Evaluation of dissociation constant of a protein-dna interaction by thermodynamic modelling and simulation.

Thermodynamics in biochemical
engineering
BT5031
Presented By:

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1. Introduction:

Heat is either emitted or absorbed when a protein interacts with a ligand. The measurement of this heat provides crucial understanding of an interaction's process. The only method is ITC. It quantifies this heat transfer and subsequently provides comprehensive thermodynamic details regarding the binding process. Since the active compounds frequently have comparable values for affinity, evaluating compound affinity alone may not be able to give a clear signal for compound selection and optimization throughout the drug development process. Knowing a reaction's thermodynamics, including key elements like entropy change and enthalpy change, might be useful information for lead finding and optimization decision-making.

1.1. Theoretical Background:

When two molecules like molecule A (say DNA) and molecule B (say ligand or a protein) interact to form a macromolecular complex AB, the reaction can be written as:

A+B=AB   ...(1)

The association constant K_a between the molecules A and B can be written as:

$$K_a = \frac{[AB]}{[A][B]} \qquad\qquad ...(2)$$

Where [AB] = concentration of AB,

[A] = concentration of A and [B] = concentration of B.

The dissociation constant K_d is often the inverse of Ka and is written as:

$$K_d = \frac{[A][B]}{[AB]} = \frac{1}{K_a} \qquad\qquad ...(3)$$

According to principles of thermodynamics,

The change is Gibb's free energy ΔG during the interaction of A and B can be written as:

$$\Delta G = \Delta H - T\Delta S \qquad\qquad ...(4)$$

Where ΔH = enthalpy of the association of A and B, T = absolute temperature of the reaction and ΔS = entropy of the reaction.

On the other hand, the relationship between Gibbs' free energy ΔG and the association constant K_a can be written as:

$$\Delta G =- RTlnK_a \qquad \qquad ...(5)$$

Where R = universal gas constant = 8.314 J/Kmol

From equations (4) and (5), we get

$$\Delta H - T\Delta S =- RTlnK_a \qquad \qquad ...(6)$$

We can calculate ΔH of the association between A and B from Isothermal Titration Calorimetry (ITC) and Ka from fitting of the ITC data.

Then, we can calculate ΔS of the reaction.

Also, from K_a, we can also obtain K_d as dissociation constant is the inverse of association constant.

Generally, the value of K_d is in the nanomolar/micromolar/millimolar range. A nanomolar value of K_d means strong interaction, a micromolar value means medium-strength association and a nanomolar value means weak association.

2. Principle:

2.1 One-Ligand-One-Binding Site (1:1):

For a system having a ligand 'B' binding to DNA 'A', the equation for tis binding can be written as:
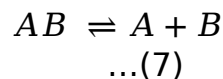
$$AB \rightleftharpoons A + B$$
...(7)

$$K_d = \frac{[A]_{free}[B]_{free}}{[AB]} = \frac{([AB]_{Total}-[AB])X([B]_{Total}-[AB])}{[AB]}$$
...(8)

$$[AB]K_d = ([AB]_{Total} - [AB])X([B]_{Total} - [AB])$$
...(9)

$$[AB]K_d = ([A]_{Total} - [B]_{Total}) - ([AB]X[A]_{Total}) - ([AB]X[B]_{Total}) + [AB]^2$$
...(10)

$$[AB]^2 - ([A]_{Total} + [B]_{Total} + K_d)X[AB] + [A]_{Total}X[B]_{Total} = 0$$
...(11)

Equation (11) is of the form of $ax^2 + bx + c = 0$, a quadratic equation

Solving for [AB], we get

$$[AB] = ([A]_{Total} + [B]_{Total} - K_d) - \frac{\sqrt{([A]_{Total}+[B]_{Total}+K_d)^2-4[A]_{Total}[B]_{Total}}}{2}$$
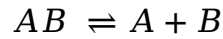...(12)

2.2 Model Used in this Project:

We have used Langmuir Model in this project.

