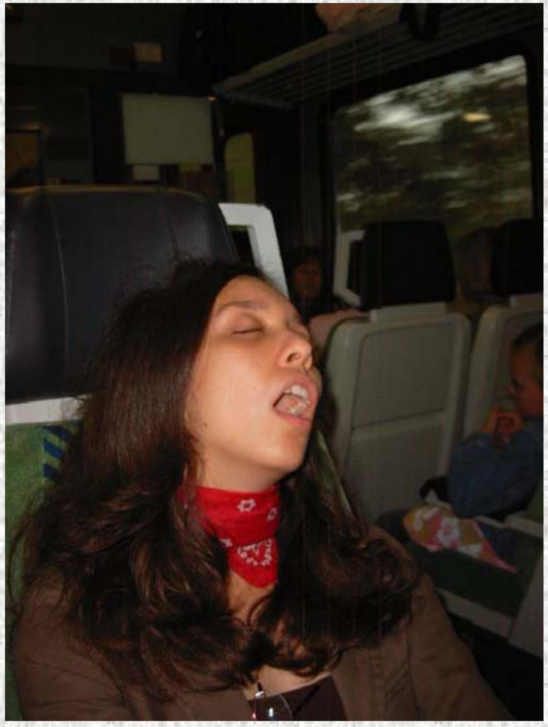
Student Session 12/09/2005

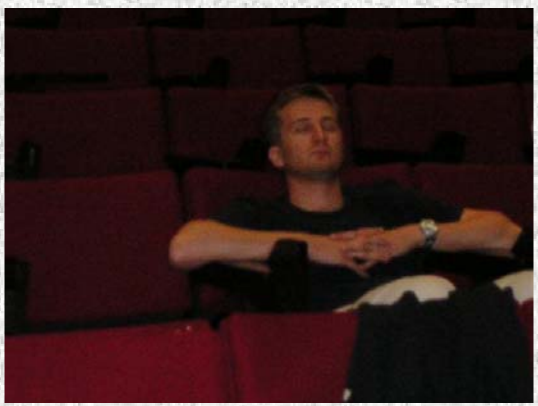
Determination of the metal-to-protein stoichiometry in metalloproteins via the quantification of the fluorescence radiation.

