Supporting Information

Modelling of multivalent ligand-receptor binding measured by kinITC

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Data sets used for comparison with the model derived here were part of a series of previously presented and evaluated measurements as described below.[3] The following section gives the reader a short introduction into the ITC and kinITC and provides plots to give additional information on the evaluation.

Short Introduction into ITC

An ITC experiment is a thermal analysis method which allows to study physical binding interactions e.g. of ligand and receptors by detecting the evolution/absorption of heat.[1] Typically, the evolution/absorption of heat takes place with the injection of a ligand solution into the sample cell, filled with the receptor solution. When heat (Q) is absorbed or evolved during injection, the instrument automatically balances the temperature between the sample and the control cell (into a constant) and detects heat-time profile. In particular, the thermal power $P(t)$ for an injection number is measured as a function of time:

$$P(t) = \frac{dQ}{dt} \tag{1}$$

Thus, ITC is used to determine thermodynamic quantities. Each heat profile within one titration can be used to determine ΔH.

$$P(t) = \frac{dQ}{dt} = -\Delta H V_0 c'_{LP}(t), \tag{2}$$

where c_{LP} is the molar concentration of the complex and c' is its time derivative, which depends on the injection number i and thus the volume and concentration of titrated L. $P(t)$ is the time-dependent thermal power, ΔH is the molar enthalpy (the heat produced

during or at ith injection) and V_0 the starting volume of the solution in the sample cell, respectively.[2]

KinITC experiments

In an ITC experiment, the thermal signal after the injection period can also carry kinetic information in terms of relaxation kinetics that is initiated by a reactant titrated into the sample cell. Kinetic constants, k_{on} and k_{off}, for a formal bimolecular binding process (following the shown reaction scheme), can be extracted from the heat-time profiles that are detected upon the injection of a ligand.

$$L + P \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} LP \qquad (3)$$

Figure S1 shows the exemplary titration obtained by 28 sequential injections of known amounts and defined volumes of a macromolecular ligand at every injection into the sample cell, which contained the protein, here ConA.[3] In this case, ITC data for one titration experiment gives exponential injection signals. For the kinetic signal to occur, the overall binding process has to be sufficiently slower than the instrumental response function. An example is given in Figure S2 for the ligand Man(1,3,5,7,9)-S9 binding to tetrameric ConA (LBB, pH 7.4). Figure S2 compares the signals of the 2nd, 4th, 10th, 20th injections and the instrumental response (note that the intensity of each signal is normalized and the time set to t=0).

