Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics


# ELECTRONIC SUPPLEMENTARY INFORMATION

## An intriguing pH-triggered FRET-based biosensor emission of pyrazoline-doxorubicin couple and application in living cells

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## Experimental Section

Ethyl-1-(4-bromophenyl)-3-(4-cyano-3-methyl-1phenyl-1Hpyrazole-5-yl)-4,5-dihydro-1H pyrazole-5-carboxylate was synthesized using the method described earlier.[1] The anticancer drug Doxorubicin was purchased from Fluka (Oakville, Canada) and used without any further purification. Buffer solutions were prepared by mixing appropriate volumes of aqueous solutions of $Na_2HPO_4$ and $NaH_2PO_4$ (0.1M each) and different pH were adjusted by adding appropriate amount of dilute HCl or NaOH solution. The pH of the solutions was measured by pH meter (model ELICO LI614). Millipore water was used for the preparation of aqueous solutions.

A Shimadzu (model UV-1700) UV-vis spectrophotometer and a Fluorolog FIIA spectrofluorimeter (Spex Inc, N. J., USA) with an external slit width of 2.5 mm were used to collect absorption and fluorescence spectra, respectively. All measurements were done repeatedly and reproducible results were obtained. All fluorescence spectra were corrected for the instrumental response. $^1$H NMR spectra of PYZ in $CDCl_3$ were recorded on a Brüker 300 MHz NMR spectrometer. The fluorescence quantum yield ($\Phi_f$) was measured relative to quinine sulphate ($\Phi_f$ = 0.54 in 0.1 M $H_2SO_4$).[2] PYZ concentration was kept $8 \times 10^{-6}$ mol dm$^3$ throughout the experiment.

## Cell culture

The human hepatocarcinoma cell line HepG2 was cultured as monolayers in DMEM medium containing 110 mg/l sodium pyruvate, 0.37% (w/v) of $Na_2HCO_3$ and 10% fetal bovine serum (FBS). Cells were immobilized on poly-L-Lysine coated cover slips before treatment.

## Live cell imaging

HepG2 monolayers were harvested, washed with PBS-glucose at 48 °C, and allowed to adhere to polylysine-coated slides. Adhered cells were overlayed with staining solution, and after 5–15 min, were observed under a Leica DMIRB confocal microscope. Staining solutions contained PBS-glucose and Mitotracker Deep Red (1 mM) or Rhodamine 123 (0.2 mg/ml). Illumination laser wavelength was 405 nm (PYZ). Control and experimental specimens were scanned and analyzed under identical gain and other settings. Image capture, processing and quantification of fluorescence were carried out using Leica SP2 confocal software.

