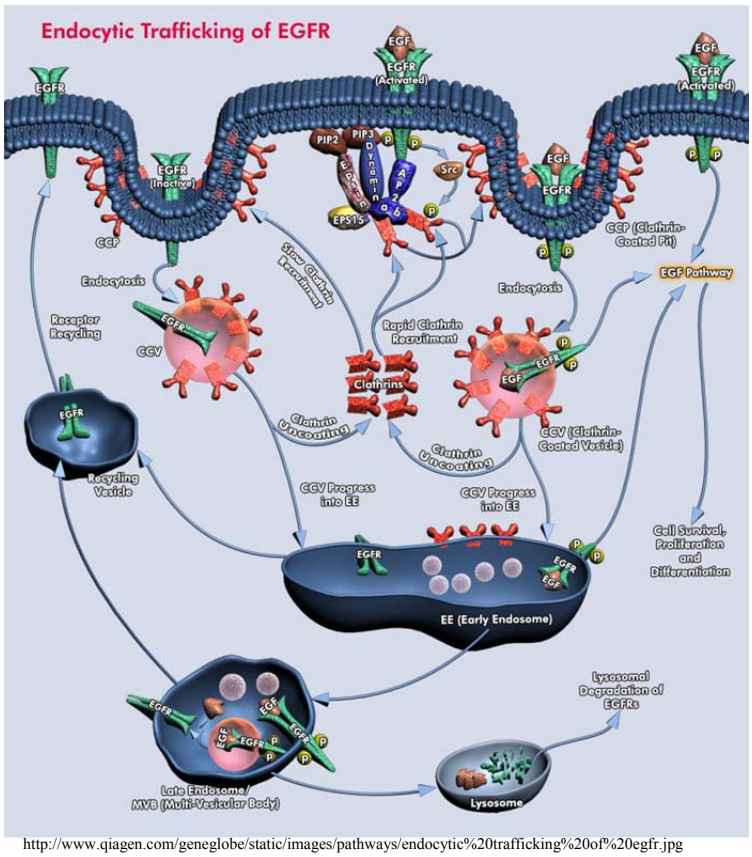
Cellular Bioengineering Project 1 – DUE February 13, 2014

For this first project, you will develop a model of receptor-ligand binding based on published reports. The goals of the project are 1) for you to demonstrate that you understand the principles of describing receptor-ligand binding and related processes we have been discussing in class and 2) to use this model to describe or answer questions about the system in question. As you can see from the figure below of EGFR trafficking, these systems can get really complicated really quickly!



http://www.qiagen.com/geneglobe/static/images/pathways/endocytic%20trafficking%20of%20egfr.jpg

For this project, you also have options. The first option is to follow a relatively prescribed process to look at the mechanism of inhibition of an HIV inhibitor. The second option is to reproduce a model of slow and fast transferrin sorting as described by Scheff *et al.* and discussed in class. The third option, only for the strong of heart, is to choose your own system from the literature and work up a model for it.

Details

This project should be fairly straightforward and it should not eat your life. If are lost or are writing pages of equations, talk to me. Also, unless you have a compelling reason otherwise, assume first order, reversible binding.

Write up your results, including the story line, relevant equations, graphs, or schematics in an ~4 page report following IEEE conventions (templates are on the course website, appendices are also encouraged and can include hand written calculations if relevant). A draft is due February 6th. The final paper is due in class on February 13.*

Your final product should reflect your own understanding of the material, but, in addition to asking me questions, feel free to consult classmates, the text book, and notes but, for option 1, not the Guo paper (I'll provide it afterwards if you are interested).

This project will be worth 15% of your grade and will be assessed on your demonstration of: Quantitative analysis (ability to use the modeling we have been discussing), Qualitative analysis

*A 20%/day grade decrease will be applied late papers. Requests for extensions will be generously considered if they are made in a timely manner.

(ability to make conclusions about graphs and data), and Communication (ability to present your work and thoughts clearly).

Option 1 – Mechanism of Action of an HIV Inhibition Drug

In general, viral infection involves travel to the target cell, binding, entry, and expression of viral genes. These steps are amenable to the types of modeling we have been discussing. For this first project, we will focus on the initial binding step and drugs developed to inhibit this step.

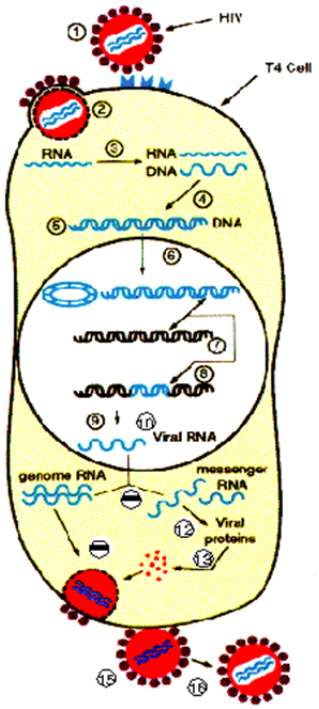


Figure 1. Schematic of the viral cycle of HIV [1].

