Supporting information

Avidin and streptavidin ligands based on the glycoluril bicyclic system

Pedro Hidalgo-Fernández, Eva Ayet, Ivan Canal, and Joan-Antoni Farrera*

Departament de Quimica Organica, Facultat de Química,Universitat de Barcelona, Martí i Franqués 1, 08028-Barcelona, Spain. E-mail: jfarrera@ub.edu;

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

$$[I] = \frac{I_t}{1 + K_{IS} \times [S]} \qquad [IS] = K_{IS} \times [S] \times [I]$$

$$[L] = \frac{L_t}{1 + K_{LS} \times [S]} \qquad [LS] = K_{LS} \times [L] \times [S]$$

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

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).

$$S\text{-}I + L_1 \rightleftharpoons S\text{-}L_1 + I$$

$$S\text{-}I + L_2 \rightleftharpoons S\text{-}L_2 + I$$

$$[I] = \frac{I_t}{1 + K_{IS} \times [S]} \qquad [IS] = K_{IS} \times [S] \times [I]$$

$$[L_1] = \frac{L_{1t}}{1 + K_{L_1S} \times [S]} \qquad [L_1S] = K_{L_1S} \times [S] \times [L_1]$$

$$[L_2] = \frac{L_{2t}}{1 + K_{L_2S} \times [S]} \qquad [L_2S] = K_{L_2S} \times [S] \times [L_2]$$

$$[S] = \frac{S_t}{1 + K_{IS} \times [I] + K_{L_1S} \times [L_1] + K_{L_2S} \times [L_2]}$$

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

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_{L1S} = Binding constant of ligand 1

K_{L2S} = Binding constant of ligand 2

c. Binding isotherms form the competitive spectrophotometric titrations of the (S)Av-HABA complex with glycoluril-type ligands.

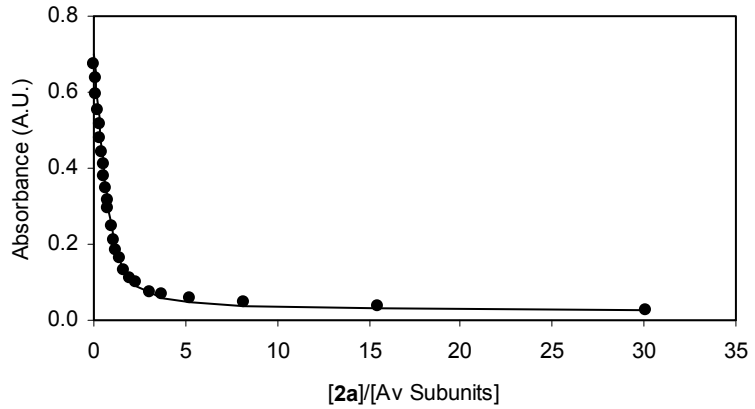

Figure 1. Absorption change at 500 nm in the titration of Av 5.7 μM (tetramer) and HABA 49.4 μM with ligand **2a** in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.

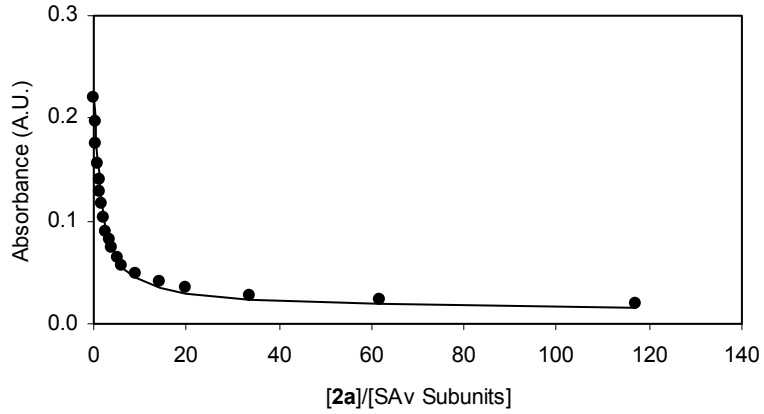

Figure 2. Absorption change at 500 nm in the titration of Sav 10.6 μM (tetramer) and HABA 35.7 μM with ligand **2a** in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.

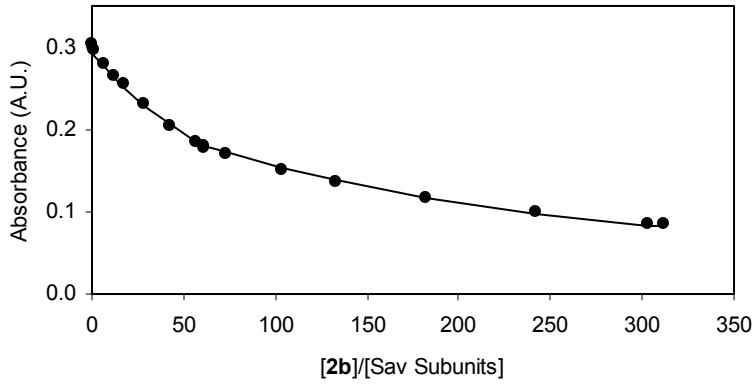

Figure 3. Absorption change at 500 nm in the titration of Sav 11.2 μM (tetramer) and HABA 38.5 μM with ligand **2b** in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.

