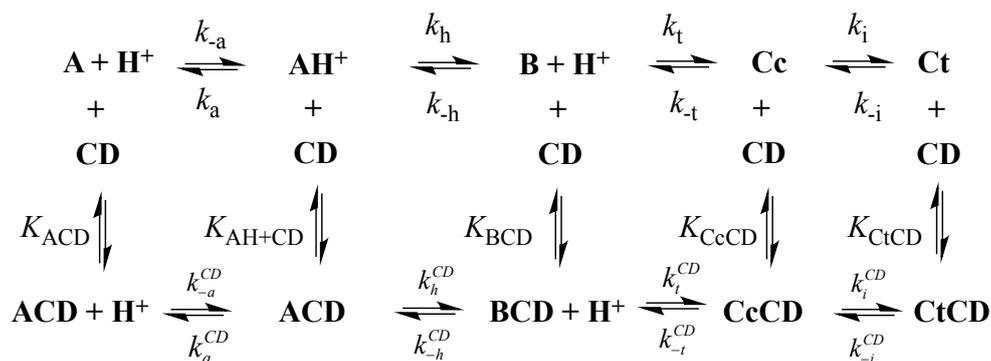


Appendix 1

1. Equilibrium

In Scheme A1 the network of chemical reactions of a flavylum compound taking place in the presence of the host (2-Hydroxypropyl)- β -cyclodextrin is shown.



Scheme A1

With the following acid-base and equilibrium constants

$$K_a = \frac{[\text{A}][\text{H}^+]}{[\text{AH}^+]}; \quad K_h = \frac{[\text{B}][\text{H}^+]}{[\text{AH}^+]}; \quad K_t = \frac{[\text{Cc}]}{[\text{B}]}; \quad K_i = \frac{[\text{Ct}]}{[\text{Cc}]}$$

$$K_a^{\text{CD}} = \frac{[\text{ACD}][\text{H}^+]}{[\text{AH}^+\text{CD}]}; \quad K_h^{\text{CD}} = \frac{[\text{BCD}][\text{H}^+]}{[\text{AH}^+\text{CD}]}; \quad K_t^{\text{CD}} = \frac{[\text{CcCD}]}{[\text{BCD}]}; \quad K_i^{\text{CD}} = \frac{[\text{CtCD}]}{[\text{CcCD}]}; \quad K_{\text{ACD}} = \frac{[\text{ACD}]}{[\text{A}][\text{CD}]};$$

as well as the association constants

$$K_{\text{ACD}} = \frac{[\text{ACD}]}{[\text{A}][\text{CD}]}; \quad K_{\text{AH}^+\text{CD}} = \frac{[\text{AH}^+\text{CD}]}{[\text{AH}^+][\text{CD}]}; \quad K_{\text{BCD}} = \frac{[\text{BCD}]}{[\text{B}][\text{CD}]}; \quad K_{\text{CcCD}} = \frac{[\text{CcCD}]}{[\text{Cc}][\text{CD}]}$$

$$K_{\text{CtCD}} = \frac{[\text{CtCD}]}{[\text{Ct}][\text{CD}]}$$

From the above relations

$$K_a^{CD} = \frac{K_a K_{ACD}}{K_{AH+CD}}; K_h^{CD} = \frac{K_h K_{BCD}}{K_{AH+CD}}; K_t^{CD} = \frac{K_t K_{CcCD}}{K_{BCD}}; K_i^{CD} = \frac{K_i K_{CiCD}}{K_{CcCD}}$$

For the subsequent calculations the following expression is important

$$K_h^{CD} K_t^{CD} K_i^{CD} = K_h K_t K_i \frac{K_{CiCD}}{K_{AH+CD}}$$

The mass balance gives:

$$C_0 = [AH^+] + [A] + [B] + [Cc] + [Ct] + [AH^+CD] + [ACD] + [BCD] + [CcCD] + [CiCD] \quad (A1)$$

By using the equilibrium constants from Scheme 1

$$C_0 = [AH^+] \left\{ 1 + \frac{K_a}{[H^+]} + \frac{K_h}{[H^+]} + \frac{K_h K_t}{[H^+]} + \frac{K_h K_t K_i}{[H^+]} + [CD] (K_{AH+CD} + K_{ACD} \frac{K_a}{[H^+]} + K_{BCD} \frac{K_h}{[H^+]} + K_{CcCD} \frac{K_h K_t}{[H^+]} + K_{CiCD} \frac{K_h K_t K_i}{[H^+]}) \right\}$$

simplifying

$$C_0 = [AH^+] \left\{ 1 + K_{AH+CD} [CD] + \frac{K'_a}{[H^+]} + \frac{K'_{aCD} [CD]}{[H^+]} \right\} \quad (A2)$$

