# Electronic Supplementary Information

## Lysine triggered ratiometric conversion of dynamic to static excimer of a pyrene derivative: aggregation-induced emission, nanomolar detection and human breast cancer cell (MCF7) imaging

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### 1. Materials and methods

High-purity HEPES, pyrene-1-carbaldehyde and phenylpropanolamine are purchased from Sigma Aldrich (India). Amino acids are purchased from Merck (India). Solvents used are of spectroscopic grade. Other chemicals are of analytical reagent grade and has been used without further purification except when specified. Mili-Q Milipore 18.2 MΩ $cm^{-1}$ water has been used throughout all the experiments. A Shimadzu Multi Spec 2450 spectrophotometer is used for recording UV-Vis spectra. FTIR spectra are recorded on a Shimadzu FTIR (model IR Prestige 21 CE) spectrophotometer. Mass spectra have been recorded using a QTOF 60 Micro YA 263 mass spectrometer in ES positive mode. $^1$H NMR titration in $CD_3OD$-$D_2O$ has been performed using a Bruker Avance 500 (500 MHz).$^{13}$C NMR spectrum of A3 is recorded using a Bruker Advance 400 (100 MHz). $^1$H NMR spectra of A3 with other representative amino acids are recorded using a Bruker Avance 300 (300 MHz). Elemental analyses are performed on a Perkin Elmer 2400 CHN analyzer. The steady state emission and excitation spectra are recorded with a Hitachi F-4500 spectrofluorimeter. Systronics digital pH meter (model 335) is used for measurement of solution pH. Dilute HCl or NaOH (50 μM) are used for pH adjustment. The imaging system is composed of an inverted fluorescence microscope (Dewinter, Italy). Images are captured through an attached CCD camera equipped with BioWizard 4.2 software. The microscope is equipped with a 50 W mercury arc lamp. All spectra are recorded at room temperature.

**2. General method of UV-Vis and fluorescence titration**

Path length of the cells used for absorption and emission studies is 1 cm. Stock solutions of A3 and Lys are prepared in $CH_3OH$/ water (4/1, v/v). Working solutions of A3 and Lys are prepared from their respective stock solutions. Fluorescence measurements are performed using 2.5 nm x 2.5 nm slit width.

**3. Determination of binding constant**

The binding constant of A3 for Lys is determined using a modified Benesi–Hildebrand equation[1]: $(I_{max} - I_0)/(I_x - I_0) = 1 + (1/K)\ (1/[C]^n)$ where $I_{max}$, $I_0$ and $I_x$ are emission intensity values for A3 in the presence of Lys at saturation, in the absence of Lys and at any intermediate Lys concentrations, respectively. A plot of $(I_{max} - I_0)/(I_x - I_0) = 1 + (1/K)\ (1/[C]^n)$ (here n = 0.5) yields the binding constant value from the slope.

**4. *In vitro* cell imaging**

Human breast cancer cell line MCF7 is grown in DMEM (Sigma, St. Louis, USA) supplemented with 10% fetal bovine serum (Sigma, St. Louis, USA), 2 mM glutamine, 100 U m/L penicillin-streptomycin solution (Gibco, Invitrogen, USA) in the presence of 5% $CO_2$ at 37 °C. For *in vitro* imaging studies, cells are seeded in 6 well culture plates with a seeding density of $10^5$ cells per well. After reaching 60–70% confluence, the previous media is replaced with serum free media, supplemented by Lys and A3 at 50 μM and 20 μM, and incubated for 2 h to facilitate the Lys or A3 uptake by the cells. Then the cells were placed under an inverted microscope (Dewinter, Italy) at different magnifications to examine any adverse effect on cellular morphology.A3 treated cells are then incubated with Lys for 15–30 min and observed under an inverted fluorescence microscope at different magnifications with a blue filter.

**Synthesis of (*E*)-2-((pyren-3-yl)methyleneamino)-1-phenylpropan-1-ol (A3)**

A methanol solution of pyrene 1-carboxaldehyde (230 mg, 1.0 mmol) is added drop wise under stirring condition to a methanol solution of 2-amino-1-phenylpropan-1-ol hydrochloride (187.6 mg, 1.0 mmol) in presence of $NaHCO_3$ (Scheme 1). Stirring is continued for further 30 min followed by reflux for 8 h. Upon removal of excess $NaHCO_3$ through filtration, an orange solution is obtained which on slow evaporation gives an orange residue with a yield of 93%. The residue is then recrystallized to get orange crystals suitable

for SC-XRD analysis. ESI-MS (m/z) (Fig.S1, ESI): $[M + H]^+$ 364.2, $[M + Na]^+$ 386.2. $^1H$ NMR ($CD_3OD$, 500 MHz) (Fig.S2, ESI): δ 1.56 (d, J= 5 Hz, 3H), δ 3.85 (m, J= 5 Hz, 1H), 4.77 (t, 1H), 7.20 (t, 1H, benzene),7.31 (t, J = 5 Hz, 2H, benzene), 7.42 (d, J = 10 Hz, 2H, benzene), 8.04 (m, J = 5 Hz, 3H, pyrene), 8.12 (m, J = 5 Hz, 3H, pyrene), 8.21 (m, J = 5 Hz, 3H, pyrene), 9.10 (s, 1H, CH=N) ppm. $^{13}C$ NMR ($CDCl_3$, 400 MHz) (Fig. S3, ESI): δ 16.48, 71.99, 77.26, 122.30-132.72, 141.16, 159.28 ppm. Elemental analysis for $C_{26}H_{21}NO$ (%): calculated, C 85.92, H 5.82, N 3.85, O 4.40; found C 85.84, H 5.91, N 3.90, O 4.32.

