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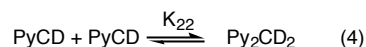
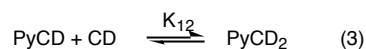
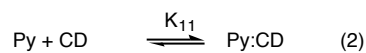
### Dynamics for the Assembly of Pyrene- $\beta$ -Cyclodextrin Host-Guest Complexes.

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#### Determination of the stoichiometry for the pyrene- $\beta$ -cyclodextrin (CD) complexes.

The complexation of pyrene to  $\beta$ -CD leads to various CD complexes, but no consensus exists as to the stoichiometry of these complexes.<sup>1-3</sup> A combination of steady-state fluorescence, quenching studies and time-resolved fluorescence experiments were performed to determine the binding stoichiometries of pyrene to  $\beta$ -CD. Our experiments are consistent with the model proposed by Hamai:<sup>3</sup>



A PTI QM2 fluorimeter was employed for steady-state fluorescence measurements at  $20.0 \pm 0.2$  °C. The excitation and emission slits were set such that the bandpass for each was 3 nm. Samples were excited at 331 nm when acquiring fluorescence spectra, whereas for excitation spectra the emission was collected at 383 nm for the monomer fluorescence and 473 nm for the excimer emission. All spectra were corrected by subtracting the spectra collected containing all components except pyrene. This baseline spectrum contained the Raman emission of the solvent and weakly emitting impurities from CD.

Time-resolved fluorescence decays were measured with a PTI LS-1 time-correlated single photon counter at  $20.0 \pm 0.1$  °C. The excitation wavelength was 331 nm and the monomer and excimer emissions were measured respectively at 383 nm and 473 nm. The slit widths were set to bandpasses between 8 and 26 nm in order to maximize the emission intensity being detected. Collection of the excimer emission at wavelengths longer than 480 nm lead to higher noise levels. A total of 10,000 counts were collected for the maximum channel. The instrument response function was measured using scattering at 331 nm from a silica gel suspension. The decay was fitted to a sum of exponentials by deconvolution from the instrument response function (PTI analysis software). Fits were considered acceptable based on the following criteria: (i)  $\chi^2$  values in the range of 0.9 to 1.2, (ii) randomness of the residuals and the autocorrelation function, (iii) Durbin-Watson parameters greater than 1.7, 1.75 and 1.8, respectively for mono-exponential, sum of two and the sum of three exponentials and (iv) Run Test (Z) parameter, which indicates a level of confidence of 95 % for  $Z > -1.96$ .<sup>4</sup>

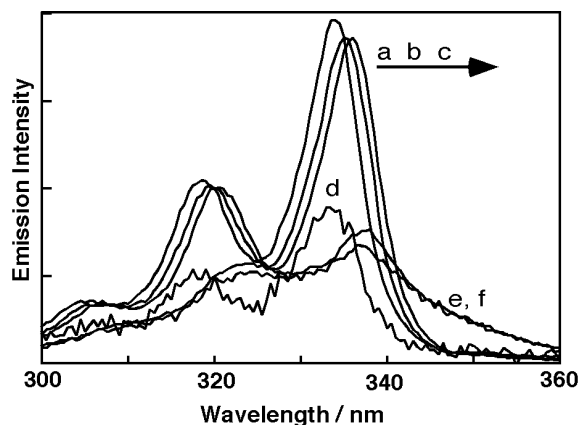
The R(I/III) values for the pyrene monomer emission were determined by measuring the emission intensity maxima for the peaks close to 371 nm and 383 nm, because the position of the maxima changes slightly ( $\leq 2$  nm) with the addition of  $\beta$ -CD. For the E/M (excimer-to-monomer) ratios the monomer intensity was measured for the peak close to 383 nm (peak III). The excimer intensities were measured at 473 nm and were corrected for the residual monomer emission by:

$$E_{\text{cor}} = I_{473} - I_{383} \frac{I_{473}(\text{aq})}{I_{383}(\text{aq})} \quad (\text{S1})$$

Where  $I_{473}$  is the uncorrected intensity measured and the intensity at 383 nm ( $I_{383}$ ) is multiplied by the ratio of intensities at 473 nm and 383 nm determined for the pyrene monomer emission in water. This correction is necessary because there is always a residual monomer emission at 473 nm.