with $K'_a = K_a + K_h + K_h K_t + K_h K_t K_i$

and $K'_{aCD} = K_{ACD} K_a + K_{BCD} K_h + K_{CcCD} K_h K_t + K_{CiCD} K_h K_t K_i$

leading to the mole fraction distribution of all the species at the equilibrium:

$$X_{AH^+} = \frac{1}{\left\{ [H^+] + \frac{1 + K_{AH+CD} [CD]}{1 + K_{AH+CD} [CD]} [H^+] \right\}} \quad (A3)$$

$$X_{AH^+} = \frac{\frac{K_{AH+CD}[CD]}{1+K_{AH+CD}[CD]}[H^+]}{\left\{ [H^+] + \frac{K'_a + K'_{aCD}[CD]}{1+K_{AH+CD}[CD]} \right\}} \quad (\text{A4})$$

$$X_A = \frac{\frac{K_a}{1+K_{AH+CD}[CD]}[H^+]}{\left\{ [H^+] + \frac{K'_a + K'_{aCD}[CD]}{1+K_{AH+CD}[CD]} \right\}} \quad (\text{A5})$$

$$X_{ACD} = \frac{\frac{K_a K_{ACD}}{1+K_{AH+CD}[CD]}[H^+]}{\left\{ [H^+] + \frac{K'_a + K'_{aCD}[CD]}{1+K_{AH+CD}[CD]} \right\}} \quad (\text{A6})$$

$$X_B = \frac{\frac{K_h}{1+K_{AH+CD}[CD]}[H^+]}{\left\{ [H^+] + \frac{K'_a + K'_{aCD}[CD]}{1+K_{AH+CD}[CD]} \right\}} \quad (\text{A7})$$

$$X_{BCD} = \frac{\frac{K_h K_{BCD}}{1+K_{AH+CD}[CD]}[H^+]}{\left\{ [H^+] + \frac{K'_a + K'_{aCD}[CD]}{1+K_{AH+CD}[CD]} \right\}} \quad (\text{A8})$$

$$X_{Cc} = \frac{\frac{K_h K_t}{1+K_{AH+CD}[CD]}[H^+]}{\left\{ [H^+] + \frac{K'_a + K'_{aCD}[CD]}{1+K_{AH+CD}[CD]} \right\}} \quad (\text{A9})$$

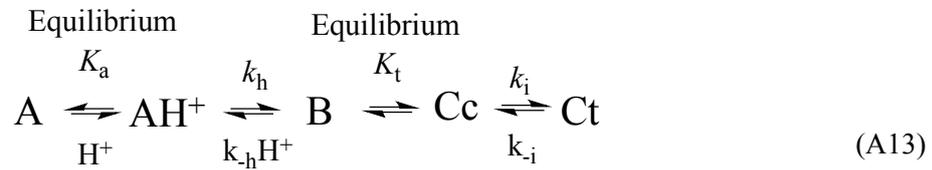
$$X_{CcCB} = \frac{\frac{K_h K_t K_{CcCD}}{1+K_{AH+CD}[CD]}[H^+]}{\left\{ [H^+] + \frac{K'_a + K'_{aCD}[CD]}{1+K_{AH+CD}[CD]} \right\}} \quad (\text{A10})$$

$$X_{Ct} = \frac{\frac{K_h K_t K_i}{1+K_{AH+CD}[CD]}[H^+]}{\left\{ [H^+] + \frac{K'_a + K'_{aCD}[CD]}{1+K_{AH+CD}[CD]} \right\}} \quad (\text{A11})$$

$$X_{CtCD} = \frac{\frac{K_h K_t K_i K_{CtCD} [H^+]}{1 + K_{AH+CD} [CD]}}{\left\{ [H^+] + \frac{K'_a + K'_{aCD} [CD]}{1 + K_{AH+CD} [CD]} \right\}} \quad (\text{A12})$$

2. Kinetics

In the case of flavylum networks lacking a *cis-trans* isomerization barrier as dracoflavylum, the equilibrium between AH^+ and A as well B and Cc is reached during the kinetic process, and the following kinetic scheme can be used.



This is equivalent to the following equilibrium



Where the equilibrium between AH^+ and A is assigned to X, the equilibrium between B and Cc to Y and Ct to Z.

Assuming the steady state for Y, the observed rate constant is given by eq.(A16)

$$k_{obs} = \eta_{Y(direct)}k_2 + \eta_{Y(reverse)}k_{-1} \quad (A15)$$

where $\eta_{Y(direct)} = \frac{k_1}{k_{-1} + k_2}$ is the formation efficiency of Y during the direct process and k_2 , the rate of Z formation during the same process, $\eta_{Y(reverse)} = \frac{k_{-2}}{k_{-1} + k_2}$ the formation efficiency of Y in the reverse process and k_{-1} the rate of X formation in this last process.