2.2.1 Langmuir Model:

For a system having a ligand 'B' binding to DNA 'A', the equation for tis binding can be written as (from equation 7):

$$AB \rightleftharpoons A + B$$

$K_d = \frac{[A][B]}{[AB]}$ (from equation (3))

$[AB] = K_d[A][B]$ ...(13)

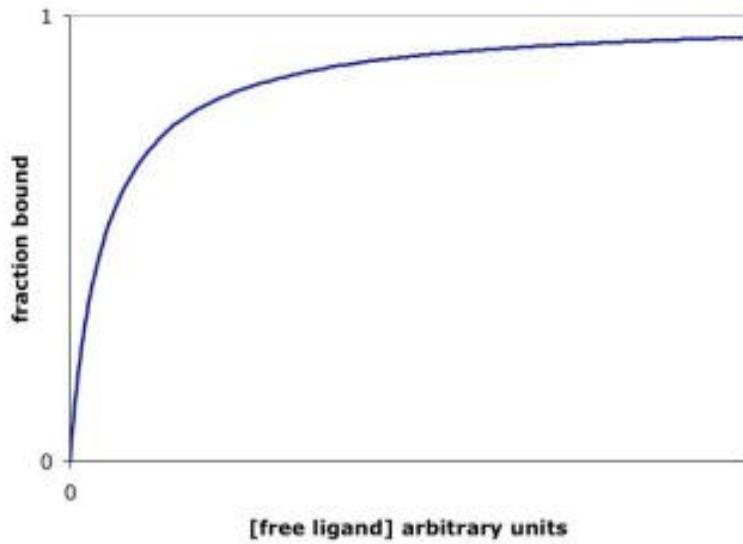Let us call the fraction of molecules bound to protein as θ.

$$\theta = \frac{[AB]}{[A]_{Total}} = \frac{[AB]}{[A]_{free}+[AB]}$$

$$\theta = \frac{K_d[A]_{free}[B]_{free}}{[A]_{free}+K_d[A]_{free}[B]_{free}}$$
...(14)

$$\theta = \frac{K_d[B]_{free}}{1+K_d[B]_{free}}$$
...(15)

The shape of the Langmuir Isotherm is shown:

2.2.2 Kd (Dissociation Constant)

To get K_d, we consider the half maximal concentration of [AB], i.e., $\theta=1/2$

$$\theta = \frac{1}{2} = \frac{K_d[B]_{free}}{1+K_d[B]_{free}} \qquad \qquad ...(16)$$

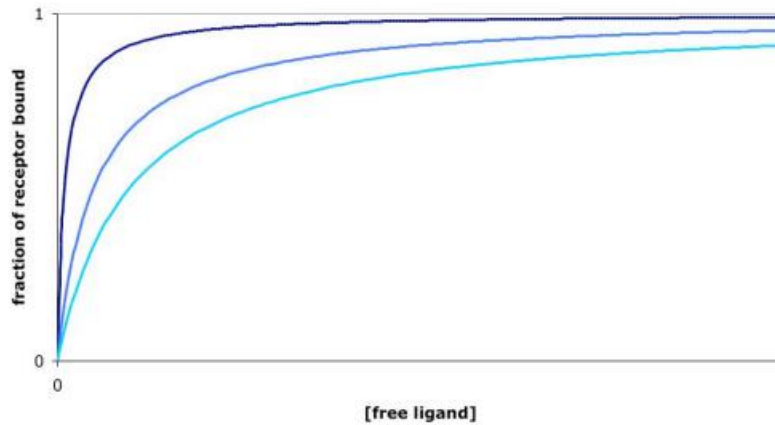Which is only possible if $K_d[B]_{free} = 1$

So, $K_d = \dfrac{1}{[B]_{free\ at\ \theta=1/2}}$

     ...(17)

Another alternative way to get K_d is from equation (15), if we assume that $K_d[B]_{free} \ll 1$

$$\theta = \frac{K_d[B]_{free}}{1+K_d[B]_{free}} = \frac{K_d[B]_{free}}{1} = K_d[B]_{free}$$

     ...(18)

From the binding curve of θ vs [B]$_{free}$, we can calculate K_d by the slope of this curve.