EMBL
European Molecular Biology Laboratory

Speaker: Dario Marrocchelli

Please don't sleep, it will not take too long…





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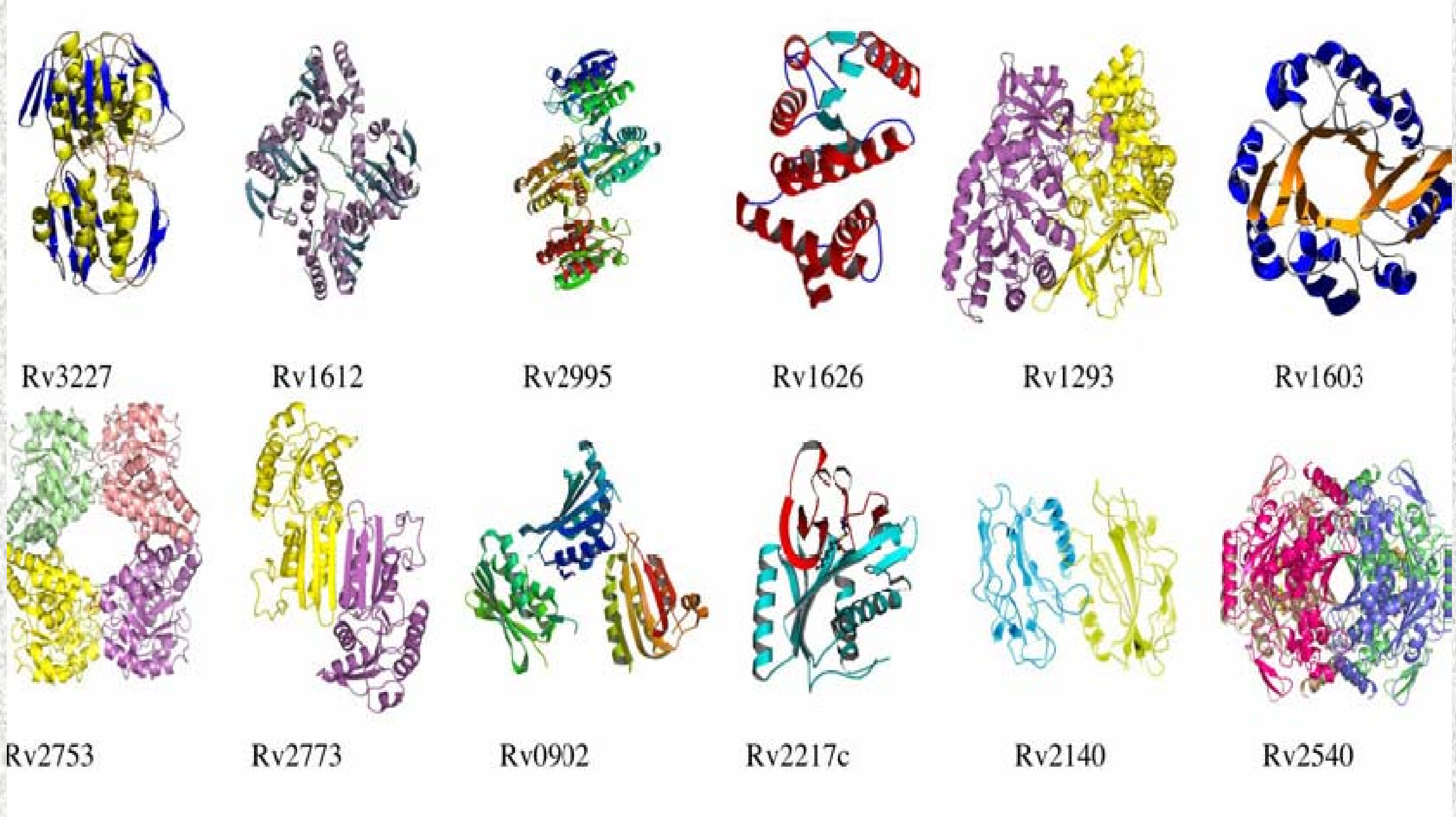
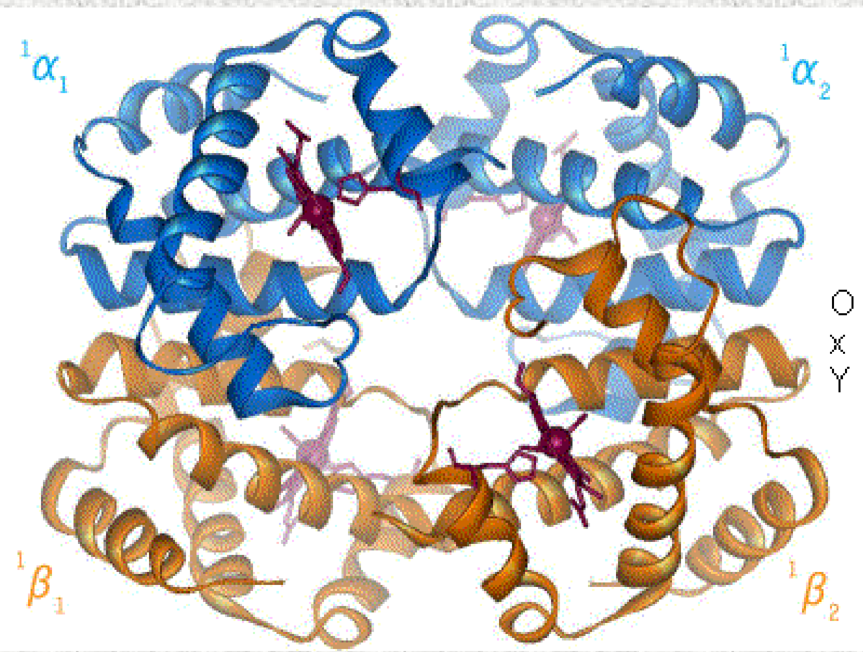
This is the subject of my project.



