Development of a Quinoxaline-based Fluorescent Probe for Quantitative Estimation of Protein Binding Site Polarity

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Supplementary Information

Experimental

Apparatus

Absorption and fluorescence spectra were measured on a Shimadzu UV-1600 and on a Hitachi F-4500 fluorescence spectrometer, respectively. Fluorescence decay measurements were performed by using a time-correlated single-photon counting method.[S1] Laser excitation at 375 nm was achieved by using a diode laser (PicoQuant, LDH-P-C-375) with a power control unit (PicoQuant, PDL 800-B) in a repetition rate of 2.5 MHz. Temporal profiles of fluorescence decay were detected by using a microchannel plate photomultiplier (Hamamatsu, R3809U) equipped with a TCSPC computer board module (Becker and Hickl, SPC630). Full-width at half-maximum (FWHM) of the instrument response function was 51 ps. The values of 2 and the Durbin–Watson parameters were used to determine the quality of the fit obtained by nonlinear regression.[S2] DMSO (for spectroscopy, Wako Chem. Japan) was used as a solvent without further purification. All measurements were carried out at room

temperature under Ar. The concentration was adjusted so that the absorption maximum of the excitation wavelength was 0.1 for each sample. ^{1}HNMR spectra were measured on a 270 MHz NMR spectrometer, EX-270. DFT calculation was carried out by using Spartan 04 for Windows.

Materials

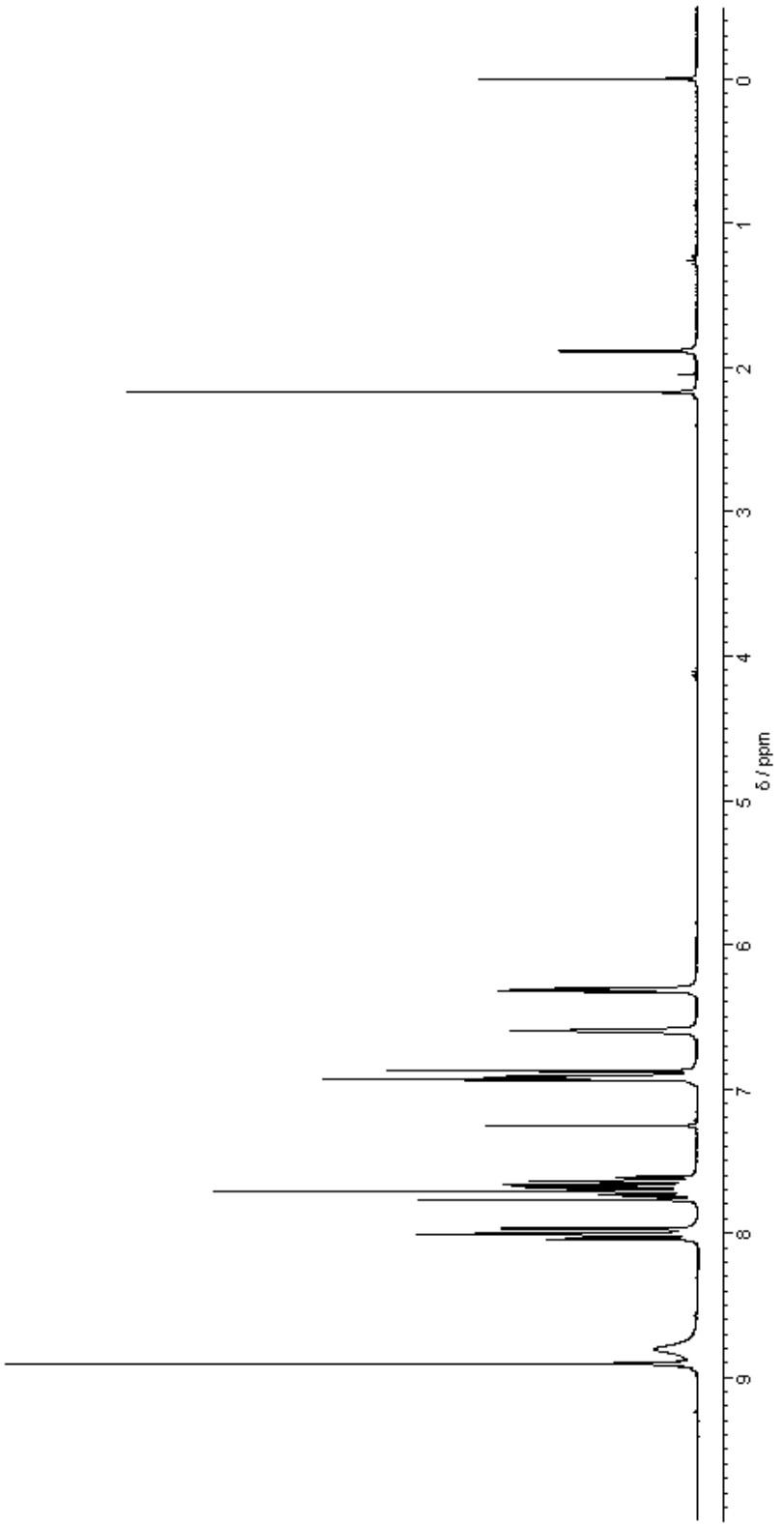
2-[(1E)-2-(1H-Pyrrol-2-yl)ethenyl]-quinoxaline (PQX)

