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SUPPORTING INFORMATION

Exploring the Synergistic Effect of Aggregation and Hydrogen Bonding: Fluorescent Probe for Dual sensing of Phytic Acid and Uric Acid

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EXPERIMENTAL SECTION

1.1 General: All chemicals (solvents, reagents, and chemicals) were purchased from best-known local chemical suppliers and used without further purification. Solvents were distilled and dried prior to use. FTIR spectra were recorded on a Perkin-Elmer FT-IR Spectrum BX system and were reported in wave numbers (cm^{-1}). On the other hand, ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance Neo 400 spectrometer operating at 400 and 100 MHz for ^{1}H and ^{13}C NMR spectroscopy, respectively. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane (TMS). Mass spectra were recorded on Shimadzu LCMS 8040.

1.2 Spectroscopic studies. The UV-vis spectroscopic studies were recorded on a JASCO (model V-650) UV-Vis spectrophotometer. The slit-width for the experiment was kept at 5 nm. Sensing was carried out by adding requisite amounts of phytic acid and uric acid (1 % DMSO) to aqueous solutions of probe **1** (10×10^{-6} M). On the other hand, fluorescence experiments were performed in FluoroLog-TM (Horiba Scientific) . The slit-width for the fluorescence experiment was kept at 5 nm (excitation) and 5 nm (emission) and the excitation wavelength was set at 340 nm. All the spectroscopic studies were repeated 3 times independently, under optimized working conditions to study its reproducibility. The relative standard deviation from 3 successive measurements are represented as error bars.

1.3 Lifetime measurements: Lifetime measurements were performed using Horiba Delta flex Modular flourescence lifetime system with following instrumental parameters: 340 nm NanoLED excitation source with an instrument response function of about 165 ps, and peak preset 10000 counts.

1.4 Detection limit determination. The method used for the calculation of the detection limit is known as the blank variability method. In this method, the calibration curve was prepared by recording fluorescence spectra of probe **1** in different amounts of phytic acid and uric acid.

From the equation obtained from the calibration plot, the added phytic acid/ uric acid concentrations were calculated. Then another calibration curve was drawn between the C_{real} (added phytic acid/uric acid, μM) vs. $C_{calc.}$ (Calculated amount of phytic acid or uric acid, μM). This afforded a value of the slope (b).

The fluorescence spectra of **1** were taken as blank reading. A total 10 replicates of the blank were measured. The standard deviation from the blank readings was calculated by fitting the fluorescence reading into the equation obtained from the first calibration curve (titration spectra). Using this standard deviation value, we calculated decision limit by this following equation.

$L_C = t_C \times s \times (1 + 1/N)1/2$...... .... (1)

where, N = the number of blank replicates taken; the value of t_c for 10 blank readings is 1.833; and s = the standard deviation value.

The detection limit (L_D) was calculated as the double of the decision limit obtained,

$L_D = 2 L_c$ .... (2)

In concentration term, the detection limit appeared as,

$x_D = 2 \times C = 2 L_C / b$ .... (3)

where, b = slope of the second calibration curve (C_{real} vs. $C_{calc.}$).

1.5 ^{1}H NMR Studies. ^{1}H NMR titration studies of compound **1** (5 mM) were performed with phytic acid (1.0 equiv) and uric acid (1.0 equiv) in DMSO-d_6. The spectra were recorded using identical parameters.

1.6 Scanning Electron Microscopy (SEM): Samples for SEM were drop casted on a silicon wafer with the required concentrations, and the solvent was allowed to evaporate overnight. The silicon wafer was then sputter-coated using a Leica Ultra Microtome EM UC7, and the stubs were loaded into an FEI Apreo LoVac to obtain images at 1 μm magnification.

1.7 Dynamic Light Scattering (DLS) Study: Zetasizer Nano S (Malvern Instruments) at 25 °C was used for DLS analysis. 2 mL of probe stock solutions (1 mM) was prepared in DMSO and diluted with water to the final concentration of 10 μM with/without phytic acid/ uric acid. Before recording, the samples were mixed thoroughly to obtain a consistent suspension in water.

