
Article:
Falconer, R.J. orcid.org/0000-0002-9912-9036 (2016) Applications of isothermal titration calorimetry - the research and technical developments from 2011 to 2015. Journal of Molecular Recognition, 29 (10). pp. 504-515. ISSN 0952-3499

https://doi.org/10.1002/jmr.2550

Journal of Molecular Recognition

Online Journal:
=Free Color
=More Color
=Faster Publication

Applications of isothermal titration calorimetry - the research and technical developments from 2011-15.

Journal:	*Journal of Molecular Recognition*
Manuscript ID	JMR-16-0004.R1
Wiley - Manuscript type:	Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Falconer, Robert; University of Sheffield, Department of Chemical and Biological Engineering
Keywords:	thermodynamics, interactions, drug discovery, enzyme kinetics, thermolysin, allostery, kinITC, ITC

Applications of isothermal titration calorimetry - the research and technical developments from 2011-15.

Robert J. Falconer*

Department of Chemical & Biological Engineering, ChELSI Institute, University of Sheffield, Sheffield, S1 3JD, England

* To whom the correspondence should be addressed. Telephone +44 114 2228253, Fax +44 114 2227501 Email r.j.falconer@sheffield.ac.uk

ABSTRACT

Isothermal titration calorimetry is a widely used biophysical technique for studying the formation or dissociation of molecular complexes. Over the last five years much work has been published on the interpretation of ITC data for single binding and multiple binding sites. As over 80% of ITC papers are on macromolecules of biological origin this interpretation is challenging. Some researchers have attempted to link the thermodynamics constants to events at the molecular level. This review highlights work done using binding sites characterised using x-ray crystallography techniques that allow speculation about individual bond formation and the displacement of individual water molecules during ligand binding and link these events to the thermodynamic constants for binding. The review also considers research conducted with synthetic binding partners where specific binding events like anion-π and π-π interactions were studied. The revival of assays that enable both thermodynamic and kinetic information to be collected from ITC data is highlighted. Lastly published criticism of ITC research from a physical chemistry perspective is appraised and practical advice provided for researchers unfamiliar with thermodynamics and its interpretation.

INTRODUCTION TO RESEARCH BETWEEN 2011-2015

Research into isothermal titration calorimetry (ITC) started around 25 years ago as high-sensitivity calorimetry instruments were developed. The publication of Ernesto Freire and coworkers' article entitled "Isothermal Titration Calorimetry" in 1990 introduced this technique to researchers interested in studying binding interactions.[1] Since 1990 there has been steady rise in research publications on ITC (Figure 1) encouraged by the release of commercial instrumentation that made this method accessible to a wide population of scientists. There are now around 600 to 700 peer-reviewed papers containing research using ITC published annually and there are no signs of this growth stopping. The field of protein chemistry has benefited most from ITC dominating the published research though synthetic chemists have increasingly found ITC useful (Figure 2). Research into lipids has used ITC to study demicellation with success and binding studies using nucleic acids, carbohydrates and synthetic molecules are also represented.

Despite the steady increase in research using ITC there have been no significant technical advances in ITC instrumentation since 2010. Robotic automated instruments were already on the market in

2010 and ITC can be considered a mature technology. There have been improvements in software making the technology increasingly user friendly. The published ITC research is dominated by simple one-site binding interactions where the mathematics and interpretation of the results are relatively simple. ITC-based techniques like thermal analysis of enzyme kinetics,[2,3] continuous ITC[4] and protein folding[5] have received minimal uptake by the research community despite their apparent value. There have been some recent advances in ITC-based techniques that are worth noting including kinITC which collects kinetic and thermodynamic information for binding interactions,[6] and advances in ITC displacement assays for high-affinity binding reactions.[7,8]

The increased use of ITC to study binding interactions with synthetic molecules is worthy of note as it provides highly defined molecules for binding studies. Protein binding studies have always been complicated by the fact that many binding sites are not well characterised and the inherent flexibility of protein molecules can make interpretation of binding site studies problematic. Progress has been made on the interpretation of ITC data since 2010. The strengths and weaknesses of ITC are also better understood. This knowledge however, has not uniformly trickled down to researchers undertaking ITC analysis where presentation of ITC data and the interpretation of thermodynamic parameters could be improved.

