Supporting Information

Differential detection of Aspartic acid in MCF-7 breast cancer cells

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1. Table S1. Performance comparison of existing methods and present method for detection of Cysteine

Analytes	Sensor type	Detection limit	Sensitivity and Selectivity	Estimation	Application	References
Aspartic acid	**Carbozole**	**46.1 nM**	**High**	**Yes**	**Yes**	**Present manuscript**
Aspartic acid, Glutaic acid	BODIPY	1 µM	Moderate	No	Yes	Dyes Pigm., 2019, 168, 111-122
Aspartic acid	Methoxysalicylaldehydethiosemicarbazone	8.7449×10^{-8} M	High	No	Yes	InorganicaChimicaActa 530 (2022) 120683
Aspartic acid, Glutaic acid	NIR cyanide fluorescent	10^{-7} M	Moderate	No	Yes	NewJ.Chem., 2014, 38, 4791
Aspartic acid	Sliver ion fabricated lanthanide complex	0.46 µM	High	No	No	Sensors and Actuators B 253 (2017) 1006–1011

1.NMR Studies

^{1}H NMR of PCF in CD_3CN:

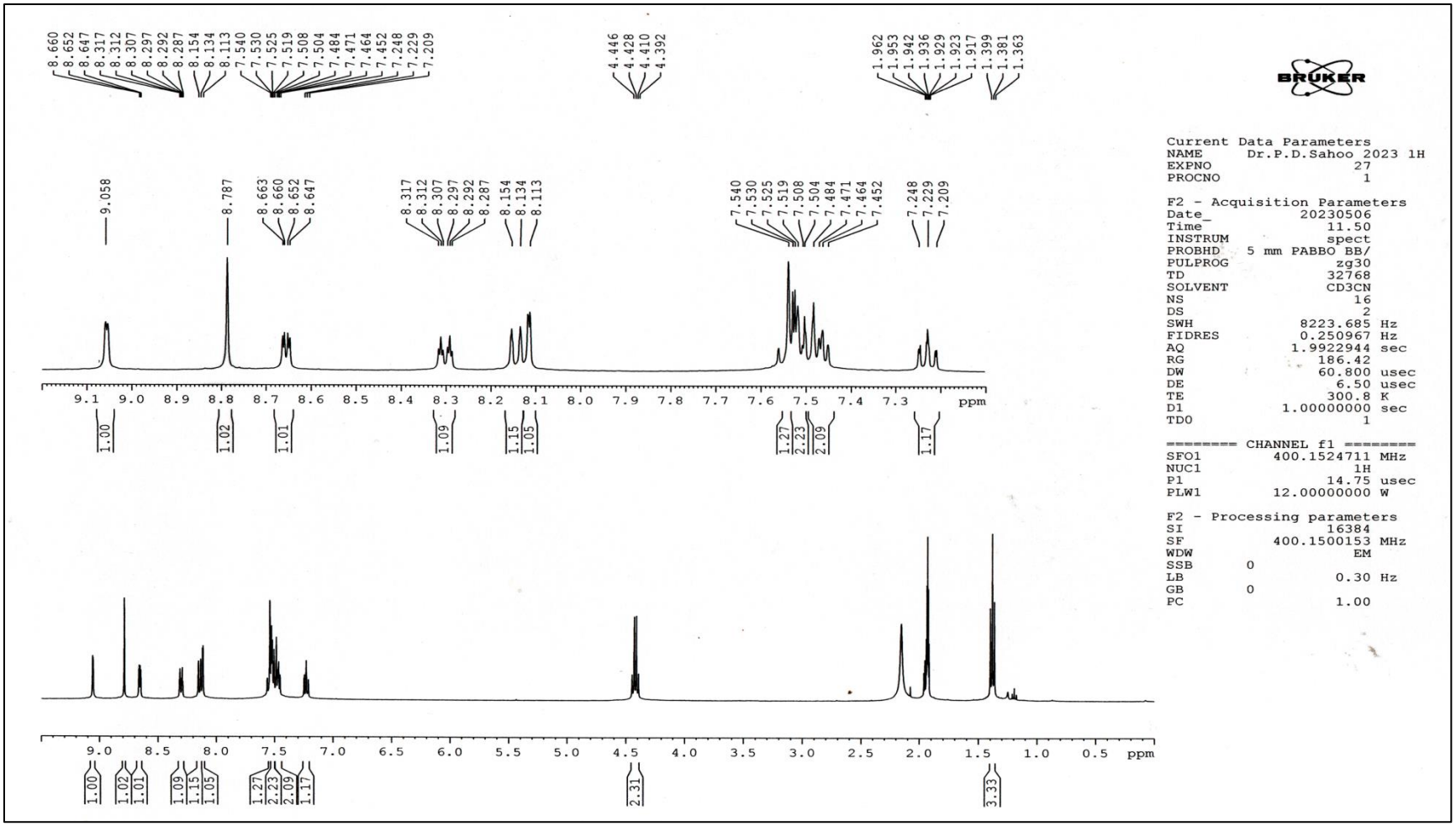

Figure S1.^{1}H NMR of P**C**F in CD_3CN (400 MHz)

^{13}C NMR of PCF in CD_3CN:

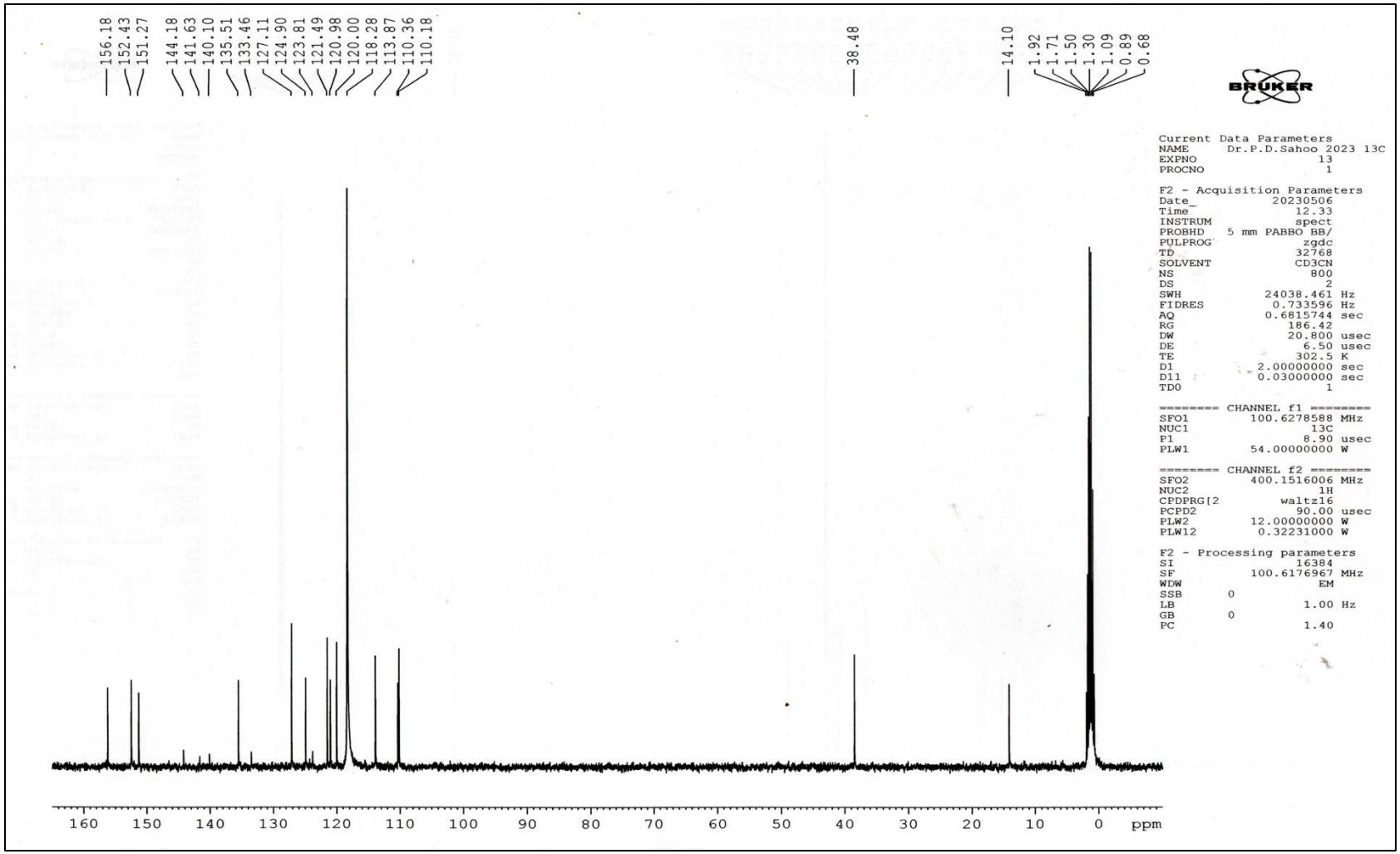

Figure S2. ^{13}C NMR of PCF in CD_3CN (100 MHz).

