

### T. D. n° 3 (Protein–ligand interactions)

#### Exercise n° 1 :

The binding of  $\text{NAD}^+$  to yeast glyceraldehyde-3-phosphate dehydrogenase causes the tryptophan residues of the enzyme protein to fluoresce (which corresponds to an extinction of this fluorescence). Knowing the fluorescence quenching that accompanies the binding of one mole of  $\text{NAD}^+$ , we can calculate the concentration of  $\text{NAD}^+$  bound for each experiment.

The experiment was conducted at pH 7 in the presence of enzyme ( $1\mu\text{M}$ ). Tryptophan residues were excited at 300 nm, and fluorescence was measured at 350 nm :

<b>[NAD<sup>+</sup>] total (<math>\mu\text{M}</math>)</b>	20	15	10	8	6	5	4	3	2
<b>[NAD<sup>+</sup>] bound (<math>\mu\text{M}</math>)</b>	3.70	3.50	3.30	3.05	2.70	2.50	2.15	1.80	1.30

- Calculate the number of  $\text{NAD}^+$  binding sites per enzyme molecule and the equilibrium constants (using the KLOTZ method).

#### Exercise n°2 :

$\text{Na}^+$ ,  $\text{K}^+$ -dependent ATPase can bind rubidium ions ( $\text{Rb}^+$ ). We used  $60\mu\text{g}$  of a membrane suspension containing ATPase in 1ml of buffer containing radioactive rubidium ions at different concentrations.

When the binding equilibrium was reached, each solution was centrifuged, and the concentration of rubidium ions in the supernatant and the amount of rubidium ions bound to the membranes were measured :

<b>[Rb<sup>+</sup>] in the supernatant (<math>\mu\text{M}</math>)</b>	2	4	12	30	50
<b>Rb<sup>+</sup> membrane-bound (nanomoles)</b>	0.15	0.26	0.455	0.59	0.64

1. Give a diagram showing the location of bound and free rubidium ions after centrifugation of the membrane suspension containing ATPase.
2. Determine the maximum amount of rubidium ions that can be bound to the membranes (in nanomoles per mg of protein) and the corresponding association and dissociation constants (using the KLOTZ method).

#### Exercise n° 3 :

The binding of triiodothyronine (**T3**) to prealbumin was studied by equilibrium dialysis, using T3 labeled with iodine  $\text{I}^{125}$ . The prealbumin concentration in the dialysis bag was  $0.77\mu\text{M}$ . When equilibrium was achieved, the concentrations of (**T3**) were measured inside and outside the dialysis bag :

<b>[T3] outside the dialysis bag (nM)</b>	33	76	110	120	190	265
<b>[T3] inside the dialysis bag (nM)</b>	265	460	560	600	750	880

- Determine the number of binding sites of (**T3**) on prealbumin and the association equilibrium constant using the SCATCHARD method.

#### **Exercise n° 4 :**

Verapamil is a compound that binds to calcium channels. It was used to study these channels in membranes prepared from heart tissue.

The experiments were carried out in 200  $\mu\text{l}$  of buffer, to which 200  $\mu\text{g}$  of membrane proteins and tritiated verapamil at different concentrations were added. After equilibrium was reached, the solutions were filtered. The radioactivity present on the filters was used to calculate the amount of verapamil bound to the proteins.

Based on these data and control experiments, it was possible to determine the amount of verapamil specifically bound to the proteins :

<b>[verapamil] (nM)</b>	210	100	50	30	10
<b>Verapamil bound (pmol/ assay)</b>	0,2	0,165	0,12	0,09	0,04

- Calculate the number of binding sites for verapamil per mg of protein and their dissociation constants using the KLOTZ method.

#### **Exercise n° 5 :**

The equilibrium of calcium binding to deoxyribonuclease at pH 9 was studied using the gel filtration method. This made it possible to determine the amount of  $\text{Ca}^{++}$  bound per mole of enzyme for different concentrations of free  $\text{Ca}^{++}$ . The results of these experiments are summarized in the following table :

<b>[Ca<sup>++</sup> free] (<math>\mu\text{M}</math>)</b>	500	200	100	50	20	10	5	2.5
<b>Number of Ca<sup>++</sup> ions bound per enzyme molecule</b>	4.00	3.40	3.00	2.20	1.25	0.75	0.40	0.25

- Determine the number of calcium ion ( $\text{Ca}^{++}$ ) binding sites on the deoxyribonuclease and their equilibrium constants (using the SCATCHARD method).