1.8 Preparation of Artificial urine

Artificial urine consisted of 55 mmol/L sodium chloride, 67 mmol/L potassium chloride, 2.6 mmol/L calcium sulfate, 3.2 mmol/L magnesium sulfate, 29.6 mmol/L sodium sulfate, 19.8 mmol/L sodium dihydrogen phosphate, 310 mmol/L urea, and 9.8 mmol/L creatinine. Different amounts of uric acid (5 μM to 50 μM) were spiked into the artificial urine for quantitative uric acid detection.

SYNTHESIS AND CHARACTERIZATION

Synthesis of compound **1**

Compound **1** was synthesized according to the procedure reported in literature[1]. Briefly, to a stirred solution of Tetraphenylethylene (TPE)-Mono aldehyde in Dichloromethane, Indole was added along with a catalytic amount of $Bi(NO_3)_3 \cdot 5H_2O$ and was stirred vigorously for 20 mins at room temperature. The reaction completion was monitored by TLC, and the solvent was evaporated to give the desired product. ^1H NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 2H), 7.41-7.32 (m, 4H), 7.21 – 6.99 (m, 21H), 6.95 (d, 2H), 6.57 (d, 2H), 5.77 (s, CH, 1H). HRMS (ESI-TOF) *m/z*: Calcd for $C_{43}H_{33}N_2$ 576.2634; Found 575.2433.

The XPS analysis performed to confirm the composition of the probe. The XPS survey spectra reveals the presence of C1s, N1s peaks. As seen in Figure. S3, the core level C1s spectra involves three peaks, at 284.83, 285.5, and 287.81 which corresponds to the C-C, C=C and C-N bond respectively.