The pH of the pyrene- $\beta$ -CD solutions was raised using a NaOH solution (0.26 M). For the quenching experiments, a 2 M NaI solution was prepared in water and 10  $\mu\text{L}$  aliquots were added to the pyrene solutions in the absence or presence of  $\beta$ -CD. A 1 M tetrabutylammonium iodide stock solution was employed for the excimer quenching experiments in acetonitrile. Quenching of the steady-state fluorescence emission was studied by the sequential addition of iodide (6 concentrations). The intensities were measured at 383 nm and 473 nm for the monomer and excimer emissions, respectively. Quenching studies for the time-resolved experiments were performed at iodide concentrations of 20 mM and 60 mM and only estimated quenching rate constants were obtained.

The formation of the 2:2 complex led to the observation of the pyrene excimer emission, which occurs when an excited state pyrene interacts with a ground-state pyrene molecule. No growth was observed for the excimer emission ( $\tau_{\text{em}} = 473$  nm). The excitation spectrum collected for an emission at 473 nm was broadened when compared to the excitation spectrum measured for the monomer emission (Fig. 1S). The monomer excitation spectrum returned to the baseline at ca. 345 nm, whereas the excimer excitation spectrum tailed to 360 nm. Both these results are in line with the formation of dimers in the ground state within the 2:2 complex. In addition, we confirmed a previous result<sup>3</sup> that the excimer emission disappears at pHs above the  $\text{pK}_a$  ( $>12$ ) of the  $\beta$ -CD,<sup>5</sup> as would be expected for complexes involving two CDs.



**Figure 1S.** Normalized excitation spectra of the pyrene (0.5  $\mu\text{M}$ ) emission measured at 383 nm in the absence (a) and presence of 2 mM (b) and 10 mM  $\beta$ -CD (c), and normalized excitation spectra measured at 473 nm in the absence (d) and presence of 2 mM (e) and 10 mM  $\beta$ -CD (f).

Quenching of the pyrene emission by iodide, which is a quencher that mainly resides in the aqueous phase, yielded information on how much the pyrene within the complex was protected from the interaction with ions in the aqueous phase. The ratio of intensities or lifetimes in the absence ( $I_0$  or  $\tau_0$ ) and presence of quencher ( $I$  or  $\tau$ ) is related to the quenching efficiency expressed as the quenching rate constant ( $k_q$ ) or the Stern-Volmer constant ( $K_{SV}$ ) (eq. (S2)).

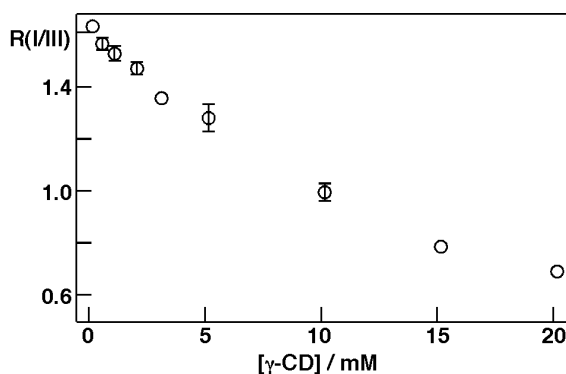
$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV} [Q] = 1 + k_q \tau_0 [Q] \quad (\text{S2})$$

The quenching of the excimer emission in the absence of CD was studied in acetonitrile due to the high concentration of pyrene required to observed excimer emission in homogenous solution. A quenching rate constant of  $5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  was observed for the quenching of the excimer by tetrabutylammonium iodide. For pyrene in water only the monomer emission was detected. A weak emission was observed at 473 nm, which corresponds to the spectral region for the excimer emission. For this reason, there is always some monomer emission in the spectral region for the excimer fluorescence, and the decay for pyrene in the presence of CD had to be fitted to the sum of two exponentials where one of the components corresponds to the emission of the pyrene monomer. The lifetime for the latter was known from independent experiments. In the presence of  $\beta$ -CD (2 or 10 mM) the excimer lifetime was  $86 \pm 8 \text{ ns}$ , and in the presence of 58 mM iodide the lifetime ratio was smaller than 1.15, leading to an upper limit of the quenching rate constant of the pyrene excimer in the CD of  $3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ . This result shows that the pyrene in the 2:2 complex is fairly well protected from aqueous quenchers.

