Supplemental Material for

“The hetero-association models of non-covalent molecular complexation”

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The sections (A)–(I) contain sets of equations required to describe practically important types of hetero-associations. The following notations are used below:

Ξ	partition function of aggregating molecular system;
Ξ_{corr}	partition function of aggregating molecular system corrected for the ‘reflected complexes’;
x_1, y_1, z_1	equilibrium monomer concentrations of $\boldsymbol{X}$, $\boldsymbol{Y}$ or $\boldsymbol{Z}$ components, respectively;
x_0, y_0, z_0	total concentrations of $\boldsymbol{X}$, $\boldsymbol{Y}$ or $\boldsymbol{Z}$ components, respectively;
K_X, K_Y, K_Z	equilibrium constants of $\boldsymbol{X}$–$\boldsymbol{X}$, $\boldsymbol{Y}$–$\boldsymbol{Y}$ or $\boldsymbol{Z}$–$\boldsymbol{Z}$ self-association, respectively;
K_h	equilibrium constant of $\boldsymbol{X}$–$\boldsymbol{Y}$ hetero-association (for two-component $\boldsymbol{X}$–$\boldsymbol{Y}$ systems (‘1:n’, ‘2:n’ and ‘m:n’) only);
K_{XY}, K_{XZ}, K_{YZ}	equilibrium constants of $\boldsymbol{X}$–$\boldsymbol{Y}$, $\boldsymbol{X}$–$\boldsymbol{Z}$ or $\boldsymbol{Y}$–$\boldsymbol{Z}$ hetero-association, respectively (for three-component $\boldsymbol{X}$–$\boldsymbol{Y}$–$\boldsymbol{Z}$ system (‘1:m:n’) only);
ξ_X	experimental observable of $\boldsymbol{X}$ component;
$\xi_X^{(m)}$	experimental observable of $\boldsymbol{X}$ monomer;
$\xi_X^{(d)}$	experimental observable of $\boldsymbol{X}$ molecule being in contact with another $\boldsymbol{X}$ molecule (homo-stack);
$\xi_{XY}^{(h)}$, $\xi_{XZ}^{(h)}$	experimental observables of $\boldsymbol{X}$ molecule being in contact with $\boldsymbol{Y}$ or $\boldsymbol{Z}$ molecule, respectively (hetero-stacks).

The sections (J) and (K) contain MATLAB code designed to fit spectrophotometric titration curves for the cases of 1:n and 1:m:n models of hetero-association, respectively.

A. ‘1:n’ hetero-association model

Partition function:

$$\Xi^{(1:n)} = x_1 + \frac{y_1}{1-K_Y y_1}\left(1 + 2K_h x_1 + \frac{K_h^2 x_1 y_1}{1-K_Y y_1}\right) \tag{A1}$$

Mass conservation law:

$$\begin{cases} x_0 = x_1\left(1+\dfrac{K_h y_1}{1-K_Y y_1}\right)^2 \\ y_0 = \dfrac{y_1}{(1-K_Y y_1)^2}\left(1+2K_h x_1 + \dfrac{K_h^2 x_1 y_1}{1-K_Y y_1}\right) \end{cases} \tag{A2}$$

Expression for experimental observable:

$$\xi_X^{(1:n)} = \xi_X^{(m)} - 2\left(\xi_X^{(m)} - \xi_{XY}^{(h)}\right)\frac{K_h x_1 y_1}{1-K_Y y_1}\left(1+\frac{K_h y_1}{1-K_Y y_1}\right)\frac{1}{x_0} \tag{A3}$$

B. ‘1:n’ hetero-association model accounting for ‘reflected complexes’

Partition function:

$$\Xi_{\mathrm{corr}}^{(1:n)} = x_1 + \frac{y_1}{1-K_Y y_1}\left(1 + K_h x_1 + \frac{K_h^2 x_1 y_1}{1-K_Y^2 y_1^2}\right) \tag{B1}$$

Mass conservation law:

$$\begin{cases} x_0 = x_1 + \dfrac{K_h x_1 y_1}{1-K_Y y_1}\left(1+\dfrac{K_h y_1}{1-K_Y^2 y_1^2}\right) \\ y_0 = \dfrac{y_1}{(1-K_Y y_1)^2}\left(1+K_h x_1\left(1+\dfrac{K_h y_1}{1-K_Y y_1}+\dfrac{K_h y_1}{(1+K_Y y_1)^2}\right)\right) \end{cases} \tag{B2}$$