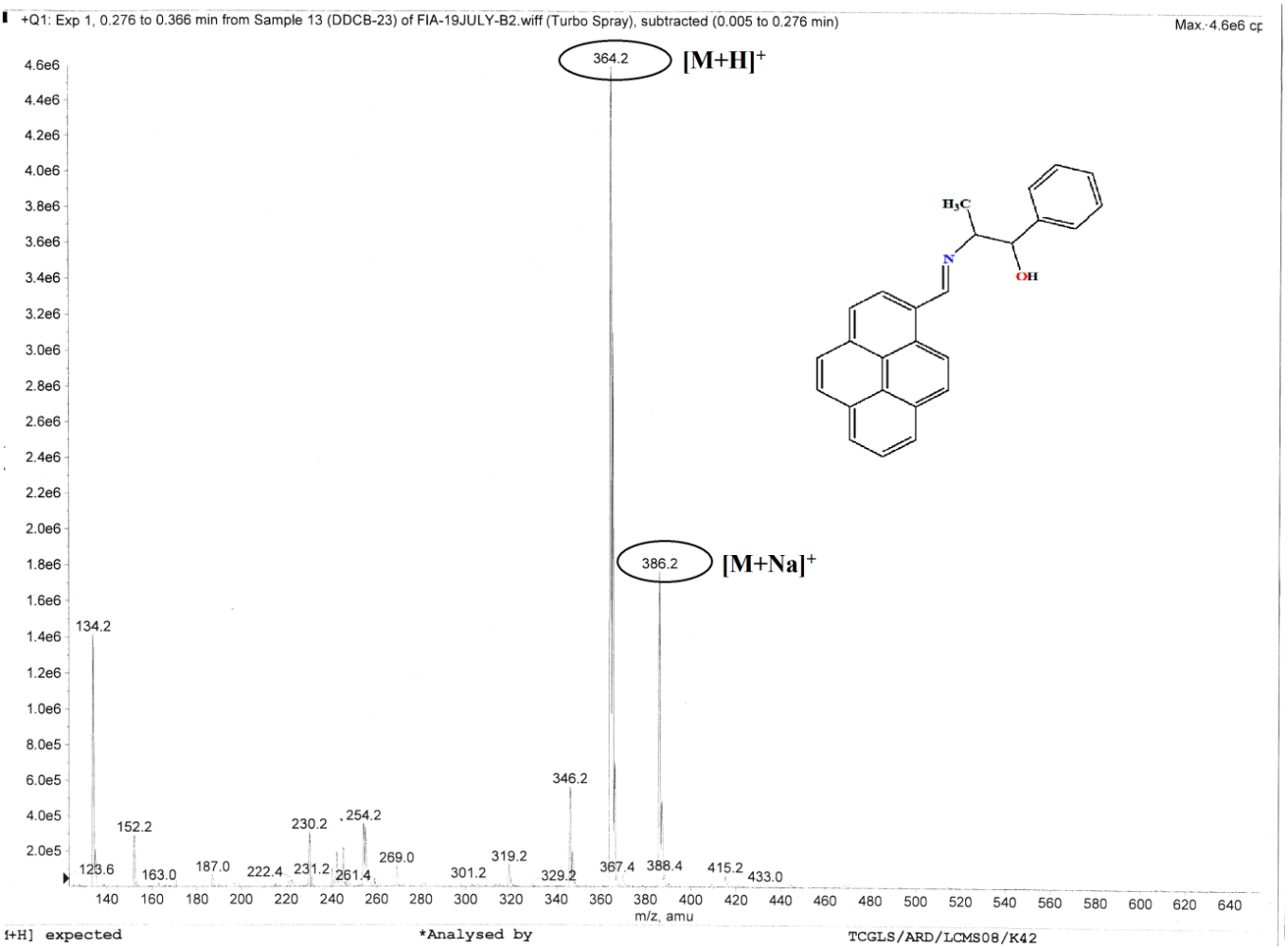


**Fig. S1** Mass spectrum of A3

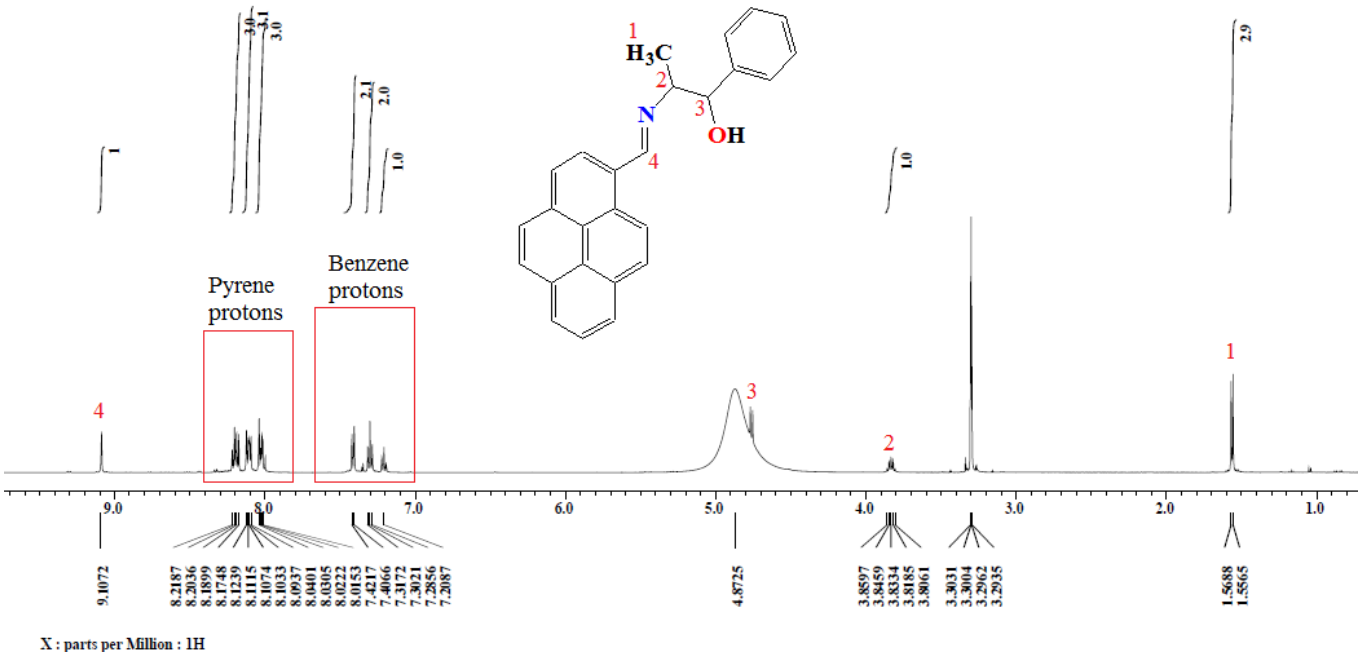


**Fig. S2** $^{1}$H NMR spectrum of A3 in $CD_3OD$

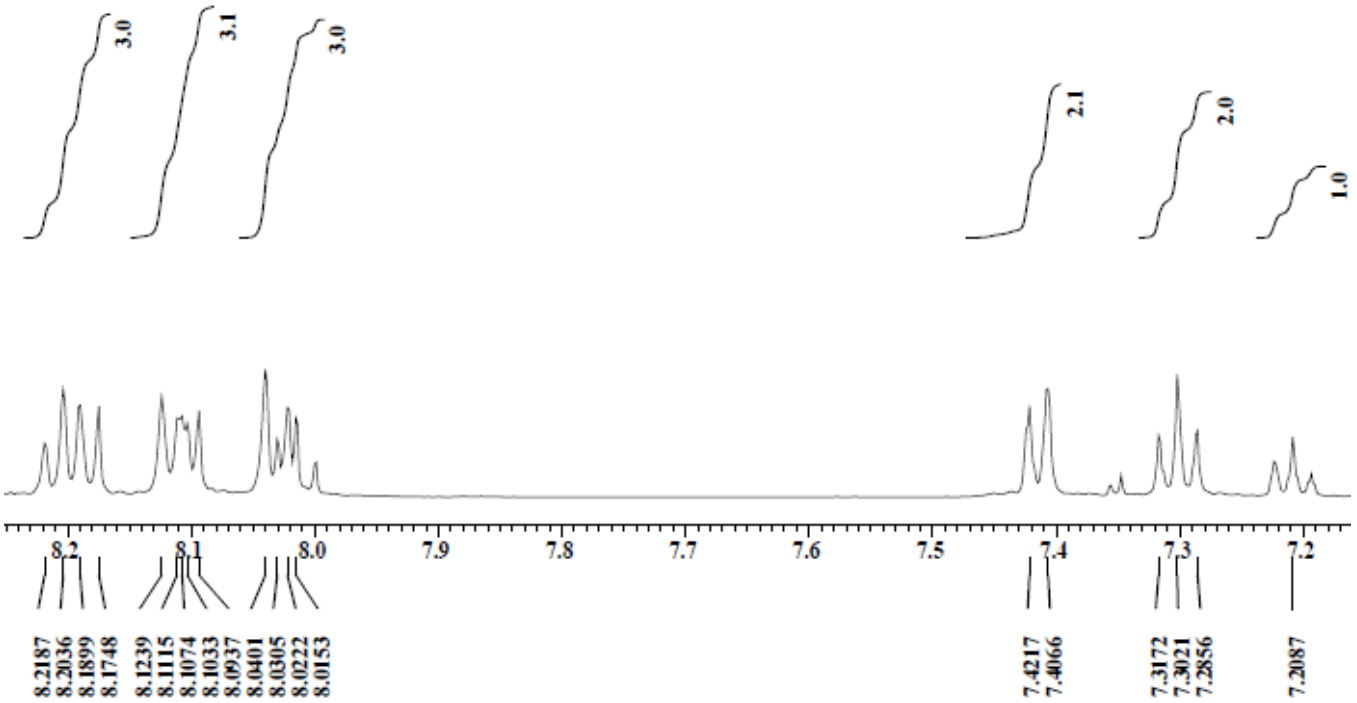

**Fig. S2**a Aromatic region of the $^{1}$H NMR spectrum of A3

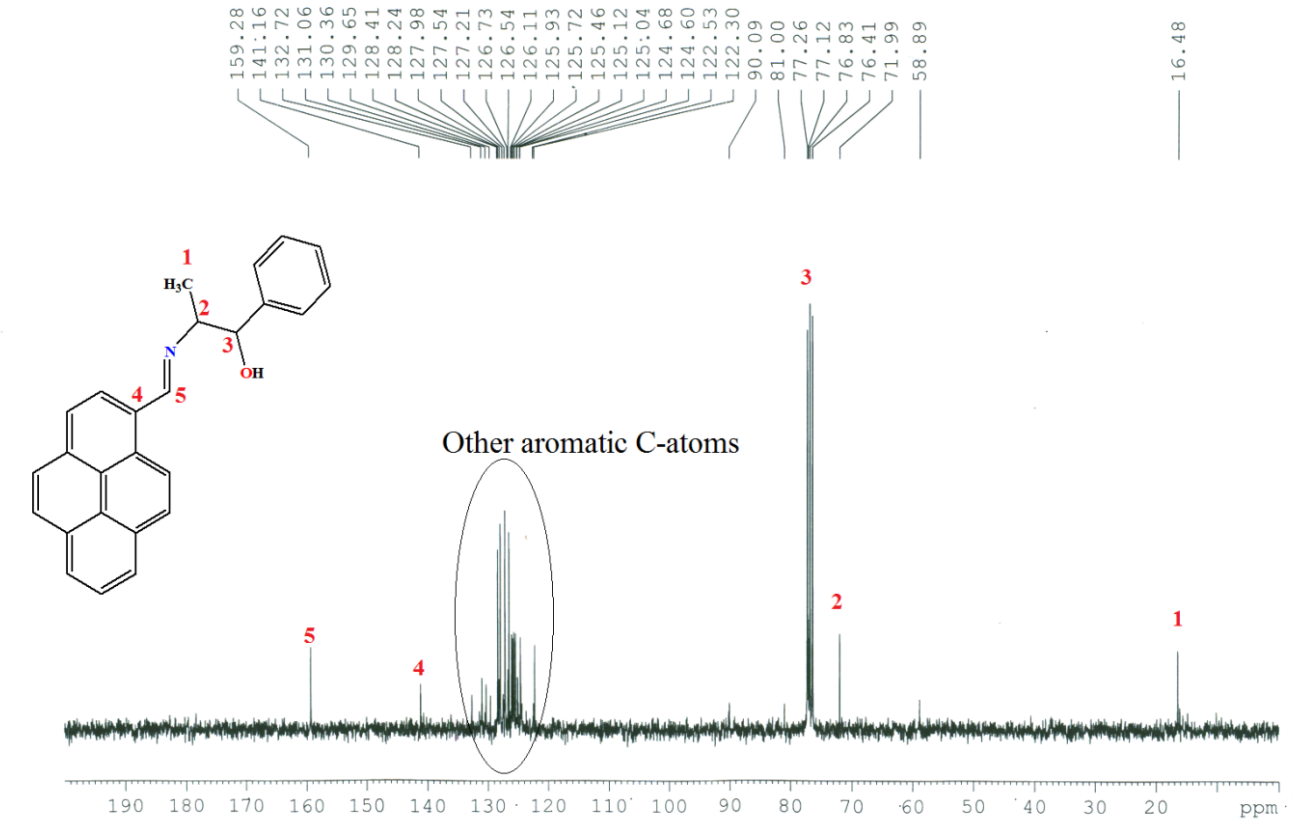


**Fig. S3** $^{13}C$ NMR spectrum of A3 in $CDCl_3$

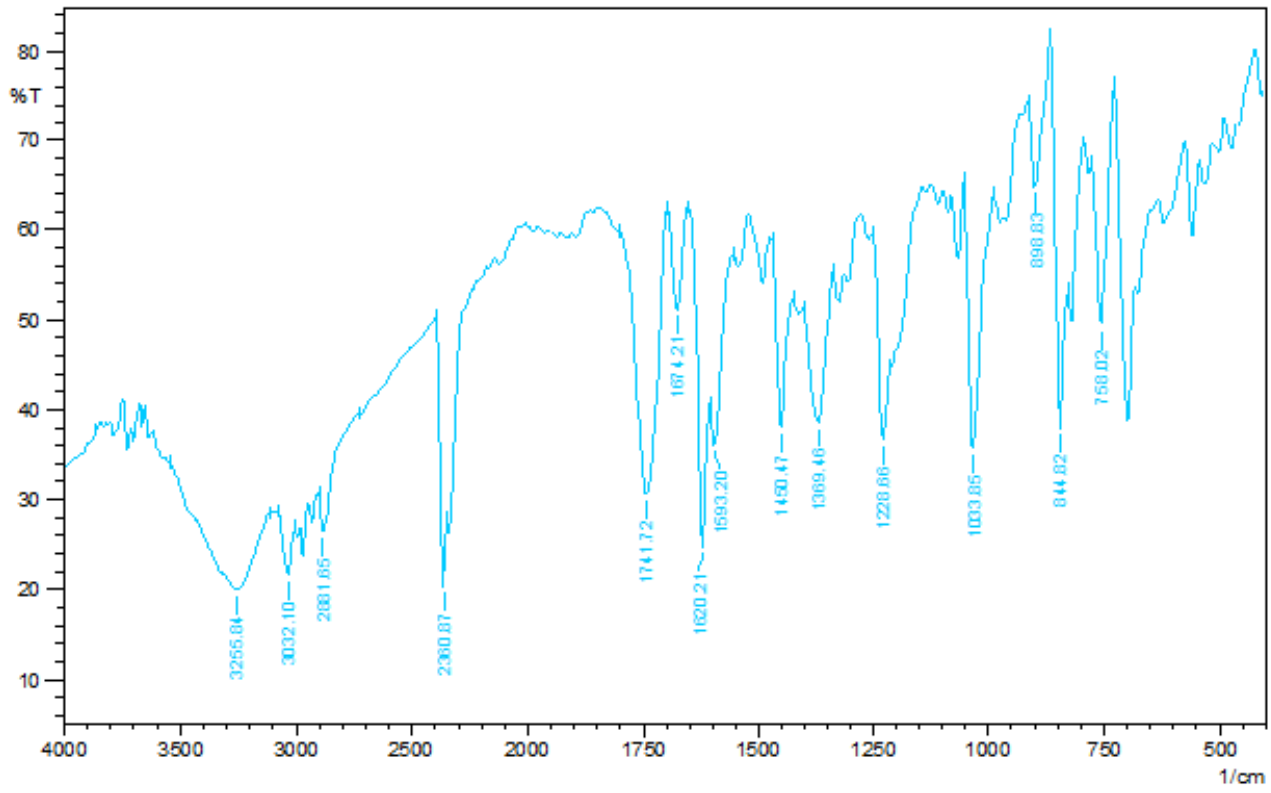


**Fig. S4** FTIR spectrum of A3

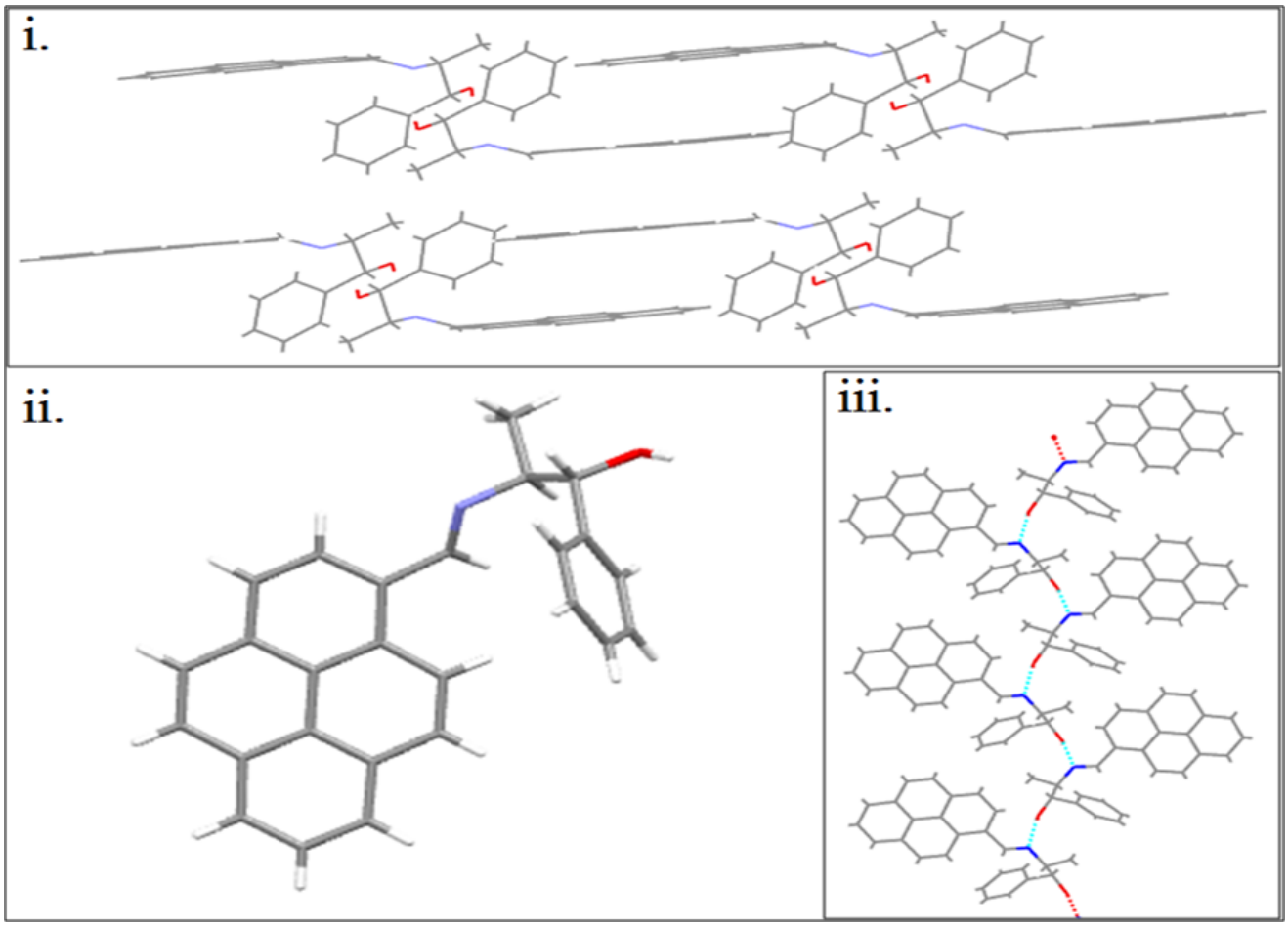