Proteins with metals…

About the 30% of proteins contain metals; one of the most famous one is hemoglobine, which contains some irons. Usally a metal is responsible for the function of the protein.



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What did I do?

I started finding a way to determine the amount of metal in a protein using fluorescence radiation.

My project can be divided into two parts:

1. Theoretical;
2. Experimental (much more difficult).

Fluorescence

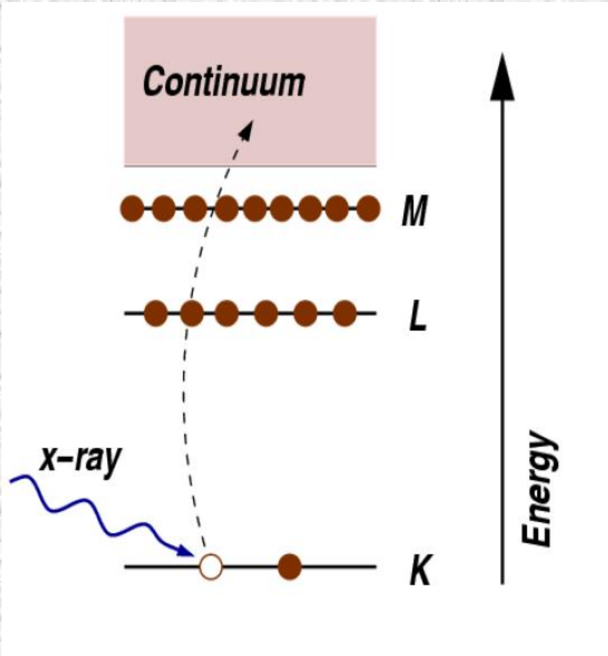
We are going to determine the metal content using fluorescence; so now Homer says:

What is fluorescence?

How can we relate fluorescence data with the amount of metal?

Fluorescence I

What we do is sending some X-rays on the sample; these can interact with an atom, pushing some electrons in the continuum.



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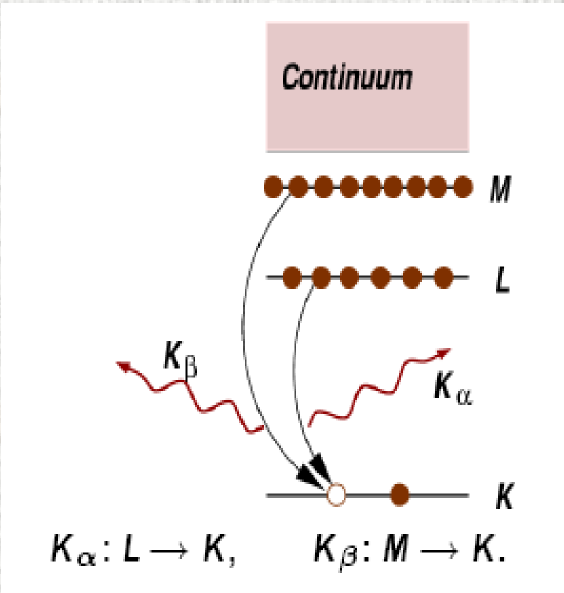
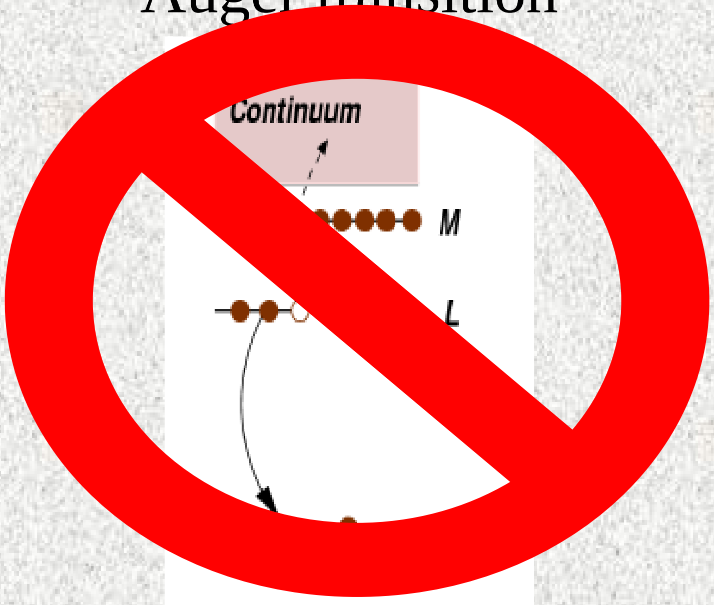
Fluorescence II

