DISCLAIMER

This workbook was written using Excel 2004 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.

If you are having difficulties using the workbook, please check the FAQs at the end of this document.

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 common K values

Conventions: Cells into which data may be entered are coloured yellow or red. Cells to adjust during solver calculations are coloured
pale green

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.
>   *Note:*   In the global fitting mode, the parameters for 'Fit Type', 'Fit with HH' and 'Fit with GG' (see below) are shared across all
>   of the sheets and should only be set by making the appropriate selections on the sheet 'Data1'

1. Start on the worksheet 'Data1'. Enter in the appropriate data for **Vol Host**, **[H] stock**, **[G] stock**, and **Vol Guest**
>   *Note 1:*   **Vol host** is the starting volume of the host solution in the NMR tube. **Vol guest** is the volume of guest
>   (cumulative) added at each point during the titration. These need to have the same units. It is assumed
>   that the guest solution contains the host at the same concentration as the starting (stock) host solution.
>   In this case, the host concentration (the chemical shifts of which are being measured) remains constant throughout
>   *Note 2:*   All of the above values are automatically copied to all other worksheets
>   *Note 3:*   The data entered on the first sheet should include the full range of concentrations used in the experiment

2. Enter in the signal name

3. Enter in the observed chemical shift data

4. Enter in the association parameters for dimerisation of the host and guest (**Khd**, **Kgd** *etc*) as appropriate
>   *Note 1:*   **δ free dim** and **δ bound dim** are the free and bound chemical shifts of the host in the titration
>   *Note 2:*   Separate dilution experiments will need to be performed on the host and guest to determine
>   their association (dimerisation) constants and complexation-induced chemical shift changes

5. The calculations that the Solver perfoms can be controlled using the 'Fit Type' drop down menu (see right):
>   *Note 1:*   If 'HG (1:1)' is selected, the concentrations of multimeric complexes will appear as '0' under a column labelled 'n/a'
>   Fits for 1:2 and 2:1 complexes can be made by selecting the appropriate option using the combo box.
>   *Note 2:*   For host and guest, checking the appropriate boxes enables parameters for dimerisation to be included during
>   regression. If you do not wish to fit dimerisation parameters, leave the boxes unchecked and the red boxes
>   at the top of the sheet empty.
>   *Note 3:*   The main function of the combo box is to allow selection of the appropriate complex stoichiometry for the curve
>   fitting procedure. However, it does also allow progressive data fits for difficult data sets to be performed - *ie* by fitting
>   a 1:1 stoichiometry first, followed by 1:2 or 2:1. This is useful when direct attempts to fit data to 2:1 or 1:2
>   isotherms generates error values.

6. Enter initial values for **K1**, **K2**, **δfree** *etc* (blue-green cells). These may be guessed based on experience, or obtained using
>   the global fitting procedure.

7. Run the Solver. There are two methods for doing this:

>   **Method A:** The traditional method. Run the Solver ('Tools' menu) to minimise the sum of least squares of the residuals. Typical parameters are shown below:
>   You may optionally run Solver to minimise the weighted sum of least squares (Target Cell: H29). Weighting is by the $1/Y^2$ method.
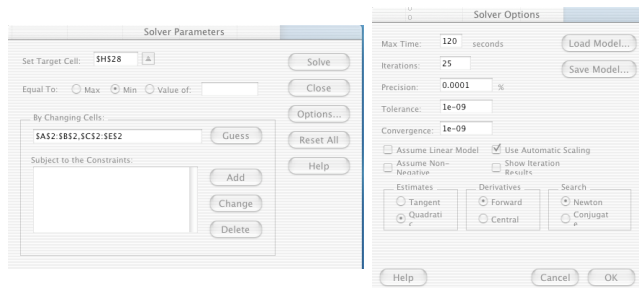

>   **Method B:** The quick method. Select the cells that you want the solver to change, choose the appropriate least squares calculation
>   from the drop down menu and click the 'Solve' button.
>   *Note 1:*   Remember that in Excel, non-contiguous cells may be selected by holding down the Apple key (Mac) or Control key (windows)
>   *Note 2:*   If the 'Reuse Solver Params.' box is checked, the solver will run with the same paramteters (Set cell, Target cells, precision *etc*)
>   as the previous run.
>   *Note 3:*   This procedure runs the solver with a standard set of values. If you want to change these, you will need to edit the 'SolveIt' macro.
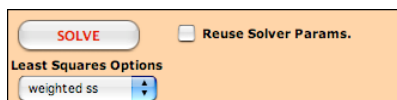
8. Set up the plot by clicking on the 'Tidy up Plot' button.
>   *Note 1:*   This will only work after the curve fitting has been performed.
>   *Note 2:*   This should produce a reasonable plot. You can also modify the colour of the data points, fit line and background
>   using the drop down menus to the right of the plot, as well as by the usual Excel methods.
>   *Note 3:*   The chart data may not appear until the worksheet is recalculated (or saved).

