Interference of Alexa Fluor 488

Alexa Fluor 488's response was observed with several metal ions shown in Figure 1. The fact that Cr^{3+} does not produce a response indicates that the interaction of Alexa Fluor 488 with Fe^{3+} is not due to a charge induced interaction. Additionally, this data shows that Cu^{2+} causes a similar amount of quenching as the Fe^{3+}.

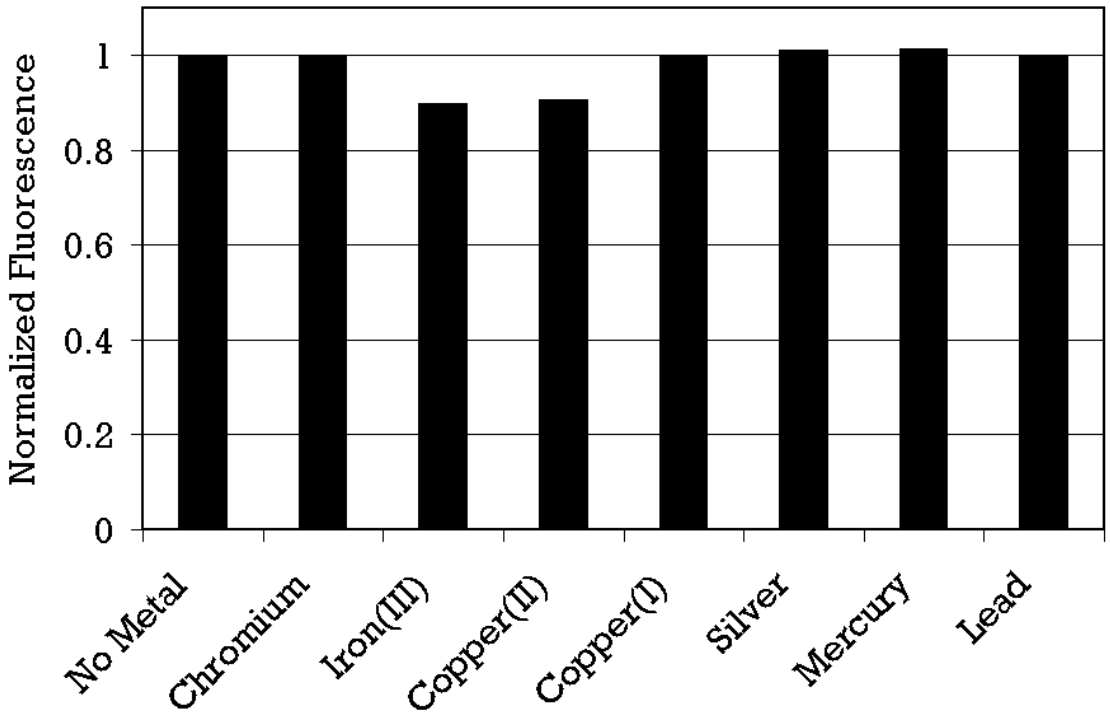

Figure 1: Effect of various metal ions on the fluorescence of Alexa Fluor 488. Each metal ion was incubated at 1 μM concentration in solution. The resulting fluorescence intensity of Alexa Fluor 488 (ex = 493 nm, em = 520 nm) was normalized by dividing the fluorescent intensity in the sample by that of the no metal sample.

Figure 2, however, shows that the sensitivity of the PEBBLE is unaffected in the presence of copper. In fact, this data suggests that changing amounts of Cu^{2+} will not compromise iron measurements.