The atom is in an excited state; there are two processes with which it comes back to the ground state:

Fluorescence



Auger transition



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Fluorescence vs Auger Transition

- **Auger transition**: the atom comes back to the ground state by emitting another electron. This processe is non radiative.
- **Fluorescence**: an electron from the outer shell fills the gap in the inner shell and the remaining energy is emitted by a photon.

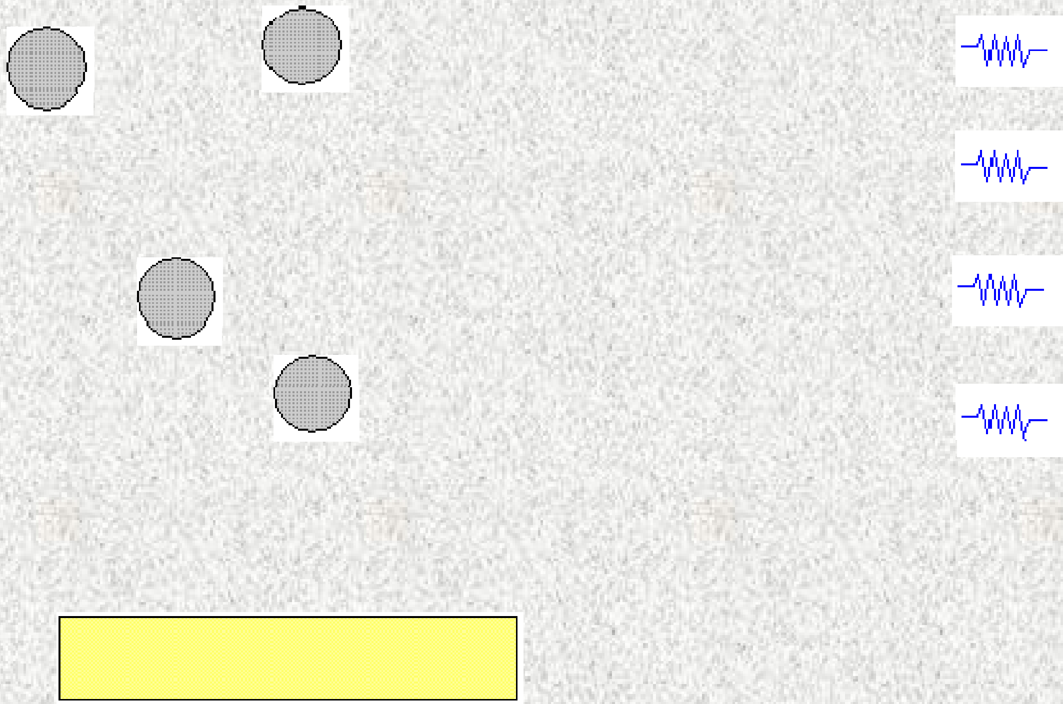
Obviously these two processes are in competition. For this reason we introduce the fluorescence/Auger yields, i.e. the relative probability for one process to happen.

Photon distribution.

So, now, what is the number of outcoming photons proportional to:

1. Number of incoming photons;
2. Cross section (i.e. the probability that a photon ionize an atom);
3. Concentration of atom (the more atoms, the bigger probability of interaction);
4. Fluorescence yield (not all the ionized atoms emit photons);
5. Many other factors: detector efficiency, absorptions.

A simple animation.