Expression for experimental observable:

$$\xi_X^{(1:n)} = \xi_X^{(m)} - \left(\xi_X^{(m)} - \xi_{XY}^{(h)}\right)\frac{K_h x_1 y_1}{1-K_Y y_1}\left(1+\frac{2K_h y_1}{1-K_Y^2 y_1^2}\right)\frac{1}{x_0} \tag{B3}$$

C. ‘2:n’ hetero-association model

Partition function:

$$\Xi^{(2:n)} = \frac{y_1}{1-K_Y y_1} + x_1\left(1+K_X x_1 + \frac{K_h^2 x_1 y_1}{1-K_Y y_1}\right)\left(1+\frac{K_h y_1}{1-K_Y y_1}\right)^2 \tag{C1}$$

Mass conservation law:

$$\begin{cases} x_0 = x_1\left(1+2K_X x_1 + \dfrac{2K_h^2 x_1 y_1}{1-K_Y y_1}\right)\left(1+\dfrac{K_h y_1}{1-K_Y y_1}\right)^2 \\ y_0 = \dfrac{y_1}{(1-K_Y y_1)^2}\left(1+K_h x_1\left(2+2K_X x_1+K_h x_1+\dfrac{3K_h^2 x_1 y_1}{1-K_Y y_1}\right)\left(1+\dfrac{K_h y_1}{1-K_Y y_1}\right)\right) \end{cases} \tag{C2}$$

Expression for experimental observable:

$$\xi_X^{(2:n)} = \xi_X^{(m)} - 2\left(\xi_X^{(m)} - \xi_X^{(d)}\right) K_X x_1^2 \left(1 + \frac{K_h y_1}{1 - K_Y y_1}\right)^2 \frac{1}{x_0} - 2\left(\xi_X^{(m)} - \xi_{XY}^{(h)}\right) \frac{K_h x_1 y_1}{1 - K_Y y_1} \left(1 + \frac{K_h y_1}{1 - K_Y y_1}\right) \left(1 + K_X x_1 + K_h x_1 + \frac{2K_h^2 x_1 y_1}{1 - K_Y y_1}\right) \frac{1}{x_0} \tag{C3}$$

D. '2:n' hetero-association model accounting for 'reflected complexes'

Partition function:

$$\Xi_{\text{corr}}^{(2:n)} = \frac{y_1}{1 - K_Y y_1} + x_1 \left(1 + K_X x_1 + \frac{K_h^2 x_1 y_1}{1 - K_Y y_1}\right) \left(1 + \frac{K_h y_1}{1 - K_Y y_1} + \frac{K_h^2 y_1^2}{(1 - K_Y y_1)^2 (1 + K_Y y_1)}\right) \tag{D1}$$

Mass conservation law:

$$\begin{cases} x_0 = x_1 \left(1 + 2K_X x_1 + \dfrac{2K_h^2 x_1 y_1}{1 - K_Y y_1}\right) \left(1 + \dfrac{K_h y_1}{1 - K_Y y_1} + \dfrac{K_h^2 y_1^2}{(1 - K_Y y_1)^2 (1 + K_Y y_1)}\right) \\ y_0 = \dfrac{y_1}{(1 - K_Y y_1)^2} \left(1 + K_h x_1 \left(1 + K_X x_1 + \dfrac{K_h^2 x_1 y_1}{1 - K_Y y_1}\right) \left(1 + \dfrac{K_h y_1}{1 - K_Y y_1} + \dfrac{K_h y_1}{(1 + K_Y y_1)^2}\right) + K_h^2 x_1^2 \left(1 + \left(1 + \dfrac{K_h y_1}{1 - K_Y^2 y_1^2}\right) \dfrac{K_h y_1}{1 - K_Y y_1}\right)\right) \end{cases} \tag{D2}$$

Expression for experimental observable:

$$\begin{aligned} \xi_X^{(2:n)} = \xi_X^{(m)} &- 2\left(\xi_X^{(m)} - \xi_X^{(d)}\right) K_X x_1^2 \left(1 + \frac{K_h y_1}{1 - K_Y y_1} + \frac{K_h^2 y_1^2}{(1 - K_Y y_1)^2 (1 + K_Y y_1)}\right) \frac{1}{x_0} \\ &- \left(\xi_X^{(m)} - \xi_{XY}^{(h)}\right) \frac{K_h x_1 y_1}{1 - K_Y y_1} \left(\left(1 + \frac{2K_h y_1}{1 - K_Y^2 y_1^2}\right) \left(1 + K_X x_1 + K_h x_1 + \frac{2K_h^2 x_1 y_1}{1 - K_Y y_1}\right) + K_h x_1 \left(1 - \frac{K_h y_1}{1 - K_Y y_1}\right)\right) \frac{1}{x_0} \end{aligned} \tag{D3}$$

