# Reference Solution - Problem Set #3: Ligand Binding

## Question 1: Antibody-Antigen Binding Analysis

Antigen was added to a 1 µM concentration of antibody. The results listed in the table below were obtained. a) Plot the data. b) How many antigen-binding sites exist per antibody molecule?

c) Estimate the dissociation constant and explain the limitations of your estimate.

|  |  |
| --- | --- |
| **Antigen added (µM)** | **Measured concentration of free antigen (µM)** |
| 0.5 | 0.005 |
| 1.0 | 0.0011 |
| 1.5 | 0.016 |
| 2.0 | 0.021 |
| 2.5 | 0.5 |
| 3.0 | 1.0 |
| 3.5 | 1.5 |
| 4.0 | 2.0 |

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### Answer to question 1

### a) Plot of the data


Figure legend: X-axis: Antigen added (μM) from 0-4 μM. Y-axis: Measured concentration of free antigen (μM) from 0-2 μM. Points show minimal free antigen until ~2μM, then a sharp linear increase

The plot shows the relationship between added antigen concentration and measured free antigen concentration. Initially, most added antigen is bound by the antibody (low free concentration). Around 2-2.5 μM of added antigen, there's a sharp increase in free antigen concentration, indicating saturation of binding sites.

### b) Number of antigen-binding sites per antibody molecule

There are **2 antigen-binding sites per antibody molecule**. This conclusion is based on the following analysis:

* The antibody concentration is 1 μM
* The binding sites become saturated at approximately 2 μM of added antigen
* The saturation point indicates that each antibody molecule can bind two antigen molecules

This stoichiometry is consistent with the typical structure of antibodies like IgG, which have two antigen-binding sites located at the tips of their Y-shaped structure.

### c) Dissociation constant estimation and limitations

The dissociation constant can be estimated through analysis of the binding data using the equilibrium binding equation:

For the reaction: Ab + 2Ag ⇌ AbAg₂ K₂\* = ([Ag]free² × [Ab]free)/[AbAg₂]

Using the data points before saturation (0.5-2.0 μM), the calculated mean Kd value can be estimated to be ~10⁻¹¹ M. However, there is a large uncertainty introduced by the high error associated with the very low concentrations of free antigen that enter these calculations.

The problem is it that the experiment has been conducted under stoichiometric binding conditions where the antibody concentration of 1 µM is much larger than Kd, i.e. Concentration (antibody) >> Kd. These conditions are good to determine the stoichiometry of binding. However, under these conditions, essentially every antigen that is added is bound to the antibody until all of its binding sites are saturated. However, the regime where Kd can be best reliably determined has similar concentrations of free antibody, free antigen and the antibody-antigen complex, which can be achieved when Concentration (antibody) << Kd. Thus, it would be better to simply give here an upper limit of Kd, i.e. Kd < 10-10 M.

## Question 2: Lac Repressor Binding Analysis

For the equilibrium binding affinity of *lac* repressor (present as a tetrameric protein complex) to its specific O1 DNA binding site in the *lac* operator a value of ∆G = -48.3 ± 6.6 kJ mol-1 has been measured in vitro.

a) What is the concentration of the O1 binding site in an *E. coli* cell?

b) Estimate how many *lac* repressor molecules would be needed to saturate the O1 binding site in an E. coli cell. State the assumptions that you make to derive your estimate.

c) How many *lac* repressor molecules would you need to get the same occupancy for an O1 binding site engineered into one human chromosome in the nucleus of a human fibroblast cell?

### Answer to question 2

### a) Concentration of the O₁ binding site in an E. coli cell

The concentration of the O₁ binding site can be calculated as follows:

* An E. coli cell has only one copy of its chromosome, which contains a single lac operon with one O₁ binding site
* The average volume of an E. coli cell is approximately 1 μm³ = 10⁻¹⁵ L
* Using Avogadro's number (6.022 × 10²³ molecules/mol):

Concentration = 1 molecule / (10⁻¹⁵ L × 6.022 × 10²³ molecules/mol) = 1.66 × 10⁻⁹ M = **1.66 nM**

### b) Number of lac repressor molecules needed for O₁ binding site saturation

To determine the number of lac repressor molecules needed to saturate the O₁ binding site, we first calculate the dissociation constant (Kd) from the Gibbs free energy:

ΔG = -RT ln(Kd)

Given:

* ΔG = -48.3 kJ/mol
* R = 8.314 J/(mol·K)
* T = 298 K (25°C)

Kd = exp(-ΔG/RT) = exp(48300/(8.314 × 298)) = 3.5 × 10⁻⁹ M

For effective saturation, the repressor concentration should be at least 10 × Kd = 3.5 × 10⁻⁸ M.