Below is a plot for three different isotherms:

fraction of receptor bound vs [free ligand]

The best way to calculate Kd:

From equation (15), we get
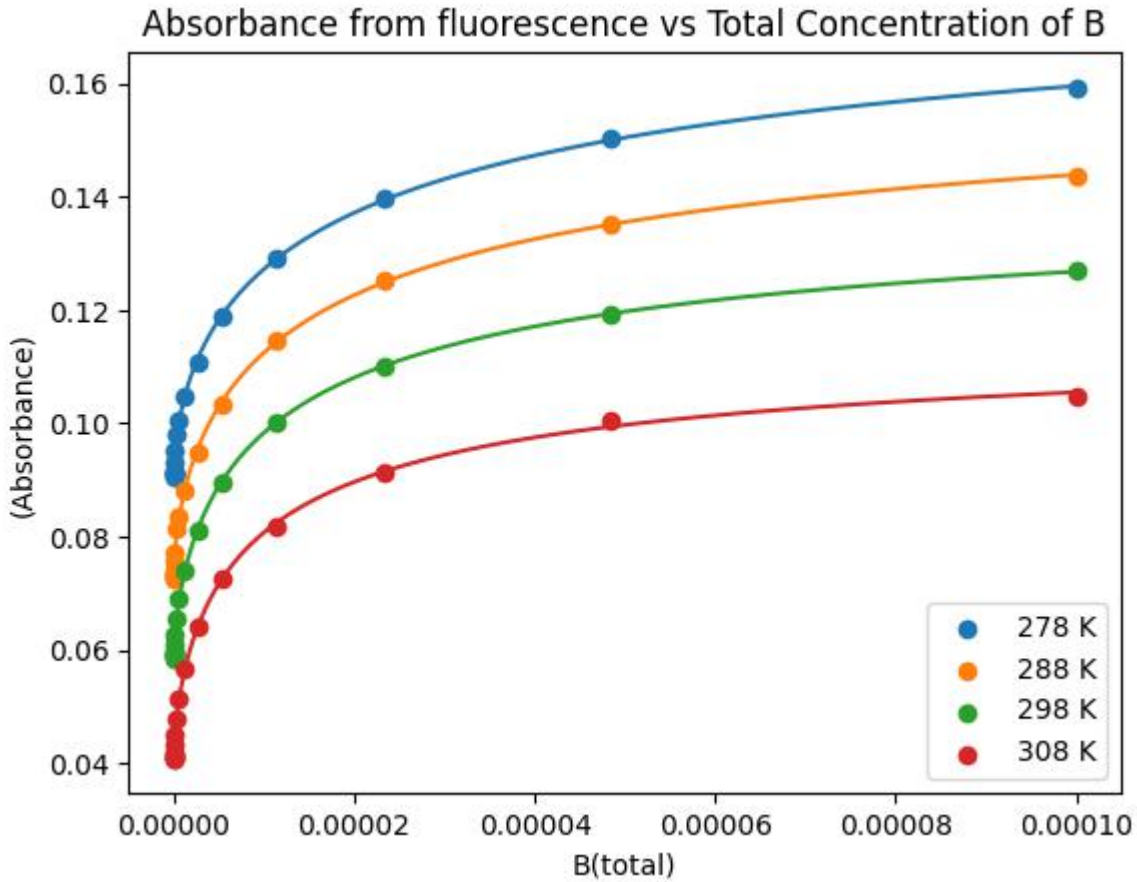
$$\theta = \frac{K_d[B]_{free}}{1+K_d[B]_{free}}$$
...(19)

$$\frac{1}{\theta} = \frac{1+K_d[B]_{free}}{K_d[B]_{free}}$$
...(20)

$$\frac{[B]_{free}}{\theta} = \frac{1}{K_d} + [B]_{free}$$
...(21)

So, if we plot $[B]_{free}/\theta$ vs $[B]_{free}$ we should get a straight line and the intercept on the y axis should be $1/K_d$.

3. Calculations:

Absorbance from fluorescence vs Total Concentration of B

3.1)# For temperature 278K

$$y_1 = 0.1882206812 + \frac{(0.0905003593 - 0.1882206812)}{1+(\frac{x}{0.0000232573})^{0.6021608752}}$$

...(22)

D1 = 0.1882206812
A1 = 0.0905003593
C1 = 0.0000232573
B1 = 0.6021608752

$$y_1 = D_1 + \frac{A_1 - D_1}{1+(\frac{x}{C_1})^{B_1}}$$ ...(23)

Total Concentration of A is 300nM ie.