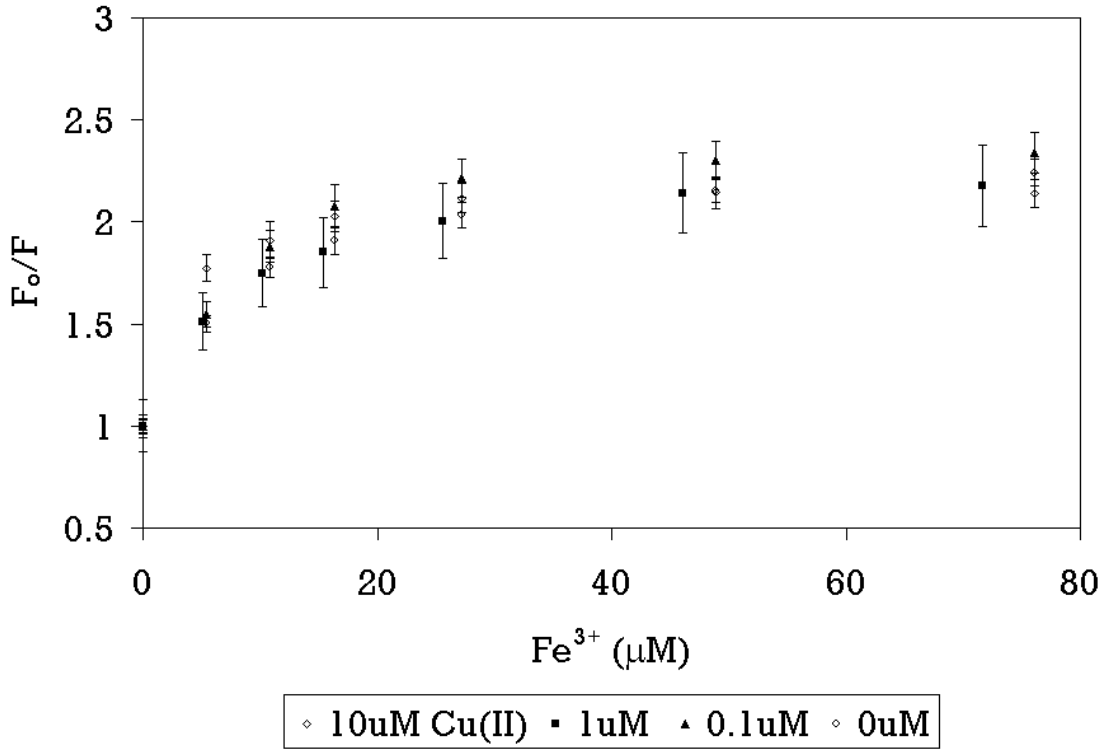

Figure 2: Dependence of the normalized fluorescence ratio of the PEBBLEs (Alexa Fluor 488 ex = 493, em = 517 nm/ Texas Red ex = 493, em = 600 nm) on iron in 10 mM MOPS pH 7.2. Four separate solutions were incubated with differing amounts of Cu^{2+} between 0 μM and 10 μM. These experiments were repeated in triplicate.

PEBBLE Spectra

Figure 3 shows a typical spectrum of a PEBBLE solution obtained by exciting at 493 nm.

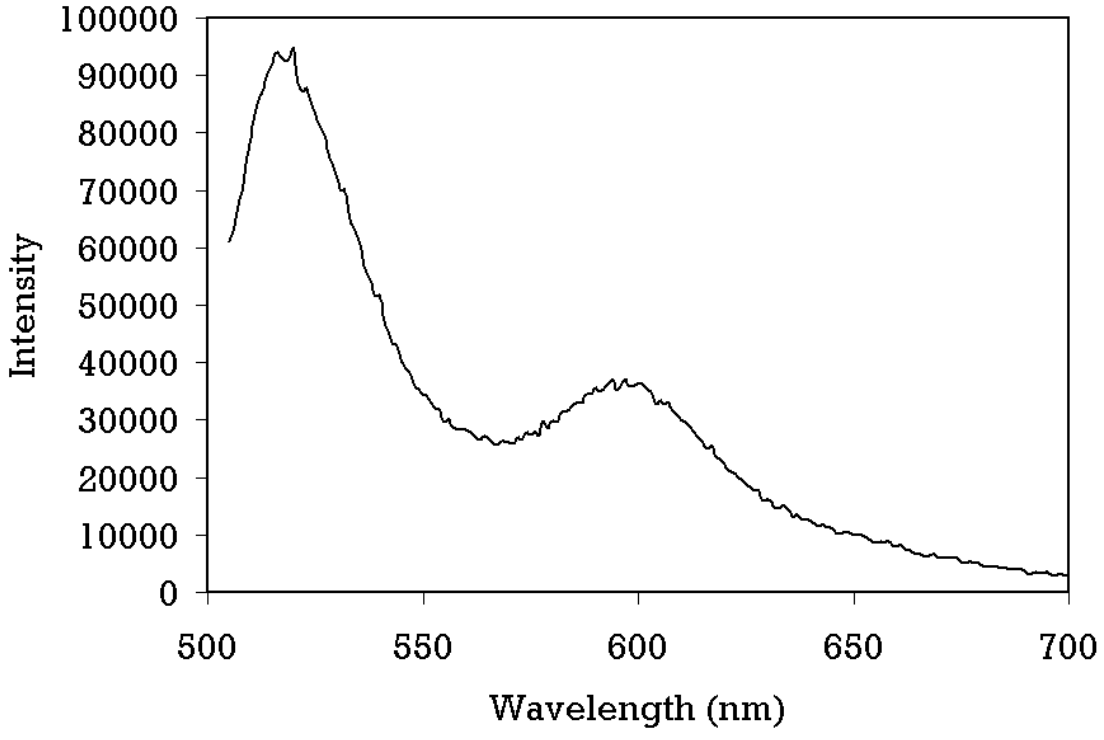

Figure 3: Spectrum of Alexa Fluor 488/Texas Red dextran PEBBLEs (Ex = 493 nm; Alexa Fluor 488 Em = 517 nm, Texas Red = 600 nm).