A recent development has been the advent of the Journal of Visualized Experiments (JoVE). JoVE is a PubMed-indexed video journal and ITC methods have been demonstrated by this journal.[9-11] This is particularly useful for researchers unfamiliar with the practical applications of ITC and can form a useful component in student or technician training.

Between 2003 and 2012 the Journal of Molecular Recognition published annual reviews of ITC research covering the years 2002 to 2010.[12-20] The authors John Ladbury, Ilian Jelesarov, Brett Collins, Robert Falconer and their co-authors not only reviewed the literature but provided expert advice on ITC use for the scientific community. The purpose of this current review is to appraise the developments from the last five years since the last annual ITC review and provide advice on the interpretation of ITC data. The author identified more than 2,500 articles reporting the use of ITC between January 2011 and December 2015, after searching the Web of Science and Scopus databases. This number of papers is impractical to cite in full so the author has selected approximately 200 that he feels best represents the field and apologises for any resulting omissions. These references have been classified into the following broad categories:

(i) References cited in the introduction.[1-20]
(ii) Review and perspective articles.[21-29]
(iii) Methods papers.[30-52]
(iv) Protein : protein interactions.[53-70]
(v) Protein interactions with other ligands.[71-143]
(vi) Lipids, micelles and membranes.[144-151]
(vii) Polysaccharides.[152-155]
(viii) Nucleic acids.[156-169]
(ix) Synthetic chemicals, polymers and nanoparticles.[170-207]
(x) Enzyme kinetics.[208-217]
(xi) Pre-2011 and non-ITC references.[218-234]

INTERPRETATION OF SINGLE BINDING SITE ITC DATA

Single binding site interactions are the simplest to study using ITC. If the c-value is between 1 and 1000 enough of the sigmoidal titration curve can be captured from which the stoichiometry, disassociation constant (K_D), change in free energy (ΔG) and change in enthalpy (ΔH) can be directly measured. From this the change in entropy (ΔS), can be calculated.[218] Note c = M_0 / K_D where M_0 is the initial concentration of the binding partner in the cell. Where the c-value is below 1 the stoichiometry and change in enthalpy (ΔH) values are problematic and where the c-value is greater than 1000 the disassociation constant (K_D) and change in free energy (ΔG) values are inaccurate. It is also worth noting that the change in entropy value (ΔS) is calculated from the equation $\Delta G = \Delta H - T\Delta S$ and will contain any errors from both the ΔG and ΔH measurements. An excellent paper by Joel Tellinghuisen written in 2012 provides further guidance for researchers designing ITC protocols to generate precise thermodynamic data.[48]

The first hurdle many researchers face is understanding the thermodynamic terms. While the definitions for change in enthalpy and change in free energy definitions are fairly obvious and there are some excellent text books on the subject.[219-220] The concept of entropy can be difficult to comprehend. Entropy can be described as a measure of disorder within a system as well as the energy state of a system.[221] For the interpretation of aqueous systems many authors rely on the concept of entropy being the movement from ordered to disordered states (and vice versa) whereas the idea of moving from a high energy state to a lower energy state is probably more accurate and avoids the need to attribute structures to water that are questionable. The water around methyl groups is an example where structural attributes have been used to describe water at the interface. In the past these structures were described as being ice-like[222] and more recently they have been described as clathrate-like cages[223] or networks.[24] A simpler way of describing the water at the interface with a methyl group is water that cannot hydrogen bond with the methyl group; this water has a higher energy state than water surrounded by water where it can exchange protons freely. The calculated entropy from ITC data is the sum of the entropies within the sample being studied and will involve the ligand, its target, the water and any co-solutes (buffer, salts, etc.) within the sample. This complexity makes it difficult to ascribe individual changes during binding (like displacement of individual water molecules) to changes in entropy.[24] For further reading on the interpretation of entropy that is written in a highly accessible manner try Frank Lambert's paper "A modern view of entropy".[224]