E. 'm:n' hetero-association model

Partition function:

$$\Xi^{(m:n)} = \frac{x_1 (1 - K_Y y_1 + K_h y_1) + y_1 (1 - K_X x_1 + K_h x_1)}{(1 - K_X x_1)(1 - K_Y y_1) - K_h^2 x_1 y_1} \tag{E1}$$

Mass conservation law:

$$\begin{cases} x_0 = x_1 \left(\dfrac{1 - K_Y y_1 + K_h y_1}{(1 - K_X x_1)(1 - K_Y y_1) - K_h^2 x_1 y_1}\right)^2 \\ y_0 = y_1 \left(\dfrac{1 - K_X x_1 + K_h x_1}{(1 - K_X x_1)(1 - K_Y y_1) - K_h^2 x_1 y_1}\right)^2 \end{cases} \tag{E2}$$

Expression for experimental observable:

$$\xi_X^{(m:n)} = \xi_X^{(m)} - 2\left(\xi_X^{(m)} - \xi_X^{(d)}\right) K_X x_1 - \left(\xi_X^{(m)} - \xi_{XY}^{(h)}\right) K_h y_1 \frac{1 - K_X x_1 + K_h x_1}{1 - K_Y y_1 + K_h y_1} \tag{E3}$$

F. 'm:n' hetero-association model accounting for 'reflected complexes'

Partition function:

$$\Xi_{\text{corr}}^{(m:n)} = \frac{1}{2}\left(\frac{x_1(1-K_Y y_1+K_h y_1)+y_1(1-K_X x_1+K_h x_1)}{(1-K_X x_1)(1-K_Y y_1)-K_h^2 x_1 y_1}+\frac{x_1(1+K_X x_1)(1-K_Y^2 y_1^2+K_h^2 y_1^2)+y_1(1+K_Y y_1)(1-K_X^2 x_1^2+K_h^2 x_1^2)}{(1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2}\right) \tag{F1}$$

Mass conservation law:

$$\begin{cases} x_0 = \dfrac{x_1}{2}\left(\left(\dfrac{1-K_Y y_1+K_h y_1}{(1-K_X x_1)(1-K_Y y_1)-K_h^2 x_1 y_1}\right)^2+\dfrac{1+K_X x_1}{1-K_X x_1}\dfrac{1-K_Y^2 y_1^2+K_h^2 y_1^2}{(1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2}\right) \\ \quad + x_1\dfrac{K_h^2 x_1 y_1(1-K_Y^2 y_1^2+K_h^2 y_1^2)}{((1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2)^2}\left(1+K_Y y_1+\dfrac{K_h^2 x_1 y_1}{1-K_X x_1}\right) \\ y_0 = \dfrac{y_1}{2}\left(\left(\dfrac{1-K_X x_1+K_h x_1}{(1-K_X x_1)(1-K_Y y_1)-K_h^2 x_1 y_1}\right)^2+\dfrac{1+K_Y y_1}{1-K_Y y_1}\dfrac{1-K_X^2 x_1^2+K_h^2 x_1^2}{(1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2}\right) \\ \quad + y_1\dfrac{K_h^2 x_1 y_1(1-K_X^2 x_1^2+K_h^2 x_1^2)}{((1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2)^2}\left(1+K_X x_1+\dfrac{K_h^2 x_1 y_1}{1-K_Y y_1}\right) \end{cases} \tag{F2}$$