2.Selectivity

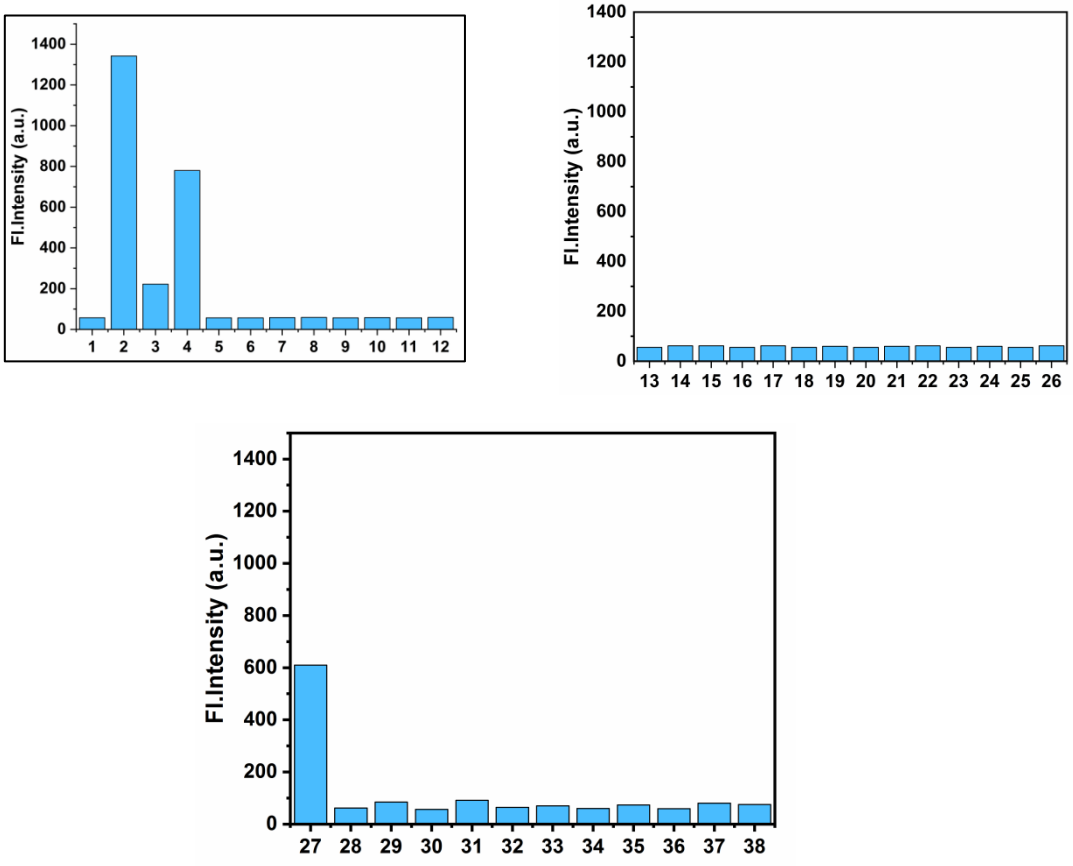

Figure S3. Fluorescence spectra of PCF in presence of different analytesat434 nm (λ_{ex}= 300 nm) in H_2O-CH_3CN (2:1, v/v) at neutral pH.1) Blank, 2) Aspartic acid, 3) Serine, 4) Glutamic acid, 5) L-proline,6) Tyrosine,7) Methionine,8) Leucine,9) Lysine, 10) Alanine, 11) Phenyl alanine 12) Histidine13) Threonine, 14)Valine, 15) Zn^{2+}, 16) Cu^{2+}, 17) Pb^{2+}, 18) Mg^{2+}, 19) Ca^{2+}, 21) H_2O_2, 22) Fe^{3+}, 23) Sn^{2+}, 24) Al^{3+},25) Na^{+} 26) K^{+}, 27)cysteine (Cys), 28) glycine (Gly), 29) glutamine (Gln), 30)valine (Val), 31) threonine (Thr), 32) methionine (Met), 33) arginine (Arg), 34) isoleucine (Iso), 35) asparagine (Asn), 36) ornithine (Orn) 37) citrulline (Cit), 38) sarcosine (Sar).