Figure 4 shows the pH dependence of Alexa Fluor dye's response to iron(III). There is an increase in the sensitivity of the dye at pH 6; the response decreases as the pH is lowered.

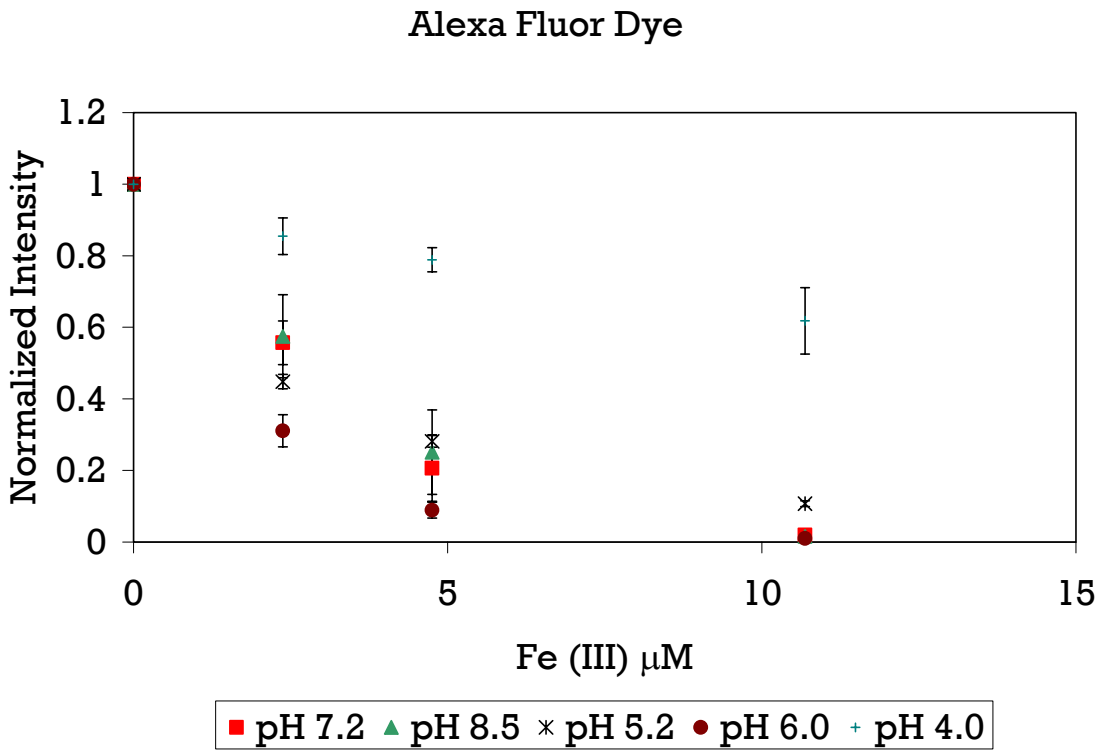

Figure 4. Dependence of Alexa Fluor dye's response to iron(III) with varying pH. The intensities were normalized by dividing resulting fluorescence from the samples by the initial fluorescence. Calibrations were completed at several pH's.

Figure 5 shows the reversibility of the Alexa Fluor 488/Texas Red PEBBLEs in the presence of desferrioxamine, a common iron chelator. After iron is added to the solution, resulting in a decrease in the intensity ratio, the chelator is added to restore the ratio to nearly 100%.