Background

During HIV infection (Fig. 1), the human immunodeficiency virus type I (HIV-I), initially binds to CD4 on T-cells of the host immune system via the HIV envelope protein, gp120. This initial binding step triggers binding of a co-receptor such as CCR5 or CXCR4 (Fig. 2). Following this, the viral particle fuses with the cell membrane in a process involving the viral protein, gp41. After fusion, the viral RNA is inserted into the cell and reverse transcribed into DNA by viral reverse transcriptase. The resulting DNA is moved to the nucleus and incorporated into the cellular genetic material. The viral cycle continues, often after a latency period when viral proteins are produced. Viral particles including proteins, RNA, and reverse transcriptase are then packaged and bud from the cell. (Summarized from [1])

<u>Part 1</u>

Consider the binding of HIV-1 via CD4 followed by CCR5. It has been shown that the co-receptor CCR5 is necessary for binding and variation in CCR5 has been proposed as the reason for some individuals having natural immunity to HIV infection[2]. Considering only the interactions of CD4, CCR5, and gp120:

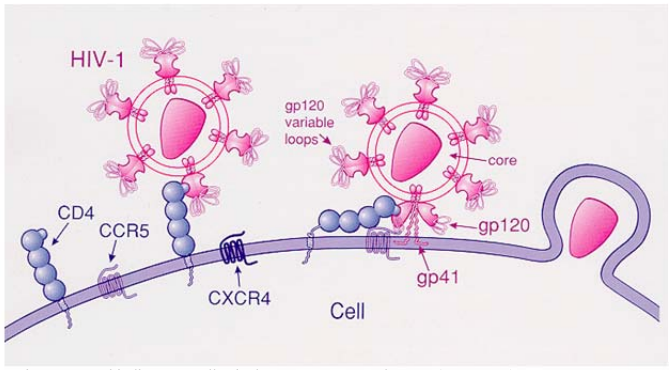


Figure 2 HIV binding to T-cells via the CD4 receptor and CCR5 (or CXCR4) [1].

1) Re-draw and clearly label the binding interaction using the customs introduced in class. (Assume that CD4 and CCR5 both bind to gp120, at separate sites. Hint: it is probably useful to choose gp120 as the receptor and the others as ligands.)
2) Write the differential and all mass balance equations representing this interaction
3) What does assuming steady state and an excess of CD4 and CCR5 do to these equations?
4) Solve for the CD4-gp120-CCR5 complex as a function of CD4 concentration.
5) Plot your solution and consider the effects of different K_D values. (Hint: As a start it may be convenient for you to assume 10^6 gp120 molecules/cell, equilibrium binding constants ~10 nM, and CD4 and CCR5 concentrations up to 10 nM.) Play and discuss.

Part 2

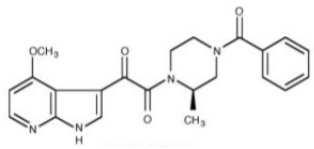


Figure 3. BMS-378806 structure (from [5]).

BMS-378806, shown in Fig. 3, is a compound that was originally developed by Bristol-Myers Squibb and has shown a great deal of promise in animal trials in the prevention of HIV transmission. A recent publication in indicates that when BMS-378806 is given with other drugs in a vaginal gel that it is efficacious at preventing HIV transmission in macaque monkeys[3]. Furthermore Bristol-Myers Squibb and Merck (who makes another drug used in this study) have agreed to provide these drugs for free (although it doesn't seem like much progress has been made since 2005).

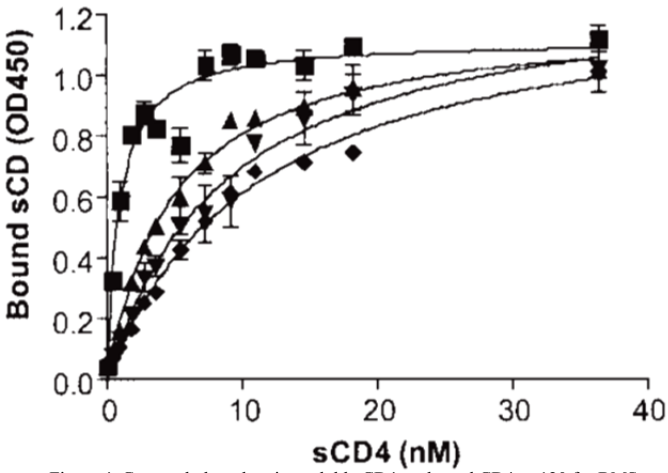


Figure 4. Guo et al, data showing soluble CD4 vs. bound CD4-gp120 for BMS-378806 concentrations of 0 (■), 0.8 (▲), 1.6 (▼), and 3.2 (♦) mM [5].

