PROTOCOL

1. At the end of an ITC experiment the user will initially integrate the heat released or absorbed in each injection.

2. Following integration, a text file should be saved in ASCII format containing data series for the injection volume (V_i), macromolecule concentration (M_T), molar ratio (X_T/M_T), and heat of injection (ndh). E.g., xyz.DAT

3. The file generated in step 2, along with the active cell volume (V_0) and syringe concentration of ligand (X_0), are used as inputs into the Matlab fitting macro (ITCFit.m), which fits the data to a modified single class of sites binding model as discussed above. Initial estimates of the apparent binding association equilibrium constant (K_A) and number of binding sites (n) must be provided.

1. One set of binding sites model with offset

Syntax for LM based: ITCFit(fname,V0,SyC,T,npd,startka,startn,corrected,ptype)

Syntax for nlin based: ITCFitnlin(fname,V0,SyC,T,npd,startka,startn,corrected,ptype)

Example MATLAB command:

```
ITCFit('xyz.DAT',1.42747,0.3,15,2,8e5,1,'y','ko')
```

INPUTS:

    fname: filename obtained from ITC instrument in ASCII text format ['xyz.DAT']
    V0 (V_0): Volume of Cell in ml [1.42747]
    Syc (X_0): Syringe concentration in mM [0.3]
    T: Temperature of the ITC experiment in C [15]
    npd: Number of initial points to discard [2]
    startka: initial estimate of K_A [8e5]
    startn: initial estimate of n [1]
    corrected: flag for correcting the isotherm using last 4 injection points ['y']
    ptype: plot type in figure for the data points ['ko']

OUTPUTS:

    Kd: fitted Dissociation constant
    err_Kd: error in the K_d
    Ka: fitted Association constant (=1/K_d)
    err_Ka: error in the K_A
    n: fitted stoichiometry of ligand binding to macromolecule
    err_n: error in the n
    dH: fitted enthalpic heat of interation
    err_dH: error in the ΔH
    c: fitted offset

2. One set of binding sites model (Origin based)

Syntax for LM based: ITCFit2(fname,V0,SyC,T,npd,startka,startn,corrected,ptype)

Syntax for nlin based: ITCFitnlin2(fname,V0,SyC,T,npd,startka,startn,corrected,ptype)

Example MATLAB command:

```
ITCFit2('xyz.DAT',1.42747,0.3,15,2,8e5,1,'y','ko')
```

INPUTS:

fname: filename obtained from ITC instrument in ASCII text format ['xyz.DAT']
V0 (V_0): Volume of Cell in ml [1.42747]
SyC (X_0): Syringe concentration in mM [0.3]
T: Temperature of the ITC experiment in C [15]
npd: Number of initial points to discard [2]
startka: initial estimate of Ka [8e5]
startn: initial estimate of n [1]
corrected: flag for correcting the isotherm using last 4 injection points ['y']
ptype: plot type in figure for the data points ['ko']

OUTPUTS:

Kd: fitted Dissociation constant
err_Kd: error in the K_d
Ka: fitted Association constant (=1/K_d)
err_Ka: error in the K_A
n: fitted stoichiometry of ligand binding to macromolecule
err_n: error in the n
dH: fitted enthalpic heat of interation
err_dH: error in the ΔH