3. Optical study and Competitive selectivity in presence of other analytes

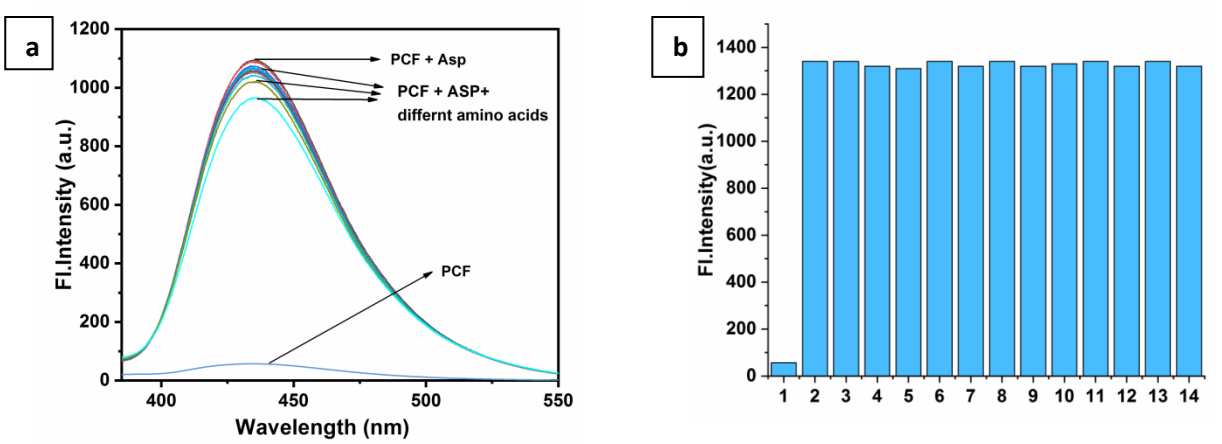

Figure S4. (a) Optical study of PCF with aspartic acid then different amino acids (Ala, Arg, Asn, Cys, Glu, Gln, Gly, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val)**(b)**Histogram representing competitive fluorescence spectra of **PCF** + **Aspartic acid** in presence of different analytes at 434 nm (λ_{ex} = 300 nm) in H_2O-CH_3CN (2:1, v/v) at pH 6.5-6.8. [1) Blank, 2) Aspartic acid, 3) Aspartic acid +Zn^{2+}, 4) Aspartic acid +Hg^{2+}, 5) Aspartic acid +Pb^{2+}, 6) Aspartic acid +Mg^{2+}, 7) Aspartic acid +Ca^{2+}, 8) Aspartic acid +Na^{+}, 9) cysteine +K^{+}, 10) Aspartic acid +H_2O_2, 11) Aspartic acid +H_2S,12) Aspartic acid +Cd^{2+}, 13) Aspartic acid+CN^{-}, 14) Aspartic acid +NO_3^{-}]

4. UV-vis and fluorescence titration studies

UV-vis spectral studies:

A stock solution of **PCF**1 × 10^{-5} M was prepared in water-acetonitrile (2:1, v/v). **Aspartic acid** solution of concentration 1 × 10^{-4} M was prepared in Millipore water. All experiments were carried out in an aqueous medium at pH 6.5-6.8. During the titration, each time a 1 × 10^{-5} M solution of **PCF** was filled in a quartz optical cell of 1 cm optical path length and **Aspartic acid** stock solution was added into the quartz optical cell gradually by using a micropipette.

Fluorescence spectral studies:

A stock solution of **PCF**1 × 10^{-5} M was prepared in water-acetonitrile (2:1, v/v). **Aspartic acid** solution of concentration 1 × 10^{-4} M was prepared in Millipore water. All experiments were carried out in aqueous medium at pH 6.5-6.8. During titration, each time a 1 × 10^{-5} M solution of **PCF** was filled in a quartz optical cell of 1 cm optical path length and **Aspartic acid** stock solution was added into the quartz optical cell gradually by using a micropipette. For all fluorescence measurements, excitations were provided at 300 nm, and emissions were collected from 390 to 550 nm.

