Model answer for T.D. no. 3
(Protein–ligand interactions)

Exercise n° 1 :

We have:

- ✓ The protein : glyceraldehyde-3-phosphate dehydrogenase with **$[P_t]$ = 1µM**
- ✓ The ligand : NAD^+
- ✓ Technique used : tryptophan residue fluorescence

$[NAD^+]$ total = $[NAD^+]$ bound + $[NAD^+]$ free so : **$[NAD^+]$ free = $[NAD^+]$ total - $[NAD^+]$bound**

The graphical representation of KLOTZ consists of plotting 1/[RL] as a function of 1/[L], thus :

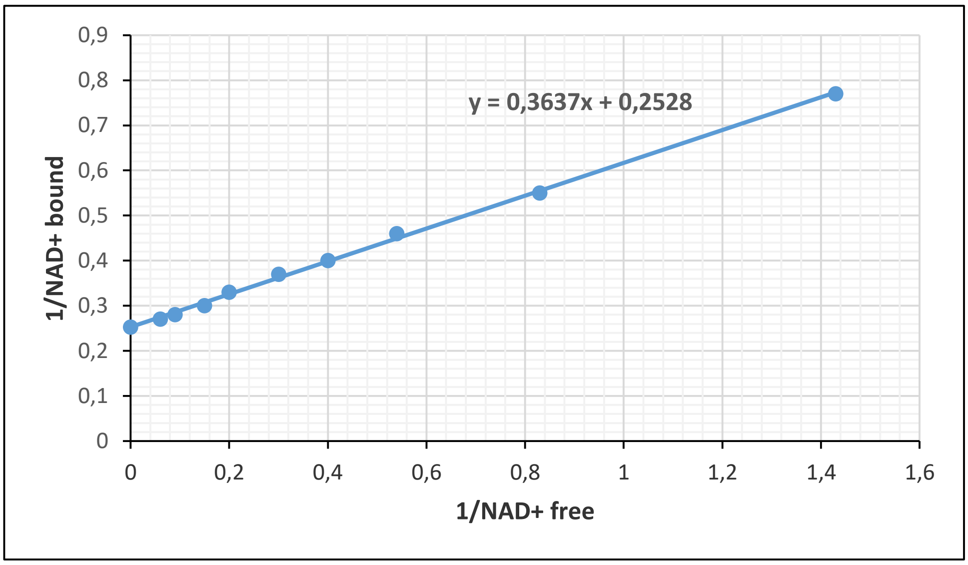
$1/[NAD^+]$ boud = f($1/[NAD^+]$ free)

$[NAD^+]$ free (µM)	16,3	11,5	6,7	4,95	3,3	2,5	1,85	1,2	0,7
$1/[NAD^+]$ bound ($µM^{-1}$)	0,27	0,28	0,30	0,33	0,37	0,40	0,46	0,55	0,77
$1/[NAD^+]$ free ($µM^{-1}$)	0,06	0,09	0,15	0,20	0,30	0,40	0,54	0,83	1,43

The KLOTZ equation : $1/[RL] = (1/[L]).(K_D/n[P_t]) + 1/n[P_t]$

So: the slope = **$K_D/n[P_t]$**

y-intercept = **$1/n[P_t]$**



Graphically :

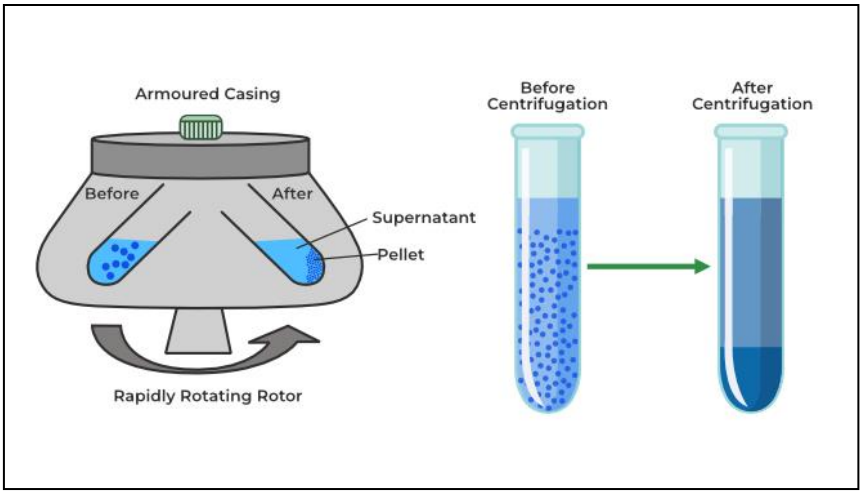
- ✓ y-intercept = 0,25 $µM^{-1}$ = $1/n[P_t]$ so : **n = 4 binding sites**
- ✓ The slope = 0,396 = $K_D/n[P_t]$ so : **K_D = 1,58 µM**
- ✓ $K_A = 1/K_D$ so : **K_A = 0,63 $µM^{-1}$**