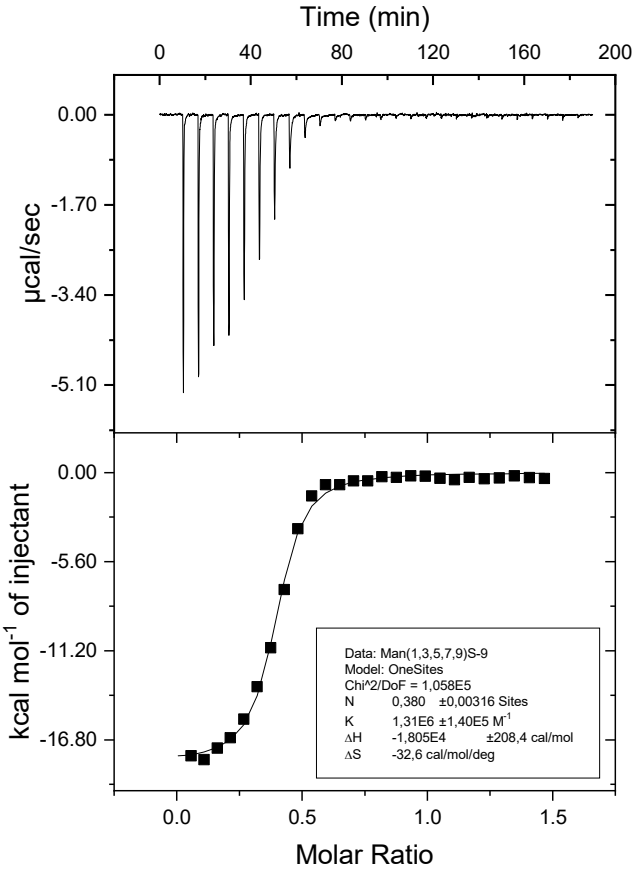

Figure S1. ITC binding isotherm for the binding of ligand Man(1,3,5,7,9)-S9 (sample 4aS in [3]) to ConA (LBB, pH 7.4) showing the heat flow signals (above, already accounted for dilution and deconvolved) and the fitted areas under the curve (below) to obtain thermodynamic data.

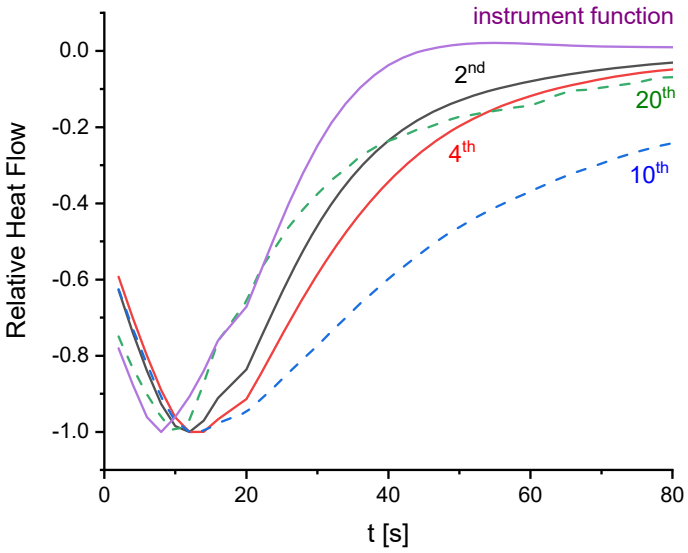

Figure S2. Comparison of the shapes of the heat flow signals for the ligand Man(1,3,5,7,9)-S9 in LBB (pH 7.4) binding to tetrameric ConA and the instrument function (k_{ITC}). For comparison, the intensities were normalized and time set to t=0.

Kinetic constants were then determined as follows [3]:

1.) Equilibrium constant K_d was determined from the titration plot, which allows to calculate the concentrations of free L, P and LP for every injection (see also Yonetani *et al.*[2]). These terms are known for every injection.

2.) According to the shape of the heat flows and the known concentrations of the free ligand, protein and complex at every injection, we used the reaction scheme

$$s_L \cdot L + s_P \cdot P \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} s_{LP} \cdot LP \qquad (4)$$

, the corresponding reaction rate equations

$$c_L'(t) = -k_{on} \cdot s_L \cdot c_L^{s_L} \cdot c_P^{s_P} + k_{off} \cdot s_L \cdot c_{LP}^{s_{LP}}, \quad c_L(0) = c_{L,0},$$

$$c_P'(t) = -k_{on} \cdot s_P \cdot c_L^{s_L} \cdot c_P^{s_P} + k_{off} \cdot s_P \cdot c_{LP}^{s_{LP}}, \quad c_P(0) = c_{P,0}, \qquad (5)$$

$$c_{LP}'(t) = +k_{on} \cdot s_{LP} \cdot c_L^{s_L} \cdot c_P^{s_P} - k_{off} \cdot s_{LP} \cdot c_{LP}^{s_{LP}}, \quad c_{LP}(0) = c_{LP,0},$$

and the formula for P(t) given above, to determine k_{on} values for every injection and one binding isotherm:

3.) We followed the relaxation time period of each heat signal evolved, thereby excluding the titration time period (injection). The procedure to determine k_{on} values for every heat flow signal is based on the rate equation of Butcher *et al.*[4], but uses another algorithm to compute the data. Specifically, the affin covariant Gauss-Newton method[5], implemented in the software code NLSCON, was used to solve the least squares minimization problem. This way from each heat profile following each injection, the k_{on} values were determined for the defined concentration range of all species involved.