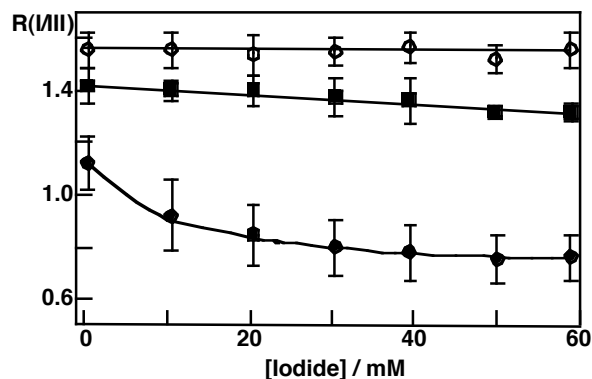
The monomer emission of pyrene is a composite of the fluorescence of pyrene in water or in CD complexes containing only one pyrene (1:1 and 1:2). The presence of two CD complexes containing monomeric pyrene with different stoichiometries was confirmed by measuring the dependence of the E/M ratio on the CD concentration, analyzing the intensity ratio ( $R(I/III)$ ) for the monomer emission at 371 nm (band I) and 383 nm (band III), and

measuring the effect of quenchers on the monomer fluorescence lifetime. The E/M ratio increases at low CD concentrations, but then decreases above 5 mM  $\beta$ -CD. At high CD concentrations the excimer intensity decreases, while the monomer intensity increases. This result suggests that at high CD concentration the amount of 2:2 complex decreased leading to the formation of a CD complex with monomeric pyrene.

The  $R(I/III)$  ratio is an indicator of the polarity around pyrene, and it has been used to characterize the binding sites of pyrene in organized systems.<sup>6,9</sup> This ratio decreased continuously (Fig. 2S). The value of 0.71 at 20 mM  $\beta$ -CD is close to the values measured in non-polar solvents such as cyclohexane (0.58-0.60).<sup>6,7</sup> Quenching experiments in the presence of  $\beta$ -CD concentration above 10 mM afforded quenching plots (eq. S2) with a downward curvature, indicating that at high iodide concentrations a pyrene species was present that was well protected from the quenchers in the aqueous phase. The  $R(I/III)$  values in the presence of 2 mM  $\beta$ -CD decreased only slightly with increasing quencher concentration, whereas in the presence of 10 mM  $\beta$ -CD the  $R(I/III)$  decreased significantly (Fig. 3S). The  $R(I/III)$  value at each  $\beta$ -CD concentration corresponds to the average of the  $R(I/III)$  values for pyrene in water and in the CD complexes taking into account the concentration and lifetime of each species. The contribution of the less protected pyrene species to the  $R(I/III)$  value decreased when the quencher concentration was increased. Therefore, the low  $R(I/III)$  observed at high CD and iodide concentrations is again consistent with a pyrene-CD species where pyrene sensed a low polarity and was well protected from quencher molecules in the aqueous phase. These results indicate that in the presence of 2 mM  $\beta$ -CD most of the complexed monomeric pyrene was in the form of a 1:1 complex, whereas at high  $\beta$ -CD concentrations the 1:2 complex was also present.



**Figure 2S.** Dependence of the  $R(I/III)$  ratio for pyrene (0.5  $\mu\text{M}$ ) in the presence of various  $\beta$ -CD concentrations. The errors correspond to the standard deviation of three trials. Error bars smaller than the symbols are not shown.



**Figure S3.** Dependence of the R(I/III) ratio for pyrene (0.5  $\mu$ M) at increasing iodide concentrations in water (O), and in the presence of 2 mM (■) and 10 mM (●)  $\beta$ -CD. The errors correspond to standard deviations of three trials.

Quenching experiments for the fluorescence lifetimes further support the assignment that two CD complexes with monomeric pyrene were present in solution at high CD concentrations. The decay for pyrene in water was monoexponential with a lifetime of 131 ns, which is similar to previously reported values.<sup>10,11</sup> The pyrene monomer emission was efficiently quenched by iodide (eq. S2,  $1.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ). In the presence of 2 mM  $\beta$ -CD, the decay was fitted to the sum of two exponentials, where the short lifetime corresponded to that of pyrene in water. The longer lived component (192 ns) was quenched less efficiently by iodide, with an estimated quenching rate constant of  $2.7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ . At higher  $\beta$ -CD concentrations (10 mM) the decay in the absence of iodide was adequately fitted to the sum of two exponentials, where the longer lifetime was 278 ns. This results shows that a pyrene species with a longer lifetime than at 2 mM  $\beta$ -CD was present. It was not possible to resolve the two lifetimes for the CD complexes in the presence of 10 mM  $\beta$ -CD, probably because their lifetimes were not very different. However, in the presence of iodide the decay at 10 mM  $\beta$ -CD could only be fitted by the sum of three exponentials. The fastest component was quenched with the same efficiency as pyrene in water, while the second component was quenched with a similar efficiency as the long lived decay in the presence of 2 mM  $\beta$ -CD. The third component corresponded to a pyrene species that was better protected than the species at 2 mM, because its lifetime decreased much less in the presence of equivalent iodide concentrations. No quenching rate constant could be determined because the lifetime in the absence of quencher could not be determined for this long lived species, which was assigned to the 1:2 complex.