Exercise n°2 :

We have :

- ✓ The protein : Na^+/K^+-dependent ATPase
- ✓ The ligand : rubidium ion (Rb^+)
- ✓ The technique used : centrifugation

The experiment design :



The maximum amount of Rb^+ bound to membranes (nmol/mg) :

KLOTZ's graphical representation consists of plotting 1/[RL] as a function of 1/[L] so :

1/[Rb^+] bound = f(1/[Rb^+] free)

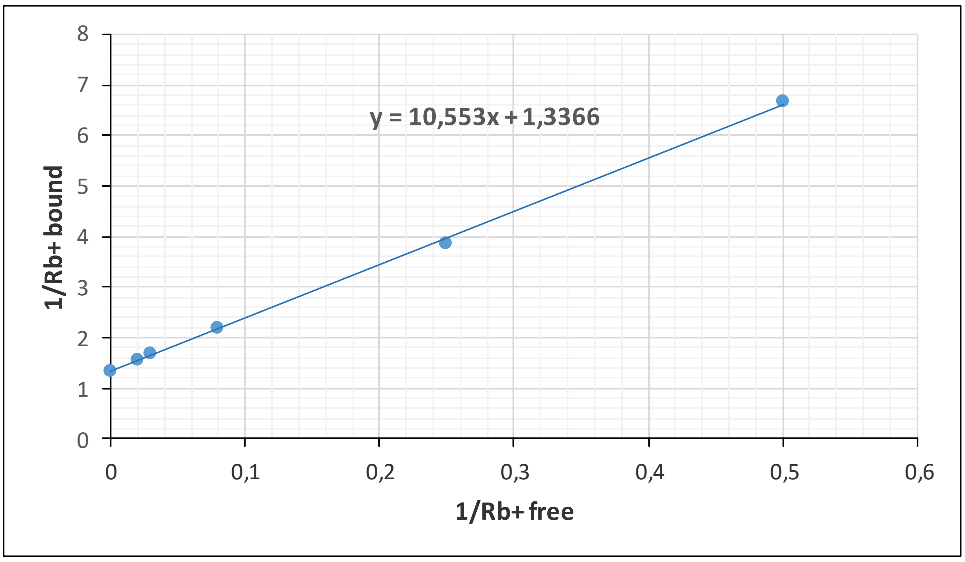
Knowing that : [Rb^+] in the supernatant = [Rb^+] free

1/[Rb^+] free (μM^{-1})	0,50	0,25	0,08	0,03	0,02
1/Rb^+ bound ($nmol^{-1}$)	6,67	3,85	2,20	1,69	1,56

The **KLOTZ** equation : $1/[RL] = (1/[L]).(K_D/n[P_t]) + 1/n[P_t]$

So : the slope = $\mathbf{K_D/n[P_t]}$

y-intercept = $\mathbf{1/n[P_t]}$



Graphically :

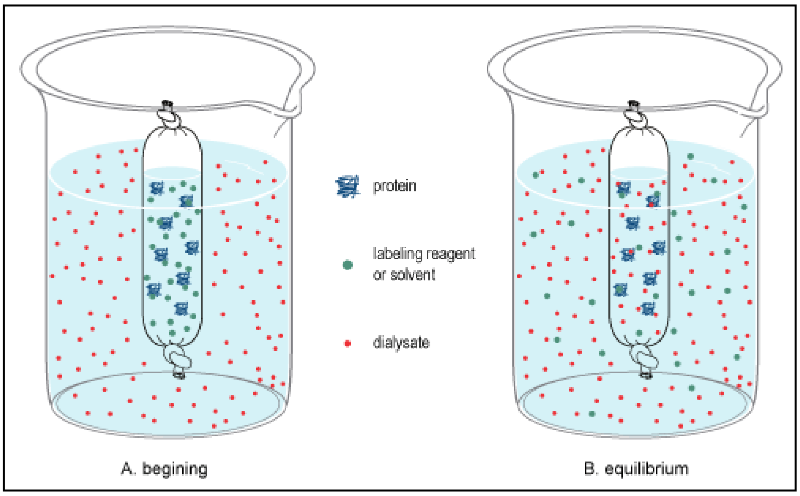
- y-intercept = 1,35 $nmol^{-1}$ = $1/n[P_t]$
- We have $[P_t]$ = 60 µg = 0,06mg
- so n = 1/1,35x0,06 from which **n = 12,3 nmol/mg protein**
- The slope = 10,5 $nmol^{-1}$.µM = $K_D/n[P_t]$ so : **K_D = 7,75 µM**
- We know that $K_A = 1/K_D$ so : **K_A = 0,13 μM^{-1}**

