
Vrashali S. Kalyani, a Dipalee D. Malkhede a,* and Jyotirmayee Mohanty b,c,*  

aDepartment of Chemistry, Savitribai Phule Pune University, Pune 411 007, India  
bRadiation & Photochemistry Division, Bhabha Atomic Research Centre, Mumbai 400 085, India.  
cHomi Bhabha National Institute, Training School Complex, Anushaktinagar, Mumbai 400 094, India  

OH  

OH  

O  

OH  

O  

OH  

O  

Calix[4]arene  

Pyrene butyric acid  

EDC / HOBr  

PCX4  

Scheme S1: Synthetic route for the synthesis of PCX4.  

Experimental methods  

Absorption spectra were recorded with a Jasco V-650 UV-vis spectrophotometer (Tokyo, Japan). Steady-state fluorescence spectra were recorded using a Hitachi F-4500 spectrofluorometer (Tokyo, Japan). The samples were excited at 345 nm, where the changes in the optical density were nominal in the absorption spectra. The time-resolved fluorescence measurements were carried out using a time-correlated single photon counting (TCSPC) set-up from Horiba Scientific (UK). In the present work, a 339 nm LED (<1ns, 1 MHz repetition rate) was used for excitation. A reconvolution procedure was used to analyze the observed decays, which could be satisfactorily fitted by mono- or biexponential decay functions. The fluorescence decays \( I(t) \) were analyzed in general as a sum of exponentials:  

\[
I(t) = \sum B_i \exp(-t/\tau_i) \tag{S1}
\]

where, \( B_i \) and \( \tau_i \) are the pre-exponential factor and fluorescence lifetime for the \( i^{th} \) component, respectively. Reduced chi-square \( (\chi^2) \) values (within 1.00-1.20) and random distribution of the weighted residuals among data channels were used to judge the acceptance of the fits.  

For anisotropy measurements, samples were excited with a vertically polarized excitation beam and the vertically and horizontally polarized fluorescence decays were collected with a large spectral bandwidth of ~32 nm. Using these polarized fluorescence decays, the anisotropy decay function, \( r(t) \), was constructed as follows:  

\[
r(t) = \frac{I_v(t) - G I_h(t)}{I_v(t) + 2G I_h(t)} \tag{S2}
\]

\( I_v(t) \) and \( I_h(t) \) are the vertically and horizontally polarized decays, respectively, and \( G \) is the correction factor for the polarization bias of the detection setup. The \( G \) factor was determined independently by using a horizontally polarized excitation beam and measuring the two perpendicularly polarized fluorescence decays.  

Method M1:  

In the present study, the binding constants \( (K_{eq}) \) for the \( \beta\)-CD:PCX4 complex was determined by using the fluorescence titration method, according to a 1:1 binding model (eq. S3).  

\[
\beta\text{-CD} + \text{PCX4} \rightarrow \text{PCX4}\beta\text{-CD} \tag{S3}
\]

Taking \( [\text{PCX4}]_0 \) and \( [\beta\text{-CD}]_0 \) as the total concentrations of PCX4 and \( \beta\text{-CD} \), respectively, eq. S4 applies for the concentration of free (uncomplexed) PCX4 in equilibrium:  

\[
[\text{PCX4}]_{\text{eq}} = (K_{eq}[\text{PCX4}]_0 - K_{eq}[\beta\text{-CD}]_0 - 1 + \sqrt{(K_{eq}[\text{PCX4}]_0 + K_{eq}[\beta\text{-CD}]_0 + 1)^2 - 4K_{eq}[\text{PCX4}]_0[\beta\text{-CD}]_0})/2K_{eq} \tag{S4}
\]

where \( K_{eq} \) represents the binding constant for the PCX4 with the host. Since the interconversion of the free and complexed dye in solution (cf. equilibrium 1) occurs at a much slower rate (in microseconds) than the excited-state lifetimes of the dyes (sub-nanosecond to nanoseconds), it can be safely assumed that during the fluorescence measurements there is effectively no ex-
change between the free and complexed dyes. Thus, the observed results in the fluorescence measurements can be attributed simply to the excited-state processes, assuming that the initial populations of the excited free and complexed dyes are determined by the binding constant of the dye as given by equilibrium 1 and the absorption coefficients of the two species at the excitation wavelength. The total fluorescence intensity can be expressed as

\[
I_{\text{total}} = I_{\text{PCX4}} \left[ \frac{[\text{PCX4}]_0}{[\text{PCX4}]} \right] + I_{\beta-\text{CD}: \text{PCX4}} \left[ \frac{[\beta-\text{CD}: \text{PCX4}]_0}{[\text{PCX4}]} \right] \quad (S5)
\]

where, \( I_{\text{PCX4}} \) is the fluorescence intensity in the absence of \( \beta-\text{CD} \) and \( I_{\beta-\text{CD}: \text{PCX4}} \) is the fluorescence intensity of the complex when all the PCX4 molecules in the solution are complexed.

Rearranging eq. S5, the changes in fluorescence intensity can be written as,

\[
\Delta I = \left( 1 - \frac{[\text{PCX4}]}{[\text{PCX4}]_0} \right) \left( I_{\beta-\text{CD}: \text{PCX4}} - I_{\text{PCX4}} \right) \quad (S6a)
\]

In the absorption titrations, we have employed the changes in the optical density of PCX4 with the \( \beta-\text{CD} \) host which can be written as

\[
\Delta OD = \left( 1 - \frac{[\text{PCX4}]}{[\text{PCX4}]_0} \right) \left( OD_{\beta-\text{CD}: \text{PCX4}} - OD_{\text{PCX4}} \right) \quad (S6b)
\]

The \( K_{\text{eq}} \) values were obtained by non-linear curve fittings according to eq. S6(a&b).

---

**Figure S1**: \( ^1\text{H-NMR of PCX4 in CDCl}_3 \).
Figure S2. $^1$H NMR titrations of PCX4 at different concentrations of γ-CD (A) and β-CD (B) in DMSO-d6.
**Figure S3.** Absorption spectra of PCX4 in water containing 1.5 mM β-CD (A) and 18 mM γ-CD at different pHs.

**Figure S4.** AFM images of PCX4 in the presence of 3.0 mM γ-CD (A), 1.5 mM of γ-CD (B) and 1.5 mM β-CD (C).
Figure S5: $^{13}$C-NMR Spectra of PCX4.

Figure S6: HR-MS spectra of PCX4.
Figure S7: FT-IR spectra of PCX4.

References