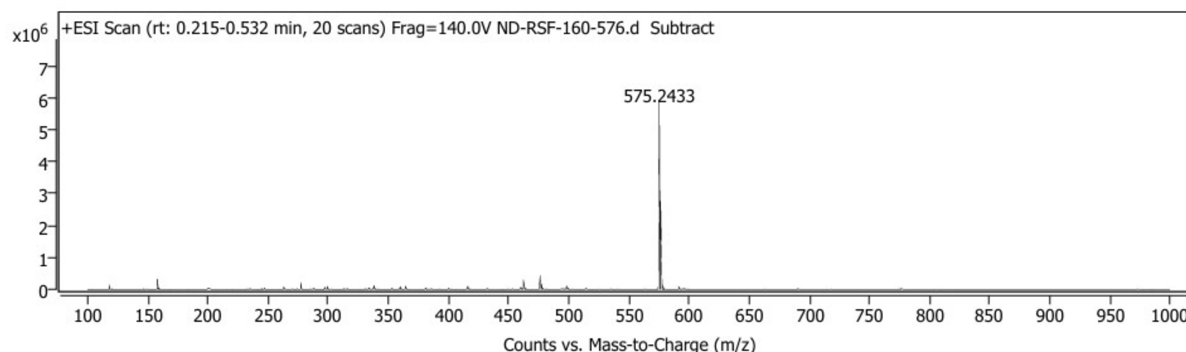


Figure. S1: HRMS of compound **1**

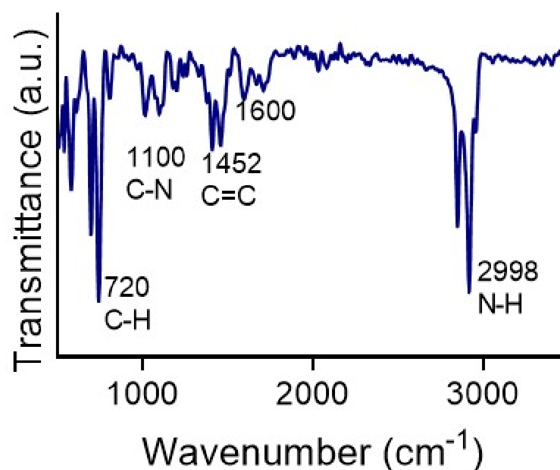


Figure. S2: FTIR spectra of compound 1

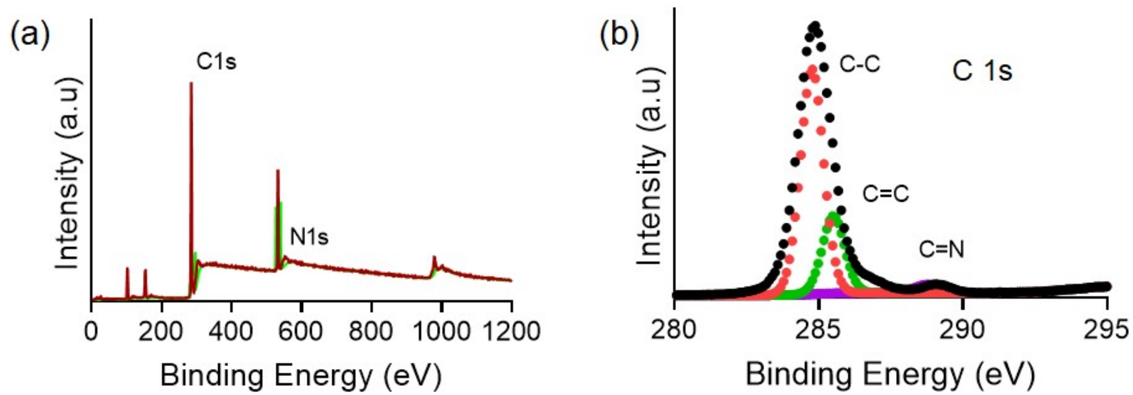


Figure. S3: (a) XPS survey spectra and (b) C1s XPS spectra of compound 1.

ADDITIONAL SPECTROSCOPIC DATA

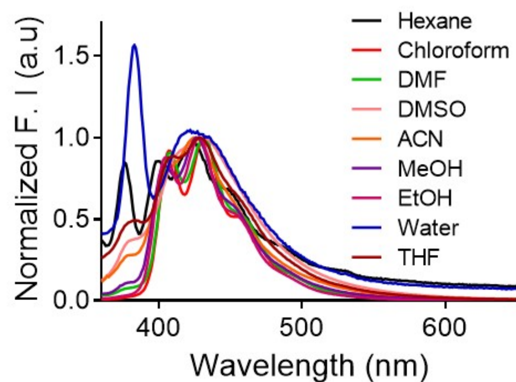


Figure. **S4**. Normalized fluorescence spectra of probe **1** (10 µM, λ_{ex} = 340 nm) in different solvents.

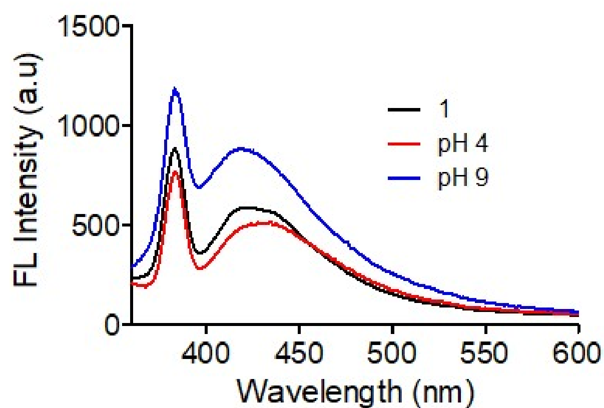


Figure. **S5**. Fluorescence spectra of probe **1** (10 µM, λ_{ex} = 340 nm) in pH 6 (black line), pH 4 (red line) and pH 9 (blue line).