The work using ITC to study drug candidates' interaction with drug targets has made researchers in this field increasingly aware of the complexity that is occurring at drug binding sites. This has been helped by the known crystal structure of some of the drug targets that were studied.[24] This enabled speculation about the specific bond formation occurring and the displacement of specific water molecules during binding.

During a binding interaction between a protein and a ligand the following occurs:

1. The ligand has to penetrate the protein's hydration layer (which may present an energetic barrier)
2. There is displacement of water from the part of the protein's and the ligand's surface where the binding occurs (desolvation).

3. There is also displacement of any co-solutes present at the protein surface. This is particularly important where electrostatic interaction plays a role as charged co-solutes are often present at the binding site and may need displacing.
4. Short-range bond formation (hydrogen bonding, van der Waal's interaction, pi-cation interactions, etc.) between the protein and the ligand will occur.
5. There is the possibility of proton exchange between both binding partners and the buffer.
6. There is the possibility of conformational change of the protein; this is particularly important where allostery plays a role in the protein's function.
7. Finally there will be a rearrangement of the water adjacent to the ligand-protein interface.

Each of these events during binding will have an effect on net ΔH and ΔS values.

The role of the protein's hydration layer on binding interactions is contentious as the methods for measuring this phenomenon like terahertz spectroscopy are still specialist techniques and not familiar to most ITC users. There is evidence that a protein's hydration layer is more extensive and complex than previously believed.[225-227] It has also been shown that cosolutes can modify the hydration layer.[228-229] There is scope for future work using ITC in conjunction with low frequency analysis of water.

Gerhard Klebe's group used inhibitor binding to thermolysin[24,76-77,108] to study the important contribution of water displacement and rearrangement on the thermodynamics of inhibitor binding. This was not a trivial undertaking. Firstly the structure was defined by x-ray crystallography at the BESSY beamline in Berlin. This enabled the binding site to be well characterised and the possible location of bound water molecules determined. In one study the ligands only differed in the replacement of a methyl with a carboxyl group.[76] The difference in thermodynamics of binding was attributed to the carboxyl group disrupting the water network around the filled binding site. A second set of ligands were used with substitutions altering the ligands hydrophobicity. As the thermolysin binding site is a hydrophobic pocket, the interaction would usually be considered as an example hydrophobic interaction and would be entropy driven.[77] Interestingly, the addition of a methyl group to the ligand resulted in an enthalpy-driven improvement in binding whereas addition of further hydrophobicity to the ligand gave a predicted entropy-driven improvement. This was ascribed to changes to the water at the surface of the protein ligand complex.[77,108] The conclusion from this research was that water played a minor role in the change in free energy but had a major effect on change in enthalpy and entropy. The work did demonstrate our current inability to consistently predict the thermodynamic profiles associated with relatively simple changes in ligand structure even when the binding sitewas well characterised.[24]

The classical approach to the thermodynamics of binding would be to consider solvation as implicit within the activity coefficients of the binding partners. Brian Castellano and Daryl Eggers argued that for binding reactions in aqueous environments, the water should be treated as a coreactant. So the binding equation was proposed that took water into account, $\Delta G^0 = -RT \ln K_i - [Q]_i \Delta G_i^{H2O}$ where ΔG^0 is the standard free energy constant, $[Q]_i$ is the concentration of the complex, K_i is the association constant and ΔG_i^{H2O} is the desolvation energy, all in a specific solution (i).[175] The example used was calcium ion binding to EDTA conducted at different reactant concentrations and temperatures. When $-RT \ln K_i$ was plotted against $[Q]_i$ the y-intercept gave the ΔG^0 and the slope the ΔG_i^{H2O} values. An observation was that K_i changes with concentration and that the $\Delta G_i^{H2O}/RT$ had a near linear

relationship to 1/*T*. This research provides a method to determine values for the desolvation energy associated with binding interactions.