Exercise n° 3 :

We have :

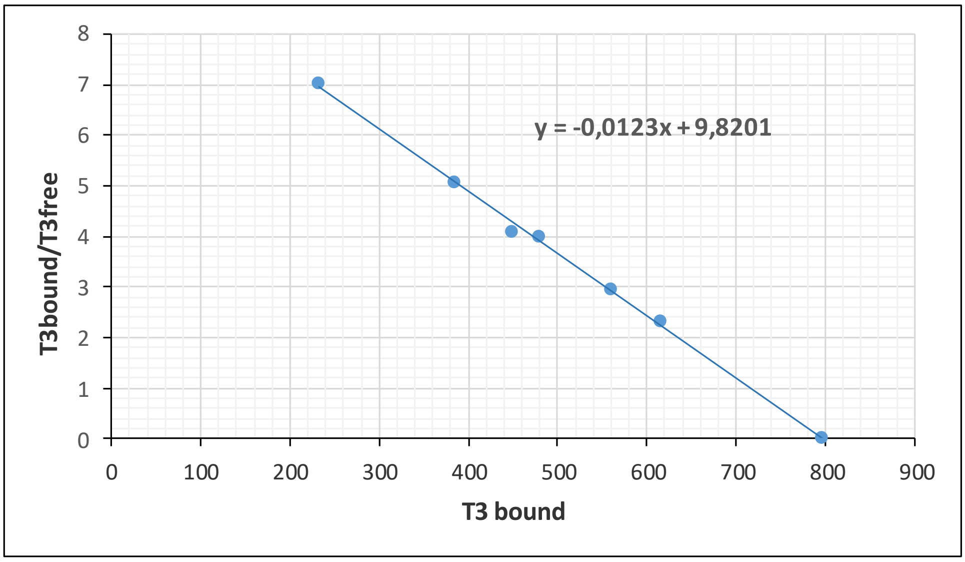
- ✓ [T3] outside the dialysis bag = **[T3] free**
- ✓ [T3] inside the dialysis bag = **[T3] bound + [T3] free**

So : **[T3] bound** = [T3] inside the dialysis bag - [T3] outside the dialysis bag



According to the SCATCHARD method : **[RL] / [L] = f([RL])**

[RL] = [T3] bound (nM)	232	384	450	480	560	615
[RL]/[L] = [T3] bound/[T3] free	7,03	5,05	4,09	4	2,95	2,32



Graphically :

- X-intercept : n [Pt] = 810nM
  Knowing that : [Pt] = 0,77µM = 770nM so : **n = 1 site**
- The slope: - K_A = - 0,012 nM^{-1} so : **K_A = 0,012 nM^{-1}**

<u>Exercise n° 4 :</u>

We have :

- The protein : calcium channels
- The ligand : verapamil
- The technique used : filtration and radioactivity

The graphical representation of **KLOTZ** consists of plotting 1/[RL] as a function of 1/[L], so :

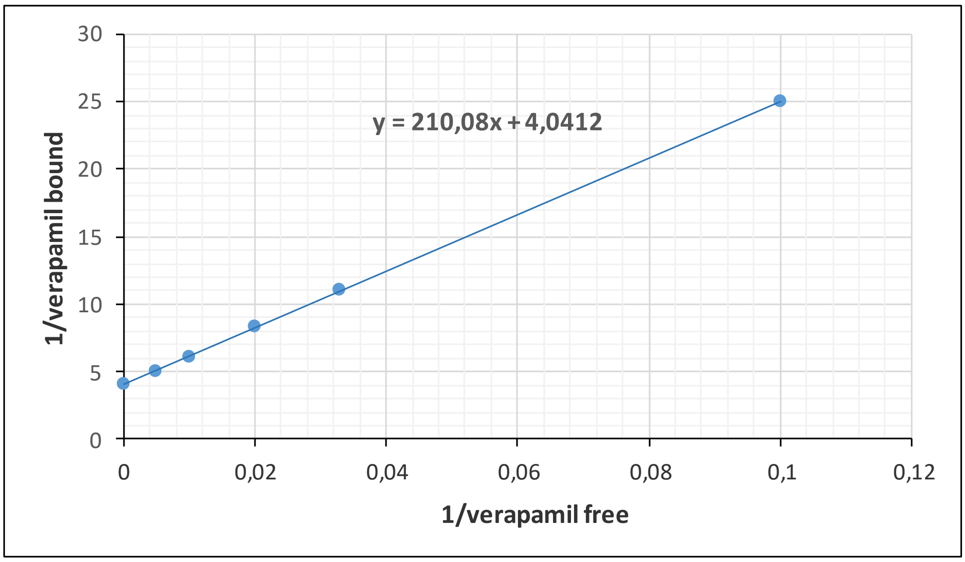
1/[verapamil] bound = f(1/[verapamil] free)