$A_t = 300 \times 10^{-9}$
$f_1 = 1/D_1$ (factor to convert y into theta as y approaches d ,

theta approaches 1)
While Fractional factor is
$\theta = f_1 * y_1$
On plotting x vs θ, we get

$y_1 = 0.5/f_1$
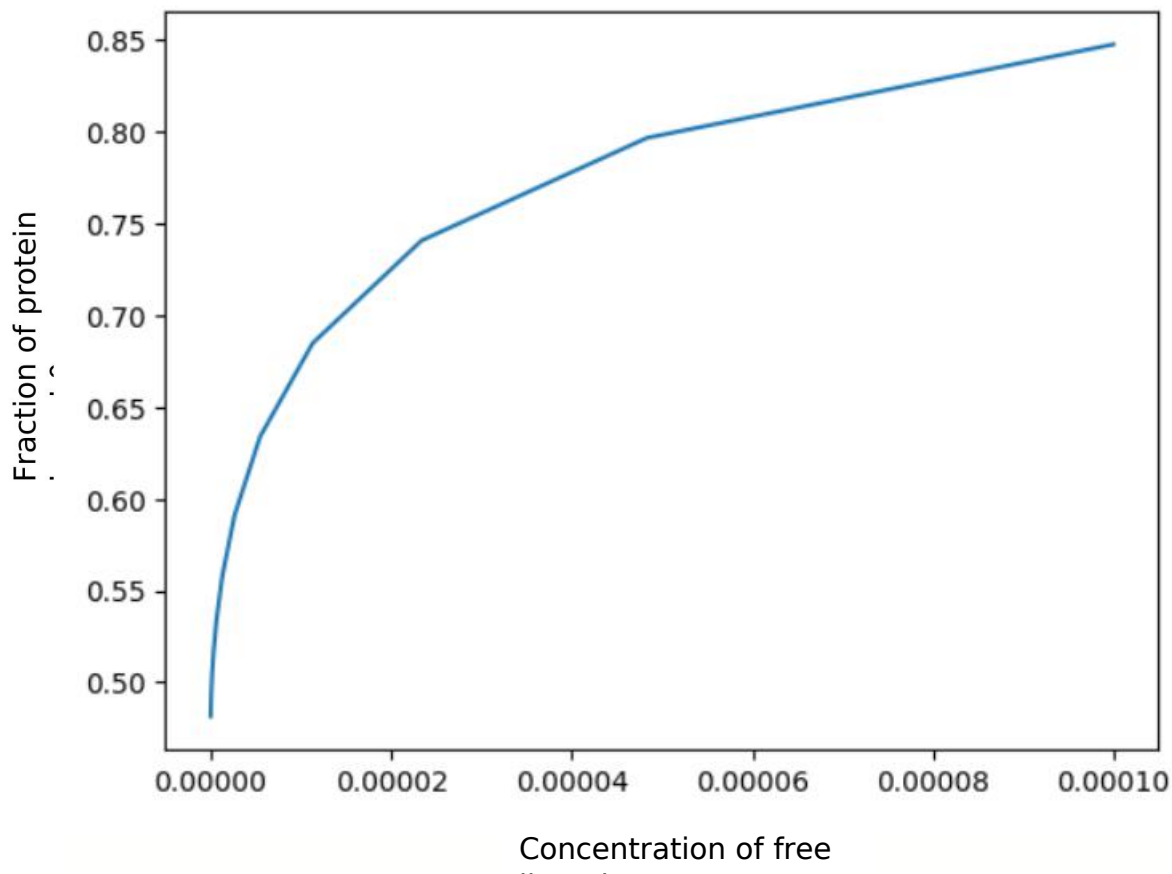The concentration of B in the X axis at which it is half is $[x]_{half1}$

$$[x]_{half1} = \frac{(C_1(A_1 - D_1)}{((y_1 - D_1) - 1)^{\frac{1}{B_1}}}$$ ...(24)

$BA = 150*10^{-9}$
$[B]_{free1} = [x]_{half1} - BA$
$Ka_1 = 1/[B]_{free1}$
$K_{d1} = 1/K_{a1}$

3.2)# For temperature 288K

$$y_2 = 0.1670661946 + \frac{(0.0726232453 - 0.1670661946)}{1 + (\frac{x}{0.0000165789})^{0.6246175511}}$$ ...(25)

$D_2 = 0.1670661946$
$A_2 = 0.0726232453$

$C_2 = 0.0000165789$
$B_2 = 0.6246175511$

$$y_2 = D_2 + \frac{A_2 - D_2}{1 + (\frac{x}{C_2})^{B_2}}$$ ...(26)

$A_t = 300*10^{-9}$
$f_2 = 1/D_2$ (factor to convert y into theta as y approaches d , theta approaches 1)