Displacement of co-solutes during binding is often overlooked during ITC studies. An example where co-solute displacement was studied used metal cation binding to the synthetic p-sulfonatocalix[4]arene (a ring structured molecule with four acidic sulpho groups where a metal ion can bind).[181] The presence of a counter ion such as sodium had a considerable effect on the thermodynamics of binding. While p-sulfonatocalix[4]arene is a synthetic molecule the principle is the same for proteins and other macromolecules where ions commonly interact with oppositely charged constituent parts. Most ITC binding studies are in buffered solutions where the co-solutes often comprise sodium chloride and a buffer that will interact with charged amino acid side chains and affect the thermodynamics of any binding that involve electrostatic interaction. George Whiteside's group used the pocket in human carbonic anhydrase II to examine the role of anions on binding.[95] This work which combined ITC with x-ray crystallography and molecular dynamic simulation suggested low charge density anions can associate with hydrophobic regions within the binding pocket, altering the charge and water structure in and round the pocket.

Proton exchange between either binding partner with the buffer received much attention before 2011.[20] A recent study of ligand binding to a t-RNA binding protein provided a good example of proton transfer during ITC experimentation having a marked effect on change in enthalpy.[119] Further analysis was able to identify which components of the binding partners were responsible for the proton exchange with the buffer.

The take home message is that interpretation of ITC data for binding interactions in aqueous systems has to take displacement of water, co-solutes and protons into consideration. Commonly used solutions like phosphate buffered saline contain the high-charge density phosphate anion which binds relatively strongly to positive charged side chains and can interfere with ligand binding to proteins (personal observation). Anyone considering selection of low charge density ions like guanidinium hydrogen chloride or iodine to improve protein solubility would be advised to read George Whiteside's paper before proceeding.[95] Chemicals like DMSO are commonly used to help solubilise ligands that have low-solubility in water but the effect of DMSO on the binding partners and their respective hydration layers has to be taken into consideration. The choice of the buffer and other cosolutes to be used during ITC experiments is very important and needs careful consideration.

INTERPRETATION OF MULTIPLE BINDING SITE ITC DATA

The study of proteins and protein complexes with multiple binding sites is of particular interest to scientists interested in allosteric regulation where the binding of one molecule to a site affects the binding of a second molecule to a separate site on the same protein or protein complex. Where the two molecules are different (heterotropic allostery) this phenomenon is easily studied using ITC as the ΔG, ΔH and ΔS values for the second binding event will be different for the protein with and without the first molecule present. An example of heterotropic allostery is the formation of the complex between mRNA containing poly(A) sequences with the translation factors polyadenylate-

binding protein-1 (PABP) and scaffolding protein eIF4G.[130] The ITC data gave clear evidence of cooperative binding of eIF4G and Poly(A) to PABP.

Allostery can also occur where the multiple bind sites on the protein or protein complex bind the same ligand (homotropic allostery). An example of this was the binding of acetyl coenzyme A to the dimeric protein aminoglycoside N-(6')-acetyltransferase-Ii.[96] This study used a combination of ITC, circular dichroism, and nuclear magnetic resonance spectroscopy to quantify the structural, dynamic and thermodynamic aspects of allostery. The ITC binding isotherms are often non-sigmoidal due to the different *ΔG* and *ΔH* values of the different binding events. Homotropic allostery presents the challenge of calculating meaningful thermodynamic constants for the multiple binding sites [31] and for detecting positive and negative cooperativity.[34] While the mathematics for calculating *ΔG*, *ΔH* and *ΔS* values for multiple binding sites has been determined and informative simulations have been undertaken[31,34] it is worth remembering that relatively small errors in the raw ITC data (especially where few titrations are present for critical parts of the thermogram) can generate plausible but misleading *ΔG*, *ΔH* and *ΔS* values for the binding sites.