4.) The determined k_{on} values at each heat profile were then summarized into one weighted average k_{on} value, which was weighted over the uncertainties as has been described in detail by Butcher *et al.*[4].

5.) From the weighted average k_{on} value and the K_d, k_{off} values were calcutated using the following expression:

$$k_{off} = k_{on} \cdot K_d \qquad (6)$$

6.) Prior to the least squares analysis for determination of k_{on} values, the raw heat flow signals were accounted for the dilution (Figure S3) and deconvolved for the instrumental response (k_{ITC}) using Tian's equation as has been described in detail in [2, 6] (Figure S4,S5).

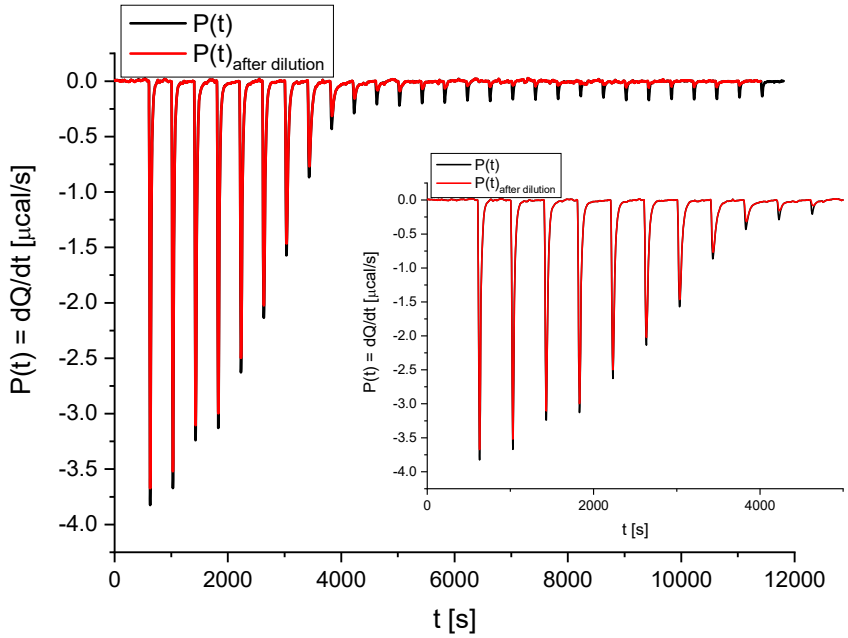

Figure S3. Measured thermal power and thermal power after accounting for dilution is shown for ligand Man(1,3,5,7,9)-S9 to ConA (LBB, pH 7.4).

Here, *Pc* corresponds to the *corrected* thermal power and k_{ITC} is the instrument time constant.[2, 4, 6] Other alternative least-squares fitting procedures to obtain k_{on} are described by Butcher *et al.*[4], Yonetani *et al.*[2] and Dumas *et al.*[6].