Expression for experimental observable:

$$\begin{aligned} \xi_X^{(m:n)} = \xi_X^{(m)} &- \left(\xi_X^{(m)}-\xi_X^{(d)}\right)K_X x_1^2\left(\begin{aligned}&\left(\frac{1-K_Y y_1+K_h y_1}{(1-K_X x_1)(1-K_Y y_1)-K_h^2 x_1 y_1}\right)^2+\frac{1-K_Y^2 y_1^2+K_h^2 y_1^2}{(1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2}\\ &+2K_X x_1\frac{K_h^2 x_1 y_1(1-K_Y^2 y_1^2+K_h^2 y_1^2)}{((1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2)^2}\left(1+K_Y y_1+\frac{K_h^2 x_1 y_1}{1-K_X x_1}\right)\end{aligned}\right)\frac{1}{x_0} \\ &- \left(\xi_X^{(m)}-\xi_{XY}^{(h)}\right)K_h x_1 y_1\left(\begin{aligned}&\frac{(1-K_X x_1+K_h x_1)(1-K_Y y_1+K_h y_1)}{((1-K_X x_1)(1-K_Y y_1)-K_h^2 x_1 y_1)^2}\\ &+\frac{K_h x_1(1+K_Y y_1)\left((1-K_X^2 x_1^2)(1-K_Y^2 y_1^2+2K_h^2 y_1^2)+K_h^4 x_1^2 y_1^2\right)}{((1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2)^2}\\ &+\frac{K_h y_1(1+K_X x_1)\left((1-K_Y^2 y_1^2)(1-K_X^2 x_1^2+2K_h^2 x_1^2)+K_h^4 x_1^2 y_1^2\right)}{((1-K_X^2 x_1^2)(1-K_Y^2 y_1^2)-K_h^4 x_1^2 y_1^2)^2}\end{aligned}\right)\frac{1}{x_0} \end{aligned} \tag{F3}$$

G. 'm:n' hetero-association model with restriction to the number of hetero-interfaces

Partition function:

$$\Xi^{(m:n)} = \frac{x_1}{1-K_X x_1} + \frac{y_1}{1-K_Y y_1} + \frac{K_h x_1 y_1}{(1-K_X x_1)(1-K_Y y_1)}\left(1 + \frac{1}{2}\frac{K_h x_1}{1-K_X x_1} + \frac{1}{2}\frac{K_h y_1}{1-K_Y y_1}\right) \tag{G1}$$

Mass conservation law:

$$\begin{cases} x_0 = \dfrac{x_1}{(1-K_X x_1)^2}\left(1 + \dfrac{K_h y_1}{1-K_Y y_1} + \dfrac{1}{2}\dfrac{K_h^2 y_1^2}{(1-K_Y y_1)^2} + \dfrac{K_h^2 x_1 y_1}{(1-K_X x_1)(1-K_Y y_1)}\right) \\ y_0 = \dfrac{y_1}{(1-K_Y y_1)^2}\left(1 + \dfrac{K_h x_1}{1-K_X x_1} + \dfrac{1}{2}\dfrac{K_h^2 x_1^2}{(1-K_X x_1)^2} + \dfrac{K_h^2 x_1 y_1}{(1-K_X x_1)(1-K_Y y_1)}\right) \end{cases} \tag{G2}$$

Expression for experimental observable:

$$\xi_X^{(m:n)} = \frac{x_1}{x_0}\left(\xi_X^{(m)}\left(2(1+K_X x_1) - \frac{1}{(1-K_X x_1)^2}\right) + 2\xi_X^{(d)}\left(\frac{1}{(1-K_X x_1)^2} - 1 - K_X x_1\right) + \xi_{XY}^{(h)}\frac{K_h y_1}{(1-K_X x_1)^2(1-K_Y y_1)}\left(1 + \frac{K_h y_1}{2(1-K_Y y_1)} + \frac{K_h x_1}{1-K_X x_1}\right)\right) \tag{G3}$$

$$\xi_X^{(m:n)} = \xi_X^{(m)} - 2\left(\xi_X^{(m)} - \xi_X^{(d)}\right)K_X x_1 - \left(\xi_X^{(m)} - \xi_{XY}^{(h)}\right)\frac{K_h x_1 y_1}{(1-K_X x_1)(1-K_Y y_1)}\left(1 + \frac{K_h x_1}{1-K_X x_1} + \frac{K_h y_1}{1-K_Y y_1}\right)\frac{1}{x_0} \tag{G4}$$

H. '1:m:n' hetero-association model

Partition function:

$$\Xi^{(1:m:n)} = x_1\left(\frac{K_{XY} y_1(1-K_Z z_1+K_{YZ} z_1) + K_{XZ} z_1(1-K_Y y_1+K_{YZ} y_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1} + 1\right)^2 + \frac{y_1(1-K_Z z_1+K_{YZ} z_1) + z_1(1-K_Y y_1+K_{YZ} y_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1} \tag{H1}$$

