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Supporting Information

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Allosterically Driven Multi-Component Assembly

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Determination of association constants by UV and fluorescence titrations. All UV and fluorescence titrations were carried out in duplo. $CHCl_3$ and CH_3CN were freshly distilled before use. To approximately 2 mL (weighed) of a 1.0-2.0 μM stock solution of the porphyrin (and the co-factor when appeasable) under investigation in a 1:1 or 4:1 (v/v) mixture of $CHCl_3$ and CH_3CN were added small amounts (25-100 μL, microsyringe) of a stock solution containing 1.0-2.0 μM of the porphyrin (and the co-factor when appeasable) under investigation and approximately 60 μM guest, so that at the end at least 10-20 equivalents of guest were present. For each titration, at least 25 data points were collected. Typically four wavelengths were monitored at the same time, two around the absorption maxima for the host and two around the absorption maxima for the complex formed. This gave four data sets from which the binding constants were obtained using a custom written global nonlinear regression analysis program within the Matlab 5.3 package[3] utilizing the Simplex algorithm.[4] It should be

stressed that the global approach, fitting all four data sets simultaneously, greatly enhances the quality of the fitting procedure. The data was fitted according to three different **binding models** (H = host, L = ligand $[X]_0$ total concentration of species X, ε = molar absorption coefficient, Δε = difference in molar absorption coefficient between species, A = measured absorbance, A_0 = measured absorbance when no ligand is present, A_{obs} = the observed absorbance at a given point in the titration and K = binding constants) as discussed further below.

Determination of association constants by NMR titrations. All NMR titrations in a similar fashion as the UV and fluorescence titrations except that the solvents were deuterated ($CDCl_3$ and CD_3CN) and for the concentrations applied, i.e. typically 1.0 mM stock solution of the porphyrin under investigation were applied. Typically four different proton resonances were monitored at the same time giving four data sets from which the binding constants were obtained using a custom written global nonlinear regression analysis program as described for the UV and fluorescence titrations.

Binding models:

(1) A simple 1:1 complexation (assuming only one binding site per molecule of porphyrin) according to equation **S-1** for UV titrations:[5]

$$A_{obs} = A_0 - \frac{\Delta\varepsilon}{2}\left\{[H]_0 + [L]_0 + 1/K - \sqrt{\{([H]_0 + [L]_0 + 1/K)^2 - 4[H]_0[L]_0\}}\right\} \quad \textbf{(S-1)}$$

For fluorescence titrations experiments indicated that the porphyrin emission of porphyrin-viologen complex was completely quenched and that no emission occurred from the viologen at the wavelengths monitored (> 600 nm). Therefore, the fluorescence data could be fitted to **S-2**[5,6]:

$$I_{obs} = I_0\left([H]_0 - \frac{1}{2}\left\{[H]_0 + [L]_0 + 1/K - \sqrt{\{([H]_0 + [L]_0 + 1/K)^2 - 4[H]_0[L]_0\}}\right\}\right) \quad \textbf{(S-2)}$$

where I_{obs} = observed emission and I_0 = emission when no ligand is present.

For NMR titrations the data was fitted to **S-3**[5,6]:

$$\delta_{obs} = \delta_H - \frac{\Delta\delta}{2[H]_0}\left\{[H]_0 + [L]_0 + 1/K - \sqrt{\{([H]_0 + [L]_0 + 1/K)^2 - 4[H]_0[L]_0\}}\right\} \quad \textbf{(S-3)}$$

where δ_{obs} = observed chemical shift of the signal under observation, δ_H = chemical shift of host in the absence of a ligand and $\Delta\delta$ = chemical shift of complex relative to host, i.e., $\delta_{complex} - \delta_{host}$.

(2) A statistical (degenerate) stepwise 2:1 (two molecules porphyrin per **DABCO** guest) complexation where the interaction parameter[6,7] $\alpha = 4*K_2/K_1 = 1$.

(3) A stepwise (non-degenerate) 2:1 (two molecules porphyrin per **DABCO** guest)binding that allows for cooperativity (negative or positive).

In both (2) and (3) the data was fitted according to the method of Taylor and Anderson.[8] For UV titrations the data was fitted to equation **S-4**:

$$\Delta A = \frac{[L]_0(\Delta\varepsilon_{11}K_1[H] + \Delta\varepsilon_{21}K_1K_2[H]^2)}{1 + K_1[H] + K_1K_2[H]^2} \quad \textbf{S-4)}$$

where $\Delta\varepsilon_{11} = \varepsilon_{HL} - \varepsilon_H$, $\Delta\varepsilon_{12} = \varepsilon_{H_2L} - \varepsilon_H$.

Whereas for NMR titrations the data was fitted to equation **S-5**:[8]

$$\Delta\delta = \frac{(\Delta_{11}K_1[H] + 2\Delta_{21}K_1K_2[H]^2)}{(1 + K_1[H] + K_1K_2[H]^2)} \quad \textbf{S-5)}$$

where abbreviations are similar to that for equations **S-3** and **S-4**.