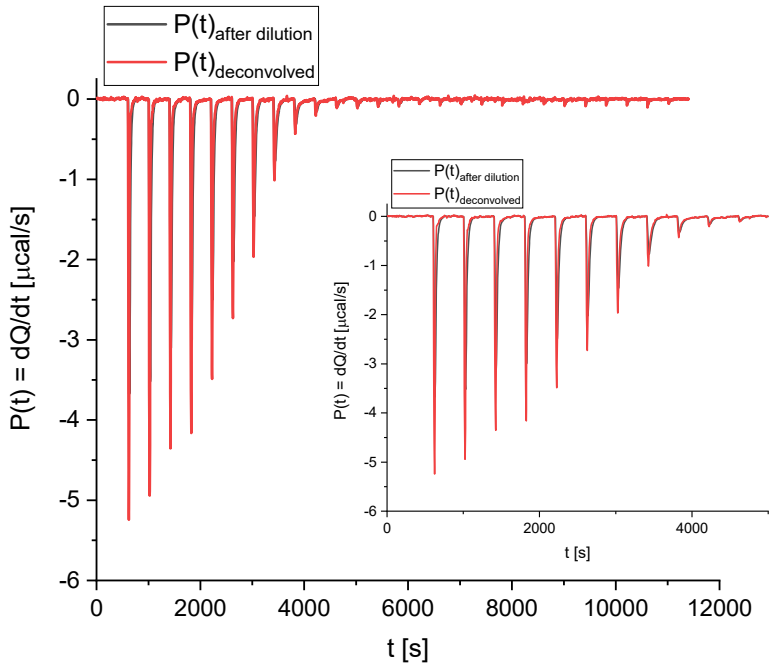

Figure S4. Thermal power after accounting for dilution for ligand Man(1,3,5,7,9)-S9 to ConA (LBB, pH 7.4) is shown compared to the signals after deconvolution using the instrumental function k_{ITC}.

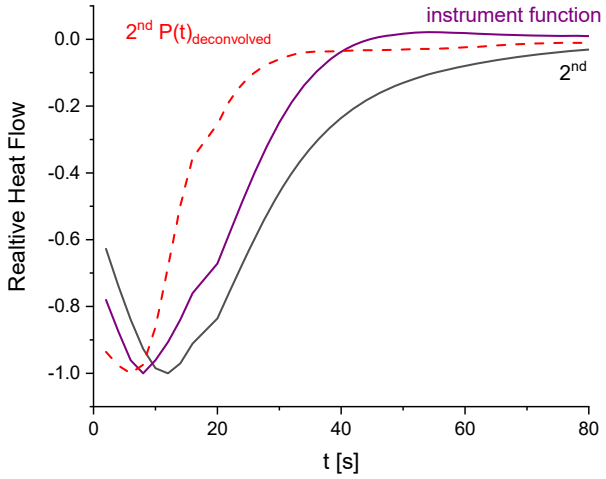

Figure S5. The second titration signal is shown and the instrument function, which yields the deconvolved heat flow signal after accounting for the instrument response. For comparison, the intensities were normalized and the time set to t=0.

Additional plots for sample Man(1,3,5)-5 (sample 3a in [3])