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9. Repeat for all signals.

10. 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 chart data, 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:guest complexes.

Global Fitting Procedure

1. On the worksheet 'Data1'. Make sure that the data for **Vol Host**, **[H] stock**, **[G] stock**, and **Vol Guest** 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

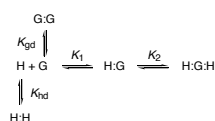**3.** Run the solver as described above

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 to a 1:1 binding isotherm, the target cells would be: \$J\$28,\$K\$30:\$L\$33

Theory

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

1. Host:Host:Guest (2:1) complexes

$$
\begin{array}{c}
\mathrm{G:G} \\
K_{gd} \updownarrow \\
\mathrm{H+G} \xrightleftharpoons{K_1} \mathrm{H:G} \xrightleftharpoons{K_2} \mathrm{H:G:H} \\
\updownarrow K_{hd} \\
\mathrm{H:H}
\end{array}
$$

where K1 and K2 are the microscopic association constants. Khd and Kgd are the association constants for dimerisation of the host and guest respectively.

The following equation is used to calculate the 2:1 binding isotherm:

$$
\delta_{obs} = \left[\frac{K_1[G](\delta_{obs1} - \delta_{free}) + 2K_2[HG](\delta_{obs2} - \delta_{free}) + 2K_{hd}[H](\delta_{di} - \delta_{free'})}{1 + K_1[G] + 2K_2[HG] + 2K_{hd}[H]} \right] + \delta_{free}
$$

where
- δ_{free} is the free chemical shift of the host in the titration
- $\delta_{free'}$ is the free chemical shift of the host obtained from the dilution experiment
- δ_{obs} is the observed chemical shift
- δ_{obs1} is the limiting complexation-induced chemical shift change of the host in the 1:1 complex
- δ_{obs2} is the limiting complexation-induced chemical shift change of the host in the 2:1 complex
- δ_{di} is the limiting complexation-induced chemical shift change of the host:host dimer
- $[H]o$ and $[G]o$ are the initial concentrations of host and guest respectively
- $[H]$ and $[G]$ are the concentrations of free (uncomplexed) host and guest respectively

The concentrations of the 1:1, 2:1, host:host and guest:guest complexes are calculated iteratively by solving the following equations:

$$
[HG] = \frac{1 + K_1([H]_o + [G]_o - 3[H_2G] - 2[H_2] - 2[G_2]) - \sqrt{\{1 + K_1([H]_o + [G]_o - 3[H_2G] - 2[H_2] - 2[G_2])\}^2 - 4K_1^2([G]_o - [H_2G] - 2[G_2])([H]_o - 2[H_2G] - 2[H_2])}}{2K_1}
$$

$$
[H_2G] = \frac{1 + K_2([H]_o + 2[G]_o - 2[G] - [HG] - 2[H_2] - 4[G_2]) - \sqrt{\{1 + K_2([H]_o + 2[G]_o - 2[G] - [HG] - 2[H_2] - 4[G_2])\}^2 - 8K_2^2([G]_o - [G] - 2[G_2])([H]_o - [HG] - 2[H_2])}}{4K_2}
$$

$$
[G_2] = \frac{1 + 4K_{gd}([G]_o - [HG] - [H_2G]) - \sqrt{\{1 + 4K_{gd}([G]_o - [HG] - [H_2G])\}^2 - 16K_{gd}^2([G]_o - [HG] - [H_2G])^2}}{8K_{gd}}
$$
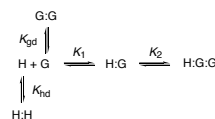
$$
[H_2] = \frac{1 + 4K_{hd}([H]_o - [HG] - 2[H_2G]) - \sqrt{\{1 + 4K_{hd}([H]_o - [HG] - 2[H_2G])\}^2 - 16K_{hd}^2([H]_o - [HG] - 2[H_2G])^2}}{8K_{hd}}
$$

The conentrations of free host and guest are calculated according to the concentration differences:

$$
[H] = [H]_o - [HG] - 2[H_2G] - 2[H_2]
$$

$$
[G] = [G]_o - [HG] - [H_2G] - 2[G_2]
$$

2. Host:Guest:Guest (1:2) complexes

$$
\begin{array}{c}
\mathrm{G:G} \\
K_{gd} \updownarrow \\
\mathrm{H+G} \xrightleftharpoons{K_1} \mathrm{H:G} \xrightleftharpoons{K_2} \mathrm{H:G:G} \\
\updownarrow K_{hd} \\
\mathrm{H:H}
\end{array}
$$

where K1 and K2 are the microscopic association constants. Khd and Kgd are the association constants for dimerisation of the host and guest respectively.