Mass conservation law:

$$\begin{cases} x_0 = x_1\left(\dfrac{K_{XY} y_1(1-K_Z z_1+K_{YZ} z_1) + K_{XZ} z_1(1-K_Y y_1+K_{YZ} y_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1} + 1\right)^2 \\ y_0 = y_1\left(2x_1\dfrac{K_{XY}(1-K_Z z_1) + K_{XZ}K_{YZ} z_1}{1-K_Z z_1+K_{YZ} z_1}\left(\dfrac{K_{XY} y_1(1-K_Z z_1+K_{YZ} z_1) + K_{XZ} z_1(1-K_Y y_1+K_{YZ} y_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1} + 1\right) + 1\right)\left(\dfrac{1-K_Z z_1+K_{YZ} z_1}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1}\right)^2 \\ z_0 = z_1\left(2x_1\dfrac{K_{XZ}(1-K_Y y_1) + K_{XY}K_{YZ} y_1}{1-K_Y y_1+K_{YZ} y_1}\left(\dfrac{K_{XY} y_1(1-K_Z z_1+K_{YZ} z_1) + K_{XZ} z_1(1-K_Y y_1+K_{YZ} y_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1} + 1\right) + 1\right)\left(\dfrac{1-K_Y y_1+K_{YZ} y_1}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1}\right)^2 \end{cases} \tag{H2}$$

Expression for experimental observable:

$$\begin{aligned} \xi_X^{(1:m:n)} = \xi_X^{(m)} &- 2\left(\xi_X^{(m)} - \xi_{XY}^{(h)}\right)\left(\frac{K_{XY} y_1(1-K_Z z_1+K_{YZ} z_1) + K_{XZ} z_1(1-K_Y y_1+K_{YZ} y_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1} + 1\right)\frac{K_{XY} x_1 y_1(1-K_Z z_1+K_{YZ} z_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1}\frac{1}{x_0} \\ &- 2\left(\xi_X^{(m)} - \xi_{XZ}^{(h)}\right)\left(\frac{K_{XY} y_1(1-K_Z z_1+K_{YZ} z_1) + K_{XZ} z_1(1-K_Y y_1+K_{YZ} y_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1} + 1\right)\frac{K_{XZ} x_1 z_1(1-K_Y y_1+K_{YZ} y_1)}{(1-K_Y y_1)(1-K_Z z_1) - K_{YZ}^2 y_1 z_1}\frac{1}{x_0} \end{aligned} \tag{H3}$$

I. '1:m:n' hetero-association model accounting for 'reflected complexes'

Partition function:

$$\Xi_{\mathrm{corr}}^{(1:m:n)} = \frac{1}{2}\left(x_1 + x_1\left(\frac{K_{XY}y_1(1-K_Zz_1+K_{YZ}z_1)+K_{XZ}z_1(1-K_Yy_1+K_{YZ}y_1)}{(1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1}+1\right)^2 + \frac{y_1(1-K_Zz_1+K_{YZ}z_1)+z_1(1-K_Yy_1+K_{YZ}y_1)}{(1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1} + \frac{y_1(1+K_Yy_1+K_{XY}^2x_1y_1)(1-K_Z^2z_1^2+K_{YZ}^2z_1^2)+z_1(1+K_Zz_1+K_{XZ}^2x_1z_1)(1-K_Y^2y_1^2+K_{YZ}^2y_1^2)}{(1-K_Y^2y_1^2)(1-K_Z^2z_1^2)-K_{YZ}^4y_1^2z_1^2}\right) \tag{I1}$$

Mass conservation law:

$$\begin{cases} x_0 = \dfrac{x_1}{2}\left(\left(\dfrac{K_{XY}y_1(1-K_Zz_1+K_{YZ}z_1)+K_{XZ}z_1(1-K_Yy_1+K_{YZ}y_1)}{(1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1}+1\right)^2 + \dfrac{K_{XY}^2y_1^2(1-K_Z^2z_1^2+K_{YZ}^2z_1^2)+K_{XZ}^2z_1^2(1-K_Y^2y_1^2+K_{YZ}^2y_1^2)}{(1-K_Y^2y_1^2)(1-K_Z^2z_1^2)-K_{YZ}^4y_1^2z_1^2}+1\right) \\ y_0 = \dfrac{y_1}{2}\left(\dfrac{2(K_{XY}y_1(1-K_Zz_1+K_{YZ}z_1)+K_{XZ}z_1(1-K_Yy_1+K_{YZ}y_1))(K_{XY}x_1(1-K_Zz_1)+K_{XZ}K_{YZ}x_1z_1)(1-K_Zz_1+K_{YZ}z_1)}{((1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1)^3} + \dfrac{((1+2K_{XY}x_1)(1-K_Zz_1)+K_{YZ}z_1(1+2K_{XZ}x_1))(1-K_Zz_1+K_{YZ}z_1)}{((1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1)^2} + \dfrac{1+K_Yy_1}{1-K_Yy_1}\dfrac{1-K_Z^2z_1^2+K_{YZ}^2z_1^2}{(1-K_Y^2y_1^2)(1-K_Z^2z_1^2)-K_{YZ}^4y_1^2z_1^2}\right) \\ \quad + \dfrac{y_1^2(1-K_Z^2z_1^2+K_{YZ}^2z_1^2)}{((1-K_Y^2y_1^2)(1-K_Z^2z_1^2)-K_{YZ}^4y_1^2z_1^2))^2}\left(K_{XY}^2x_1(1-K_Z^2z_1^2)+K_{XZ}^2K_{YZ}^2x_1z_1^2+K_{YZ}^2z_1(1+K_Zz_1)+\dfrac{K_{YZ}^4y_1z_1^2}{1-K_Yy_1}\right) \\ z_0 = \dfrac{z_1}{2}\left(\dfrac{2(K_{XY}y_1(1-K_Zz_1+K_{YZ}z_1)+K_{XZ}z_1(1-K_Yy_1+K_{YZ}y_1))(K_{XZ}x_1(1-K_Yy_1)+K_{XY}K_{YZ}x_1y_1)(1-K_Yy_1+K_{YZ}y_1)}{((1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1)^3} + \dfrac{((1+2K_{XZ}x_1)(1-K_Yy_1)+K_{YZ}y_1(1+2K_{XY}x_1))(1-K_Yy_1+K_{YZ}y_1)}{((1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1)^2} + \dfrac{1+K_Zz_1}{1-K_Zz_1}\dfrac{1-K_Y^2y_1^2+K_{YZ}^2y_1^2}{(1-K_Y^2y_1^2)(1-K_Z^2z_1^2)-K_{YZ}^4y_1^2z_1^2}\right) \\ \quad + \dfrac{z_1^2(1-K_Y^2y_1^2+K_{YZ}^2y_1^2)}{((1-K_Y^2y_1^2)(1-K_Z^2z_1^2)-K_{YZ}^4y_1^2z_1^2))^2}\left(K_{XZ}^2x_1(1-K_Y^2y_1^2)+K_{XY}^2K_{YZ}^2x_1y_1^2+K_{YZ}^2y_1(1+K_Yy_1)+\dfrac{K_{YZ}^4y_1^2z_1}{1-K_Zz_1}\right) \end{cases} \tag{I2}$$

Expression for experimental observable:

$$\begin{aligned} \xi_X^{(1:m:n)} = \xi_X^{(m)} &- \left(\xi_X^{(m)}-\xi_{XY}^{(h)}\right)K_{XY}x_1y_1\left(\left(\frac{K_{XY}y_1(1-K_Zz_1+K_{YZ}z_1)+K_{XZ}z_1(1-K_Yy_1+K_{YZ}y_1)}{(1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1}+1\right)\frac{1-K_Zz_1+K_{YZ}z_1}{(1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1} + \frac{K_{XY}y_1(1-K_Z^2z_1^2+K_{YZ}^2z_1^2)}{(1-K_Y^2y_1^2)(1-K_Z^2z_1^2)-K_{YZ}^4y_1^2z_1^2}\right)\frac{1}{x_0} \\ &- \left(\xi_X^{(m)}-\xi_{XZ}^{(h)}\right)K_{XZ}x_1z_1\left(\left(\frac{K_{XY}y_1(1-K_Zz_1+K_{YZ}z_1)+K_{XZ}z_1(1-K_Yy_1+K_{YZ}y_1)}{(1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1}+1\right)\frac{1-K_Yy_1+K_{YZ}y_1}{(1-K_Yy_1)(1-K_Zz_1)-K_{YZ}^2y_1z_1} + \frac{K_{XZ}z_1(1-K_Y^2y_1^2+K_{YZ}^2y_1^2)}{(1-K_Y^2y_1^2)(1-K_Z^2z_1^2)-K_{YZ}^4y_1^2z_1^2}\right)\frac{1}{x_0} \end{aligned} \tag{I3}$$