Non-specific binding can be easily confused with multiple binding site interactions. There are many molecules that will bind to proteins, nucleic acids and synthetic molecules, while not targeting individual binding sites. Possibly the best studied family of molecules that bind "promiscuously" to proteins are the polyphenolics.[79,107,117,143] It is believed that polyphenolics hydrogen bond with the peptide backbone of a protein. The complicating factor in studying non-specific binding of polyphenolics to proteins is their propensity to cross-link proteins which can displace water around the proteins and contribute to the recorded *ΔG* and *ΔH* values (personal observation). The ITC binding isotherms are often non-sigmoidal and could be interpreted as evidence of allostery if cross-linking was not taken into consideration.[107]

METHODOGICAL ADVANCES

kinITC assay to capture both thermodynamic and kinetic information. Burnouf et al 2012 proposed a method for collecting both kinetic and thermodynamic data from ITC experimentation that could be used for simple binding interactions and more complex processes.[6] The example they studied included the binding of the inhibitor Nevirapine to HIV-1 reverse transcriptase, and the binding of thiamine pyrophosphate (TPP) to the *Escherichia coli* riboswitch present in the 5'-UTR of the thiC mRNA which folded on binding of TPP. The paper's supplementary information provided details on instrument response time, injection times and mixing times for their Microcal ITC200 which had to be taken into account if this method was to be reliable. Work on the partial validation of kinITC used surface plasmon resonance as the gold standard method for determining the kinetic on and off constants. The collection of kinetic data using an ITC is obviously attractive as it does not require a tether to a solid support but the assay must be well validated and the instrument response time, injection times and mixing times for the instrument known.

The collection of both kinetic and thermodynamic data has also been applied to study RNA helical packing.[166] By running the assay at different temperatures they were able to calculate the Arrhenius activation energy and Eyring transition state entropy as well as the thermodynamic parameters for GAAA tetraloop–receptor interaction in magnesium and potassium solutions.

ITC assays for the quantification of high-affinity binding interactions. The standard ITC displacement assay used to study high-affinity interactions has been around since 2000 and has been used to study a range of high-affinity interactions.[230,231] This technique uses the displacement of a moderate-affinity ligand to lower the apparent affinity of a high-affinity ligand. A displacement assay using weakly binding fragments to thrombin was run in parallel with direct (low-C) assay and showed both methods yielded valid disassociation constants.[44] The direct low-C titrations, however, have highly questionable stoichiometry. The displacement assay also had a drawback that different displaced ligands affected the enthalpic values indicating that the choice of the displaced ligand was important and that experimental conditions need to be standardised so comparison can be made between different fragments. This phenomenon was ascribed to the solvation structure and protein dynamics of the initial protein–ligand complexes before displacement occured.[44] The displacement method has a serious drawback as the high-affinity ligand of interest has to be soluble at high concentrations (>100 μM). Many high-affinity drugs have low solubility in water making the traditional displacement assay impractical. The competition assay published in Krainer et al 2012 can be used to study low solubility high affinity ligands.[7] In this assay the receptor was titrated into a mixture of competing high- and moderate-affinity ligands which generated a biphasic isotherm that was be used to quantify disassociation constants (K_D) and binding enthalpies (ΔH) for both ligands. Another alternative approach was a single-experiment displacement assay.[8] The assay involved the titration of the high-affinity ligand into a solution containing the moderate-affinity ligand bound to the receptor with excess moderate-affinity ligand. The isotherm was also biphasic and was used to quantify K_D and ΔH values for both high-affinity and medium-affinity ligands competing for the same binding site. This provides three different strategies for analysing problematic high-affinity binding interactions.