While Fractional factor is
$\theta = f_2 * y_2$
On plotting x vs θ, we get

$Y_2 = 0.5/f_2$
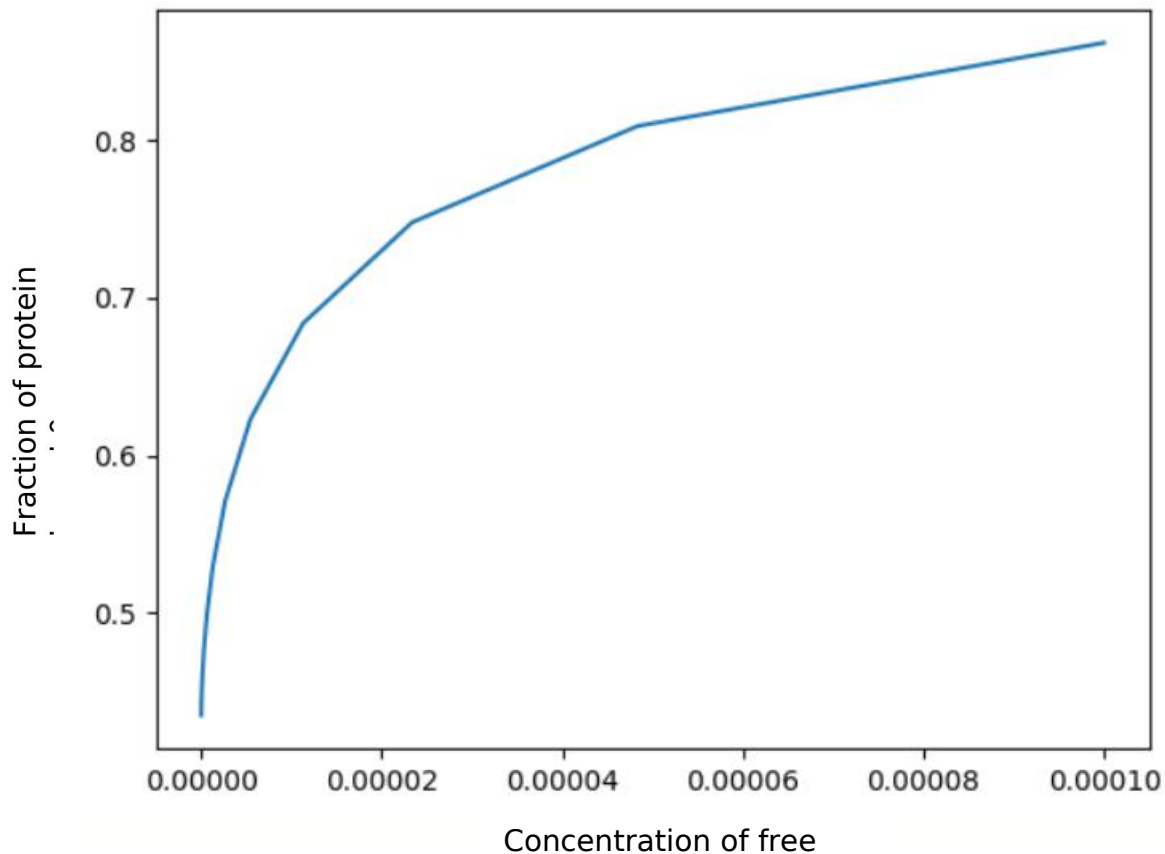The concentration of B in the X axis at which it is half is $[x]_{half2}$

$$[x]_{half2} = \frac{(C_2(A_2 - D_2)}{((y_2 - D_2) - 1)^{\frac{1}{B_2}}}$$ ...(27)

$BA = 150*10^{-9}$
$[B]_{free2} = [x]_{half2} - BA$
$K_{a2} = 1/[B]_{free2}$
$K_{d2} = 1/K_{a2}$

Fraction of protein

Concentration of free

3.3)# For temperature 298K

$$y_3 = 0.1435587436 + \frac{(0.0584934545 - 0.1435587436)}{1+(\frac{x}{0.0000121148})^{0.6652574263}}$$ ...(28)

$D_3 = 0.1435587436$
$A_3 = 0.0584934545$
$C_3 = 0.0000121148$
$B_3 = 0.6652574263$

$$y_3 = D_3 + \frac{A_3 - D_3}{1+(\frac{x}{C_3})^{B_3}}$$ ...(29)