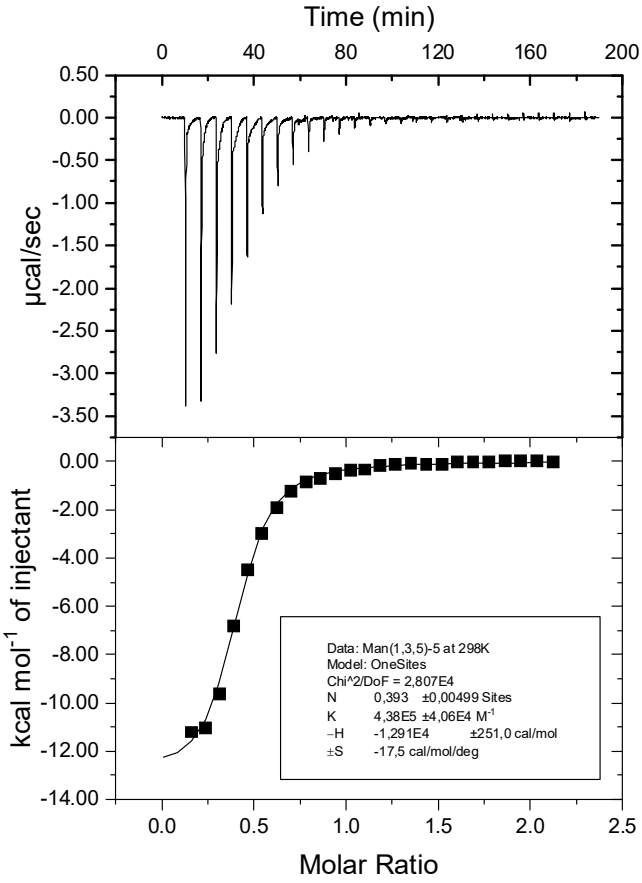

Figure S6. ITC binding isotherm for the binding of ligand Man(1,3,5)-5 (sample 3a in [3]) to ConA (LBB, pH 7.4), showing the heat flow signals (above, already accounted for dilution and deconvolved) and the fitted areas under the curve (below) to obtain thermodynamic data.

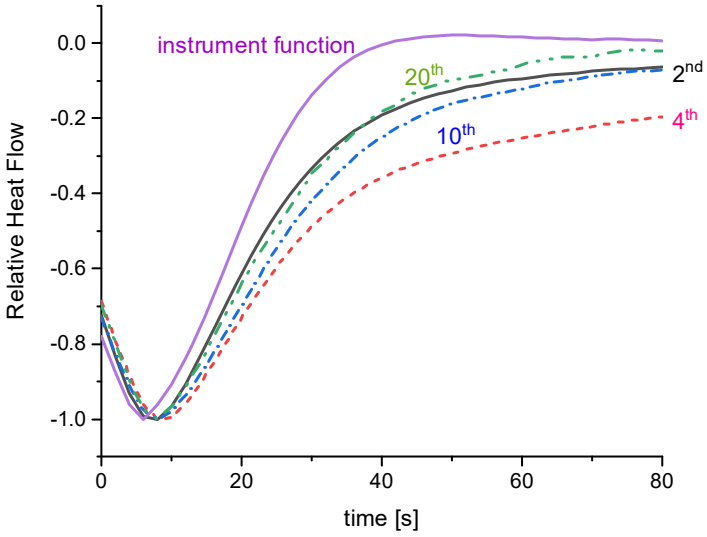

Figure S7. Comparison of the shapes of the heat flow signals for the ligand Man(1,3,5)-5 in LBB (pH 7.4) binding to tetrameric ConA and the instrument function (k_{ITC}). For comparison, the intensities were normalized and time set to t=0.

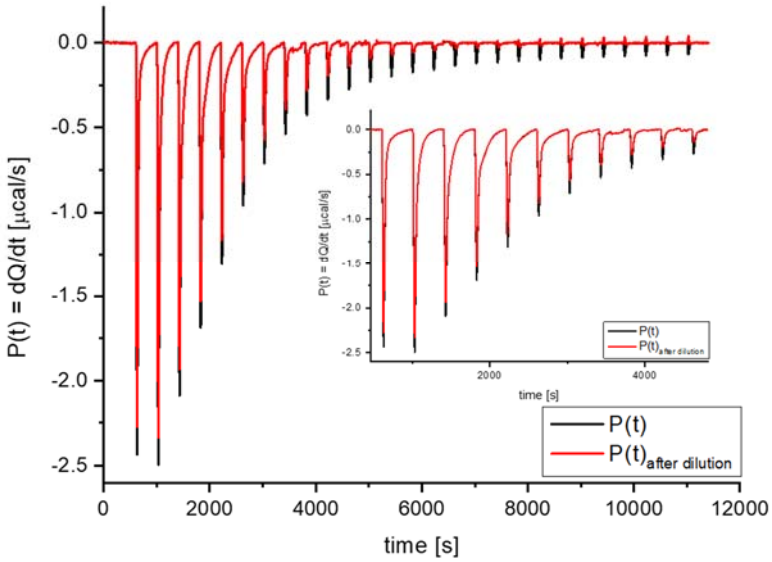

Figure S8. Measured thermal power and thermal power after accounting for dilution is shown for ligand Man(1,3,5)-5 to ConA (LBB, pH 7.4).