Software. Researchers using ITC are recommended to appraise the software NITPIC (which claims to be superior to Origin) and SEDPHAT that have been developed to assist in analysis of ITC data.[39,45,52] The program NITPIC can be downloaded for free from http://biophysics.swmed.edu/MBR/software.html. SEDPHAT can be downloaded from http://sedfitsedphat.nibib.nih.gov/software free of charge. AFFINImeter produce commercial software that can be used for analysis of displacement assays, micellization experiments, kinITC, the application of complex models for complex interactions, and ligand induced conformational change. At the time of writing this software was only suitable for MicroCal data but they were intending to release the software compatible with other brands of ITC.

SYNTHETIC MOLECULES

Over 80% of research using ITC is with macromolecules of biological importance including proteins, nucleic acids, lipids and carbohydrates. Macromolecules are poorly suited to studying specific interactions. The use of ITC with synthetic molecules provides a range of opportunities to study binding interactions using receptors that are simpler and well defined. This has enabled hypotheses regarding interactions in aqueous solutions to be tested using well defined synthetic ligands. This information can then be transferred to help our understanding of the interactions that are occurring in proteins, nucleic acids, etc.

There are several papers studying cation-π; anion-π; and π-π interactions. In one study ciprofloxacin hydrochloride was used in an aqueous solution.[197] Ciprofloxacin hydrochloride has a quinolone ring and a protonated amine. ITC was used alongside H^1 NMR spectroscopy to demonstrate one-dimensional aggregates formed by π-π stacking and dimer formation brought together by cation-π interaction. Anion-π were studied in aqueous solutions with a tren (tris(2-aminoethyl)amine) molecule attached to a nitroso-amino-pyrimidine.[173] A range of anions were shown to interact with the heteroaromatic ring. A large entropic contribution favoured association and was attributed to displacement of water around the hydrophobic pyrimidine surface during association suggesting in this case water displacement played an important contribution to this anion-π interaction. Anion-π interactions were also studied using halides (Cl−, Br−, and I−) and "two-wall" calix[4]pyrrole receptors with two six-membered aromatic rings in organic solvents.[170] The number and electron drawing character of aromatic substitutions increased the positive electrostatic surface potential of the centre of the six member ring enabling the anion-π interaction. The interaction of fullerenes to a buckycatcher (comprised of two corannulene subunits tethered together) in a range of organic solvents is an example of binding with a strong π-π interaction component.[189] In a binding interaction where solvent displacement played a significant role, the change in entropy played a minor role in driving binding which surprised the authors.

A synthetic octa-acid host with a hydrophobic pocket was used to study the effect of anions on binding of small molecule ligands.[182] In the case of low charge density anions like ClO_3^- the anion was found to enhance affinity at low concentrations and weaken it at high concentrations. At higher ClO_3^- concentrations, for the small molecule ligand to bind to the hydrophobic pocket required displacement of the anion. This supports the theory of Kim Collins that explains the behaviour of low charge density anions and protein solubility in terms of low charge density anion interaction with hydrophobic surfaces on the protein.[232] While the synthetic octa-acid host study is ongoing it does provide the opportunity to challenge or confirm the theories for low charge density anion interaction with hydrophobic pockets at a nanometer-scale and complements work undertaken with binding to proteins in the presence of low charge density anions that observe similar effects.[95] It also has the capacity to challenge theories about the activity of medium and high charge density anion indirect interaction through competition for solvent.[233]

In an interesting study, allostery was mimicked using a dual-cavity basket which had six alanine residues at the entrance of two juxtaposed cavities that was designed to trap organophosphorus nerve agents.[177] Molecular dynamic simulation and H^1 NMR spectroscopy suggested a negative homotropic cooperativity of binding in water. This is an attractive candidate for ITC studies as it could be used to validate computer simulations of negative cooperativity binding.