$A_t = 300*10^{-9}$
$f_3 = 1/D_3$ (factor to convert y into theta as y approaches d , theta approaches 1)
While Fractional factor is
$\theta = f_3 * y_3$
On plotting x vs θ, we get

$Y_3 = 0.5/f_3$
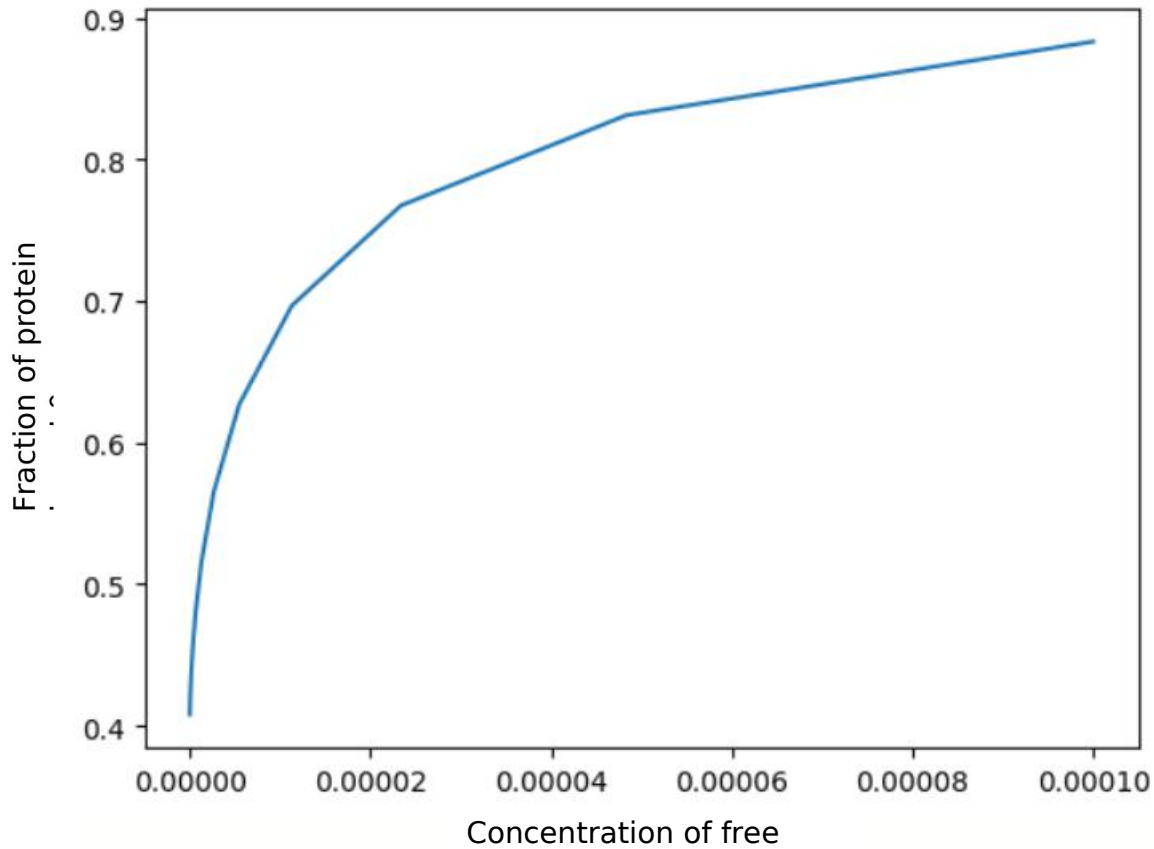The concentration of B in the X axis at which it is half is $[x]_{half2}$

$$[x]_{half3} = \frac{(C_3(A_3-D_3))}{((y_3-D_3)-1)^{\frac{1}{B_3}}}$$ ...(30)

$BA = 150*10^{-9}$
$[B]_{free3} = [x]_{half3} - BA$
$Ka_3 = 1/[B]_{free3}$
$K_{d3} = 1/K_{a3}$

Fraction of protein (y-axis)
Concentration of free ligand (x-axis)

3.4)# For temperature 308K

$$y_3 = 0.1177448238 + \frac{(0.0407385912 - 0.1177448238)}{1 + \left(\frac{x}{0.0000089570}\right)^{0.6891917260}} \quad \dots(31)$$