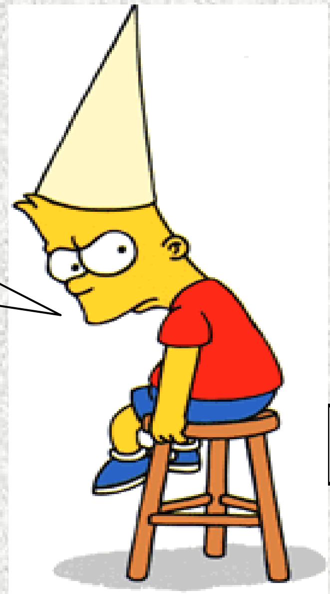


The final formula:

$$Y_0(Z) = \frac{N_{inc.} C_Z \omega_Z b_Z^{\alpha} \varepsilon_Z \mu(E) N_A}{A_Z} \int_{E_0}^{E_t} \frac{\sigma_Z(E) T_Z(E) dE}{S(E)} \times$$

$$e^{-\mu(E)_{inc} x_{inc} - \mu(E)_{out} x_{out} - \mu(E)_{air} x_{air}}$$

That's too difficult!



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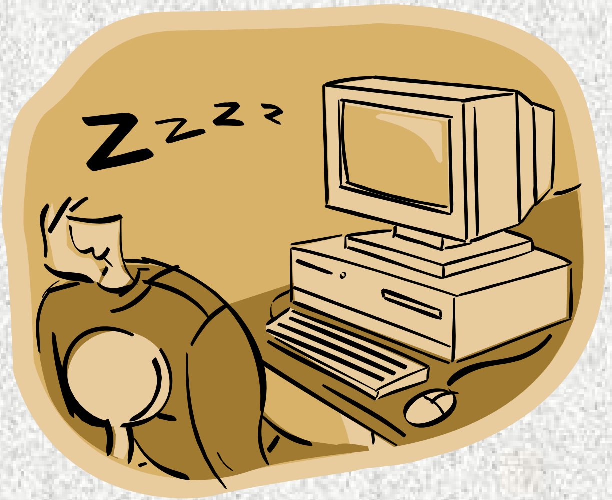
Problem I

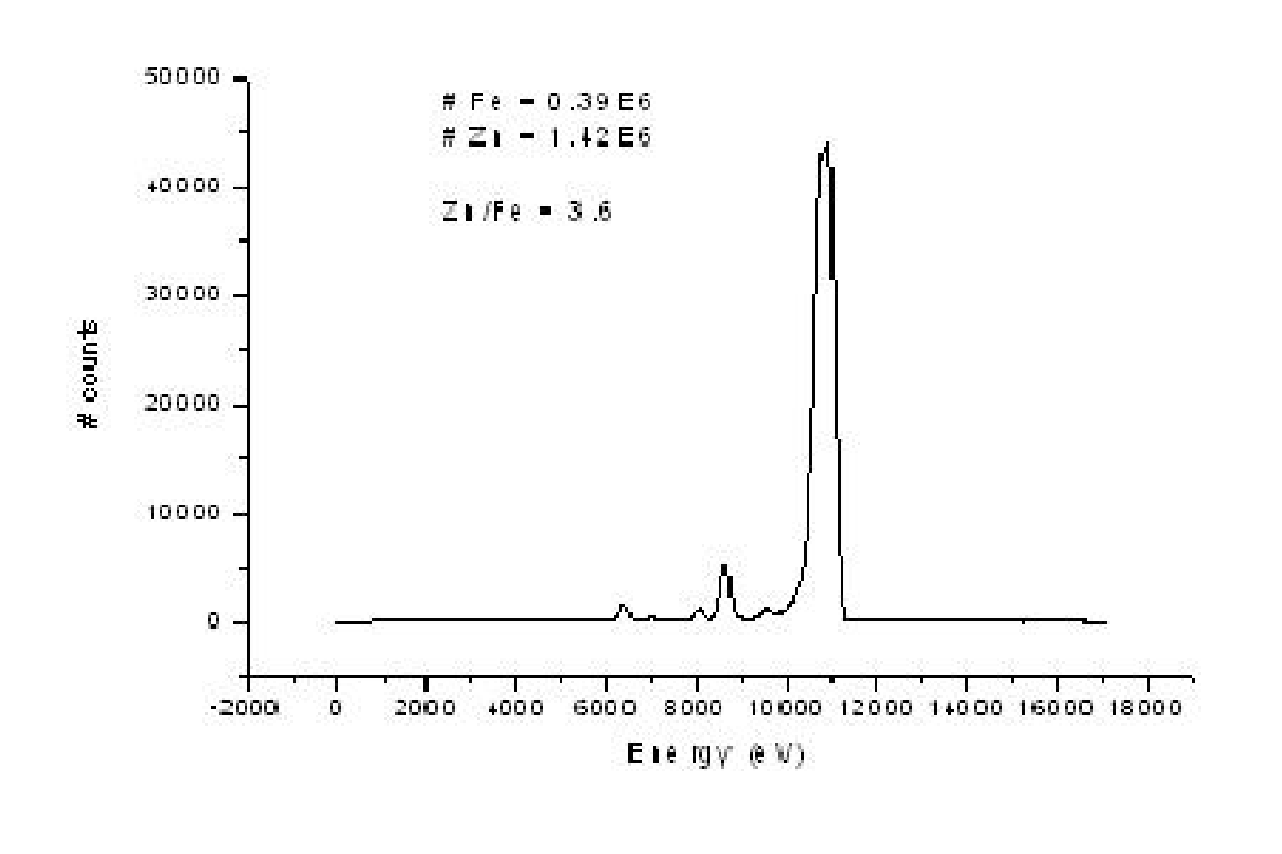
In the preceding formula everething is either measurable or known by theoretical calculations; so theoretically we “only” have to measure the number of outcoming photons and infer the metal content. But this is easier said than done; practically it is almost impossible, if you don’t use a program.