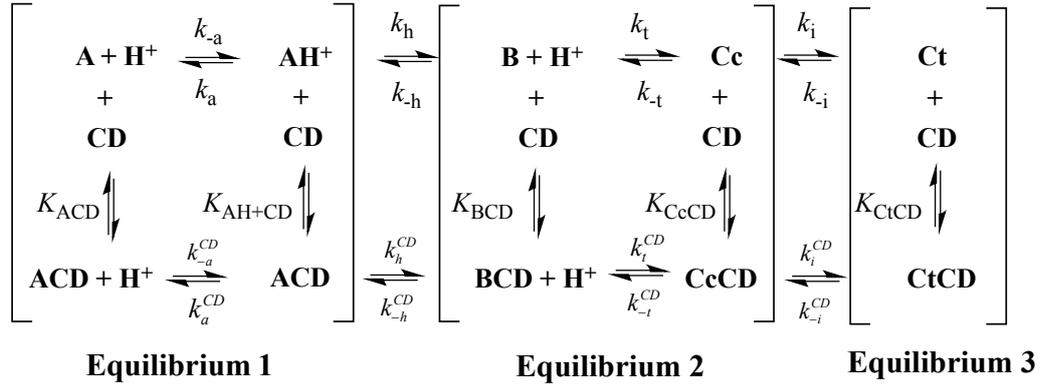
In the scheme shown in eq.(A14) k_1 is equal to $\frac{[H^+]}{[H^+] + K_a}k_h$ since k_h should be multiplied by the mole fraction of AH^+ available due to its equilibrium with A; k_2 equal to $k_i \frac{K_t}{1 + K_t}$ due to the equilibrium between B and Cc. Regarding the reverse processes, k_{-2} is identified as k_{-i} , and k_{-1} , to $k_{-h}[H^+] \frac{1}{1 + K_t}$ the last term also due to the equilibrium between B and Cc.

Substituting these constants in eq.(A15)

$$k_{obs} = \frac{\frac{[H^+]}{[H^+] + K_a}k_hk_i \frac{K_t}{1 + K_t} + k_{-i}k_{-h}[H^+] \frac{1}{1 + K_t}}{\frac{k_{-h}[H^+]}{1 + K_t} + k_i \frac{K_t}{1 + K_t}} \quad (A16)$$

$$k_{obs} = \frac{\frac{[H^+]}{[H^+] + K_a}k_hK_tk_i + k_{-i}[H^+] \frac{1}{1 + K_t}}{[H^+] + \frac{K_tk_i}{k_{-h}}} \quad (A17)$$

In the presence of the HCD Scheme A2 should be considered. The species AH^+ , A inside and outside the host are in fast equilibrium, equivalent to X in eq.(14); B, Cc inside and outside of the cyclodextrin are equivalent to Z; Ct inside and outside the cyclodextrin assigned to Z.



Scheme A2

At this point the situation is similar to eq.(A14), and it is easy to calculate the mole fraction distribution of each species in these three fast equilibria.

Equilibrium 1

$$X_{\text{AH}^+} = \frac{\frac{1}{1 + K_{\text{AH+CD}}[\text{CD}]} [H^+]}{[H^+] + \frac{K_a(1 + K_{\text{aCD}}[\text{CD}])}{1 + K_{\text{AH+CD}}[\text{CD}]}} \quad (\text{A18})$$

$$X_{\text{AH+CD}} = \frac{\frac{K_{\text{AH+CD}}[\text{CD}]}{1 + K_{\text{AH+CD}}[\text{CD}]} [H^+]}{[H^+] + \frac{K_a(1 + K_{\text{aCD}}[\text{CD}])}{1 + K_{\text{AH+CD}}[\text{CD}]}} \quad (\text{A19})$$

$$X_A = \frac{\frac{K_a}{1 + K_{\text{AH+CD}}[\text{CD}]} }{[H^+] + \frac{K_a(1 + K_{\text{aCD}}[\text{CD}])}{1 + K_{\text{AH+CD}}[\text{CD}]}} \quad (\text{A20})$$

$$X_{ACD} = \frac{\frac{K_a K_{aCD}[CD]}{1 + K_{AH+CD}[CD]}}{[H^+] + \frac{K_a(1 + K_{aCD}[CD])}{1 + K_{AH+CD}[CD]}} \quad (\text{A21})$$

Equilibrium 2

$$X_B = \frac{1}{(1 + K_t + (K_{BCD} + K_{CcCD}K_t)[CD])} \quad (\text{A22})$$

$$X_{Cc} = \frac{K_t}{(1 + K_t + (K_{BCD} + K_{CcCD}K_t)[CD])} \quad (\text{A23})$$

$$X_{BCD} = \frac{K_{BCD}[CD]}{(1 + K_t + (K_{BCD} + K_{CcCD}K_t)[CD])} \quad (\text{A24})$$

$$X_{CcCD} = \frac{K_{CcCD}K_t[CD]}{(1 + K_t + (K_{BCD} + K_{CcCD}K_t)[CD])} \quad (\text{A25})$$