CAUTIONARY NOTE

In 2015, Brian Pethica from Princeton University wrote a highly critical paper entitled "Misuse of thermodynamics in the interpretation of isothermal titration calorimetry data for ligand binding to proteins" [26] which should serve as a cautionary note for scientists who don't have a strong background in thermodynamics. Pethica's critique, however, should not dissuade researchers from using ITC to study binding as long as they are aware of the assumptions that predicate the calculation of the thermodynamic constants.

The first key assumption behind the equation $\Delta G=\Delta H-T\Delta S$ (where ΔH is the change in enthalpy, ΔG is the change in free energy and ΔS is the change in entropy) is that the binding reaction is reversible and that equilibrium has been reached. This assumption is acceptable for most binding reactions but it should be remembered that allosteric change in a binding partner could prevent the ligand returning to the solution. The most common error in published ITC data was too shorter time between titrations which does not allow the peak to return to the baseline (i.e. equilibrium was not reached before the next injection) and this key assumption was not met.

The second assumption is that the ligand and the macromolecule (protein, nucleic acid or synthetic molecule) are totally soluble. In practice many ligands such as drug candidates have low solubility in water. In some cases ligand preparations may include insoluble along with the soluble ligand. When injected into the ITC cell some of the insoluble material will dissolve and there will be a ΔH associated with this event. The use of control titrations of ligand into buffer (without the macromolecule present) and titrations of buffer (without the ligand) into the macromolecule can be used to detect this type of event occurring. The use of these controls should be a normal part of ITC experimental design.

The third assumption is that macromolecule solutions are ideal (i.e. there are no macromolecule-macromolecule interactions, no macromolecule-cosolute interactions, and no interactions between macromolecule-ligand complexes). Macromolecule solutions are not ideal. Cosolutes interact with macromolecules both by direct binding and indirectly by modifying their hydration layers. [233-235] Macromolecules similarly interact with each other or compete with each other for water for their hydration layers. [226,229] The issue of cosolutes altering the thermodynamics of ligand binding is unavoidable and the researcher has to accept that the thermodynamic constants derived from their research are for the solution conditions used and will change if different buffer, pH or temperatures are used. The issue of macromolecule-macromolecule interactions is also unavoidable. Even a target like EDTA demonstrated concentration-dependant thermodynamics of binding to calcium ions.[174] This was attributed to the desolvation of the binding partners and demonstrated that undertaking ITC at several target concentrations will provide a better understanding of the non-ideality of macromolecule solutions.

To overcome the criticism from physical chemists like Brian Pethica, the author recommends that researchers should do the following:

- Outline the assumptions behind the thermodynamic calculations in their papers.
- Make sure titration peaks do reach the baseline (achieving equilibrium).
- Run the control titrations of ligand into buffer (without the macromolecule present) and buffer (without the ligand) into the macromolecule as a standard part of the ITC experimentation then present these thermograms in the paper or as supplementary information.
- Specify the conditions used for the binding experiments including the composition of both titrant solution and the solution in the sample cell (include pH and temperature). Also include the specific titration strategy used. While this does not avoid non-ideality of macromolecule solutions it does define the experimentally derived thermodynamic constants for the precise conditions used.

- For experimentation with low solubility ligands, be careful that the ligand is totally dissolved and if chemicals like DMSO are used to improve ligand solubility, consider their potential interaction with the binding partners.

REQUEST FOR RAW DATA PUBLICATION

The author would like to suggest that editors and reviewers of articles containing ITC data should request that the raw ITC data (the experimentally derived thermograms) should be published in the paper or as supplementary material. There are many ITC papers where the calculated binding isotherms alone are published without the experimentally derived thermograms. To the experienced ITC operator the raw data contains a wealth of information and should be provided to verify that the analysis was done to a high standard. The raw data can confirm that the baseline was steady and equilibrium was reached before the next injection. The raw data can also be used to better understand the kinetics of the interaction and detect mixed interactions (e.g. rapid binding followed by slow aggregation). It is the author's opinion that much useful data is being lost and that confidence in published data is eroded due to the frequent failure to publish raw ITC data.