5. Evaluation of the association constants for the formation of PCF-Aspartic acid complex:

By Fluorescence Method:

Binding constant of the chemosensor **PCF** was calculated through emission method by using the following equation:

$$1/ (I – I_0) = 1/K(I_{max}– I_0) [G] + 1/(I_{max}– I_0) \quad ……..(ii)$$

Where I_0, I_{max}, and I represent the emission intensity of free **PCF**, the maximum emission intensity observed in the presence of added **Aspartic acid**at 434 nm (λ_{ex}= 300 nm), [G] is the concentration of the guest**Aspartic acid** and the emission intensity at a certain concentration of the **Aspartic acid**, respectively. [H] is the concentration of the host **PCF**.

Binding constant calculation graph (Fluorescence method):

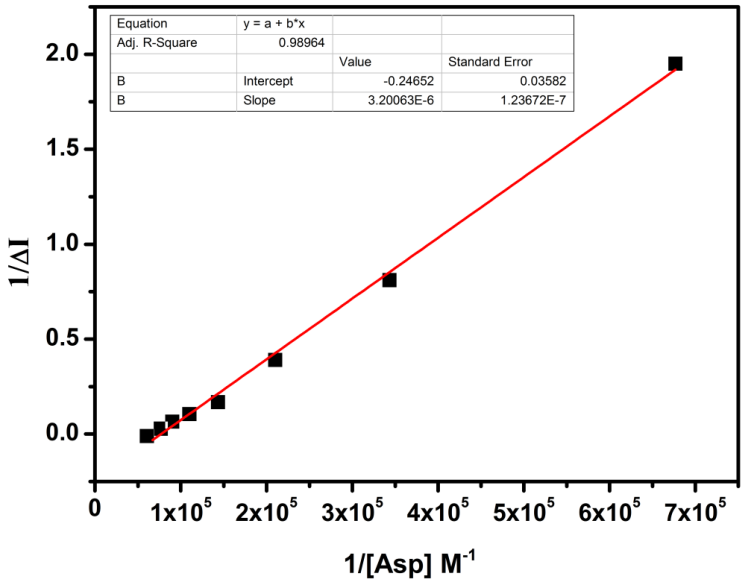

Fig. S5. Linear regression analysis (1/[G] vs 1/ΔI) for the calculation of association constant value by fluorescence titration method.

The association const. (K_a) of **PCF** for sensing **Aspartic acid**was determined from the equation: K_a = intercept/slope. From the linear fit graph, we get intercept =0.24652, slope = 3.20063× 10^{-6}. Thus, we get, K_a = 0.24652/ (3.20063 × 10^{-6}) = 7.7 × $10^4 M^{-1}$.

6. Job's plot

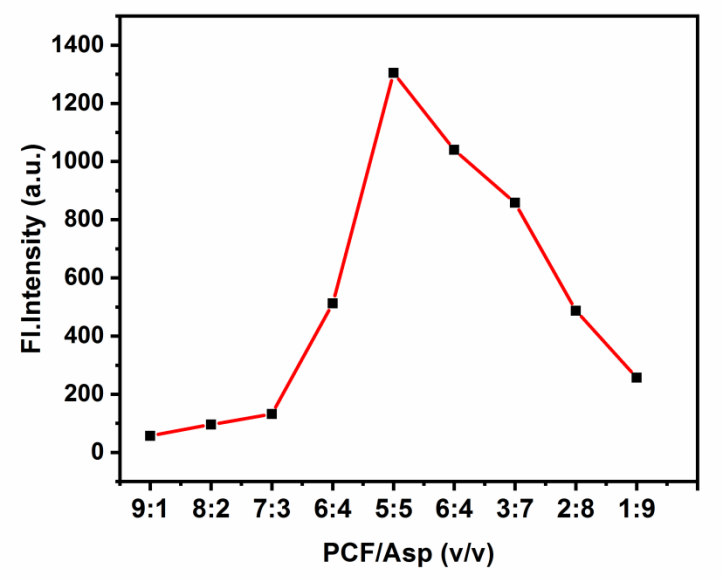

Figure S6. Job's plot of **PCF**(10μM) with **Aspartic acid** in acetonitrile-water (1:2, v/v), at neutral pH, by fluorescence method, which indicate 1:1 stoichiometry for **PCF** with **Aspartic acid**. Standard deviations are represented by error bar (n=3).