$D_4 = 0.1177448238$
$A_4 = 0.0407385912$
$C_4 = 0.0000089570$
$B_4 = 0.6891917260$

$$y_4 = D_4 + \frac{A_4 - D_4}{1 + \left(\frac{x}{C_4}\right)^{B_4}} \quad \dots(32)$$

$A_t = 300 * 10^{-9}$
$f_4 = 1/D_4$ (factor to convert y into theta as y approaches d, theta approaches 1)
While Fractional factor is
$\theta = f_4 * y_4$
On plotting x vs θ, we get

$Y_4 = 0.5/f_4$
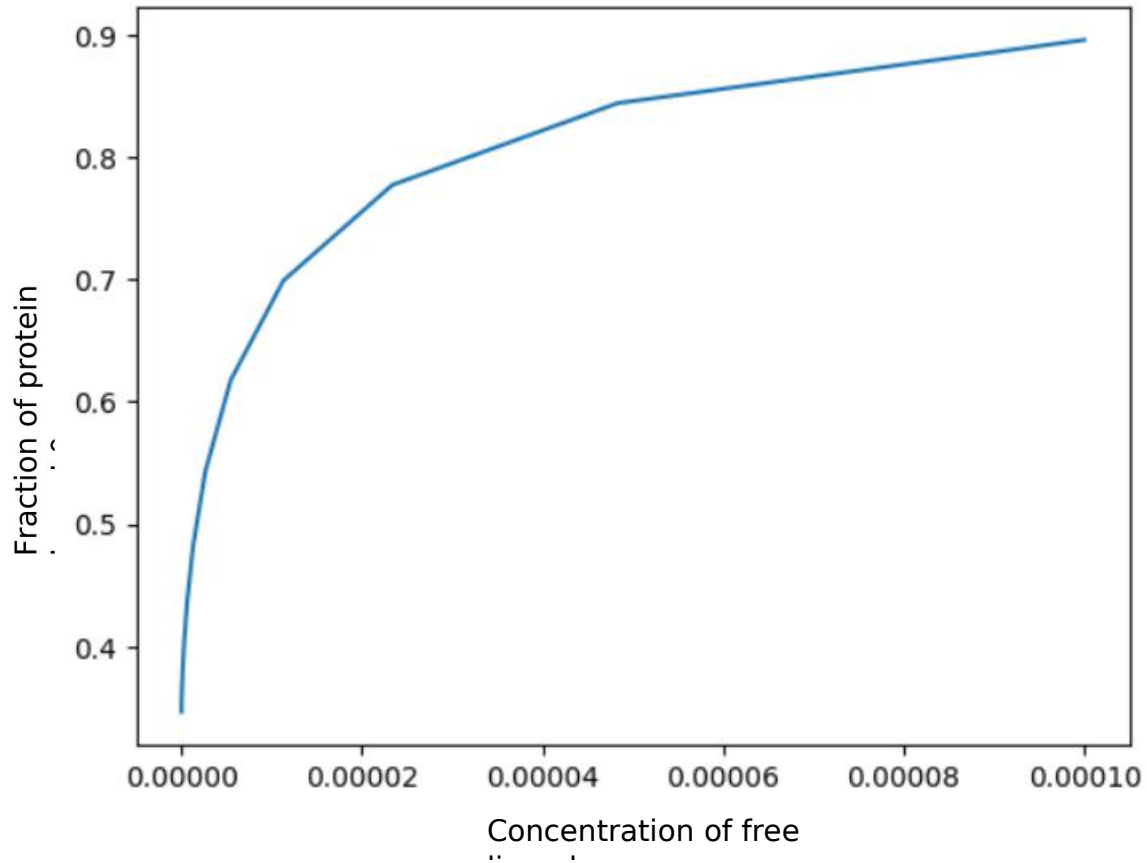The concentration of B in the X axis at which it is half is $[x]_{half2}$

$$[x]_{half4} = \frac{(C_4(A_4-D_4)}{((y_4-D_4)-1)^{\frac{1}{B_4}}}$$ ...(33)

BA= 150*10^{-9}
[B]$_{free4}$= [x]$_{half4}$-BA
Ka_4 =1/[B]$_{free4}$
K_{d4} = 1/K_{a4}

4. Results:

4.1) For Temperature 278K:
K_{a1}= -21490842.32671216 (M)
K_{d1}= -4.6531447432241616X10^{-8} (M)