Problem II



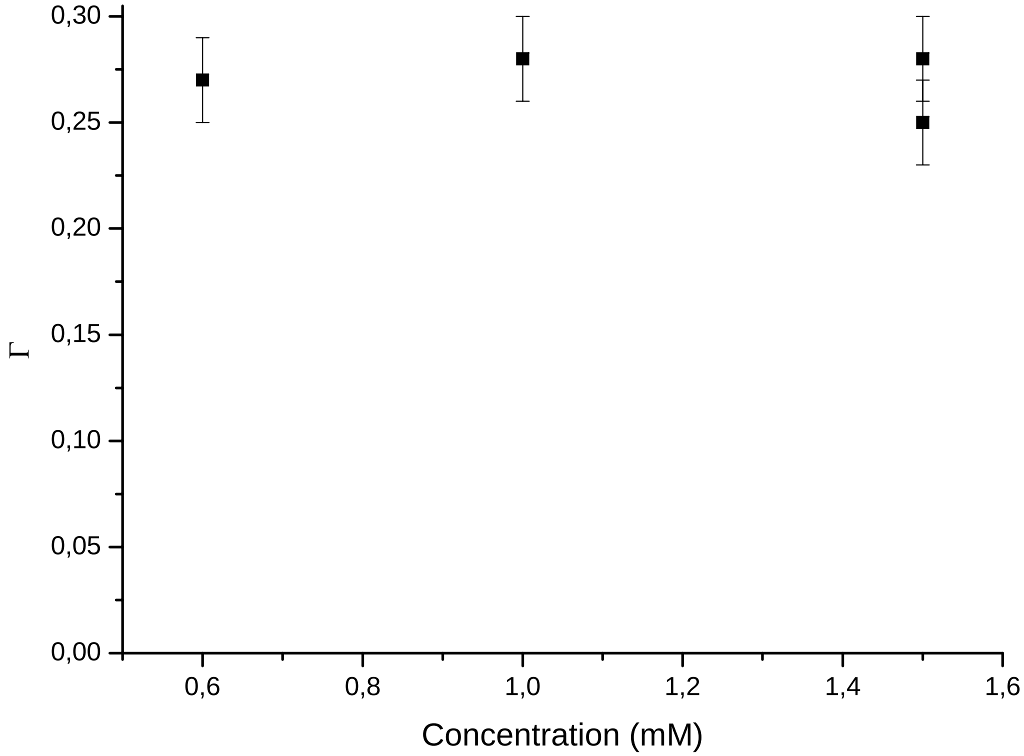
I am lazy and I hate programming!!