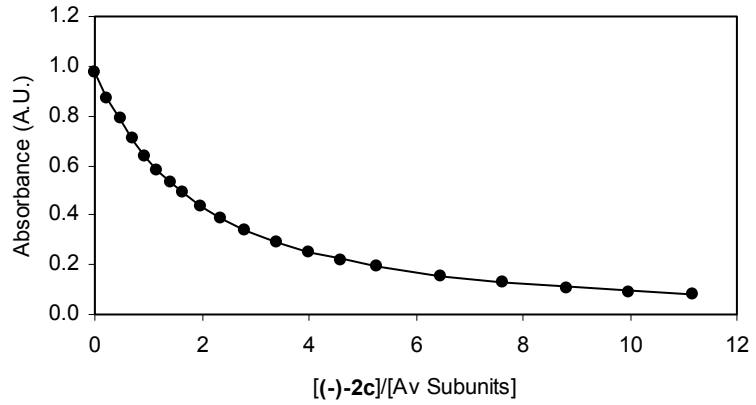

Figure 4. Absorption change at 500 nm in the titration of Av 9.3 μM (tetramer) and HABA 43.6 μM with ligand **(-)-2c** in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.

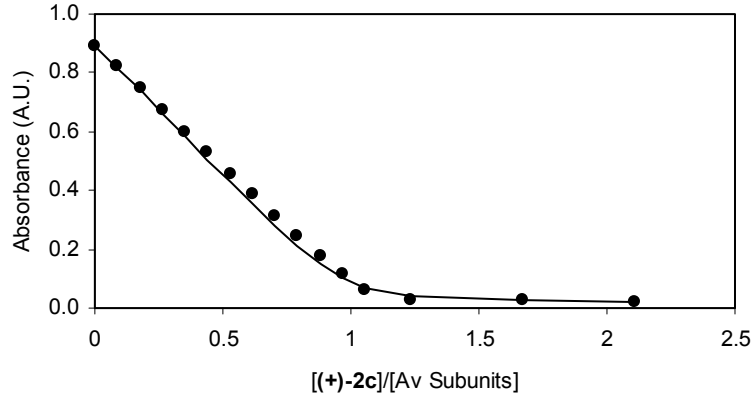

Figure 5. Absorption change at 500 nm in the titration of Av 8.7 μM (tetramer) and HABA 37.5 μM with ligand **(+)-2c** in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model.

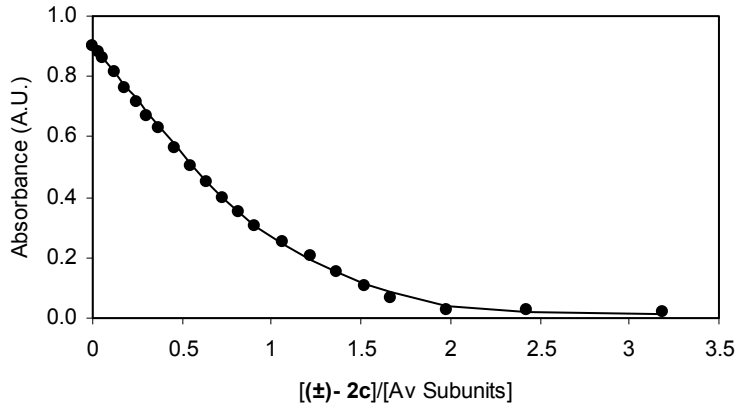

Figure 6. Absorption change at 500 nm in the titration of Av 12.5 μM (tetramer) and HABA 31.5 μM with racemic **2c** in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model considering different binding constants for each enantiomer.

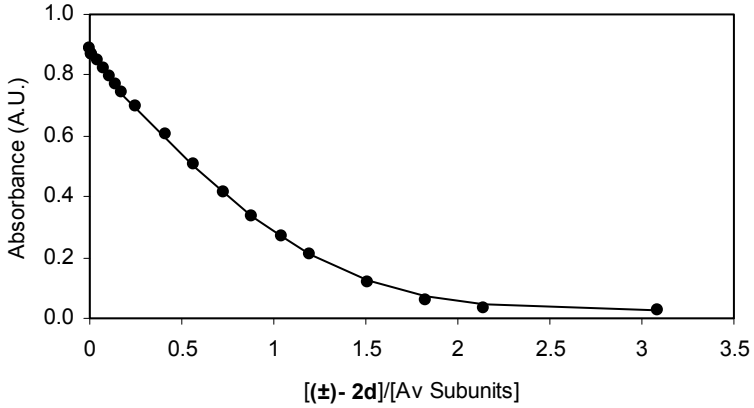

Figure 7. Absorption change at 500 nm in the titration of Av 9.3 μM (tetramer) and HABA 38.3 μM with racemic **2d** in phosphate buffer 0.1 M, pH = 7.0. The solid line represents the fit of the data to the (1:1) binding model considering different binding constants for each enantiomer.