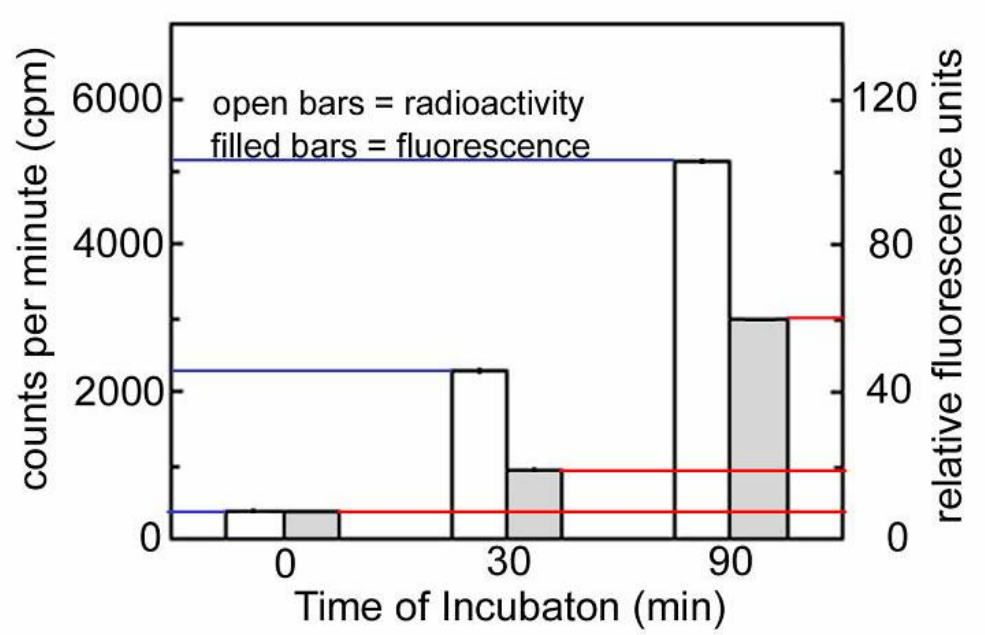
Key to Homework 2

Calculation of number of macrophages bound.

- From the graph estimate the cpm and rfu for all of the samples (see figure and cpm/rfu column in table)
- Use the 0 time point and subtract from the other values (see blank column in table).
- Use the conversion factors to convert cpm or rfu into parasites (see parasites column in table).
- Divide by the amount of sample analyzed to determine the number of parasites per ml (see per ml column in table).
- Divide by 10^5 macrophages/ml to calculate number of parasites bound per macrophage (see per MΦ column in table).



sample	cpm/rfu	blank	parasites	per ml	per MΦ
0 rad	300	-300	÷0.078	÷0.5	÷105
30 rad	2200	1900	2.4x104	4.8x104	**0.5**
90 rad	5100	4800	6.2x104	1.2x105	**1.2**
0 fluor	7	-7	÷0.018	÷0.1	
30 fluor	20	14	778	7780	**0.08**
90 fluor	60	53	2944	2.9x104	**0.3**

Explanation of differences between methods.

- The 2 methods gave substantially different results.
- Both methods can have problems with quenching which would interfere with determining the exact value.
- It is unlikely that either method would give you an artificially high value so one would tend to favor the method that gave higher numbers.
- One possible explanation involves how the parasites are prepared in the two methods.
    - Incubating the parasites with ^{3}H-thymidine would have little effect on the overall physiology of the parasites.
    - Modifying the surface proteins of the parasite with FITC could have an affect on the binding of the parasites to macrophages or affect their overall physiology.
- The simplest way to determine the actual levels of binding would be to count the number of bound parasites using microscopy.

Other analyses using fluorescent parasites

- Fluorescent microscopy could be used. The results would be similar to using conventional microscopy except that detecting the parasites may be easier.
- Flow cytometry can evaluate individual macrophages and determine the number of parasites bound to the individual macrophages. Fluorometry just gives an average of number of parasites bound per macrophage and does not give any information about the ranges in terms of number of macrophages bound.