Lin, et al., published one of the initial reports showing that BMS-378806 inhibited HIV infection of cells in culture by blocking the CD4-mediated binding[4] and then the same group further characterized the inhibitory mechanism of BMS-378806[5]. We will be discussing their data (Fig. 4) for Part 2.

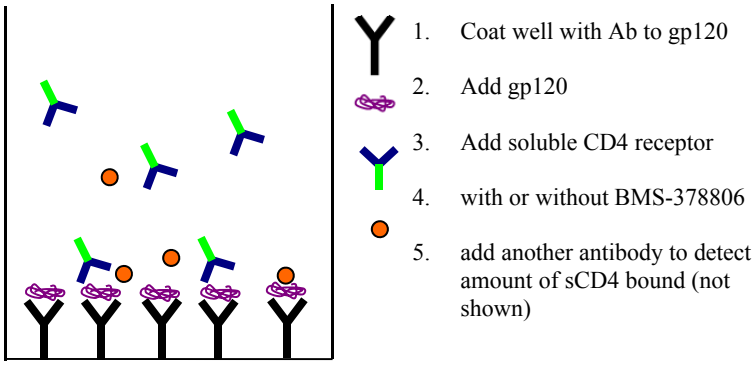


Figure 5. Schematic of measurement technique used in [5].

To understand what is in Figure 4, you should know how they obtained their results (Fig. 5). Specifically, they used gp120 and CD4 isolated from their respective virus and cell to measure binding between them with or without BMS-378806 in the solution. First, they immobilized gp120 on a surface (Fig. 5, steps 1-2, using an antibody, but we ignore that antibody). Then they added soluble CD4 (sCD4, step 3) with or without BMS-378806 (0.8, 1.6, or 3.2 μM, step 4) and waited a sufficient time to achieve equilibrium. Finally, after washing away excess unbound molecules, they measured the amount of bound sCD4 using a labeled antibody to CD4 (step 5). The amount of bound sCD4 (in absorbance units from the labeled antibody signal) vs. sCD4 added (in nM) is shown in Fig. 4.

It has been proposed that BMS-378806 is a competitive inhibitor (meaning it binds to gp120 at or near the CD4 binding site to block it). Considering this binding event (neglecting CCR5 binding):

1) Draw and clearly label receptor-ligand schematics.
2) Write the representative differential and all mass balance equations.
3) Solve for the CD4-gp120 complex as a function of CD4 and BMS-378806 concentrations, explicitly stating and justifying any assumptions you make.
4) It is up to you to decide whether BMS-378806 exhibits competitive or non-competitive inhibition. Use the data in Table 1 and your solution. Provide values of key parameters R_T and K_D.

Table 1. Colormetric readings of bound sCD4 as a function of BMS concentration (made to match data from [Error! Bookmark not defined.**]).**

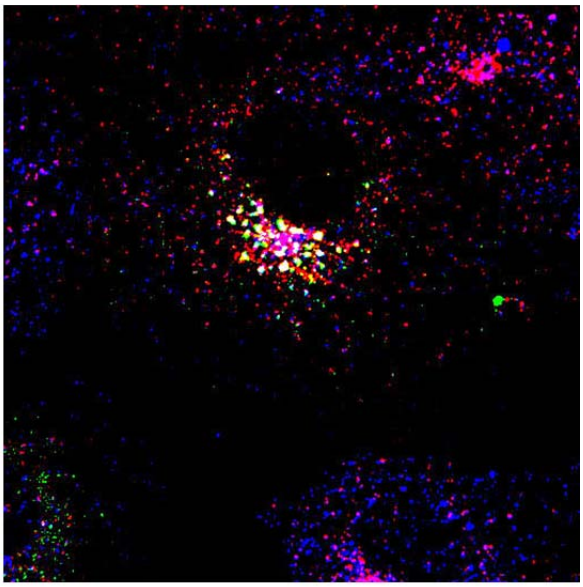
BMS (uM)	0	0.8	1.6	3.2
sCD4 (nM)				
1	0.680	0.283	0.182	0.105
2	0.880	0.500	0.320	0.210
3	1.008	0.600	0.450	0.267
4	1.076	0.720	0.524	0.348
5	1.100	0.790	0.605	0.395
6	1.200	0.816	0.618	0.456
7	1.176	0.903	0.707	0.504
8	1.224	0.936	0.792	0.560
9	1.206	0.990	0.810	0.603
10	1.210	1.000	0.810	0.610
15	1.260	1.065	0.975	0.780
20	1.320	1.180	1.000	0.860
30	1.320	1.260	1.080	1.020
40	1.280	1.200	1.160	1.000

Part 3

Another class of inhibitors interferes with CCR5 binding. They are thought to act through an allosteric (noncompetitive) mechanism. This means that the inhibitors bind at a site remote from the gp120 binding site on CCR5, but change the affinity for gp120. For this class of inhibitors:

1) Draw and clearly label receptor-ligand schematics of this process (including CD4). (Note: CCR5 and the inhibitor can bind and unbind regardless of whether the other is bound. Assume that neither can bind until CD4 is bound.)
2) Write the differential and all mass balance equations representing this interaction.
3) Discuss the approach you would take to solve this problem or implement this system of equations in a program of your choice (e.g. Maple).