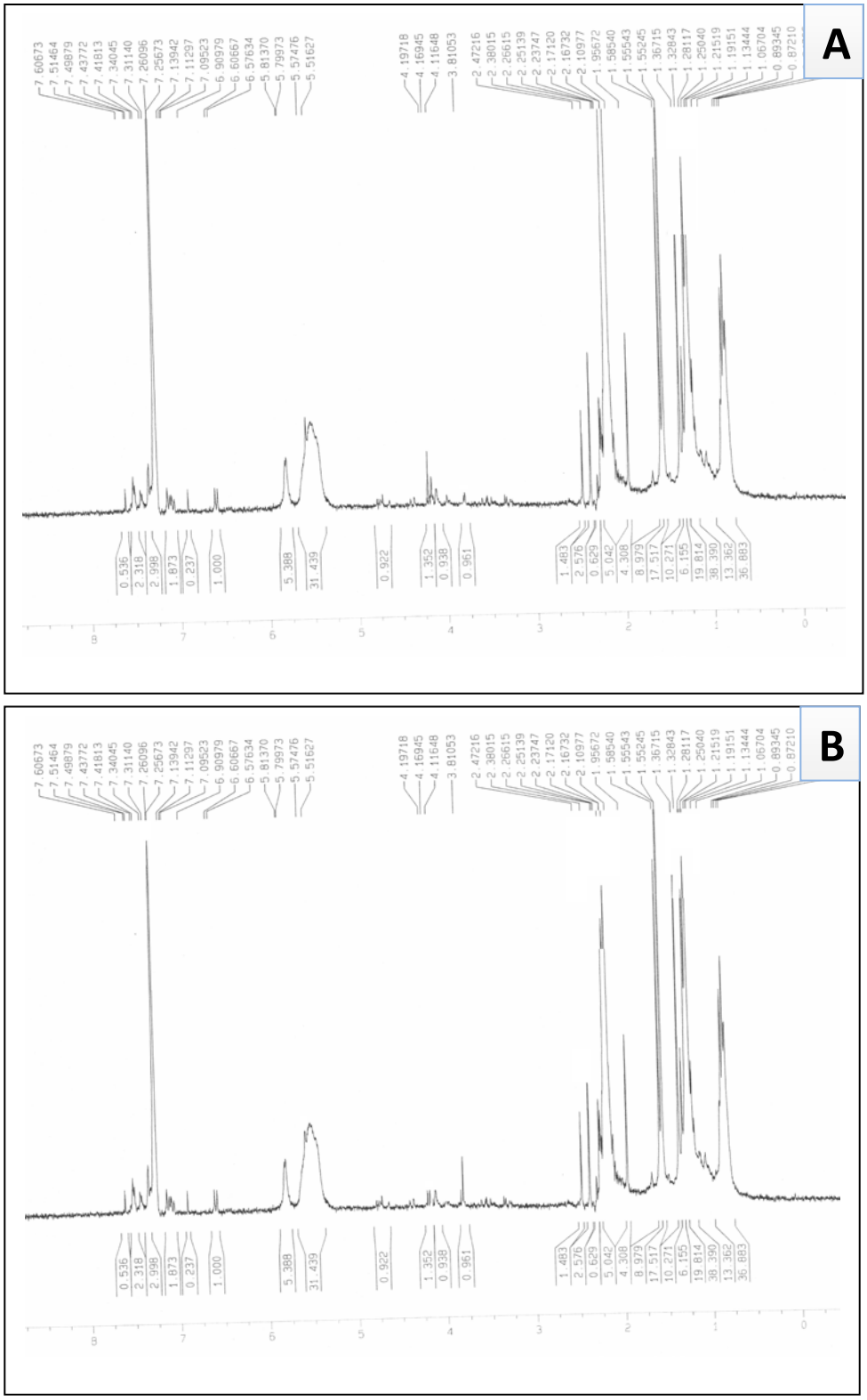


**Figure S1.** NMR spectrum of PYZ. (A) Blank; (B) With NaOD.

## Emission Behavior of PYZ

In order to get insight whether the -CN group is responsible or not for spectral shift in basic pH, the ester moiety of PYZ was replaced by phenyl group. The PYZ derivative which is devoid of ethyl ester moiety shows subtle interaction both in ground and excited state as there is no change in the wavelength maxima of the corresponding spectra with increase in pH. From which we can conclude that the -CN group do not hydrolyse in the experimental condition.

## Absorption Behavior of DOX

DOX exhibits two absorption bands, one strong band around 480 nm wavelength region, and other weak shoulder around 346 nm wavelength region. Gradual increase in pH of buffer solution changes the absorption spectrum drastically. Increase of pH leads to disappearance of the absorption band at 480 nm along with the formation of a new band at 550 nm which can be attributed to the deprotonated form. The solution of DOX at pH 7.0 exhibits an orange color while the solution at pH 9.5 exhibits a purple color corresponding, indicating that the conformational change of the DOX. The isosbestic points at 512 nm for DOX indicate a ground state acid–base equilibrium involving two species. From the inflexion point of the spectrophotometric titration curves the determined ground state pKa-value is 9.88.

Ratios of the absorbance at 550 nm and 480 nm increased from 0.48 to 1.79 from pH 2 to pH 13 (Figure S2). According to these pH dependent ratios, typical application range of DOX can be found to be from pH 7.0 to 11.0.