**Fig. S5** Crystal packing (i), molecular structure (ii) and H-bonding network (iii) of A3

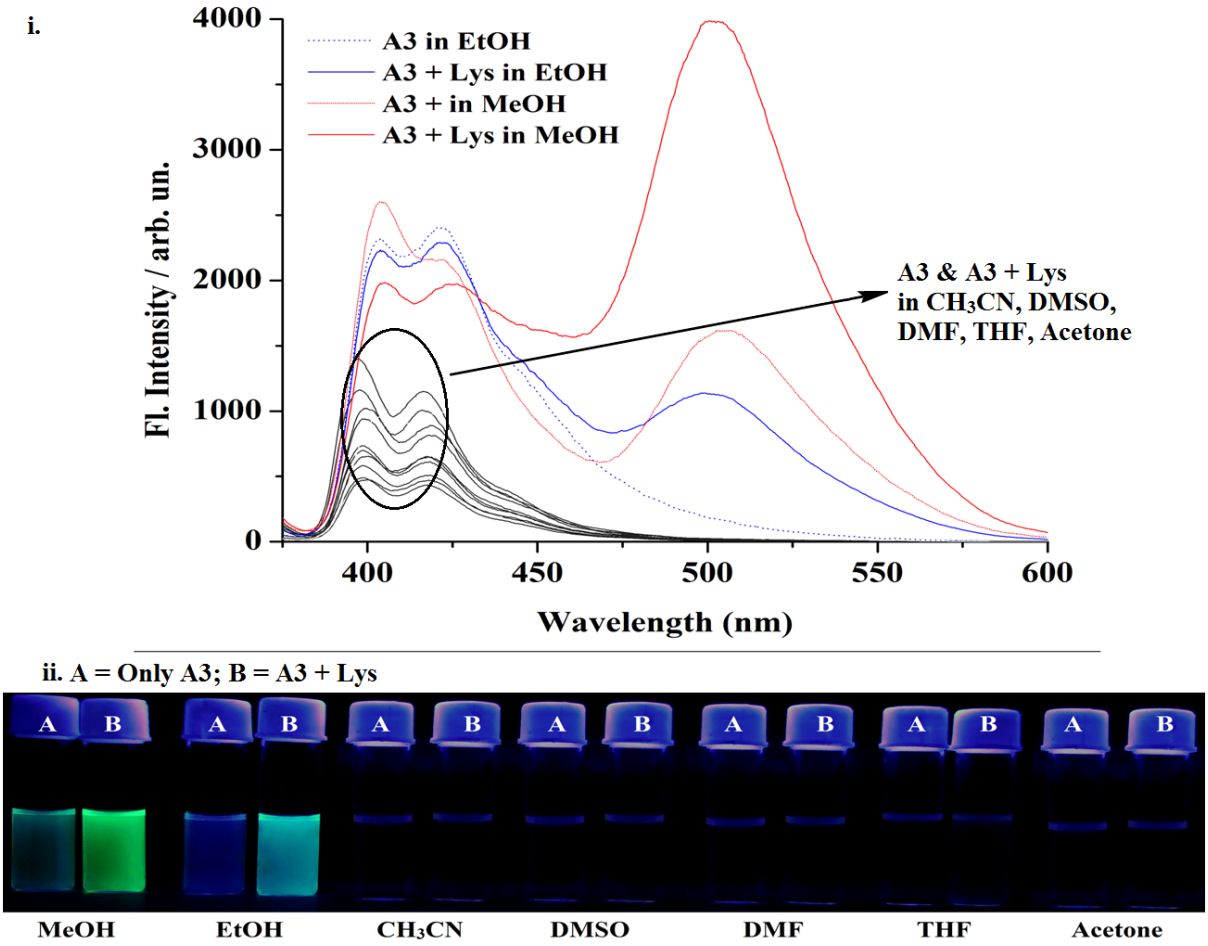


**Fig. S6** (i) Emission spectra and (ii) UV light exposed colors of **A3** (10 μM) in absence and presence of Lys (20 μM) in different solvents, $\lambda_{ex}$ = 360 nm

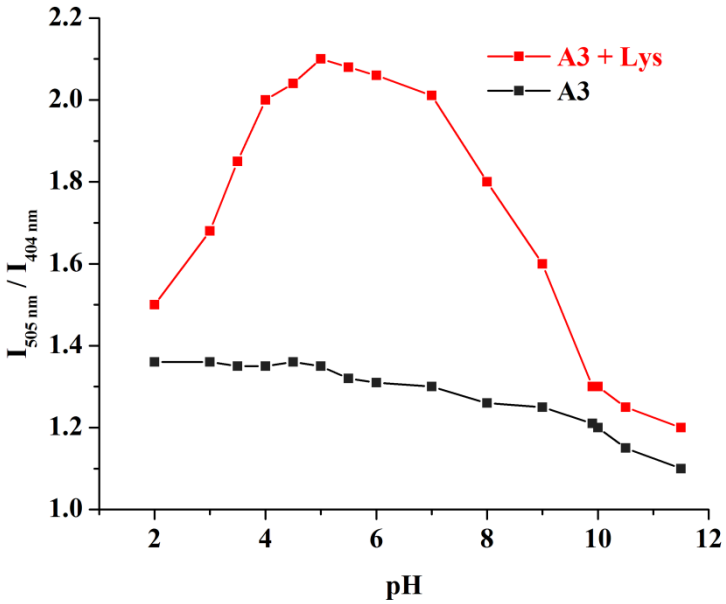


**Fig.S7** Effect of pH on the emission intensities of **A3** (10 μM) before and after addition of Lys (2 equiv.), $\lambda_{ex}$ = 360 nm

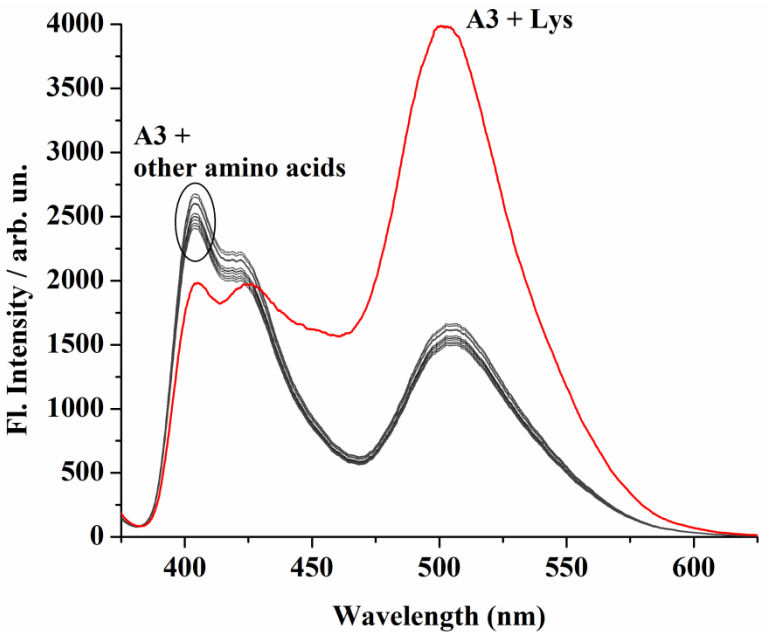