J. MATLAB code for the case of '1:n' model of hetero-association

The code was designed to fit spectrophotometric titration curves for the case of '1:n' model of hetero-association and was tested by means of MATLAB software (version 9.2, MathWorks Inc.).

```
function [p, ps] = het_1n
str = uigetfile('*.dat','File Selector');
DE = load(str); X0 = 0.0126; Em = DE(size(DE,1),2)/X0;
switch str
    case 'pf-caf.dat'
        Ky = 0.012; Kh0 = 0.14; Eh0 = 22;
    case 'pf-nmd.dat'
        Ky = 0.001; Kh0 = 0.03; Eh0 = 25;
end
[p,d] = lsqcurvefit(@(x,xdata)obsparam(x,xdata,Ky,X0,Em),[Kh0;Eh0],DE(:,1),DE(:,2));
c = 0:0.1:DE(1,1); r = obsparam(p,c,Ky,X0,Em);
plot(DE(:,1),DE(:,2),'.r',c,r,'b');
p = [p; 1-d/sum((DE(:,2)-mean(DE(:,2))).^2)]; ps = [c.' r];

function F = obsparam(x,xdata,Ky,X0,Em)
Kh = x(1); Eh = x(2); c = zeros(length(xdata),2);
for i = 1:length(xdata)
    c(i,:) = fsolve(@(c)mcl(c,xdata(i),Kh,Ky,X0),[0.01 0.01],optimset('Display','off'));
end
X = c(:,1); Y = c(:,2);
Txy = Kh*X.*Y./(1-Ky*Y).*(1+2*Kh*Y./(1-Ky^2*Y.^2));
F = Em*X0-(Em-Eh)*Txy;

function y = mcl(c,Yi,Kh,Ky,X0)
X  = c(1); Y  = c(2);
y(1) = X*(1+Kh*Y/(1-Ky*Y)+Kh^2*Y^2/(1-Ky*Y)^2/(1+Ky*Y))-X0;
y(2) = Y/(1-Ky*Y)^2*(1+Kh*X+Kh^2*X*Y/(1-Ky*Y)+Kh^2*X*Y/(1+Ky*Y)^2)-Yi;
```

`pf-caf.dat` contains the following data

CAF concentration, mM	**Absorbance, a.u.**
60.000000	0.335884
42.000000	0.341133
29.400000	0.348753
20.580000	0.356714
14.406000	0.365886
10.084000	0.376255
8.0673600	0.382377
6.0505200	0.390826
3.9328380	0.402018
1.9664190	0.416763
0.9832095	0.425175
0.7865676	0.427154
0.5505973	0.429362
0.0000000	0.437506

`pf-nmd.dat` contains the following data

NMD concentration, mM	**Absorbance, a.u.**
80.000000	0.418656
60.000000	0.419901
42.000000	0.423586
29.400000	0.426195
20.580000	0.428955
14.406000	0.430713
10.084000	0.432000
8.0673600	0.432814
6.0505200	0.433334
3.9328380	0.434392
1.9664190	0.435408
0.9832095	0.435953
0.7865676	0.435865
0.5505973	0.435900
0.0000000	0.436921