7. Calculation of limit of detection (LOD) of PCF with Aspartic acid:

The detection limit of the chemosensor **PCF** for **Aspartic acid** was calculated on the basis of fluorescence titration. To determine the standard deviation for the fluorescence intensity, the emission intensity of four individual receptors without **Aspartic acid** was measured by 10 times and the standard deviation of blank measurements was calculated.

The limit of detection (LOD) of **PCF** for sensing **Aspartic acid** was determined from the following equation[2-3]:

$$LOD = K \times SD/S$$

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor solution; S is the slope of the calibration curve.

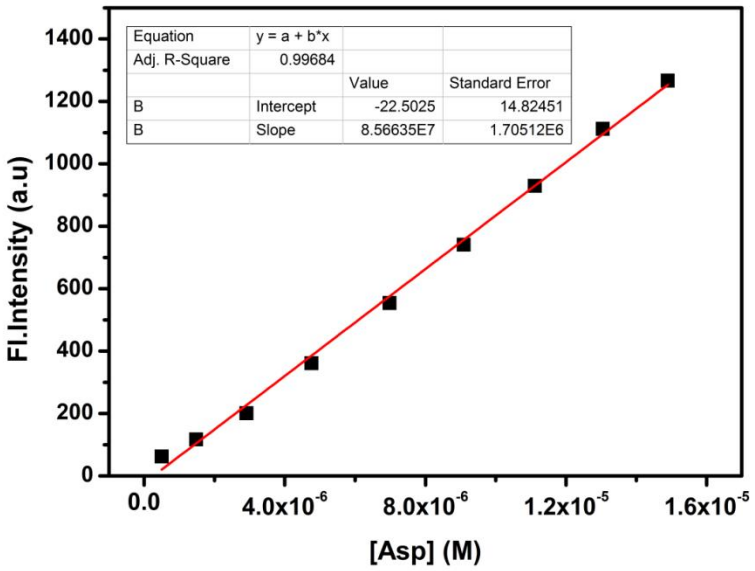

Figure S7. Linear fit curve of **PCF**at434 nm with respect to **Aspartic acid** concentration

For **PCF** with **Aspartic acid:**
From the linear fit graph, we get slope = 8.56635×10^{7}, and SD value is 1.3189665.
Thus, using the above formula, we get the Limit of Detection = 4.61×10^{-8}M. Therefore,**PCF** can detect **Aspartic acid** up to this very lower concentration by fluorescence technique.

8. Time-dependent fluorescence spectra of PCF in the presence of Aspartic acid

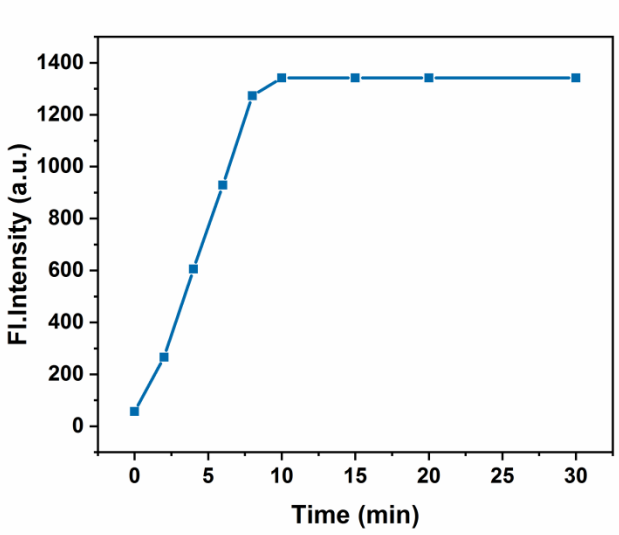

Figure S8. Time-dependent fluorescence spectra of PCF in the presence of **Aspartic acid.**