A mixture of 2-bromomethyl quinoxaline[S3] (500 mg, 2.24 mmol) and triethylphosphite (1.85 g, 11.2 mmol) was refluxed for 15 h. After cooling, the excess triethylphosphite was removed *in vacuo* to leave red residue. This crude product diethyl (quinoxalin-2-ylmethyl)phosphonate was used in the next step without further purification.

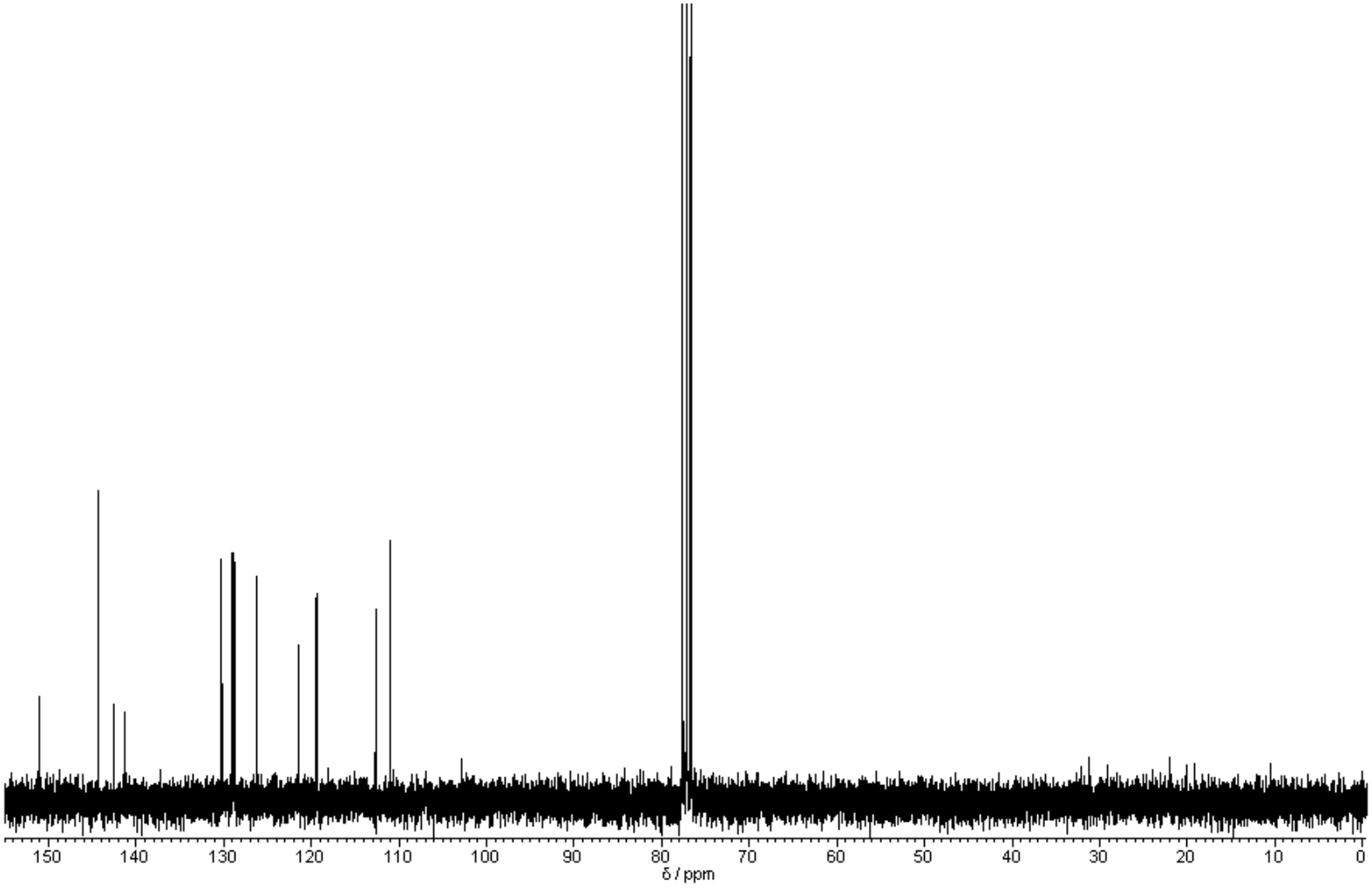
To a mixture of sodium hydride (135 mg, 5.63 mmol) and formyl pyrrole (335mg, 3.52mmol) in dry THF (5mL) was added diethyl (quinoxalin-2-ylmethyl)phosphonate dissolved in dry THF (10 mL), and the mixture was stirred at 70 °C for 1.5 h, after which time the reaction was quenched by addition of water (0.5 mL). The solvent was evaporated and the residue was dissolved in chloroform (100 mL), washed with brine, dried over $MgSO_4$, filtered and evaporated. The residue was purified by silica gel