The free host concentration [H] is obtained from the cubic equation **S-6**:[5]

$$K_1K_2[H]^3 + \{K_1(2K_2[L]_0 - K_2[H]_0 + 1)\}[H]^2 - \{K_1([L]_0 - [H]_0) + 1\}[H] + [H]_0 = 0 \quad \textbf{(S-6)}$$

This cubic equation is solved directly in Matlab[3] and the results put into equations **S-4** and **S-5**.

The only difference in treating the statistical (2) and cooperative 1:2 (3) binding models is if the program is allowed to optimize both K_1 and K_2. In the statistical approach, (2) only K_1 is varied and K_2 fixed as $K_2 = K_1/4$ while in the cooperative model (3), the program is allowed to optimize both binding constants.

The results from these three different binding models were then compared based on both quantitative analysis of goodness of the fit (RMS and covariance of the fit) and inspection of the scatter diagram residual plots.[9] In all cases, the experimental data was found to show the best fit to the cooperative 2:1 stepwise binding model, except for **ZnP**:**DABCO** system as detailed below.

Determination of the interaction parameter α for the ZnP**:**DABCO **complexation:**

The data for the **ZnP**:**DABCO** system could not be easily fitted in a straightforward fashion to any of the binding models above. The data did though seem to fit best with a negatively cooperative binding model. In the absence of the viologen (**V**) co-factor, no reliable fitting could be obtained from UV titration data, however, the NMR data was somewhat more promising. As pointed out earlier,[8] NMR binding data cannot be fitted directly to 2:1 binding system without prior knowledge of either the K_1 or K_2 binding constant. No reliable estimate of K_1 could be obtained from UV titration data, instead a grid search was carried out a range of fixed K_1 values and the data fitted to equation **S-5**. The resulting K_2, $\Delta\varepsilon_{11}$ and $\Delta\varepsilon_{12}$ were then tabulated (e.g., as Table S-1). Next, data from NMR experiments were the co-factor **V** was presented was collected, where most of the signals under observations had moved from the fast to the slow exchange regime (as the binding strength had increased by an order of magnitude, *vide supra*), allowing a direct estimation of $\Delta\varepsilon_{12}$ (the $\Delta\varepsilon_{11}$ values cannot be easily detected). These $\Delta\varepsilon_{12}$ values were then used to analyse the tables obtained from the grid search to identify the most probable solution for K_2 and hence α as the example in Table S-1 shows.

Table S-1. Example of a grid search for K_2 using NMR data for the complexation of **ZnP** to **DABCO**.

K_1 (fixed) / M^{-1}	K_2 (obtained) / M^{-1}	$\alpha = 4K_2/K_1$	$\Delta\varepsilon_{12}$[a] / ppm	$\Delta\varepsilon_{12}$[b] / ppm
30000	82	0.01	-1.67	-2.32
40000	124	0.01	-1.14	-1.59
45000	284	0.03	-0.55	-0.76
48000	482	0.04	-0.36	-0.49
50000	613	0.05	-0.30	-0.41
52000	743	0.06	-0.26	-0.35
60000	1261	0.08	-0.18	-0.25
80000	2559	0.13	-0.13	-0.23
100000	3872	0.15	-0.11	-0.14
200000	10613	0.21	-0.08	-0.10
Determined in the slow exchange regime (with V)			**-0.32**	**-0.52**

[a]The signal followed is one of the β-pyrrolic H of **ZnP**.
[b]The signal followed is one of the *meso*-aryl H of **ZnP**.

Inspection of Table S-1 shows that K_1 must be around 50000 M^{-1} and α = 0.05 to fit with the $\Delta\varepsilon_{12}$ values obtained in the slow exchange regime.

To determine a for the binding of ZnP to DABCO in the presence of the co-factor V a combination of NMR and UV data was used. Again, the experimental UV titration data could not be fitted directly to equation **S-4**. As noted above most of the NMR signals were in the slow exchange and this allowed the concentrations of the different species to be determined by integration. The method of Sanders and co-workers could then be used to calculate the ratio between K_1 and K_2 according to equation **S-7**:[10]

$$\frac{K_1}{K_2} = \frac{[HL]^2}{[H_2L][L]} \qquad \textbf{(S-7)}$$

The UV titration experimental data was then fitted to equation **S-4** after fixing the ratio between K_1 and K_2 (and hence the interaction parameter α) to the value obtained from equation **S-7**.

Calculation of free energy changes:

The free energy of the stepwise binding processes was then calculated as in our previous work,[11] i.e. after taking the appropriate statistical factors into account we

find that $K_1 = 2*K_{1i}$ (K_{1i} = first microscopic binding constant) and $K_2 = K_{2i}/2$.[6] The free energy change in each step was then calculated according to equation **S-4**:

$$\Delta G_x = -RT\ln K_{xi} \qquad \textbf{(S-4)}$$

where K_{xi} = the stepwise microscopic binding constants for step x.

It is clear from this that in a statistical (de-generate) 1:2 system (model (2) above), $\Delta G_1 = \Delta G_2$.

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