9. PCF lifetime in presence and absence of aspartic acid

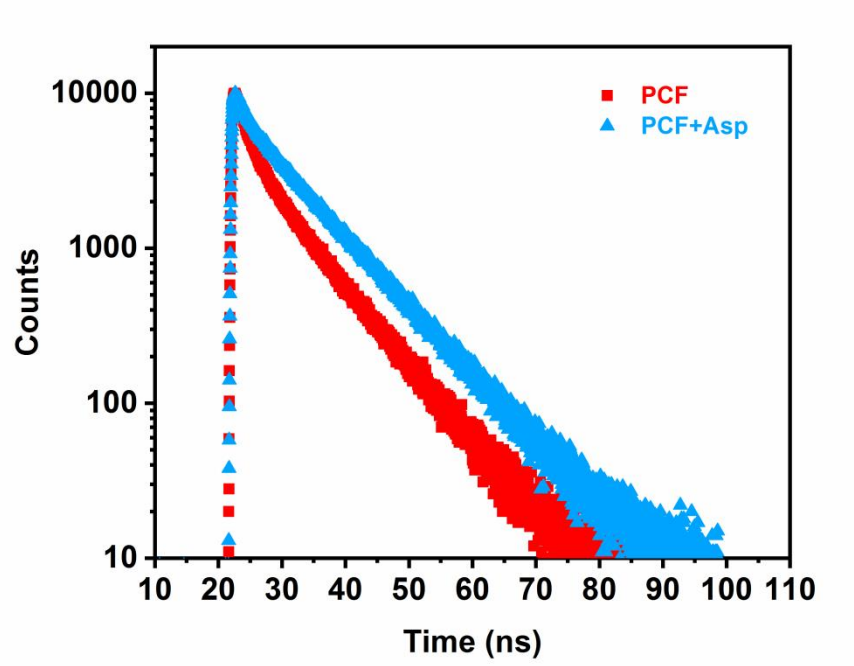

Figure S9. PCF lifetime in presence and absence of aspartic acid

Table S2. Decay time components of PCF and PCF + Asp

System	**b_1**	**τ_1**	**b_2**	**τ_2**	**$<\tau>=b_1\tau_1+b_2\tau_2$**
PCF	0.430738	5.42E-01	0.029351	1.75E+00	0.28 ns
PCF + Asp	0.280081	1.45E-01	0.449727	5.48E+00	2.50 ns

10. Reversibility analysis of PCF

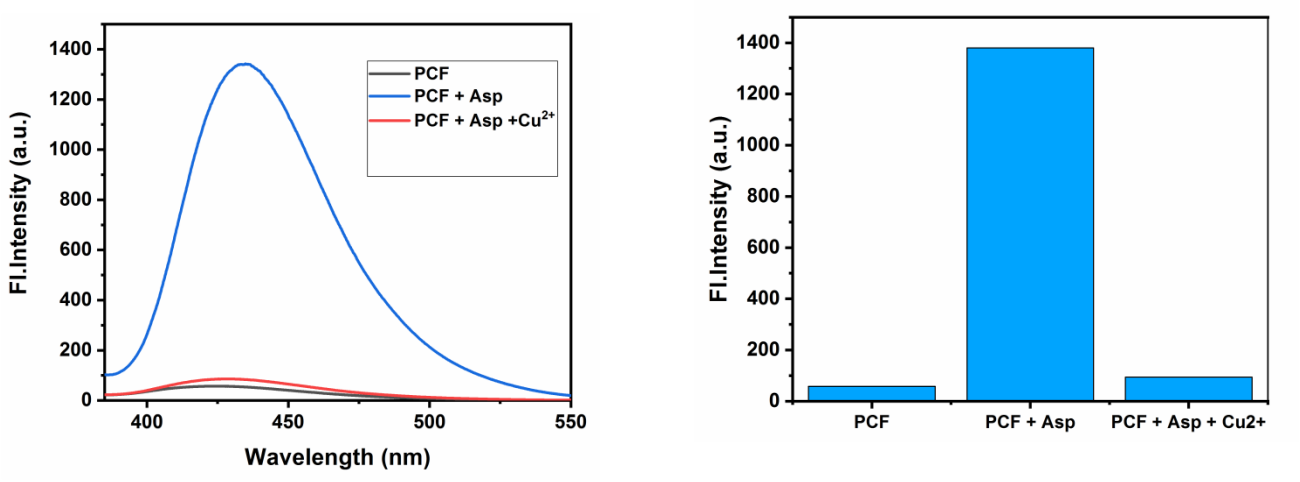

Figure S10. Reversibility analysis of PCF (10^{-5} M) with Aspartic acid (10^{-4} M) in presence of Cu^{2+} (10^{-4} M)**.**