**Fig.S8** Emission spectra of **A3** (10 μM) in presence of different amino acids (20 μM) in HEPES buffered (0.1 M; MeOH: $H_2O$, 4:1 v/v, pH 7.4; $\lambda_{ex}$ = 360 nm) solution

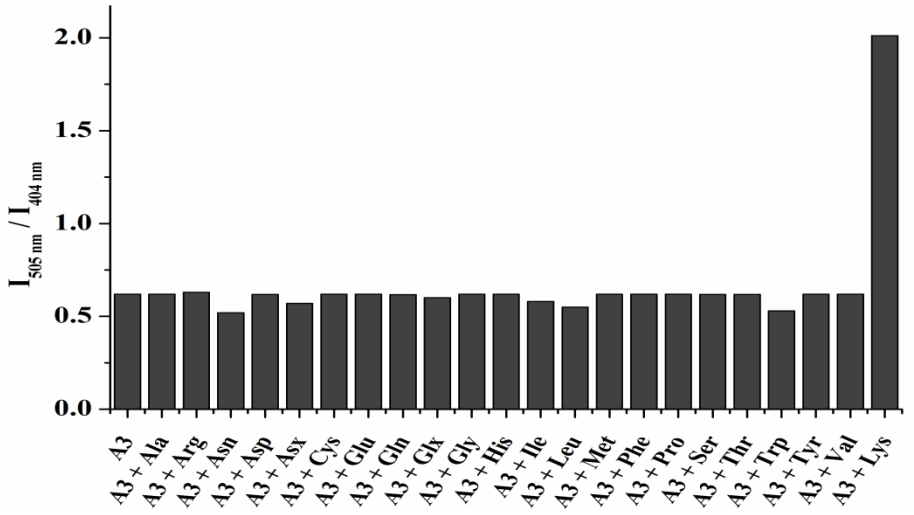


**Fig. S9** Changes in $I_{505\ nm}$ / $I_{404\ nm}$ value of A3 (10 μM) in presence of different amino acids (20 μM) in HEPES buffered (0.1 M; MeOH: $H_2O$, 4:1 v/v; pH 7.4; $\lambda_{ex}$ = 360 nm) solution