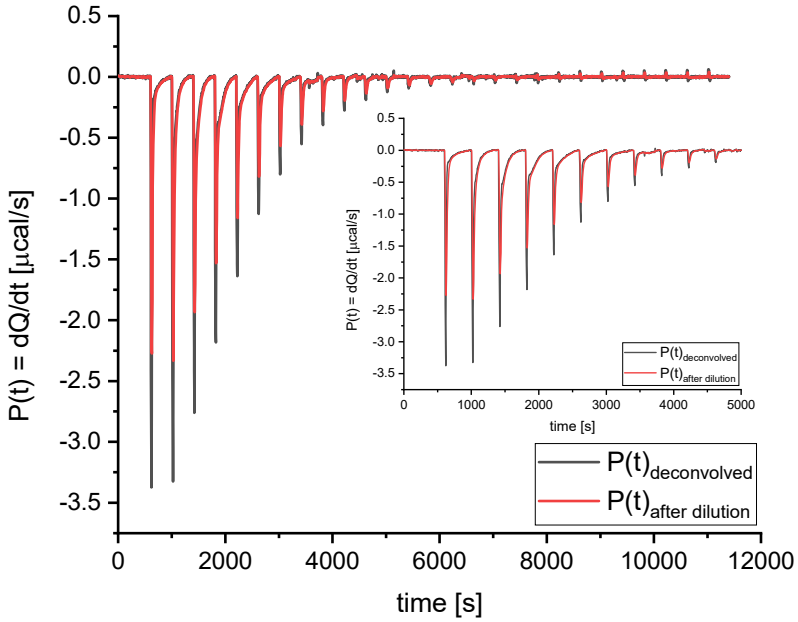

Figure S9. Thermal power after accounting for dilution for ligand Man(1,3,5)-5 to ConA (LBB, pH 7.4) is shown compared to the signals after deconvolution using the instrumental function k_{ITC}.

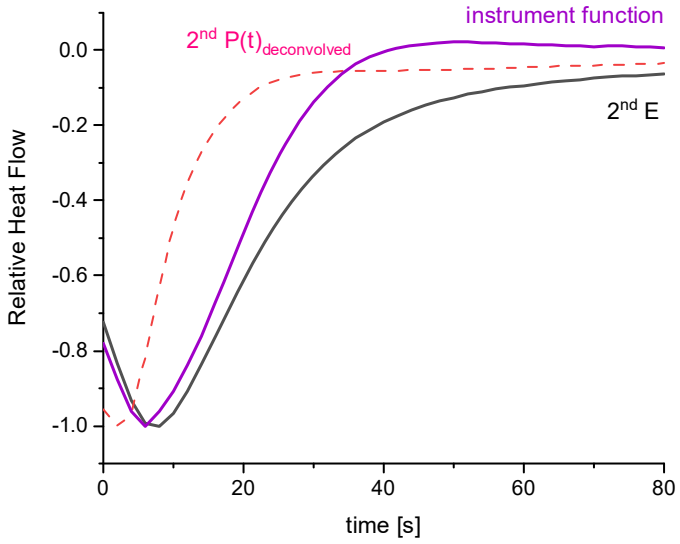

Figure S10. The second titration signal is shown and the instrument function, which yields the deconvolved heat flow signal after accounting for the instrument response. For comparison, the intensities were normalized and the time set to t=0.

