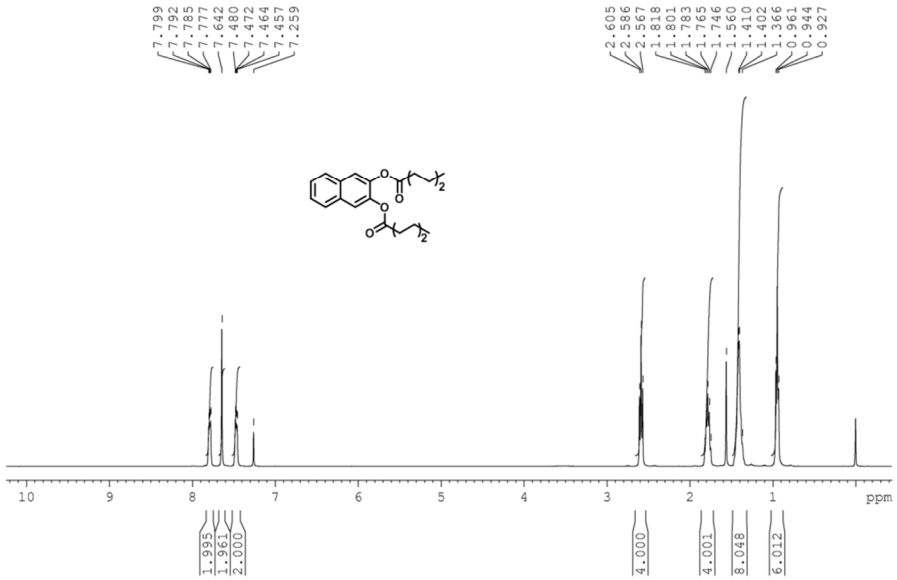
^{1}H NMR (400 MHz, $CDCl_3$) of Compound **4**



^{13}C NMR (100 MHz, $CDCl_3$) of Compound **4**



^{1}H NMR (400 MHz, $CDCl_3$) of Compound **5**



^{13}C NMR (100 MHz, $CDCl_3$) of Compound **5**