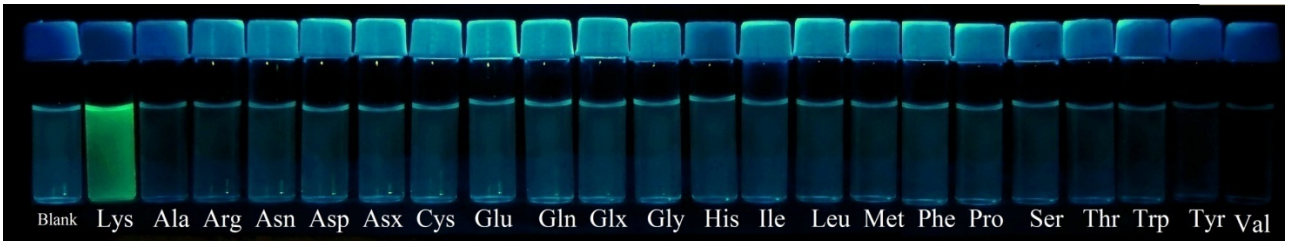


**Fig S10** UV light exposed colors of **A3** (10 μM) in presence of different amino acids (20 μM) in HEPES buffered (0.1 M; MeOH: $H_2O$, 4:1 v/v; pH 7.4)

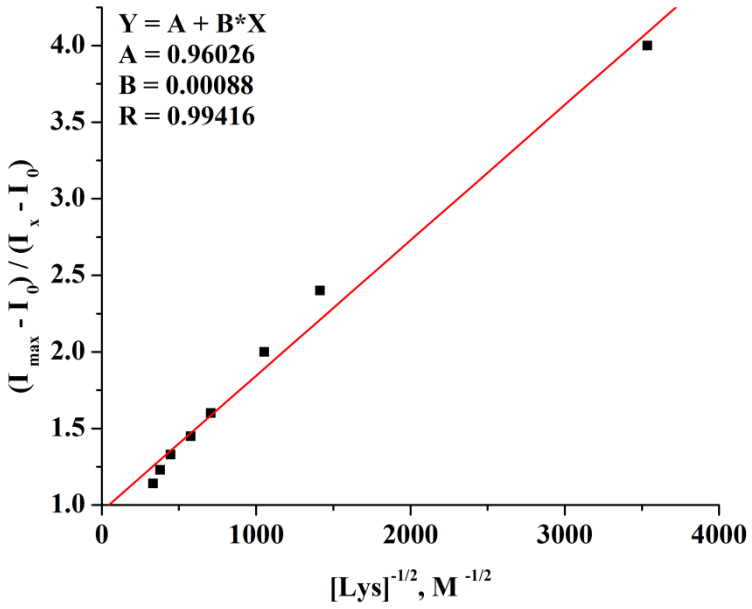


**Fig S11** Benesi-Hildebrand plot for determination of the association constant of **A3** with Lys

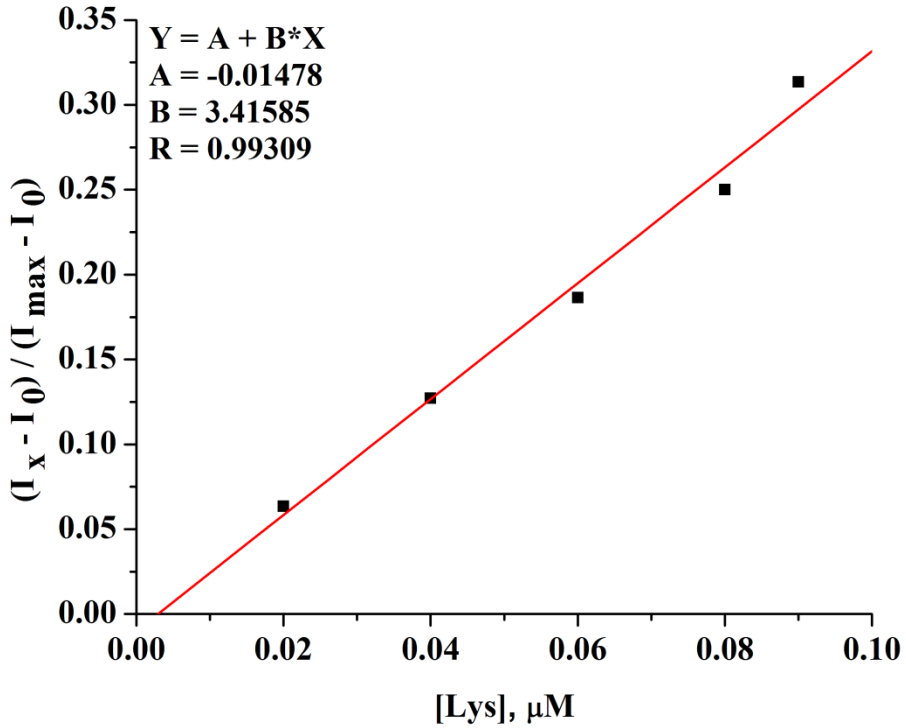


**Fig S12** Emission intensities of **A3** (10 μM) as a function of externally added [Lys] in HEPES buffered (0.1 M; MeOH: $H_2O$, 4:1 v/v; pH 7.4; $\lambda_{ex}$ = 360 nm, $\lambda_{em}$ = 505 nm) solution. The data obtained from fluorescence titration in Fig.2

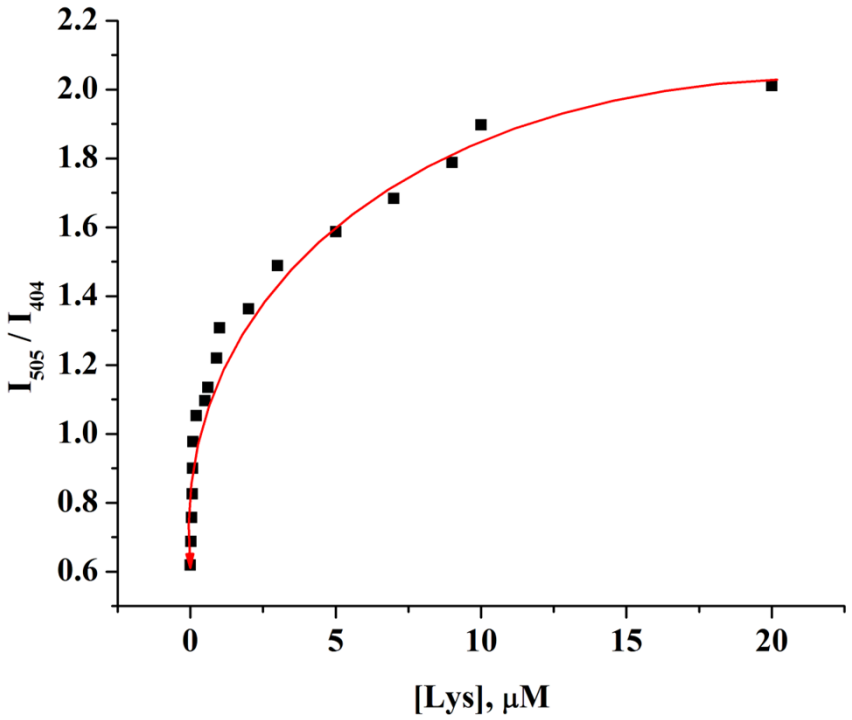


**Fig S13** Changes in $I_{505\ nm}$ / $I_{404\ nm}$ of A3 (10 μM, $\lambda_{ex}$ = 360 nm) as a function of externally added Lys in HEPES buffered (0.1 M; MeOH: $H_2O$, 4:1, v/v; pH 7.4) solution

