Integrative Molecular and Cellular Bioengineering, SPRING '07
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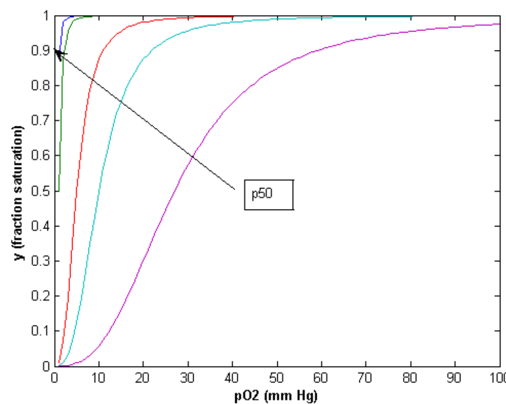
Assignment 3: Due March 2, 2007

1. The following figure shows the O_2 binding curve for bovine hemoglobin encapsulated in a "lipogel" consisting of a liposome entrapping a photopolymerized gel. The authors are investigating the effect of photopolymerization time on the O_2 binding curve.
a) Is the affinity of these carriers greater or less than that of extracted hemoglobin? How will this impact their oxygen carrying functionality?

The affinity is greater. The curves shift to the left, meaning that less O2 is required for the same amount of binding; hence, the affinity is greater.

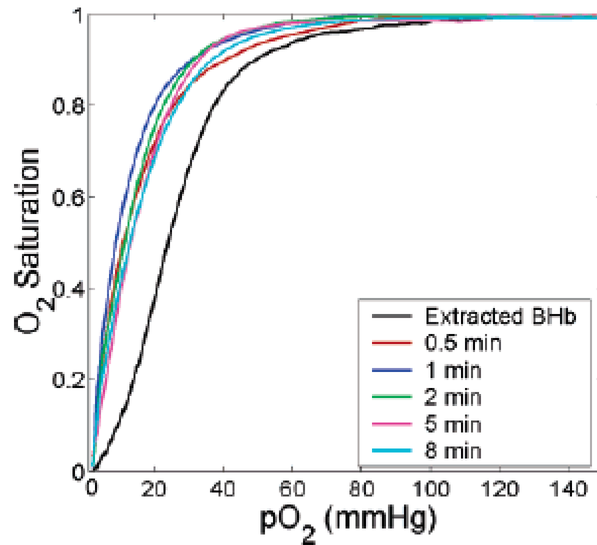
b) The authors claim that the cooperativity is not affected by entrapment in a photopolymerized gel. Is this plausible based on the data presented?

Yes. The new curves appear less cooperative, but it is just because the affinity is higher. I verified this by plotting the function $y = \frac{p_{O_2}^{\ n}}{p_{50}^n + p_{O_2}^{\ n}}$ for various values of p_{50} holding n=2.8 constant. The results are as follows and verify that the observed curves are plausible for constant n.



c) Discuss the design features that would be important in developing a blood substitute based on entrapment of hemoglobin in a polymeric or liposomal carrier.

The main reasons for using a carrier are to prolong circulation time and to maintain hemoglobin concentration so that it remains intact and functional. Therefore, circulation time in humans would be an important design feature. For functionality, the polymer would have to allow transport of O_2 into and out of its boundaries, and it would have to allow for the efficient entrapment of high concentrations of hemoglobin. In this environment, hemoglobin would have to retain its ability to bind much oxygen at high O_2 partial pressures and exhibit dissociation (unloading) at low partial pressures. Furthermore, the carrier would have to be non-toxic and would have to eventually degrade or be cleared without inducing any other harmful effects (blood pressure, immune system activation, etc.)



2. Suppose that I want to use siRNA to inhibit the expression of the PTEN gene in breast cancer cells. I have designed an appropriate siRNA sequence and selected a lipid-based delivery agent. Describe the controls that I would use to ensure that I am achieving sequence-specific siRNA-mediated knockdown of gene expression.

Assume that I have a quantitative measure of gene expression (e.g., real-time PCR). We want to have a negative control (cells without treatment) and would like to have a positive control (another cell line with low or no expression of PTEN), though this is not always feasible. In addition, we need to test lipid with non-target siRNA sequence and siRNA without lipid to ensure the specificity of the siRNA and whether the lipid is necessary, respectively. Showing the same effect in another cell type would strengthen the case even further.

3. In the Boder paper, do you think that the authors could achieve another order of magnitude improvement in affinity? What are some limitations that might prevent this?

Based on the fact that they achieved an order of magnitude improvement in each round of selection, one could extrapolate and assume that further improvement is possible. However, we noted in class that the last round resulted in a large number of mutations relative to the second to last round. That is a sign that achieving improvements is becoming more and more difficult. Furthermore, the last round of selection required days for dissociation. To characterize an antibody with another order of magnitude slower dissociation would mean monitoring it for weeks to months, which may be infeasible.