K. MATLAB code for the case of '1:m:n' model of hetero-association

The code was designed to fit spectrophotometric titration curves for the case of '1:m:n' model of hetero-association and was tested by means of MATLAB software (version 9.2, MathWorks Inc.).

```
function [p, ps] = het_1mn
DE = load('pf-nmd-caf.dat');
X0 = 0.0126; Em = DE(size(DE,1),2)/X0; Y0 = 10; Ky = 0.001; Kz = 0.012;
K0 = 0.007; Khy = 0.0316; Khz = 0.1399; Ehy = 33.7101; Ehz = 30.2462;
[p,d] = lsqcurvefit(@(x,xdata)obsparam(x,xdata,Ky,Kz,X0,Y0,Em,Ehy,Ehz),[K0;Khy;Khz;Ehy;Ehz],DE(:,1),DE(:,2));
c = 0:0.1:DE(1,1); r = obsparam(p,c,Ky,Kz,X0,Y0,Em,Ehy,Ehz);
plot(DE(:,1),DE(:,2),'.r',c,r,'b');
p = [p; 1-d/sum((DE(:,2)-mean(DE(:,2))).^2)]; ps = [c.' r];

function F = obsparam(x,xdata,Ky,Kz,X0,Y0,Em,Ehy,Ehz)
K0 = x(1); Khy = x(2); Khz = x(3); %Ehy = x(4); Ehz = x(5);
c = zeros(length(xdata),3);
for i = 1:length(xdata)
    c(i,:) = fsolve(@(c)mcl(c,xdata(i),K0,Ky,Kz,X0,Y0,Khy,Khz),[0.01 0.01 xdata(i)],optimset('Display','off'));
end
X = c(:,1); Y = c(:,2); Z = c(:,3);
B = Y./(1-Ky.*Y); Bs = Y.^2./(1-Ky^2*Y.^2);
C = Z./(1-Kz.*Z); Cs = Z.^2./(1-Kz^2*Z.^2);
Txy = Khy*X.*(((Khy*B.*(1+K0*C)+Khz*C.*(1+K0*B))./(1-K0^2*B.*C)+1).*...
      B.*(1+K0*C)./(1-K0^2*B.*C)+Khy*Bs.*(1+K0^2*Cs)./(1-K0^4*Bs.*Cs));
Txz = Khz*X.*(((Khy*B.*(1+K0*C)+Khz*C.*(1+K0*B))./(1-K0^2*B.*C)+1).*...
      C.*(1+K0*B)./(1-K0^2*B.*C)+Khz*Cs.*(1+K0^2*Bs)./(1-K0^4*Bs.*Cs));
F = Em*X0-(Em-Ehy)*Txy-(Em-Ehz)*Txz;

function y = mcl(c,Zi,K0,Ky,Kz,X0,Y0,Khy,Khz)
X = c(1); Y = c(2); Z = c(3);
B = Y/(1-Ky*Y); Bs = Y^2/(1-Ky^2*Y^2);
C = Z/(1-Kz*Z); Cs = Z^2/(1-Kz^2*Z^2);
y(1) = X/2*(((Khy*B*(1+K0*C)+Khz*C*(1+K0*B))/(1-K0^2*B*C)+1)^2+...
       (Khy^2*Bs*(1+K0^2*Cs)+Khz^2*Cs*(1+K0^2*Bs))/(1-K0^4*Bs*Cs)+1)-X0;
y(2) = Y/(1-Ky*Y)^2/2*(2*X*(Khy*B*(1+K0*C)+Khz*C*(1+K0*B))*(Khy+Khz*K0*C)*(1+K0*C)/(1-K0^2*B*C)^3+...
       (1+K0*C+2*X*(Khy+Khz*K0*C))*(1+K0*C)/(1-K0^2*B*C)^2+(1+K0^2*Cs)/(1-K0^4*Bs*Cs))+(Y/(1-Ky^2*Y^2))^2*...
       (X*(Khy^2+Khz^2*K0^2*Cs)+K0^2*(C+K0^2*B*Cs))*(1+K0^2*Cs)/(1-K0^4*Bs*Cs)^2-Y0;
y(3) = Z/(1-Kz*Z)^2/2*(2*X*(Khy*B*(1+K0*C)+Khz*C*(1+K0*B))*(Khz+Khy*K0*B)*(1+K0*B)/(1-K0^2*B*C)^3+...
       (1+K0*B+2*X*(Khz+Khy*K0*B))*(1+K0*B)/(1-K0^2*B*C)^2+(1+K0^2*Bs)/(1-K0^4*Bs*Cs))+(Z/(1-Kz^2*Z^2))^2*...
       (X*(Khz^2+Khy^2*K0^2*Bs)+K0^2*(B+K0^2*Bs*C))*(1+K0^2*Bs)/(1-K0^4*Bs*Cs)^2-Zi;
```

`pf-nmd-caf.dat` contains the following data

CAF concentration, mM	**Absorbance, a.u.**
30.000000	0.353788
21.000000	0.360417
14.700000	0.368995
10.290000	0.378697
7.2030000	0.388950
5.0421000	0.398328
4.0336800	0.404413
3.0252600	0.411276
1.9664190	0.418559
0.9832095	0.428156
0.4916048	0.432835
0.3932838	0.433544
0.2752987	0.435273
0.0000000	0.437428