4.2) For Temperature 288K:

K_{a2}= 2052852.2830802025 (M)
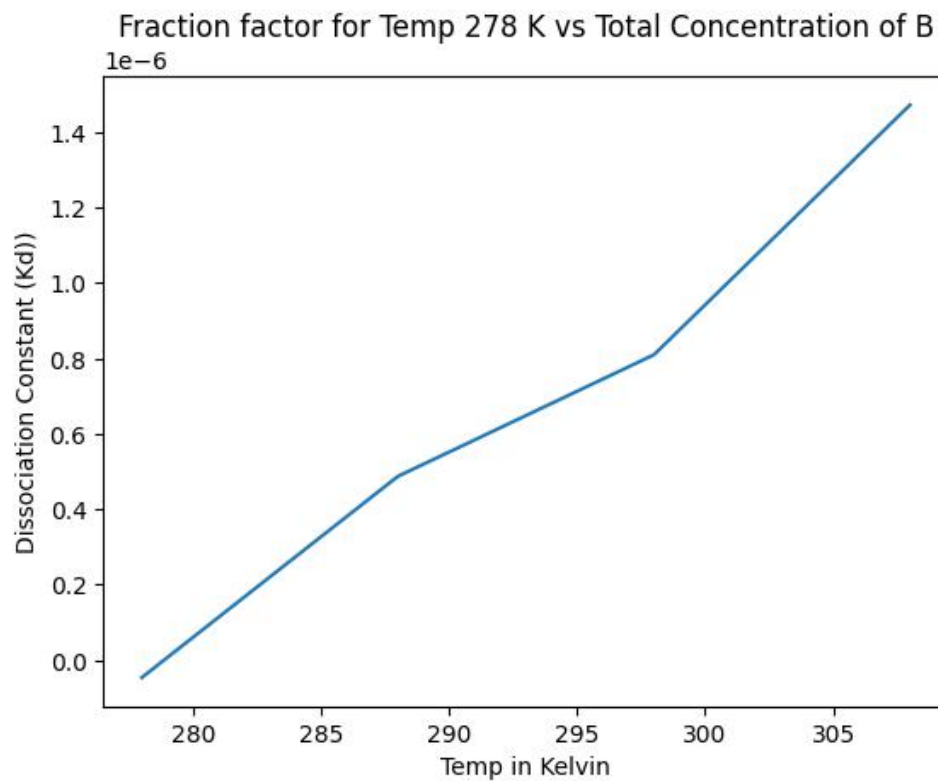K_{d2}= 4.871271100420094X10^{-7} (M)

4.3) For Temperature 298K:

K_{a3}= 1235225.1314783688 (M)
K_{d3}= 8.095690206716879X10^{-7} (M)

4.4) For Temperature 308K:

K_{a4}= 679260.9254699461 (M)
K_{d4}= 1.4721883189558575X10^{-6} (M)

Fraction factor for Temp 278 K vs Total Concentration of B

5. Inference:

From the curves we can deduce that with increase in temperature there is increase in Disscociation constant which is linear in nature as shown by graph and values calculated, also as the dissociation constant has values in 10^{-6} range we can infer that the Ligand and substrate have high affinity for the substrate and less amount of ligand-substrate dissociate from each other as the temperature increases, the K_d value increases which means increase in temperature results in more dissociation of the ligand-substrate here protein from the DNA.

In further analysis the DNA molecule could have high affinity protein binding sites on it and as the curve doesn't follow $Y=a*e^{(-b*x)}+c$ this molecule could have more than 1 protein binding site. Also the algothing used for the curve fitting is 4LP which we were unable to get working in matlab thus python code is attached.

References:

1. Lin, Kui, and Geng Wu. "Isothermal titration calorimetry assays to measure binding affinities in vitro." *The Hippo Pathway* (2019): 257-272.
2. Bligh, SW Annie, Terry Haley, and Peter N. Lowe. "Measurement of dissociation constants of inhibitors binding to Src SH2 domain protein by non-covalent electrospray ionization mass spectrometry." *Journal of Molecular Recognition* 16.3 (2003): 139-148.
3. http://www.bio.brandeis.edu/classes/biochem104/langmuir.pdf
4. Molecular Driving Force, Ken A Dill.
5. Dissociation Constant - Chemistry LibreTexts