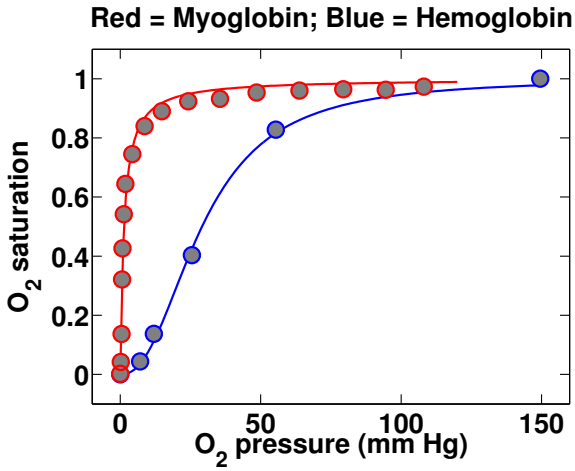
Solutions to problem set 3

Problem 1

Oxygen binding by myoglobin.
The fact that myoglobin is a monomer with only one heme suggests that it can only bind one oxygen molecule. Therefore, binding is not cooperative and we expect a binding curve of the form

$$Y = \frac{[O_2]}{K_d + [O_2]} \tag{1}$$

i.e. a standard binding curve that does not have the sigmoidal shape typical of the cooperative binding of hemoglobin.
The fact that myoglobin "takes over" the oxygen from hemoglobin in the muscle tissue suggests that at concentrations typical for tissue, where hemoglobin has a low fractional binding and let's go of the bound oxygen, myoglobin should still have a significant affinity for oxygen. In other words, the binding curve for myoglobin should be to the left of the hemoglobin curve. See below a plot for actual data, taken from Kelly *et al.*, *Appl. Spectr.* (1991) for myoglobin and from Bohr *et al.*, *Skand. Arch. Physiol* (1904) for hemoglobin.



Problem 2

Estimates of hemoglobin in the body. We are told that there are roughly $5 \cdot 10^6$ red blood cells per μl of blood, that there are roughly 15 g of hemoglobin for every deciliter of blood, and that an average adult has about 5 L of blood in total.

a) The total number of red blood cells for an average adult:
$5 \cdot 10^6/\mu l \cdot 10^6 \mu l/l \cdot 5l = 2.5 \cdot 10^{13}$

b) The total amount (mass) of hemoglobin in the body:
15 g/dl · 10 dl/l · 5 l = 750 g

c) Given molecular mass of hemoglobin, ≈ 64 kDa, the number of hemoglobin molecules in the body is
0.75 kg / ($1.66 \cdot 10^{-27}$ kg · 64000) = $7 \cdot 10^{21}$

d) The number of hemoglobin molecules in one cell:
$7 \cdot 10^{21}$ / ($2.5 \cdot 10^{13}$) ≈ $3 \cdot 10^{8}$

e) The volume of a red blood cell is very roughly $5 \cdot 5 \cdot 2\ \mu\text{m}^3 \approx 50\ \mu\text{m}^3$. The average spacing is generally given by $d = (V/N)^{1/3} = c^{-1/3}$ (see problem set 1, problem 1). Therefore, we find for the spacing of hemoglobin in a cell:
$d = (50\mu\text{m}^3\ /\ (3 \cdot 10^8))^{1/3} \approx 6$ nm. This average spacing is not much larger than the size of hemoglobin, ≈ 2 nm; in other words, hemoglobin is very densely packed in the cell.

Problem 3

Free vs. bound ligand. This problem follows essentially Problem 6.3 or 6.4, respectively, from the first or second edition of *Physical Biology of the Cell* by Phillips *et al.*

a) To derive an expression for the fraction bound as a function of $[L]_{tot}$ and $[R]_{tot}$, we can start with the definition

$$Y = \frac{[RL]}{[R]_{tot}} = \frac{[RL]}{[R] + [RL]} = \frac{[L]/K_d}{1 + [L]/K_d} \tag{2}$$

where we have used the definition of the dissociation constant

$$K_d = \frac{[L] \cdot [R]}{[RL]} \Rightarrow [RL] = \frac{[L] \cdot [R]}{K_d} \tag{3}$$

in the last step in equation 2.