**Definition of the equations used for the numerical fitting of the change in the excimer intensity with the  $\beta$ -CD concentration using Scientist:**

**IndVars:** H0, G0 (The independent variables are the total CD (H0) and total pyrene (G0) concentrations)

**DepVars:** Iob, H, G, C11, C12, C22 (The dependent variables are the free CD (H), free pyrene (G), the 1:1 complex (C11), the 1:2 complex (C12), and the 2:2 complex (C22) concentrations)

**Params:** K11, K12, K22, Phi (The parameters are the equilibrium constants for the 1:1 (K11), 1:2 (K12) and 2:2 (K22) complexes and the relative quantum yield for the excimer emission (Phi))

**Definitions:**

$$C11 = K11 * G * H$$

$$C12 = K12 * C11 * H$$

$$C22 = K22 * C11 * C11$$

$$H = H0 - C11 - 2 * C12 - 2 * C22$$

$$G = G0 - C11 - C12 - 2 * C22$$

$$Iob = \Phi * C22$$

**Ranges for dependent variables:**

$$0 < H < 0.025 \text{ M}$$

$$0 < G < 1 \times 10^{-5} \text{ M}$$

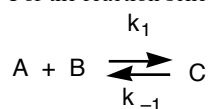
$$0 < C11 < 1 \times 10^{-4} \text{ M}$$

$$0 < C12 < 1 \times 10^{-4} \text{ M}$$

$$0 < C22 < 1 \times 10^{-4} \text{ M}$$

**Derivation for the relaxation kinetics<sup>12</sup> of complex formation.**

For the reaction scheme



the rates are defined as:

$$-\frac{d[A]}{dt} = -\frac{d[B]}{dt} = \frac{d[C]}{dt} = k_1[A][B] - k_{-1}[C] \quad (S3)$$

At equilibrium:

$$K_1 = \frac{k_1}{k_{-1}} = \frac{[C]_{eq}}{[A]_{eq}[B]_{eq}}; \quad k_1[A]_{eq}[B]_{eq} = k_{-1}[C]_{eq} \quad (S4)$$

During a relaxation experiment the system is perturbed and relaxes to a new final equilibrium defined by the concentrations  $[A]_{eq}^F$ ,  $[B]_{eq}^F$ ,  $[C]_{eq}^F$ .

The concentrations at a given time ( $[A]$ ,  $[B]$ ,  $[C]$ ) and the deviations from the final equilibrium ( $\Delta[A]$ ,  $\Delta[B]$ ,  $\Delta[C]$ ) are related by:

$$[A] = [A]_{eq}^F + \Delta A \quad (S5)$$

$$[B] = [B]_{eq}^F + \Delta B \quad (S6)$$

$$[C] = [C]_{eq}^F + \Delta C \quad (S7)$$

According to the principle of mass balance:

$$\Delta A = -\Delta C$$

$$\Delta B = -\Delta C$$

We define the change in concentration as “x”:

$$\Delta A = \Delta B = -\Delta C = x \quad (\text{S8})$$

From equation S5:

$$\frac{d[A]}{dt} = \frac{d([A]_{\text{eq}}^F + \Delta A)}{dt} = \frac{d\Delta A}{dt}, \text{ since } \frac{d[A]_{\text{eq}}^F}{dt} = 0$$

therefore:

$$\frac{d[A]}{dt} = \frac{dx}{dt} = -(k_1[A][B] - k_{-1}[C])$$

Substituting equations S5-S7 and S8 into the equation above:

$$\begin{aligned} \frac{dx}{dt} &= -[k_1([A]_{\text{eq}}^F + x)([B]_{\text{eq}}^F + x) - k_{-1}([C]_{\text{eq}}^F - x)] = \\ &= -k_1[A]_{\text{eq}}^F[B]_{\text{eq}}^F + k_{-1}[C]_{\text{eq}}^F - [k_1([A]_{\text{eq}}^F + [B]_{\text{eq}}^F) + k_{-1}]x - k_{-1}x^2 \end{aligned}$$

The first two terms cancel out (see eq. (S4)):

$$\frac{dx}{dt} = -[k_1([A]_{\text{eq}}^F + [B]_{\text{eq}}^F) + k_{-1}]x - k_{-1}x^2 \quad (\text{S9})$$