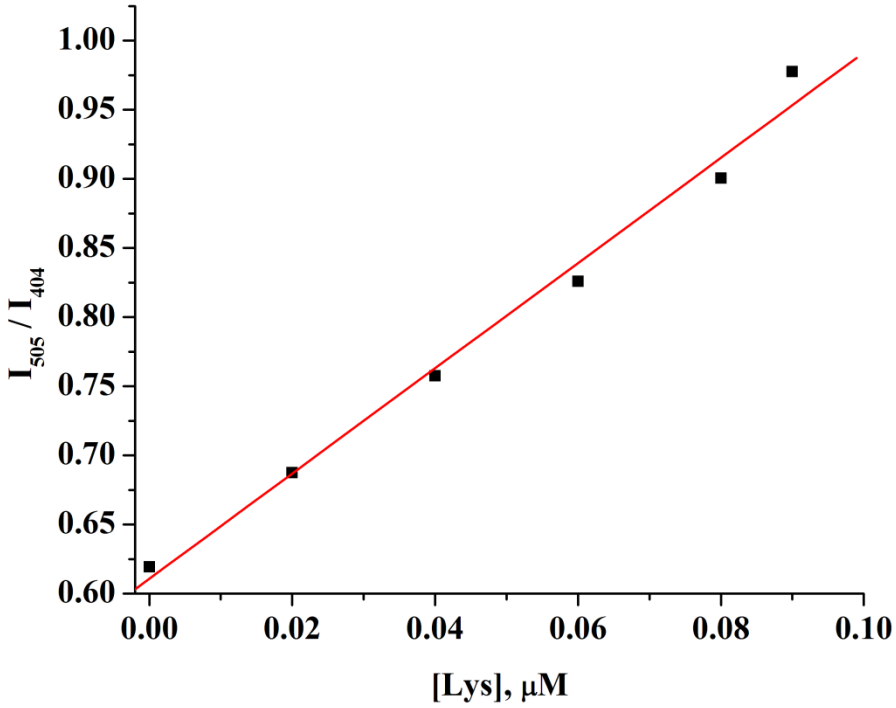


**Fig S14** Linear portion of the $I_{505\ nm}$ / $I_{404\ nm}$ of A3 (10 μM, $\lambda_{ex}$ = 360 nm) as a function of externally added Lys in HEPES buffered (0.1 M; MeOH: $H_2O$, 4:1, v/v; pH 7.4)