column chromatography [eluent: hexane/ethyl acetate (1/1)] to give 1 as a yellow solid (303 mg, 61%).

^{1}H NMR (270 MHz $CDCl_3$): δ 8.91 (s, 1H), 8.89 (s, 1H), 8.81-7.97 (m, 2H), 7.74-7.61 (m, 3H), 6.94-6.88 (m, 2H), 6.61-5.86 (m, 1H), 6.33-6.30 (m, 1H); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 151.0, 144.3, 142.5, 141.2, 130.3, 130.1, 129.1, 128.9, 128.8, 126.2, 121.4, 119.5, 112.7, 111.0; Anal. Calcd. for $C_{14}H_{11}N_3$: C, 76.00; H, 5.01; N, 18.99; Found: C, 76.09; H, 5.16; N, 19.02.



^{1}H NMR of **PQX** (270 MHz) Solvent: $CDCl_3$



^{13}C NMR of **PQX** (67.8 MHz) Solvent: $CDCl_3$

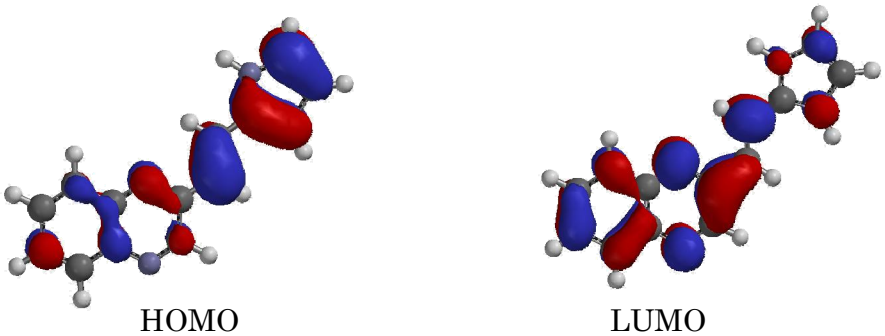


Fig. S1 Frontier molecular orbitals of **PQX** calculated by density functional method (DFT) at B3LYP/6-31G* level.