Number of molecules = 3.5 × 10⁻⁸ mol/L × 10⁻¹⁵ L × 6.022 × 10²³ molecules/mol ≈ **21 molecules**

Assumptions made:

* The lac repressor binds as a tetramer to the O₁ site
* Binding follows a simple 1:1 binding model
* Non-specific binding to the genome is negligible
* The cell environment does not significantly alter binding constants from in vitro measurements
* 90% saturation is considered sufficient (using 10 × Kd)

### c) Lac repressor molecules needed in a human fibroblast nucleus

For a human fibroblast nucleus:

* The nucleus volume is approximately 500 μm³ = 5 × 10⁻¹³ L (500 times larger than an E. coli cell)
* Using the same binding parameters and saturation threshold:

Number of molecules = 3.5 × 10⁻⁸ mol/L × 5 × 10⁻¹³ L × 6.022 × 10²³ molecules/mol ≈ **10,500 molecules**

This large number reflects the dilution effect of the much larger nuclear volume compared to the bacterial cell.

## Question 3: Oxygen Binding to Hemoglobin

The figure below describes the binding of oxygen to hemoglobin. The experimental data are fitted to the Hill equation or to the Monod-Wyman-Changeaux (MWC) model. The parameters that gave the best fit of the model to the data are listed adjacent to the plot.



a) Explain the meaning of the parameters *α*H (Hill equation) and *R*, *c* and *K*R (MWC model). How do the two models compare concerning the number of fit parameters and how well they describe the experimental data?

b) How is the cooperativity of O2 binding described by the two models and which model is more informative about the molecular mechanism of cooperativity?

c) What is the fraction of proteins in the *R* state at half saturation of bindings sites for the MWC model for the parameters given in the figure?

### Answer to question 3

### a) Parameters interpretation and model comparison

**Hill equation parameters:**

* α₍ₕ₎ = 2.8: The Hill coefficient represents the degree of cooperativity in binding. A value greater than 1 indicates positive cooperativity, meaning that binding of one oxygen molecule increases the affinity for subsequent oxygen molecules.

**MWC model parameters:**

* R = 9000: The allosteric constant, representing the ratio of proteins in the T (tense) state to the R (relaxed) state in the absence of ligand
* c = 0.014: The ratio of binding constants K₍ᵣ₎/K₍ₜ₎, indicating that the R state has approximately 71× higher affinity for oxygen than the T state
* K₍ᵣ₎ = 2.8: The dissociation constant for oxygen binding to the R state

**Comparison of models:** The Hill equation is simpler, using only one parameter (α₍ₕ₎) to describe cooperativity. The MWC model uses three parameters (R, c, K₍ᵣ₎) to provide a more mechanistic description of the binding process. The plot shows that while both models fit the data reasonably well, the MWC model provides a slightly better fit, particularly at low and high oxygen concentrations where the Hill equation deviates more from the experimental data.

### b) Cooperativity description in the two models

**Hill equation:** The Hill equation describes cooperativity empirically through the Hill coefficient (α₍ₕ₎). The value of 2.8 indicates strong positive cooperativity but doesn't provide insight into the molecular mechanism. The model assumes that binding is "all-or-none," which is not physically realistic.

**MWC model:** The MWC model provides a more mechanistic description of cooperativity based on the two-state model of protein conformation:

* It assumes hemoglobin exists in two states: T (tense, low affinity) and R (relaxed, high affinity)
* Binding of oxygen shifts the equilibrium toward the R state
* The parameters R, c, and K₍ᵣ₎ describe the equilibrium between states and binding affinities
* Cooperativity arises naturally from the shift in population between the two states

The MWC model is more informative about the molecular mechanism because it:

* Explains cooperativity through a plausible physical mechanism (conformational change)
* Accounts for the sigmoidal shape of the binding curve as a transition between two hyperbolic binding curves
* Provides parameters with physical interpretations related to protein structure and energetics

### c) Fraction of proteins in the R state at half saturation

To calculate the fraction of proteins in the R state at half saturation (θ = 0.5), we need to:

* Determine the oxygen concentration at half saturation
* Calculate the fraction of proteins in the R state at this concentration

The fraction of proteins in the R state is given by: R̄ = (1 + α)ⁿ / [(1 + α)ⁿ + R(1 + cα)ⁿ]

Where:

* α is the normalized oxygen concentration
* n = 4 (number of binding sites in hemoglobin)
* R = 9000
* c = 0.014

At half saturation, α ≈ 10 (corresponding to approximately 30 torr pO₂)

Substituting these values: R̄ = (1 + 10)⁴ / [(1 + 10)⁴ + 9000(1 + 0.014×10)⁴] ≈ **0.2** or **20%**

This indicates that at half saturation of binding sites, approximately 20% of hemoglobin molecules are in the R state, highlighting the transition from predominantly T state to predominantly R state that occurs during oxygen binding.