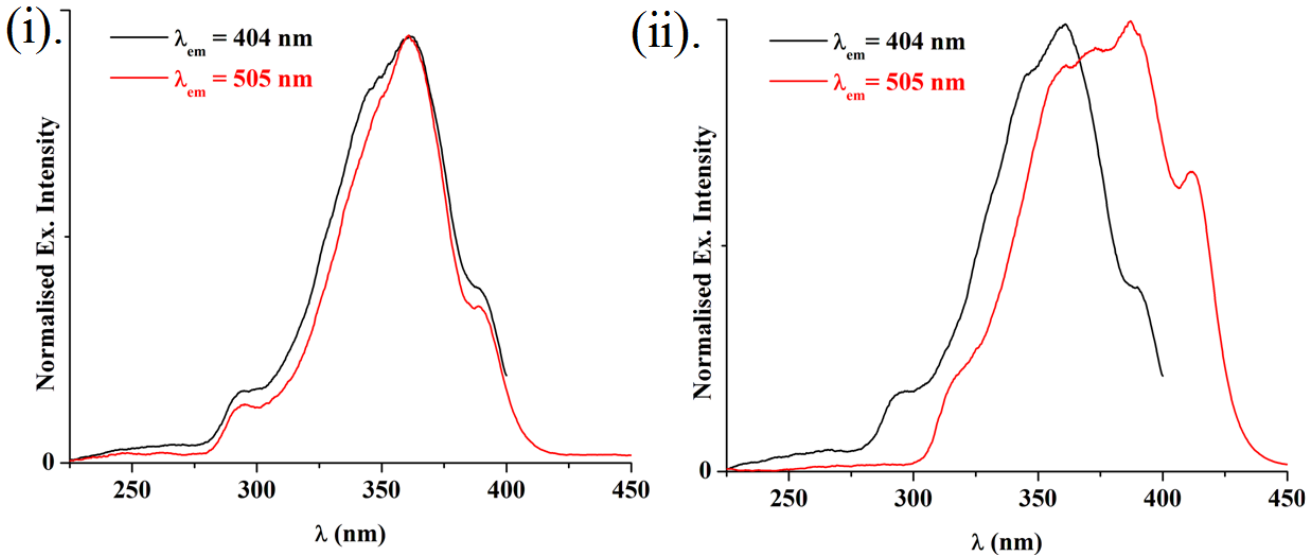


**Fig S15** Normalized excitation spectra of **A3** (10 μM) (left) and **A3** (10 μM) + 2 equiv. Lys (right).

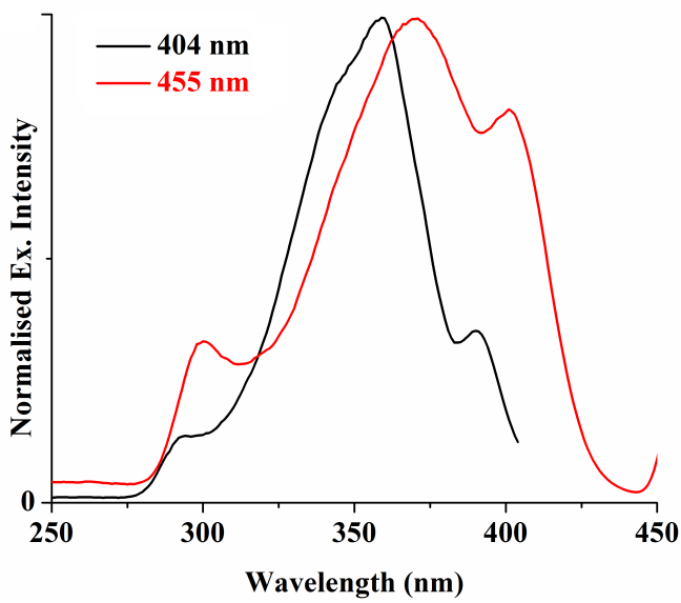


**Fig S15a** Normalized excitation spectra of **A3** (10 μM) + 5 equiv. Lys.

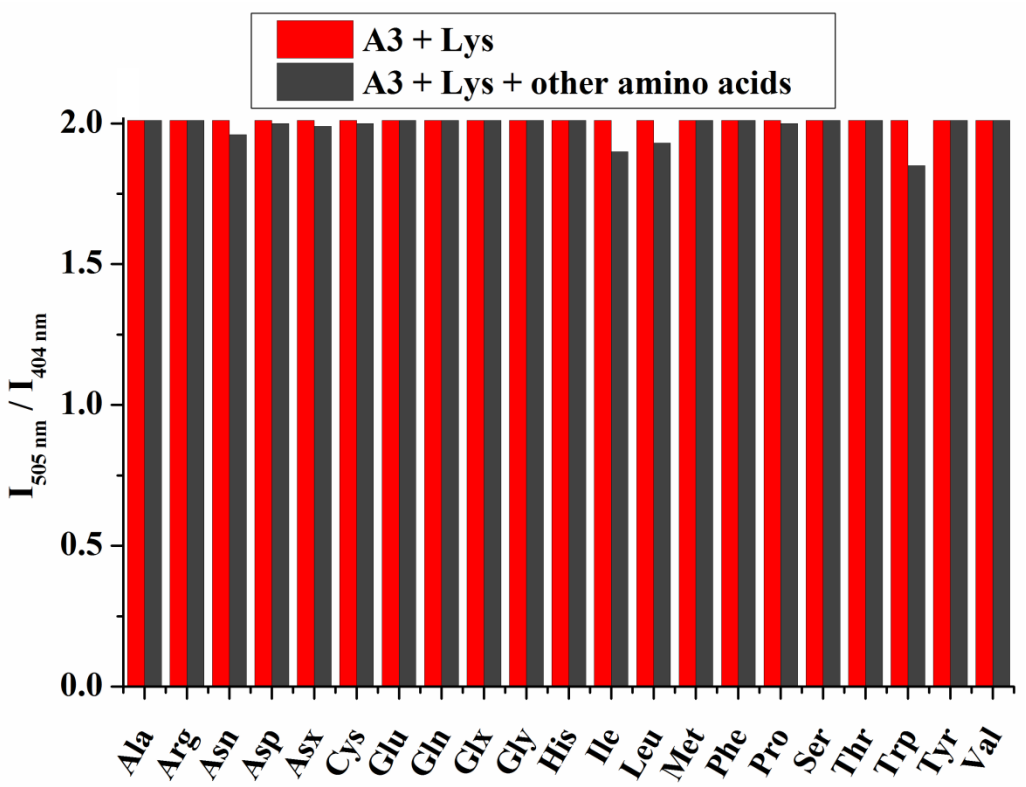


**Fig S16** The ratio $I_{505nm}$ / $I_{404}$nm for **[**A3 + Lys] system in presence of other amino acids

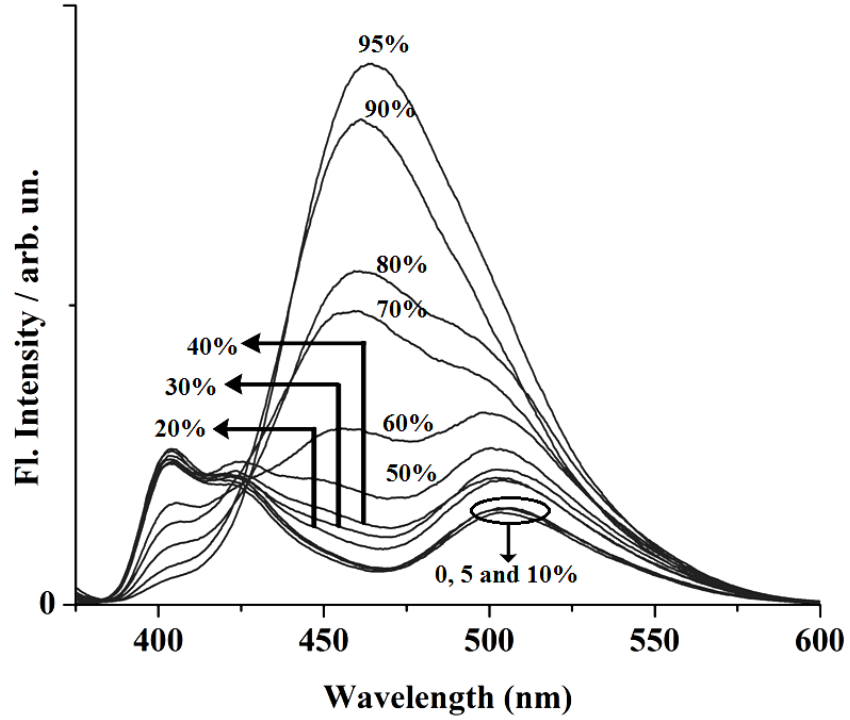


**Fig S17a** Changes in the emission spectra of A3 (10 μM, $CH_3OH$) with varying water percentage

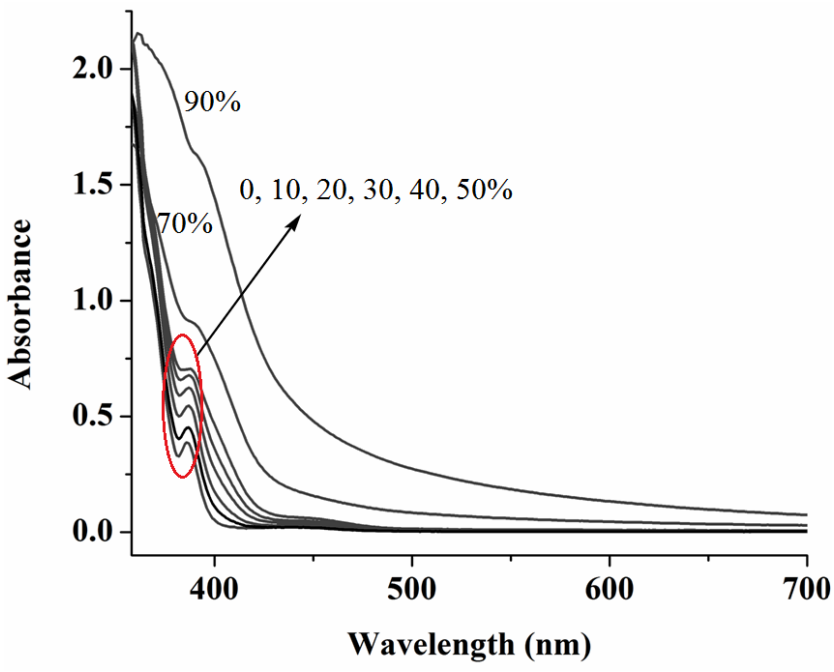


**Fig S17b** Changes in the absorption spectra of A3 (10 μM, $CH_3OH$) with varying water percentage

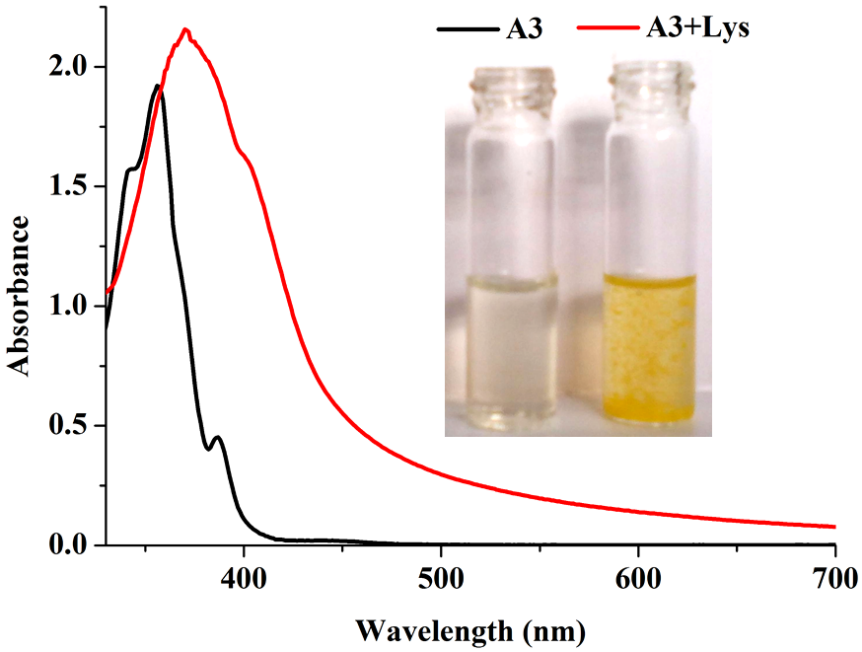


**Fig S18a** Changes in the absorption spectra of A3 (30 μM) after addition of Lys (1800 μM) in MeOH: $H_2O$, 4:1, v/v (inset show the visible colors of the respective solutions)

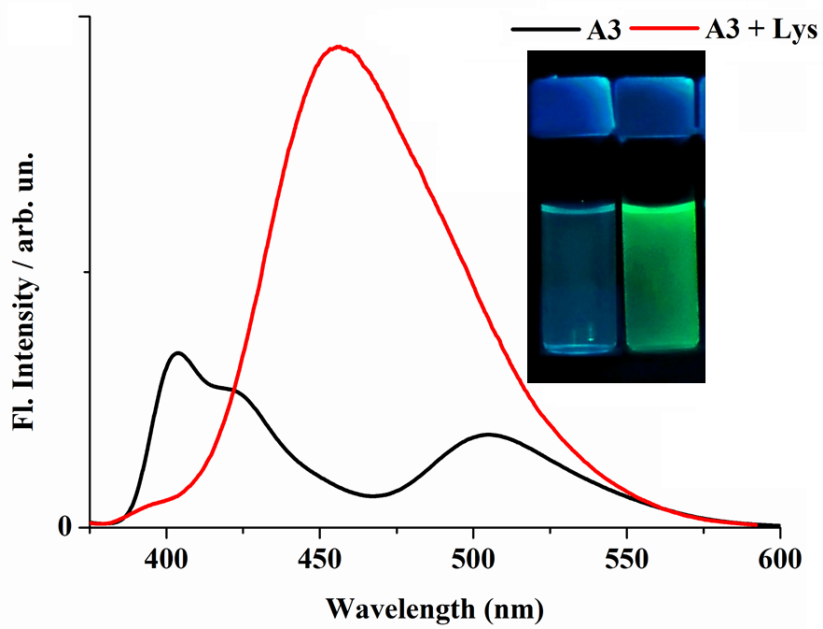


**Fig S18b** Changes in the emission spectra of A3 (30 µM) after addition of Lys (1800 µM) in MeOH: $H_2O$, 4:1, v/v (inset shows the UV light exposed colors of the respective solutions)

