Load the instruction files:

File>>open>>constrants>>kens\_constants\_jun14\_2005.TCM

**To get free Ca2, Mg2 and MgATP:**

Calculations>>type>>select total (Why total? We know the final free Ca2 and Mg concentration, we need to calculate total amounts need to add)

Free Metal>>adjust temp, pH and ionic strength >>OK>>select ATP, CRPHOSP, EGTA, Ca2, Mg2>>OK>>input the free amounts you want >>ok (you need to repeat this step with various ATP concentration so that we have 1mM Mg2 and 4mM MgATP free whatever amount of Ca2 you want to).

Save this result file and don’t close this result file

Without deleting the result window, calculation>>2D>>select Ca2>>low and high free Ca2 values (since we have selected type of calcuation as "total", input needs to be free amount we want)

Click “Edit”>>”To output”>>

Save this result file

Oen matlab "process.m" file. Change file location and output location and name on line 3 and 5. Run the code.

**Finding Ionic Strength of Immidazoline**

On max chelator pH Buffers>>other.bcm>>addjust paramenters>>select IMIDAZOLE>>input 0.020 in buffer amount, (yellow box) and hit "Calc Ionic" button, the value under 3 is the ionic strength.