# 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 (Vi), macromolecule concentration (MT), molar ratio (XT/MT), and heat of injection (ndh). E.g., xyz.DAT

3. The file generated in step 2, along with the active cell volume (V0) and syringe concentration of ligand (X0), 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 (KA) 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 (V0): Volume of Cell in ml [1.42747]

Syc (X0): 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 Kd

Ka: fitted Association constant (=1/Kd)

err\_Ka: error in the KA

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 (V0): Volume of Cell in ml [1.42747]

SyC (X0): 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 Kd

Ka: fitted Association constant (=1/Kd)

err\_Ka: error in the KA

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