

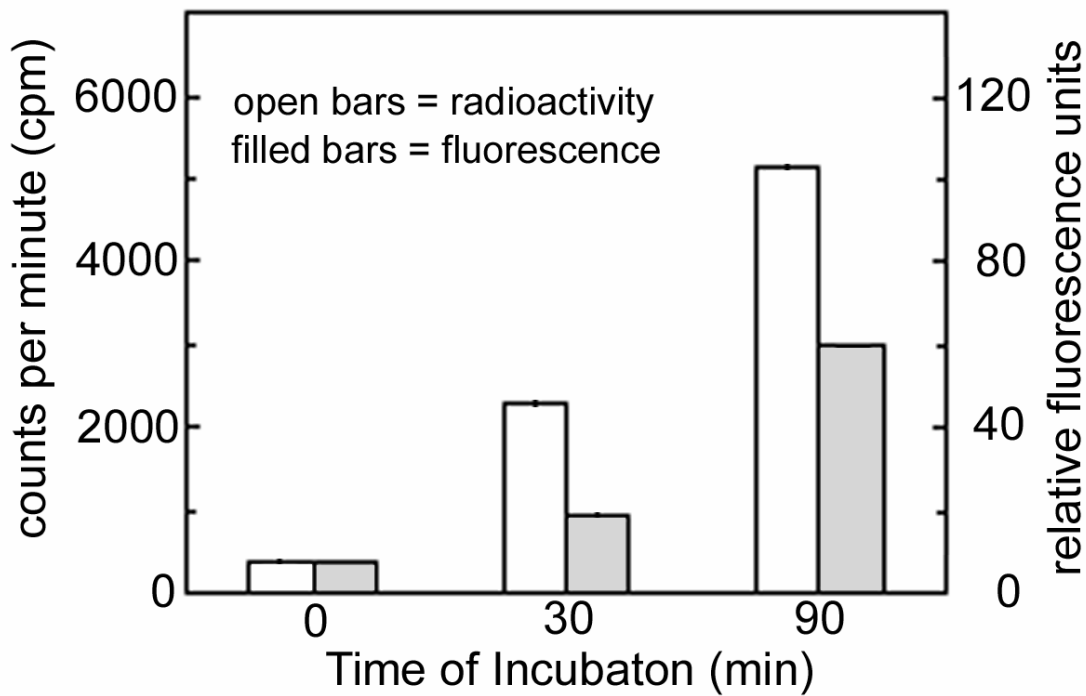
## Homework #2 (due 1/30/08)

You are trying to assess the interaction of *Leishmania* parasites with macrophages and want to develop an assay to quantify how many parasites bind to macrophages. Your initial experiment is to label the parasite (i.e., promastigotes) with either radioactivity or a fluorescent probe. These labeled parasites are then incubated with macrophages and the number of parasites bound to the macrophages is determined either by counting the radioactivity by scintillation spectrophotometry or by measuring the amount of fluorescence with a fluorometer. A detailed protocol is in the box below. Results and questions are on the following page.

1. Preparing tritium-labeled parasites. *Leishmania* parasites were cultured in standard medium containing  $^3\text{H}$ -thymidine. The radioactive thymidine is incorporated into the parasite DNA. At the end of the culture period the parasites are extensively washed to remove all of the thymidine which is not incorporated into DNA.
2. Preparing FITC-labeled parasites. *Leishmania* parasites were cultured as described above in step 1 except without the  $^3\text{H}$ -thymidine. The parasites were then incubated with FITC. FITC is a fluorescent compound which chemically reacts with proteins and forms a covalent bond with proteins on the surface of the parasites. The parasites are extensively washed to remove all of the free FITC not attached to parasite surface.
3. Preparation of macrophages and binding experiment. An adherent macrophage cell line was added to 35 mm Petri dishes at a density of  **$10^5$  macrophages per plate**. Following an incubation to allow the macrophages to completely attach to the Petri dishes,  **$10^6$  parasites** labeled with either radioactivity (step 1) or fluorescence (step 2) were added to each of the Petri dishes and incubated for either 0, 30, or 90 minutes. In other words, there are 6 total Petri dishes. The 0 min time point is a blank in which the parasites were added and then quickly removed to measure the background. Following the incubation the adhered macrophages were extensively washed to remove any unbound parasites.
4. Determination of parasite binding. **One ml of a solubilization buffer** which completely dissolves the macrophages and any bound parasites was added to each of the Petri dishes following the last wash. The radioactive samples were analyzed by adding **0.5 ml of the solubilized samples to a scintillation cocktail** and determining the cpm in each of the 3 samples. The fluorescent samples were analyzed by adding **100  $\mu\text{l}$  of solubilized samples** to a cuvette containing 0.9 ml of water and measuring the amount of fluorescence expressed in relative fluorescence units with a fluorometer.

5. Preparation of calibration controls. A sample containing  $10^5$  **radioactive parasites** was analyzed for radioactivity under conditions exactly the same as described in step 4 and had **7800 cpm**. A sample containing  $10^4$  **fluorescent parasites** was analyzed with a fluorometer exactly as described in step 4 and exhibited **180 relative fluorescence units**.

For each of the samples (3 radioactive and 3 fluorescent) you plotted either the counts per minute (open bars and left legend) or the relative fluorescence units (filled bars and right legend) vs. incubation time as shown below:



Answer the following questions:

- 1) Using the information in the detailed protocol calculate the number of parasites bound per macrophage for both methods (radioactivity and fluorescence) after 30 and 90 minutes.
- 2) Do you feel that one technique (i.e., radioactivity vs. fluorescence) provides a more accurate number for the calculation of parasites per macrophage than the other? Briefly discuss why or why not. What could be done to check the accuracy of these two methods?
- 3) What is another method(s) besides fluorometry that can be used to evaluate the experiment with the fluorescence-labeled parasites? Briefly discuss the additional information that might be obtained with this method(s).