Equilibrium 3

$$X_{Ct} = \frac{1}{1 + K_{CtCD}[CD]} \quad (\text{A26})$$

$$X_{CtCD} = \frac{K_{CtCD}[CD]}{1 + K_{CtCD}[CD]} \quad (\text{A27})$$

The strategy is the same: i) we have to consider the mole fraction of flavylum cation in water and multiply by k_h , ii) the mole fraction of B and multiply by $k_{-h}[H^+]$, iii) the mole fraction of Cc and multiply by k_i and also iv) the mole fraction of Ct multiplied by k_{-i} .

$$k_{obs} = \frac{\frac{1}{(1+K_{AH+CD}[CD])}[H^+]}{[H^+] + \frac{K_a(1+K_{ACD}[CD])}{(1+K_{AH+CD}[CD])}} k_h k_i \frac{K_t}{(1+K_t + (K_{BCD} + K_{CCD}K_t)[CD])} + \frac{k_{-i}}{1+K_{CCD}[CD]} k_{-h}[H^+] \frac{1}{(1+K_t + (K_{BCD} + K_{CCD}K_t)[CD])}}{k_{-h}[H^+] \frac{1}{(1+K_t + (K_{BCD} + K_{CCD}K_t)[CD])} + k_i \frac{K_t}{(1+K_t + (K_{BCD} + K_{CCD}K_t)[CD])}} \quad (A28)$$

$$k_{obs} = \frac{\frac{1}{(1+K_{AH+CD}[CD])}[H^+]}{[H^+] + \frac{K_a(1+K_{ACD}[CD])}{(1+K_{AH+CD}[CD])}} K_h k_i K_t + \frac{k_{-i}}{1+K_{CCD}} [H^+] }{[H^+] + k_i \frac{K_t}{k_{-h}}} \quad (A29)$$

The same *mutatis mutandis* for the species inside the host.

$$K_{obsCD} = \frac{\frac{K_{AH+CD}[CD]}{1+K_{AH+CD}[CD]} [H^+]}{[H^+] + \frac{K_a(1+K_{ACD}[CD])}{1+K_{AH+CD}[CD]}} K_h^{CD} K_t^{CD} k_i^{CD} + \frac{K_{CCD}[CD]}{1+K_{CCD}[CD]} k_{-i}^{CD} [H^+] }{[H^+] + \frac{k_i^{CD} K_t^{CD}}{k_{-h}^{CD}}} \quad (A30)$$

3. Flash Photolysis

3.1 In pure water

Identically to the case of the quantum yields, the pH dependence of the flash photolysis can be controlled by hydration or tautomerization

4.1.1 Control by hydration.

The data show the change of regime. At higher pH jumps the rate determining step is the hydration reaction and eq.(A31) is followed.

$$k_{obs} = \frac{1}{1+K_t} k_{-h} [H^+] + \frac{K_t}{1+K_t} k_i \quad (A31)$$

3.1.2. Control by tautomerization

At lower pH values the hydration becomes faster than tautomerization (it is directly proportional to $[H^+]$) and eq.(A32) should be used

$$k_{obs} = k_{-t} + k_i + k^H [H^+] + k^{OH} [OH^-] \quad (A32)$$

3.2. In the presence of HCD

3.2.1 Control by hydration in water in the presence of HCB

$$k_{obs} = \frac{1}{1+K_t + (K_{BCD} + K_{CcCD} K_t)[CD]} k_{-h} [H^+] + \frac{K_t}{1+K_t + (K_{BCD} + K_{CcCD} K_t)[CD]} k_i \quad (A33)$$

3.2.2. Control by tautomerization in water in the presence of HCB

$$k_{obs} = \frac{1}{1+K_{CcCD}[CD]} (k_i + k_{-t} + k^H [H^+] + k^{OH} [OH^-]) \quad (A34)$$

3.2.3 Control by hydration inside the HCD

$$k_{obs} = \frac{K_{BCD}[CD]}{1 + K_t + (K_{BCD} + K_{CcCD}K_t)[CD]} k_{-h}^{CD} [H^+] + \frac{K_{CcCD}K_t[CD]}{1 + K_t + (K_{BCD} + K_{CcCD}K_t)[CD]} k_i^{CD} \quad (A35)$$

3.2.4. Control by tautomerization inside the HCD

$$k_{obs} = \frac{K_{CcCD}[CD]}{1 + K_{CcCD}[CD]} (k_i^{CD} + k_{-t}^{CD} + k^{CDH} [H^+] + k^{CDOH} [OH^-]) \quad (A36)$$