First let's see if the theory works, and then…



$$R = \frac{C_1}{C_2} \times \Gamma$$

At low concentrations constants are constant…



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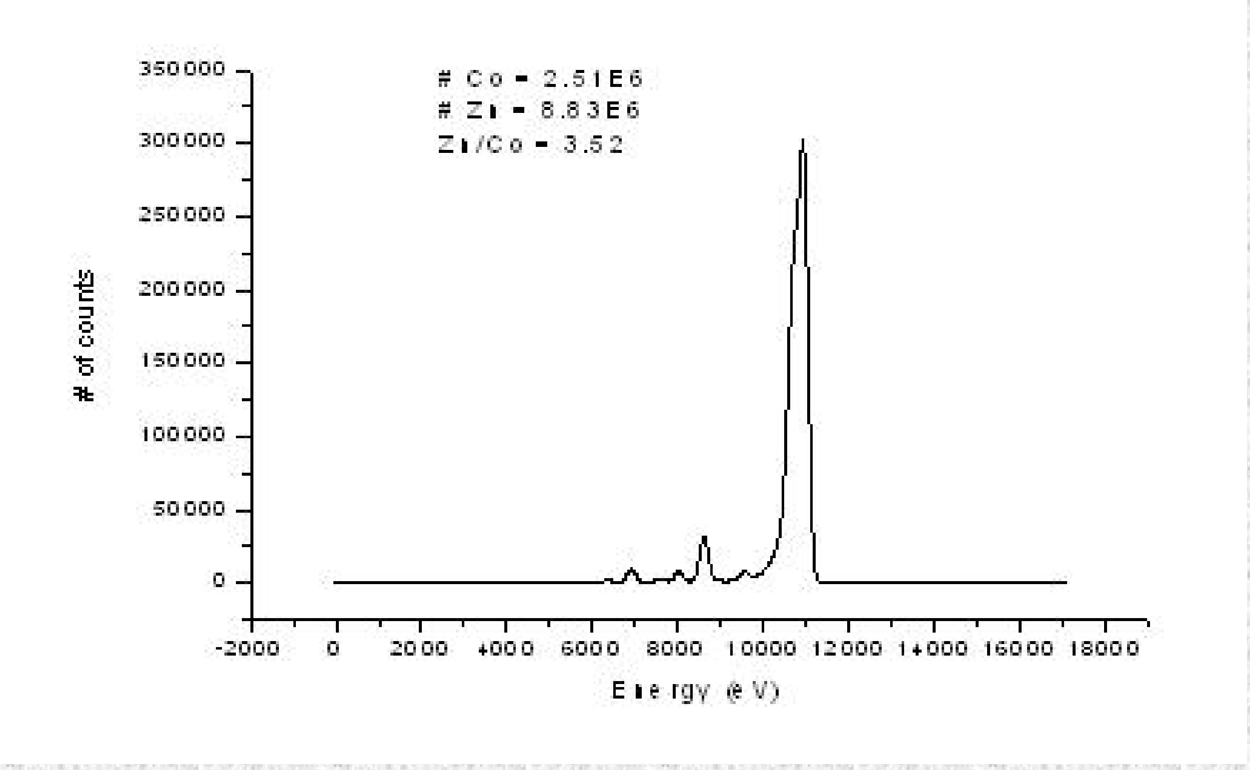
One application: MTB FurB.



$$\frac{Zn}{Co} = 1$$

In agreement with the protein praparation procedure!

How about results?

WORK IN PROGRESS

End

Results.

1. I found a theoretical approach to relate fluorescence radiation with the metal content in proteins;
2. I proved the theoretical approach to work (in a simplified case);
3. I applied this approach to a practical case (MTB FurB) and had some consistent results.

Thank you for the attention!

And remember…

Los neutrinos no tienen
massa!