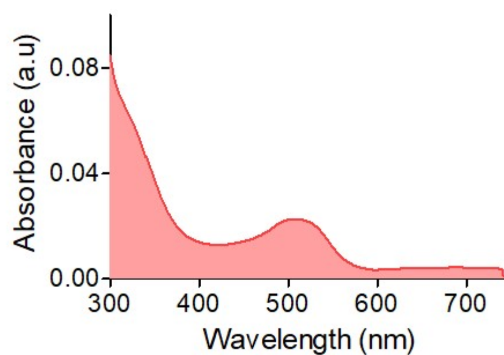


Figure. **S6**. UV-Visible spectra of probe **1** (10 µM) in water medium.

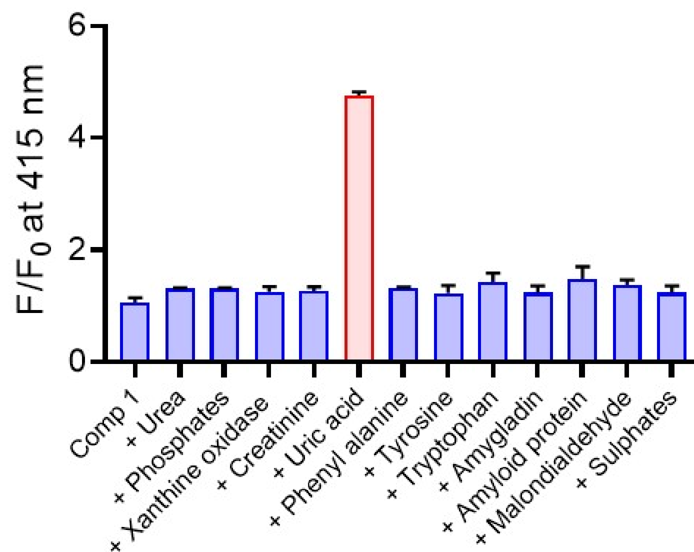


Figure. **S7**. Change in fluorescence intensity of probe **1** at 415 nm in the presence of different urinary disease biomarkers (10 equiv.) and other potent interfering analytes present in urine.

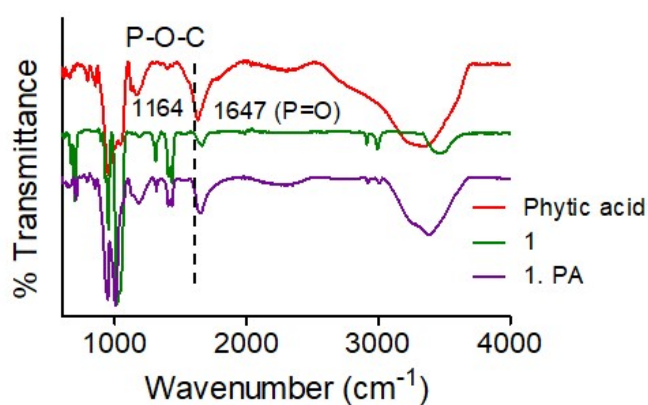


Figure. **S8**. FTIR spectra of phytic acid, probe **1** and **1**. PA

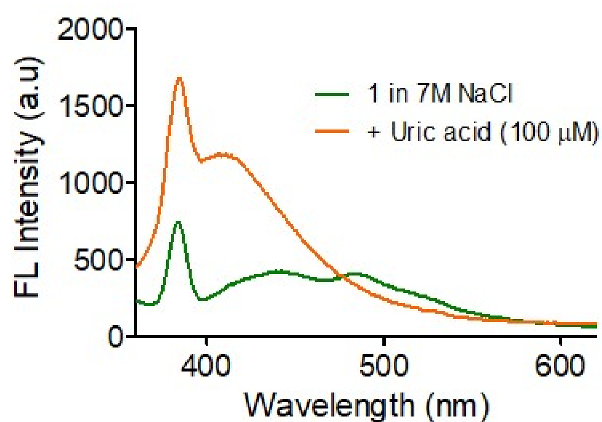


Figure. **S9**. Fluorescence spectra of probe **1** (10 µM) upon addition of uric acid in 7 M NaCl medium.