11. pH titration study:

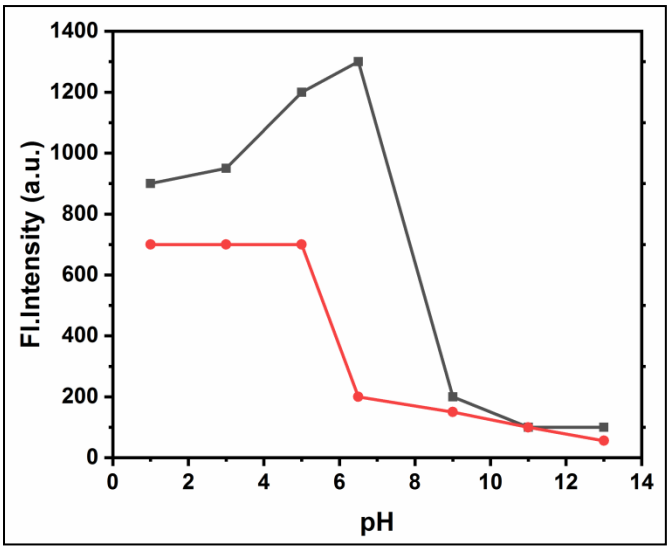

Figure S11. Effect of pH on the fluorescence intensity of **PCF** (10^{-5}M) in the absence of **Aspartic acid** (red line) and in the presence of **Aspartic acid** (10^{-4}M, black line).

12. DFT study

Table S3. Details of the geometry optimization in Gaussian 09 program

Details	PCF	Aspartic acid	PCF 1
Calculation method	B3LYP	B3LYP	B3LYP
Basis set	6-31G (d,p)	6-31G (d,p)	6-31G (d,p)
E(CAM-B3LYP) (a.u.)	-936.66	-512.32	-1449.00
Charge, Multiplicity	0, 1	0, 1	0, 1
Solvent (CPCM)	Water	Water	Water

TDDFT- Calculations

Table S4. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations of the low-lying excited states of PCF. The data were calculated by TDDFT//B3LYP/6-31G(d,p) based on the optimized ground state geometries.

Molecules	Electronic Transition	Excitation Energy[a]	f[b]	Composition[c](%)
PCF	$S_0 \rightarrow S_1$	3.2237 eV 384.60 nm	0.5287	H → L (69.1%)
PCF 1	$S_0 \rightarrow S_1$	3.0716eV 403 nm	0.4055	H → L (70 %)

[a]Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b]Oscillator strength. [c]H stands for HOMO and L stands for LUMO.

Table S5. Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

Species	E_{HOMO} (a.u.)	E_{LUMO} (a.u.)	ΔE(a.u.)	ΔE(eV)	ΔE(kcal/mol)
PCF	-0.19252	-0.05946	0.14459	3.22	74.25
PCF 1	-0.20149	-0.07434	0.14563	3.07	70.80

13. Cytotoxicity assay

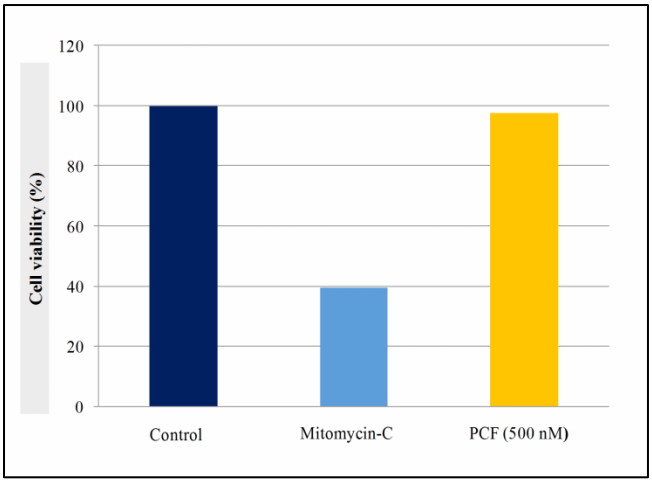

Figure S12. MTT assay is to determine the cytotoxic effect of PCF at 500 nM concentration on MCF-7 cells. Untreated control cells and Mitomycin-C treated cells are kept as negative and positive control respectively.