

DISCLAIMER

This workbook was written using Excel X on Mac OS X. Whilst it will probably work on recent versions of Excel for windows, you may experience some problems.

If you benefit from the use of this spreadsheet, please be kind enough to provide an acknowledgment in any publications resulting from its use.

How to Use This Workbook

This workbook has two operating modes:

1. 'Fit Individually', which allows each titration curve to be solved on a case-by-case basis
2. 'Global Fit', which fits all of the titration data to a common K value

By default, the workbook will open in the individual fitting mode. After the first data series on the sheet 'Data1' has been subjected to curve fitting procedures, the workbook may be toggled between the two modes using the buttons on this sheet. Data generated in each fitting mode should be maintained during the switch.

1. Start on the worksheet 'Data1'. Enter in the appropriate data for **Initial Vol**, **[H] stock**, and **Vol Added**

Note 1: **Initial Vol** is the starting solvent volume in the NMR tube. **Vol added** is the volume of host solution (cumulative) added at each point during the dilution These need to have the same units.

Note 2: All of the above values are automatically copied to all the other worksheets

Note 3: This workbook works on the assumption that a stock host solution is added progressively to the NMR tube to produce an increasing concentration - the reverse of a dilution, but experimentally easier.

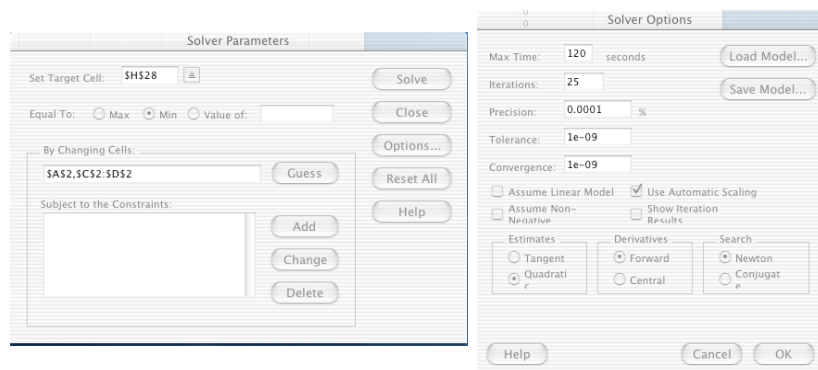
Note 4: The data entered on the first sheet should include the full range of data points without any gaps

2. Enter in the signal name

3. Enter in the observed chemical shift data

4. Enter initial values for **K**, **free** etc (blue-green cells).

5. Run the Solver ('Tools' menu) to minimise the sum of least squares of the residuals. Typical parameters are shown below:



6. You may optionally run Solver to minimise the weighted sum of least squares. Weighting is by the 1/Y² method.

7. Repeat for all signals.

8. Most of the data is automatically copied to the 'Summary' worksheet. You will need to fill in the missing fields before printing.

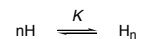
- Note 1: As with the data in the 'temp' worksheet, the fields in the 'Summary' worksheet will not be updated until the worksheet is saved or recalculated.
- Note 2: All of the worksheets, with the exception of 'temp2' are pre-formatted for printing.
- Note 3: Association constants are calculated as the mean of the signals fitted, weighted by the observed chemical shift change for each signal. Percentage bound is calculated according to the maximum calculated concentration of each of the host:host dimers.

Global Fitting Procedure

1. On the worksheet 'Data1'. Make sure that the data for **Initial Vol, [H] stock** and **Vol Added** are correct
2. Enter/modify in the chemical shift and binding constant parameters found on the sheet 'Data1' in the new table at the bottom of the sheet
3. Run the solver as described above
 - Note 1: The target cells are \$H\$26 for least squares fitting and \$H\$27 for weighted least squares fitting
 - Note 2: You will need to select the correct cells to change during the fitting. For example, to fit all the data on sheets 1 to 4 the target cells would be: \$J\$28,\$K\$30:\$L\$33

Theory

This workbook fits data to binding isotherms for the following equilibrium:



where K is the microscopic association constant and $n = 2$

The following equation is used to calculate the binding isotherm:

$$\delta_{obs} = \left[\frac{nK[H]^n(\delta_{bnd} - \delta_{free})}{[H]_o} \right] + \delta_{free}$$

where

- δ_{free} is the free chemical shift of the host
- δ_{obs} is the observed chemical shift
- δ_{bnd} is the limiting complexation-induced chemical shift change of the host in the dimeric complex
- $[H]_o$ is the initial concentration of the host
- $[H]$ is the concentration of the free (uncomplexed) host

The concentration of the dimeric complex is calculated iteratively by solving the following equations:

$$[H_n] = \frac{1 + 2nK[H]_o - \sqrt{(1 + 2nK[H]_o)^2 - 4K^2n^2[H]_o^2}}{2n^2K}$$

The concentration of free host is calculated according to the concentration difference:

$$[H] = [H]_o - n[H_n]$$

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