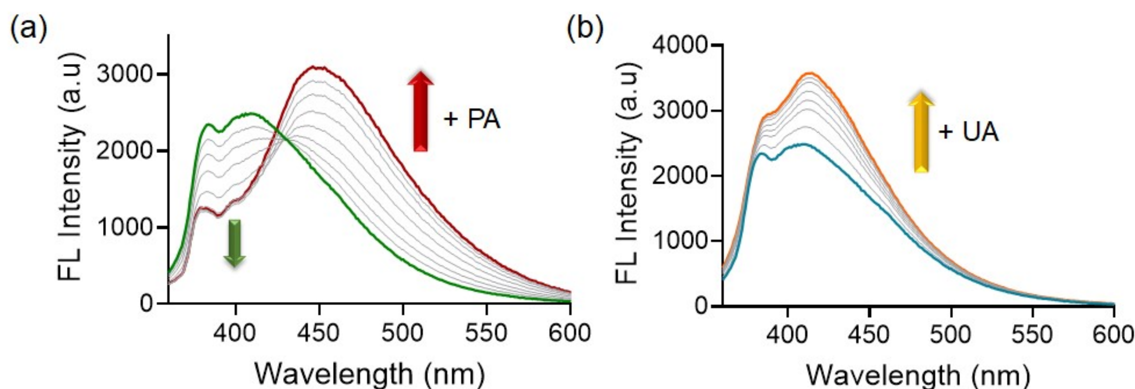
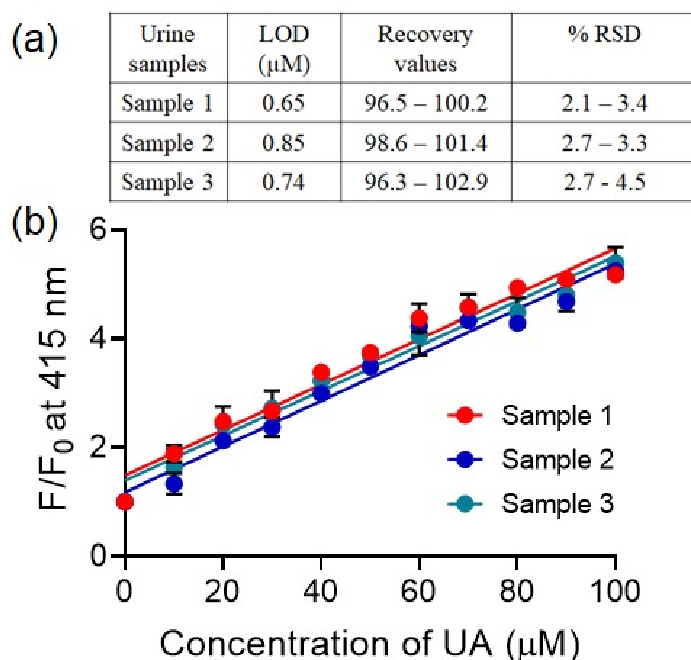


Figure. **S10**. Fluorescence spectra of probe **1** (10 µM, λ_{ex} = 340 nm) in the presence of (a) phytic acid (0-70 µM) and (b) Uric acid (0-70 µM) in 7 M Urea.



(a)

Urine samples	LOD (µM)	Recovery values	% RSD
Sample 1	0.65	96.5 – 100.2	2.1 – 3.4
Sample 2	0.85	98.6 – 101.4	2.7 – 3.3
Sample 3	0.74	96.3 – 102.9	2.7 - 4.5

Figure. S11: (a) Table displaying quantitative estimation (detection limit (in µM), recovery values and relative standard deviations) of UA in real urine samples. (b) Change in fluorescence response of **1** at 415 nm of different concentration of uric acid in diluted urine (20%, v/v) at pH 7.4.

References

1. F. Lafzi, H. Kilic, B. Ertugrul, M. Arik, N. Saracoglu, J. Lumin. 2019, **208**, 174-182.