Option 2 – Transferrin Sorting



http://www.endocytosis.org/epsinR/epsinrWT.html

Transferrin is a glycoprotein (protein with sugar chains attached) found in the blood and produced primarily in the liver whose job it is to regulate iron.[6] Transferrin can bind up to 2 iron ions with high affinity; binding affinity is greatly reduced at low pH.[6] Iron-bound transferrin (not to be confused with the unbound state, holotransferrin) binds to the transferrin receptor on the surface of a cell. As iron is required for a variety of functions including DNA synthesis, almost all cells in the body have the transferrin receptor on their surfaces, albeit at different levels for different cells.[7,8] Once iron-bound transferrin binds the transferring receptor, it is taken up via endocytosis and then the pH of the endosome is lowered, resulting in iron dissociation from transferrin and storage or use by the cell.[8] The image at the right shows colocalization of internalized transferrin (blue) with some other molecules and is just there for gratuitous purposes.

In general, as we (will) have discussed, the progression inside the cell is from early endosomes, to late endosomes, to recycling endosomes. Each of these populations is associated with certain markers which allow researchers to visualize them within cells. In general, early endosomes are associated with markers rab4 and rab5, late endosomes are associated with rab7 and rab9, and recycling endosomes are associated with rab11.[9]

In their paper, Scheff *et al.* investigate the internalization and recycling process in polarized cells.[10] Polarized cells, are cells that have an "up," or apical, and a "down," or basolateral, such as epithelial cells lining mucus membranes and endothelial cells lining blood vessels. The apical surface is typically in contact with a (e.g. blood, gastric juices) while the basolateral surface is typically in contact with an underlying extracellular matrix. Polarized cells engage in an additional active transport phenomenon called transcytosis, in which a molecule is transported across the cell via endocytosis at one surface and exocytosis at the other surface. In the Scheff paper, they investigate different compartments involved in recycling and transcytosis.

For this option of project 1, your should:

1) Succinctly summarize the background and significance of their work in your paper
2) Identify each of the relevant species to keep track of
3) Graphically represent all binding and unbinding events and include in your paper
4) Write out the relevant differential equations describing each event and explain all the terms
5) Either use the solutions provided to reproduce their model results or implement (e.g. by using Matlab with ode45 or implementing something like Euler's method in some other program)

Option 3 – Choose your own adventure

There are numerous cell systems involving receptor-ligand binding, trafficking, and signaling. Find one and write about why it is important, graphically and mathematically represent the system, and show some predictions from that model. Short description, very hard project.

REFERENCES

[1] http://www.niaid.nih.gov/daids/dtpdb/virpage1.asp and http://www.niaid.nih.gov/daids/dtpdb/attach.asp *(accessed 1/06)*

[2] Kilby JM and Eron JJ, *Novel Therapies Based on Mechanisms of HIV-1 Cell Entry*, **NEJM** 2003, 348, p. 2228-38.

[3] Veazey, *et al*., *Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion*, **Nature** 2005, 438(7064), p. 99-102.

[4] Lin, *et al*., *A small molecule HIV-1 inhibitor that targets the HIV-1 envelope and inhibits CD4 receptor binding*. **PNAS** 2003, 100(19), p. 11013-8.

[5] Guo, *et al, Biochemical and genetic characterizations of a novel human immunodeficiency virus type 1 inhibitor that blocks gp120-CD4 interactions,* **J Virol**. 2003, 77(19), p. 10528-36.

[6] "Transferrin," http://en.wikipedia.org/wiki/Transferrin, *accessed January 2, 2014*.

[7] "Transferrin Receptor," http://en.wikipedia.org/wiki/Transferrin_receptor, *accessed January 2, 2014.*

[8] Ponka P and Lok CN, *The transferrin receptor: role in health and disease,* **Int J Biochem Cell Biol.** 1999, 31(10), p. 1111-37.

[9] "Endosome," http://en.wikipedia.org/wiki/Endosome, *accessed January 2, 2014*.

[10] Sheff *et al., The receptor recycling pathway contains two distinct populations of early endosomes with different sorting functions.* **J Cell Biol** 1999, 145(1), p. 123-39.