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The following equation is used to calculate the 1:2 binding isotherm:

$$\delta_{obs} = \left[\frac{K_1[G](\delta_{obs1} - \delta_{free}) + K_1 K_2 [G]^2 (\delta_{obs2} - \delta_{free}) + 2K_{hd}[H](\delta_{di} - \delta_{free'})}{1 + K_1[G] + K_1 K_2 [G]^2 + 2K_{hd}[H]} \right] + \delta_{free}$$

where δfree is the free chemical shift of the host in the titration
δfree' is the free chemical shift of the host obtained from the dilution experiment
δobs is the observed chemical shift
δobs1 is the limiting complexation-induced chemical shift change of the host in the 1:1 complex
δobs2 is the limiting complexation-induced chemical shift change of the host in the 1:2 complex
δdi is the limiting complexation-induced chemical shift change of the host:host dimer
[H]o and [G]o are the initial concentrations of host and guest respectively
[H] and [G] are the concentrations of free (uncomplexed) host and guest respectively

The concentrations of the 1:1, 1:2, host:host and guest:guest complexes are calculated iteratively by solving the following equations:

$$[HG] = 0.5\{[H]_o + [G]_o - 3[HG_2] - 2[H_2] - 2[G_2] + (1/K_1)\} - \sqrt{0.25\{[H]_o + [G]_o - 3[HG_2] - 2[H_2] - 2[G_2] + (1/K_1)\}^2 - ([G]_o - 2[HG_2] - 2[G_2])([H]_o - [HG_2] - 2[H_2])}$$

$$[HG_2] = 0.25\{2[H]_o - 2[H] + [G]_o - [HG] - 4[H_2] - 2[G_2] + (1/K_2)\} - \sqrt{0.0625\{2[H]_o - 2[H] + [G]_o - [HG] - 4[H_2] - 2[G_2] + (1/K_2)\}^2 - 0.5([G]_o - [HG] - 2[G_2])([H]_o - [H] - 2[H_2])}$$

$$[H_2] = \frac{1 + 4K_{hd}([H]_o - [HG] - [HG_2]) - \sqrt{\{1 + 4K_{hd}([H]_o - [HG] - [HG_2])\}^2 - 16K_{hd}^2([H]_o - [HG] - [HG_2])^2}}{8K_{hd}}$$

$$[G_2] = \frac{1 + 4K_{gd}([G]_o - [HG] - 2[HG_2]) - \sqrt{\{1 + 4K_{gd}([G]_o - [HG] - 2[HG_2])\}^2 - 16K_{gd}^2([G]_o - [HG] - 2[HG_2])^2}}{8K_{gd}}$$

The conentrations of free host and guest are calculated according to the concentration differences:

$$[H] = [H]_o - [HG] - [HG_2] - 2[H_2]$$

$$[G] = [G]_o - [HG] - 2[HG_2] - 2[G_2]$$

FAQ
Q. I've entered my data and I'm trying to calculate the binding data for a 1:2 complex, but I only get errors
A. Try to fit a 1:1 isotherm first before fitting 1:2 or 2:1 isotherms

Q. The conversion to/from global fitting mode doesn't work
A. The most likely reason for this is that you have changed the location or formulae of key cells. The macro that does the conversion expects to find key data in specific locations. If you copy the raw data (in yellow boxes) and paste them into a new sheet, it should work.

Q. When performing calculations with common K values (global fits), the data converge poorly.
A. This may be a problem with the data itself, but you should make sure that you have set up the solver correctly. If you forget to change the target cells in the global mode, you will overwrite key formulae in the cells A2:E2, and you will only be performing calculations on the sheet 'Data1'. You can rescue this situation by entering the 'individual fit' mode and then returning to the global mode.

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$$\delta_{obs} = \left[\frac{K_1[G](\delta_{obs1} - \delta_{free}) + K_1 K_2 [G]^2 (\delta_{obs2} - \delta_{free}) + 2K_{hd}[H](\delta_{di} - \delta_{free'})}{1 + K_1[G] + K_1 K_2 [G]^2 + 2K_{hd}[H]} \right] + \delta_{free}$$