Additional plots for sample Man(1,5)-5 (sample 2 in [3])

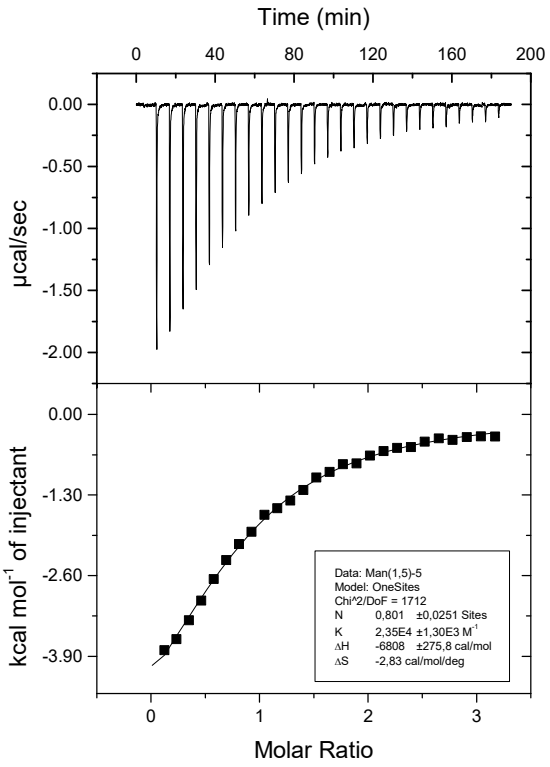

Figure S11. ITC binding isotherm for the binding of ligand Man(1,5)-5 (sample 2 in [3]) to ConA (LBB, pH 7.4), showing the heat flow signals (above, already accounted for dilution and deconvolved) and the fitted areas under the curve (below) to obtain thermodynamic data.

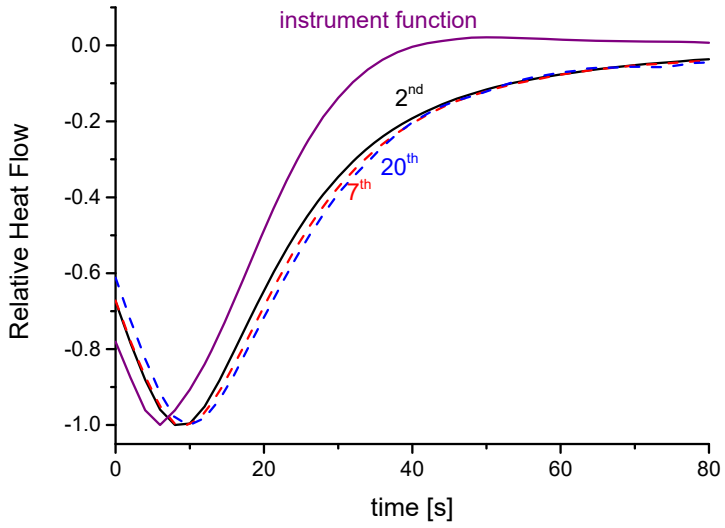

Figure S12. Comparison of the shapes of the heat flow signals for the ligand Man(1,5)-5 in LBB (pH 7.4) binding to tetrameric ConA and the instrument function (k_{ITC}). For comparison, the intensities were normalized and time set to t=0.