We now need to find expression of Y in terms of $[L]_{tot}$, $[R]_{tot}$, and K_d only. Total ligand concentration is the sum of free and bound ligand:

$$[L]_{tot} = [L] + [RL] \Rightarrow [L] = [L]_{tot} - [RL] \tag{4}$$

Similarly, total receptor is the sum of free and bound receptor:

$$[R]_{tot} = [R] + [RL] \Rightarrow [R]_{tot} = \frac{K_d[RL]}{[L]} + [RL] \tag{5}$$

In the last step, we have again used the definition of the dissociation constant. Now we plug in the last expression in equation 4 into 5:

$$[R]_{tot} = \frac{K_d[RL]}{[L]_{tot} - [RL]} + [RL] \tag{6}$$

The last expression can be rearranged to be a quadratic equation for $[RL]$, by multiplying through with the denominator and rearranging terms

$$[R]_{tot}[L]_{tot} - [R]_{tot}[RL] = K_d[RL] + [L]_{tot}[RL] - [RL]^2 \tag{7}$$

Now, we bring this into the standard form for quadratic equations:

$$[RL]^2 - ([R]_{tot} + [L]_{tot} + K_d)[RL] + [R]_{tot}[L]_{tot} = 0 \tag{8}$$

This can be solved by quadratic completion (known in German as the "p-q-formula") and gives:

$$[RL] = \frac{([R]_{tot} + [L]_{tot} + K_d) - \sqrt{([R]_{tot} + [L]_{tot} + K_d)^2 - 4[R]_{tot}[L]_{tot}}}{2} \tag{9}$$

Note that the other solution (with a + in front of the square root) gives physically meaningless results (you obtain negative dissociation constants from the fit). Finally, we recall that $Y = \frac{[RL]}{[R]_{tot}}$, the starting point of our derivation, and write

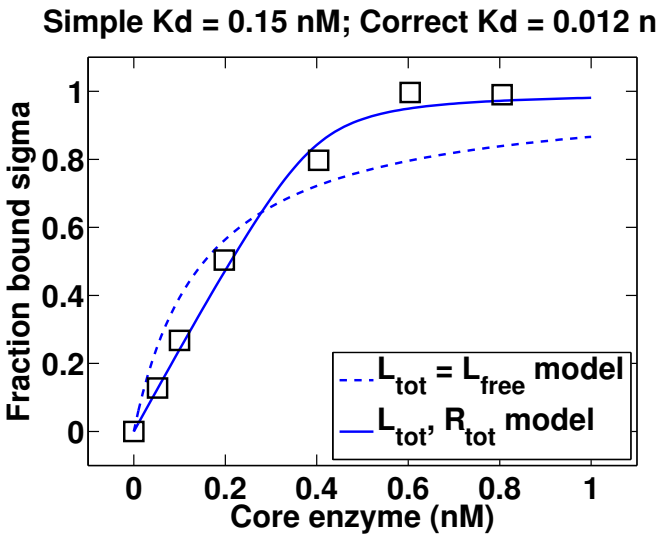
$$Y = \frac{([R]_{tot} + [L]_{tot} + K_d) - \sqrt{([R]_{tot} + [L]_{tot} + K_d)^2 - 4[R]_{tot}[L]_{tot}}}{2[R]_{tot}} \tag{10}$$

As desired, this gives us an expression of Y as a function of $[L]_{tot}$, $[R]_{tot}$, and K_d only. This expression can be used to fit K_d to the data, see the separate matlab code for part c.

b) Briefly, in the limit where $[L]_{tot} \gg [R]_{tot}$, we also have $[L]_{tot} \gg [RL]$ and thus $[L]_{tot} \approx [L]$ and we do not need to distinguish free and bound ligand concentration and can use equation 2.

c) Inspecting the data of Maeda *et al.*, *Nucleic Acids Research* (2000), for core enzyme binding to the σ^{70} subunit, we see that the core enzyme concentrations (the ligand, in this case) are in the range of 0 to 0.8 nM, while the receptor concentration (σ^{70}) is at a concentration of 0.4 nM; clearly we do not have $[L]_{tot} \gg [R]_{tot}$ and need to fit our modified model.

If we fit the standard expression (dashed line below), we obtain a relatively poor fit and an apparent dissociation constant of 0.15 nM. Fitting the correct model, we obtain a much better fit (solid line) and a significantly lower dissociation constant of 0.012 nM, corresponding to higher affinity. This is to be expected: Given the high receptor concentration, at the same total ligand concentration the free ligand concentration is always going to be lower than it would be for a lower receptor concentration. Therefore, the true affinity needs to be higher than it would have to be in the $[L]_{tot} \gg [R]_{tot}$ case to see e.g. half maximal binding at a certain concentration.



The matlab code used for fitting the two models and plotting the results is available on the course website.