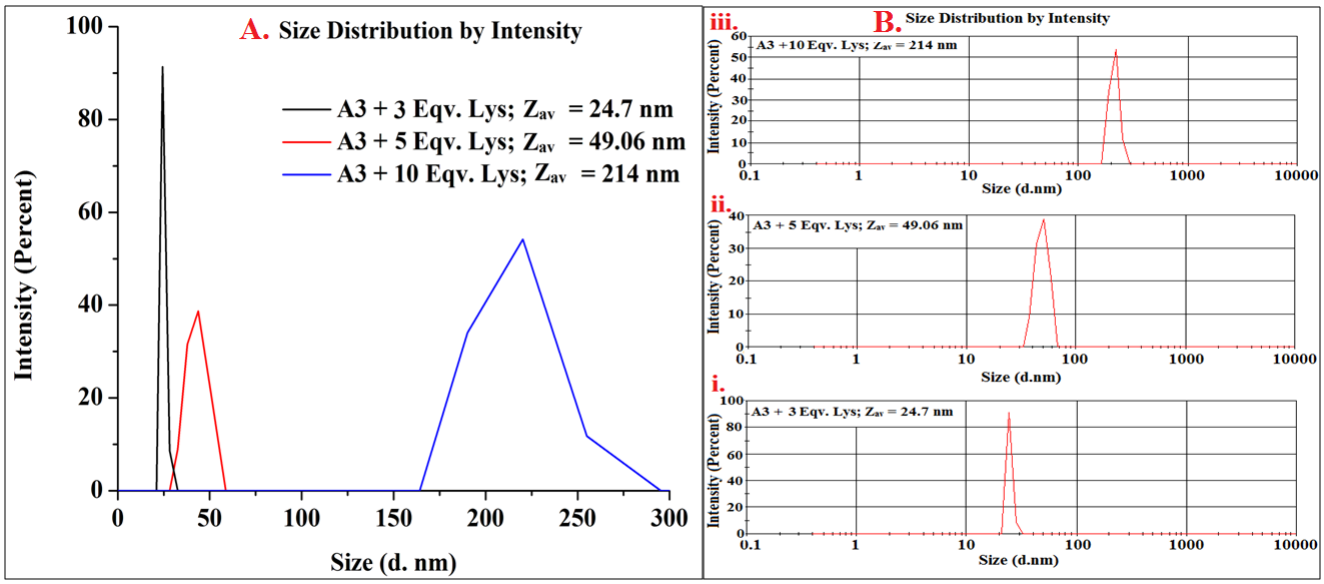


**Fig S19** DLS of A3 (10 µM) with increasing Lys content (3.0 equiv., 5.0 equiv. and 10.0 equiv.)

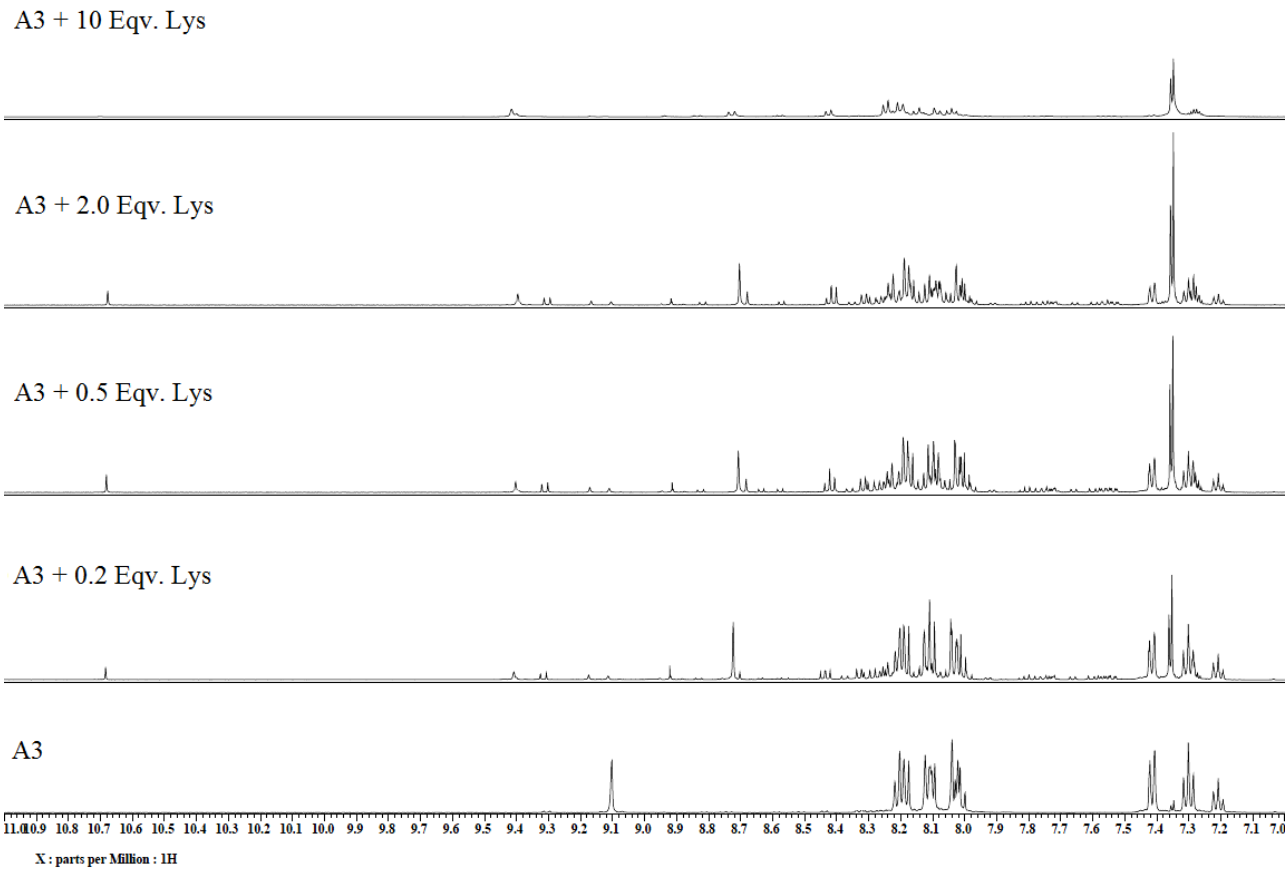


**Fig S20** $^{1}H$ NMR spectral changes of A3 ($CD_3OD$) upon gradual addition of Lys ($D_2O$)

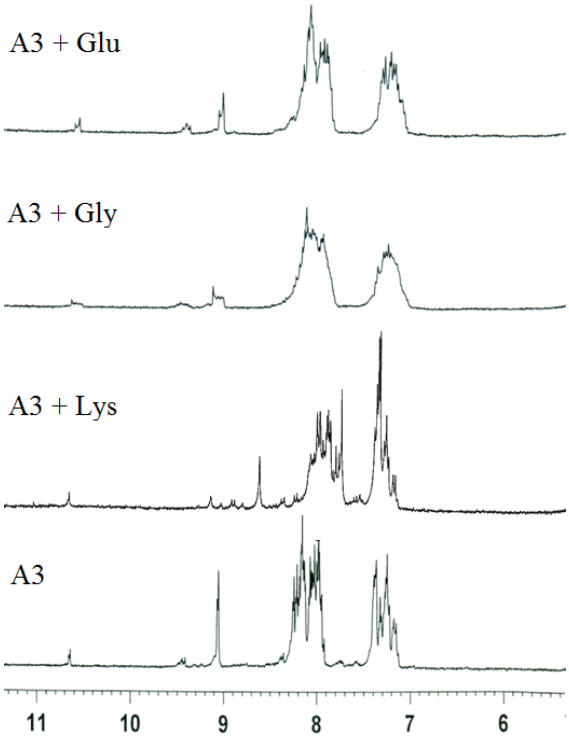


**Fig S20a** Aromatic region of the $^{1}H$ NMR spectra of A3 ($CD_3OD$) upon addition of 2.0 equiv. other amino acids ($D_2O$

**Table S1**.Crystal data and structure refinement for A3 **(CCDC No. 1007420)**

| | |
|---|---|
| Empirical formula | $C_{26}H_{21}NO$ |
| Formula weight | 363.44g/mol |
| Temperature | 296(2) K |
| Wavelength | 0.71073 Å |
| Crystal system | Monoclinic |
| Space group | *P 1 c 1* |
| Unit cell dimensions | a = 28.0546(17) Å; b = 9.6810(5) Å; c = 15.0716(8) Å; α= 90°, β= 109.781(6)°, γ = 90° |
| Volume | 3851.9(4) $Å^3$ |
| Z | 8 |
| Density (calculated) | 1.253 $g/cm^3$ |
| Absorption coefficient | 0.076 $mm^{-1}$ |
| F(000) | 1536 |
| Theta range for data collection | 2.52 to 28.48° |
| Index ranges | -37<=h<=37, -12<=k<=12, -20<=l<=19 |
| Reflections collected | 35240 |
| Independent reflections | 4832 [R(int) = 0.0775] |
| Completeness to theta = 22.21° | 99.4% |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data / restraints / parameters | 4832 / 0 / 255 |
| Goodness-of-fit on $F^2$ | 1.006 |
| Final R indices [I>2sigma(I)] | R1 = 0.0489, wR2 = 0.1161 |
| R indices (all data) | R1 = 0.1117, wR2 = 0.1454 |
| Largest diff. peak and hole | 0.185 and -0.198 $eÅ^{-3}$ |
| R.M.S. deviation from mean | 0.046 $eÅ^{-3}$ |

## References

1. H. A. Benesi and J. H. Hildebrand*, J. Am. Chem. Soc*., 1949, **71**, 2703-2707.