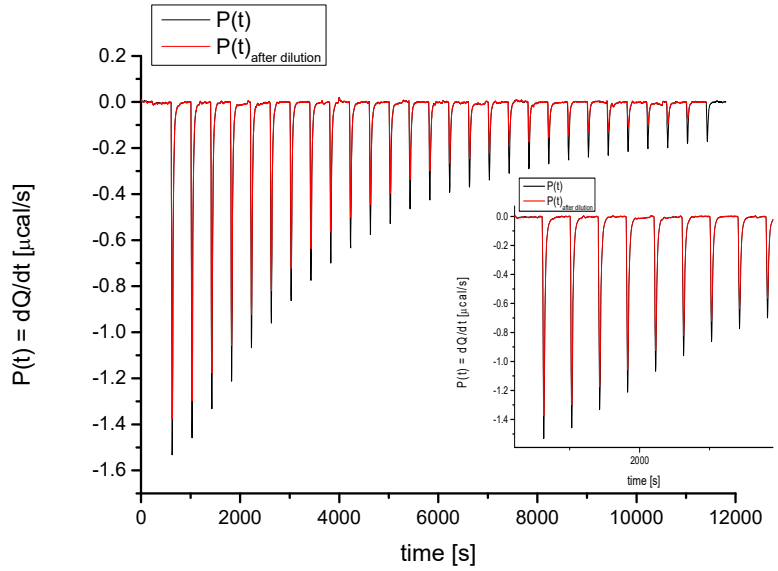

Figure S13. Measured thermal power and thermal power after accounting for dilution is shown for ligand Man(1,5)-5 to ConA (LBB, pH 7.4).

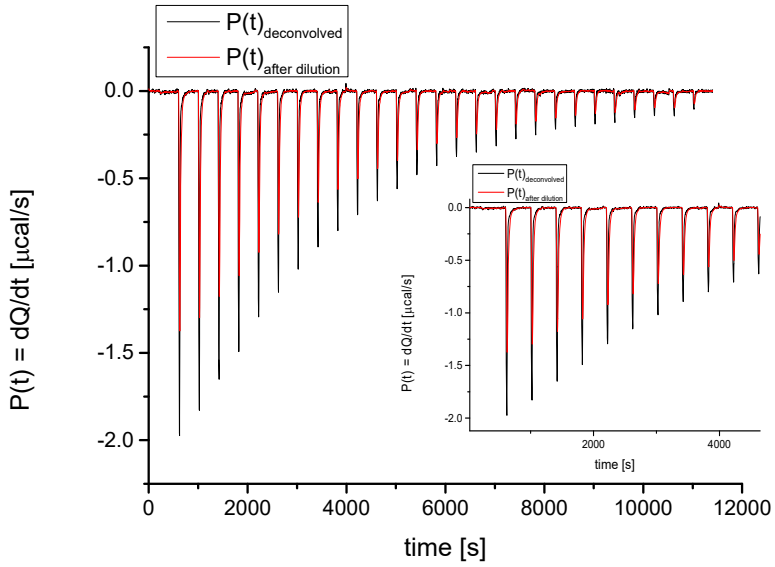

Figure S14. Thermal power after accounting for dilution for ligand Man(1,5)-5 to ConA (LBB, pH 7.4) is shown compared to the signals after deconvolution using the instrumental function k_{ITC}.

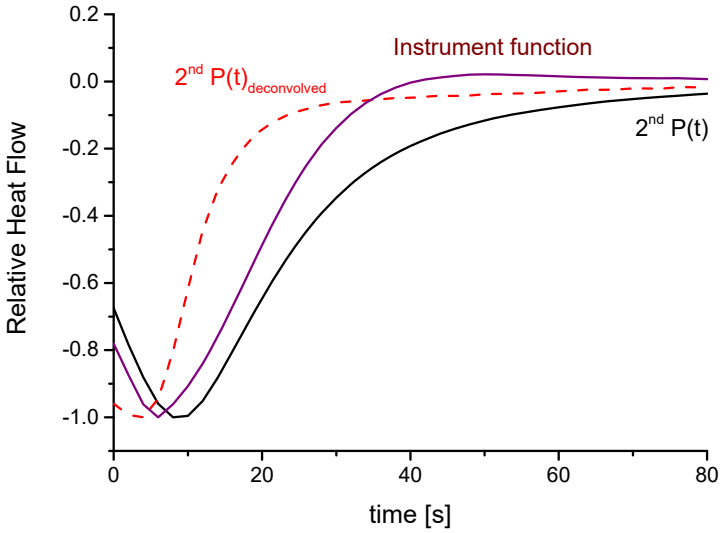

Figure S15. The second titration signal is shown and the instrument function, which yields the deconvolved heat flow signal after accounting for the instrument response. For comparison, the intensities were normalized and the time set to t=0.

REFERENCES

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