When the perturbation is small the term  $k_{-1}x^2$  is negligible and the kinetics follows pseudo-first order behavior:

$$\frac{dx}{dt} = -[k_1([A]_{\text{eq}}^F + [B]_{\text{eq}}^F) + k_{-1}]x$$

where the observed rate constant is given by:

$$k_{\text{obs}} = \frac{1}{\Delta_{\text{obs}}} = k_1([A]_{\text{eq}}^F + [B]_{\text{eq}}^F) + k_{-1} \quad (\text{S10})$$

When one of the components is present in excess, e.g.  $[B] \gg [A]$ , its concentration does not change after the perturbation and eq. S10 is simplified to:

$$k_{\text{obs}} = k_1[B] + k_{-1} \quad (\text{S11})$$

The formation of a dimer is a special case where  $A = B$

$$-\frac{1}{2} \frac{d[A]}{dt} = \frac{d[C]}{dt} = k_1[A]^2 - k_{-1}[C] \quad (\text{S12})$$

At equilibrium:

$$k_1[A]_{\text{eq}}^2 = k_{-1}[C]_{\text{eq}} \quad (\text{S13})$$

The concentrations of A and C are given by S5 and S7.

According to the principle of mass balance, and by defining that  $\Delta A = x$ :

$$\Delta A = -2\Delta C = x \quad (\text{S14})$$

From equation S5:

$$\frac{d[A]}{dt} = \frac{d([A]_{\text{eq}}^F + \Delta A)}{dt} = \frac{d\Delta A}{dt}, \text{ since } \frac{d[A]_{\text{eq}}^F}{dt} = 0$$

therefore:

$$\frac{d[A]}{dt} = \frac{dx}{dt} = -(2k_1[A]^2 - 2k_{-1}[C])$$

Substituting equations S5, S7 and S14 into the equation above:

$$\begin{aligned} \frac{dx}{dt} &= -[2k_1([A]_{\text{eq}}^F + x)^2 - 2k_{-1}[C]_{\text{eq}}^F - \frac{x}{2}] = \\ &= -[2k_1([A]_{\text{eq}}^F)^2 - 2k_{-1}[C]_{\text{eq}}^F + (4k_1[A]_{\text{eq}}^F + k_{-1})x + 2k_1x^2] \end{aligned}$$

The first two terms cancel out (see eq. (S13)):

$$\frac{dx}{dt} = -(4k_1[A]_{\text{eq}}^F + k_{-1})x - 2k_1x^2$$

When the perturbation is small the term  $2k_1x^2$  is negligible and the kinetics follows pseudo-first order behavior:

$$\frac{dx}{dt} = -(4k_1[A]_{\text{eq}}^F + k_{-1})x$$

where the observed rate constant is given by:

$$k_{\text{obs}} = 4k_1[A]_{\text{eq}}^F + k_{-1} \quad (\text{S15})$$

## References.

- (1) Kobayashi, N.; Saito, R.; Hino, H.; Hino, Y.; Ueno, A.; Osa, T. *J. Chem. Soc. Perkin Trans. II* **1983**, 1031- 1035.
- (2) Kano, K.; Takenoshita, I.; Ogawa, T. *Chem. Lett.* **1982**, 321- 324.
- (3) Hamai, S. *J. Phys. Chem.* **1989**, 93, 6527-6529.
- (4) Bohne, C.; Redmond, R. W.; Scaiano, J. C. Use of Photophysical Techniques in the Study of Organized Assemblies. In *Photochemistry in Organized and Constrained Media*; Ramamurthy, V., Ed. VCH Publishers: New York, 1991; pp 79-132.
- (5) Connors, K. A. *Chem. Rev.* **1997**, 97, 1325-1357.
- (6) Dong, D. C.; Winnik, M. A. *Photochem. Photobiol.* **1982**, 35, 17-21.
- (7) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, 99, 2039-2044.
- (8) Kalyanasundaram, K. *Photochemistry in Microheterogeneous Systems*; Academic Press: Orlando, 1987.
- (9) Ramamurthy, V. *Photochemistry in Organized and Constrained Media*; VCH Publishers: New York, 1991.
- (10) Yang, H.; Bohne, C. *J. Photochem. Photobiol. A: Chem.* **1995**, 86, 209-217.
- (11) Xu, W.; Demas, J. N.; DeGraff, B.; Whaley, M. *J. Phys. Chem.* **1993**, 97, 6546-6554.
- (12) Bernasconi, C. F. *Relaxation Kinetics*; Academic Press, Inc.: New York, 1976.