Supporting information

Avidin and streptavidin ligands based on the glycoluril bicyclic system

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a. Binding model and equations for a competitive titration with an enantiomerically pure ligand (single ligand).

\[ S-I + L \rightleftharpoons S-L + I \]

\[ [I] = \frac{I_t}{1 + K_{IS} [S]} \quad [IS] = K_{IS} [S] [I] \]

\[ [L] = \frac{L_t}{1 + K_{LS} [S]} \quad [LS] = K_{LS} [L] [S] \]

\[ [S] = \frac{S_t}{1 + K_{IS} [I] + K_{LS} [L]} \]

Where

\( I_t \) : Total concentration of indicator (HABA)
\( S_t \) : Total concentration of protein subunits
\( L_t \) : Total concentration of ligand

\( [I] \) : Concentration of free indicator (HABA)
\( [S] \) : Concentration of free protein subunits
\( [L] \) : Concentration of free ligand

\( [IS] \) : Concentration of complexed indicator
\( [LS] \) : Concentration of complexed ligand

\( K_{IS} \) : Binding constant of the indicator
\( K_{LS} \) : Binding constant of the ligand
b. Binding model and equations for a competitive titration with a racemic ligand (or a mixture of two ligands).

\[
\begin{align*}
S - I + L_1 & \rightleftharpoons S - L_1 + I \\
S - I + L_2 & \rightleftharpoons S - L_2 + I
\end{align*}
\]

Where \( I_t, S_t, [S], [IS] \) and \( K_{IS} \) are as in part b of the supporting information, and

\[
\begin{align*}
[I] &= \frac{I_t}{1 + K_{IS} \times [S]} \\
[IS] &= K_{IS} \times [S] \times [I] \\
[L_1] &= \frac{L_{1t}}{1 + K_{L_1S} \times [S]} \\
[L_1S] &= K_{L_1S} \times [S] \times [L_1] \\
[L_2] &= \frac{L_{2t}}{1 + K_{L_2S} \times [S]} \\
[L_2S] &= K_{L_2S} \times [S] \times [L_2] \\
[S] &= \frac{S_t}{1 + K_{IS} \times [I] + K_{L_1S} \times [L_1] + K_{L_2S} \times [L_2]}
\end{align*}
\]

\( L_{1t} = \) Total concentration of ligand 1 (or enantiomer 1)
\( L_{2t} = \) Total concentration of ligand 2 (or enantiomer 2)

\([L_1S] = \) Concentration of complexed ligand 1
\([L_2S] = \) Concentration of complexed ligand 2

\( K_{L_1S} = \) Binding constant of ligand 1
\( K_{L_2S} = \) Binding constant of ligand 2
c. Binding isotherms form the competitive spectrophotometric titrations of the (S)Av-HABA complex with glycoluril-type ligands.

Figure 1. Absorption change at 500 nm in the titration of Av 5.7 µM (tetramer) and HABA 49.4 µM with ligand 2a in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.

Figure 2. Absorption change at 500 nm in the titration of Sav 10.6 µM (tetramer) and HABA 35.7 µM with ligand 2a in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.
Figure 3. Absorption change at 500 nm in the titration of Sav 11.2 µM (tetramer) and HABA 38.5 µM with ligand 2b in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.

Figure 4. Absorption change at 500 nm in the titration of Av 9.3 µM (tetramer) and HABA 43.6 µM with ligand (-)-2c in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.

Figure 5. Absorption change at 500 nm in the titration of Av 8.7 µM (tetramer) and HABA 37.5 µM with ligand (+)-2c in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.
Figure 6. Absorption change at 500 nm in the titration of Av 12.5 μM (tetramer) and HABA 31.5 μM with racemic 2c in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model considering different binding constants for each enantiomer.

Figure 7. Absorption change at 500 nm in the titration of Av 9.3 μM (tetramer) and HABA 38.3 μM with racemic 2d in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model considering different binding constants for each enantiomer.