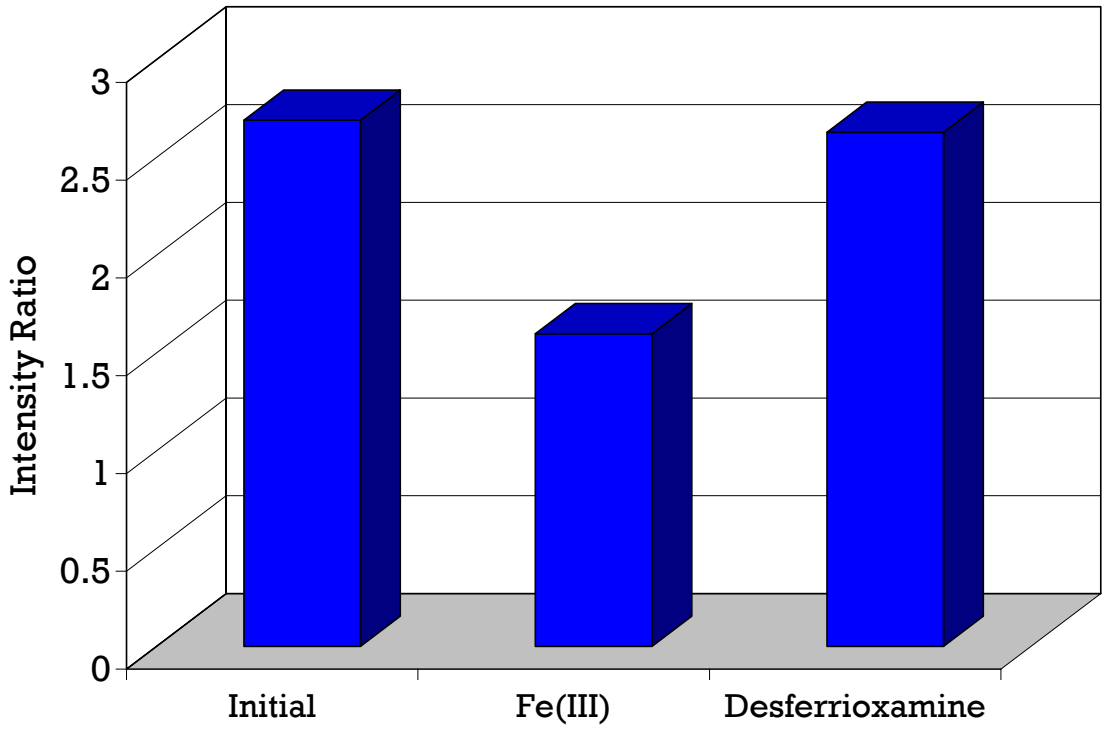

Figure 5. The fluorescence ratio of the Alexa Fluor 488/Texas Red PEBBLE was monitored as Fe^{3+} and desferrioxamine was added. The solution was allowed to equilibrate with 10 μM Fe^{3+}, then 10 μM desferrioxamine was added to complex the iron(III).

Alexa Fluor 488 dye was tested in the presence of glucose, as shown in Figure 6. The data show that glucose does not interfere with the binding of iron(III) to the Alexa Fluor 488.

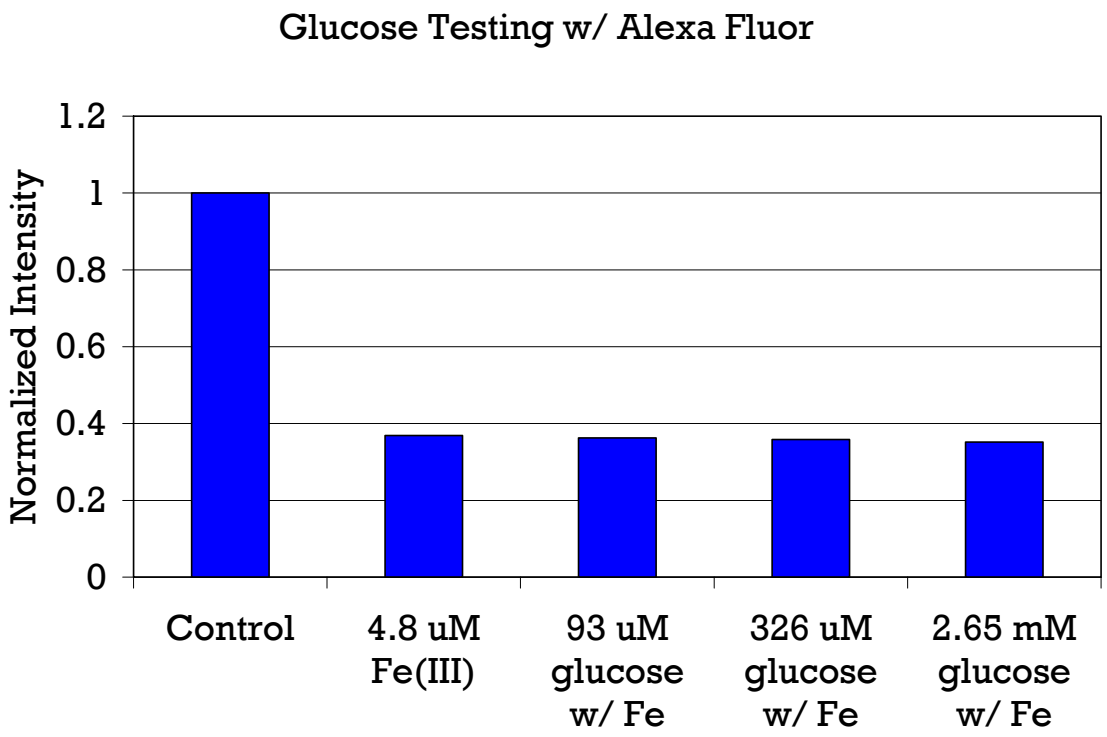

Figure 6. The effect of iron(III) on the fluorescence emission of Alexa Fluor 488, in the presence of varying glucose concentrations. The samples containing glucose also contained 4.8 μM Fe(III). The intensities were normalized to the initial fluorescence from a sample with neither iron nor glucose.