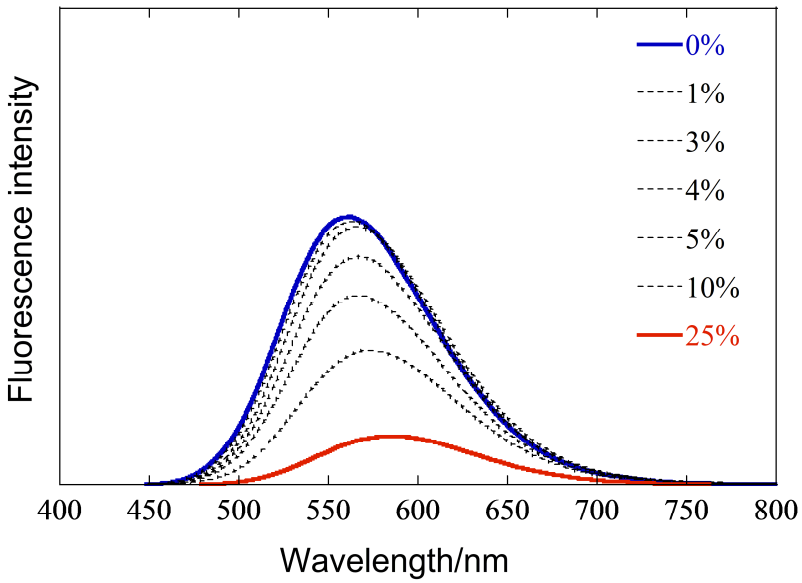


Fig. S2 Change in the fluorescence spectrum of **PQX** upon addition of water to a solution in DMSO.

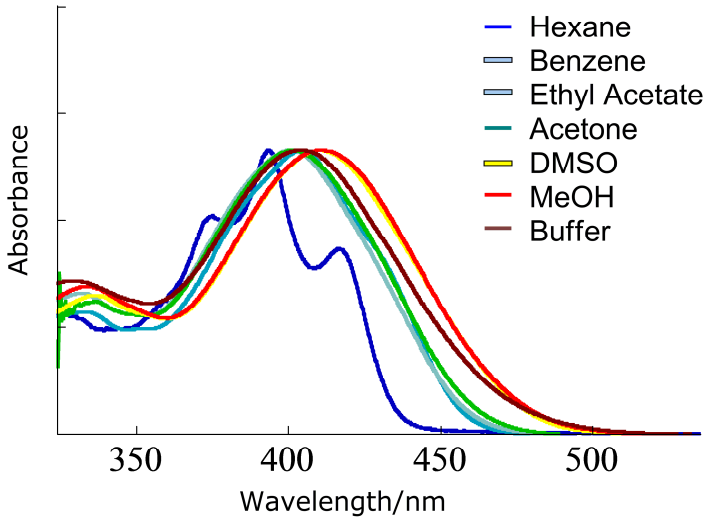


Fig. S3 Absorption spectra of **PQX** in various solvents.

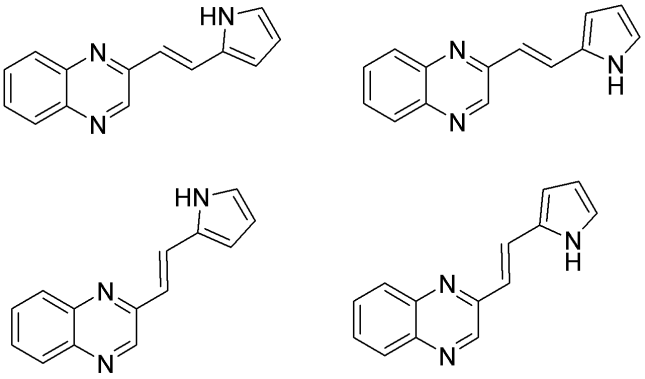


Fig. S4 Structures of rotational isomers of **PQX.**

References

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(S2) Boens, N.; Tamai, N.; Yamazaki, I.; Yamazaki, T., *Photochem. Photobiol.* 1990, *52*, 911.
(S3) Hegedus, L. S.; Greenberg, M. M.; Wendling, J. J.; Bullock, J. P., *J. Org. Chem.*, 2003, *68,* 4179.