1/[verapamil]free (nM^{-1})	0,005	0,010	0,020	0,033	0,100
1/Verapamil bound ($pmol^{-1}$)	5,00	6,06	8,33	11,11	25,00

The **KLOTZ** equation : $1/[RL] = 1/[L].(K_D/n[P_t]) + 1/n[P_t]$

So : the slope = $\mathbf{K_D/n[P_t]}$

The y-intercept = $\mathbf{1/n[P_t]}$



Graphically :

- y-intercept = $1/n[P_t]$ = 3,8 $pmol^{-1}$
- we have : $[P_t]$ = 0,2 mg so **n = 1,31 pmol/mg**
- The slope = $K_D/n[P_t]$ = 200 $pmol^{-1}.nM^{-1}$ so $\mathbf{K_D}$ **= 52,4 nM**

Exercise n° 5 :

We have :

- ✓ The protein : deoxyribonuclease (DNAse)
- ✓ The ligand : calcium ion Ca^{2+}
- ✓ Technique used : gel filtration

The SCATCHARD graph plots [RL]/[L] as a function of [RL], as follows :

$$[Ca^{2+}]bound/[Ca^{2+}]free = f([Ca^{2+}]bound)$$

Note: the number of Ca^{2+} ions bound per enzyme molecule = **$[Ca^{2+}]$ bound $/[P_t]$**

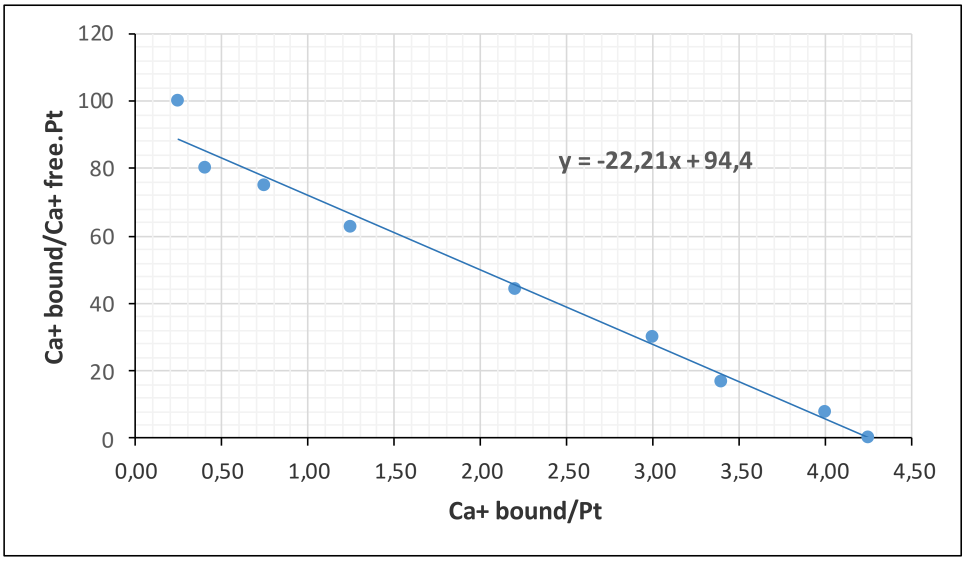
$[Ca^{2+}]bound/[P_t]$	4,00	3,40	3,00	2,20	1,25	0,75	0,40	0,25
$[Ca^{2+}]bound/[Ca^{2+}]free.[P_t]$ $(x10^{-3})$ (μM^{-1})	8	17	30	44	62,5	75	80	100

The SCATCHARD equation is written as : **$[RL]/[L] = (-1/K_D)[RL] + n[P_t]/K_D$**

Since we have **$[RL]/[P_t]$**, this equation becomes : **$[RL]/[L][P_t] = (-1/K_D)[RL]/[P_t] + n/K_D$**

From which : the slope = **$-1/K_D = -K_A$**

X-intercept = **n**



Graphically :

- X-intercept = 4,4 so **n = 4 sites**
- The slope = - 20. 10^{-3} μM^{-1} so **$K_A = 20.\ 10^{-3}\ \mu M^{-1}$**
- $K_D = 1/K_A$ so **$K_D = 50\ \mu M$**