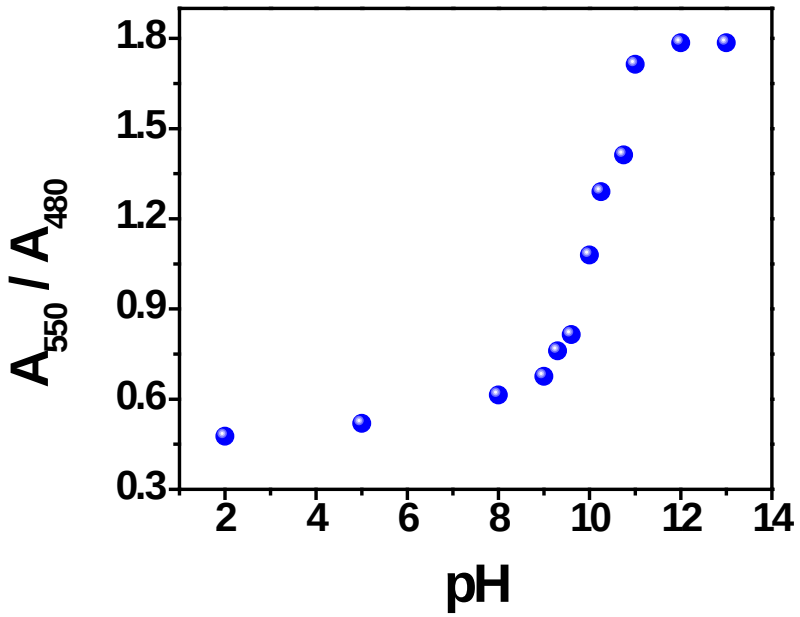


**Figure S2.** Ratiometric plot of the absorbance at 550 nm and 480 nm.

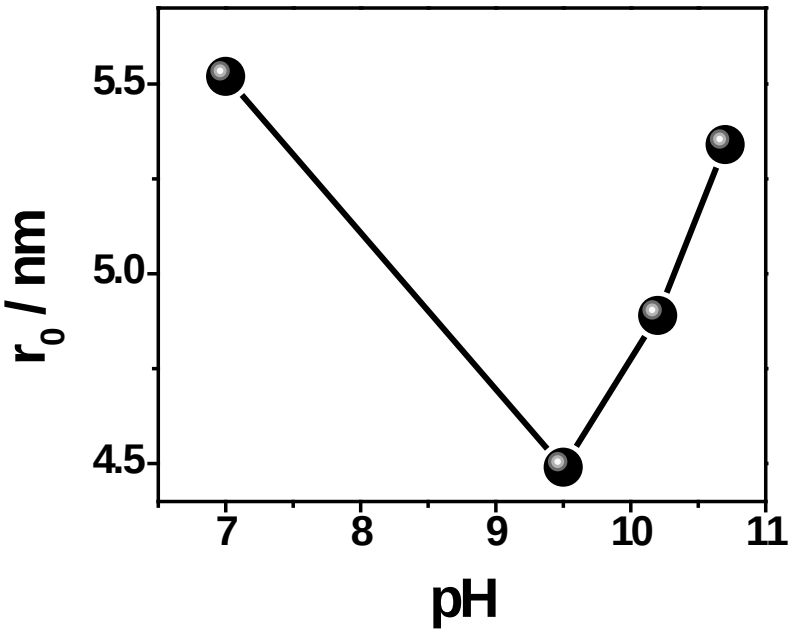


**Figure S3.** Variation of the actual donor-acceptor distance ($r_0$) as a function of medium pH.

## REFERENCES

1. A. Mukherjee and K. K. Mahalanabis, *Heterocycles*, 2009, **78**, 911.
2. S. Dhar, S. S. Roy, D. K. Rana, S. Bhattacharya, S. Bhattacharya and S. C. Bhattacharya, *J. Phys. Chem. A*, 2011, **115**, 2216.