Figure Titles

Figure 1 Articles written with isothermal titration calorimetry content since 1990 sourced from the Web of Science ™.

Figure 2 Subject material studied using isothermal titration calorimetry in 2014. Note protein related research accounted for 67% of the articles. Synthetic compounds were 17%, lipids and micelles were 6%, nucleic acids were 4%, carbohydrates were 3% and the remainder were 3% of the articles.

(i) References cited in the introduction

1. Freire E, Mayorga OL, Straume M. 1990. Isothermal titration calorimetry. *Anal. Chem*. **62**: 950A-959A.
2. Todd MJ, Gomez J. 2001. Enzyme kinetics determined using calorimetry: a general assay for enzyme activity? *Anal. Biochem*. **296**: 179–87.
3. Olsen SN. 2006. Applications of isothermal titration calorimetry to measure enzyme kinetics and activity in complex solutions. *Thermochimica Acta* **448**: 12-18.
4. Markova N, Hallén D. 2004. The development of a continuous isothermal titration calorimetric method for equilibrium studies. *Anal. Biochem*. **331**: 77-88.
5. Lopez MM, Chin DH, Baldwin RL, Makhatadze GI. 2002. The enthalpy of the alanine peptide helix measured by isothermal titration calorimetry using metal-binding to induce helix formation. *Proc. Natl Acad. Sci. USA* **99**: 1298–1302.
6. Burnouf D, Ennifar E, Guedich S, Puffer B, Hoffmann G, Bec G, Disdier F, Baltzinger M, Dumas P. 2012. kinITC: A new method for obtaining joint thermodynamic and kinetic data by isothermal titration calorimetry. *J. Am. Chem. Soc*. **134**: 559-565.
7. Krainer G, Broecker J, Vargas C, Fanghänel J, Keller S. 2012. Quantifying high-affinity binding of hydrophobic ligands by isothermal titration calorimetry. *Anal. Chem*. **84**: 10715-10722.
8. Krainer G, Keller S. 2015. Single-experiment displacement assay for quantifying high-affinity binding by isothermal titration calorimetry. *Methods* **76**: 116-123.
9. Duff MR, Grubbs J, Howell EE. 2011. Isothermal titration calorimetry for measuring macromolecule-ligand affinity. *J. Vis. Exp*. **55**: UNSP e2796.
10. Freiburger LA, Mittermaier AK, Auclair K. 2011. Collecting variable-concentration isothermal titration calorimetry datasets in order to determine binding mechanisms. *J. Vis. Exp*. **50**: UNSP e2529.
11. Mazzei L, Ciurli S, Zambelli B. 2014. Hot biological catalysis: Isothermal titration calorimetry to characterize enzymatic reactions. *J. Vis. Exp*. **86**: e51487.
12. Cliff MJ, Ladbury JE. 2003. A survey of the year 2002 literature on applications of isothermal titration calorimetry. *J. Mol. Recognit*. **16**: 383–91.
13. Cliff MJ, Gutierrez A, Ladbury JE. 2004. A survey of the year 2003 literature on applications of isothermal titration calorimetry. *J. Mol. Recognit*. **17**: 513–23.
14. Ababou A, Ladbury JE. 2006. Survey of the year 2004: literature on applications of isothermal titration calorimetry. *J. Mol. Recognit*. **19**: 79–89.
15. Ababou A, Ladbury JE. 2007. Survey of the year 2005: literature on applications of isothermal titration calorimetry. *J. Mol. Recognit*. **20**: 4–14.
16. Okhrimenko O, Jelesarov I. 2008. A survey of the year 2006 literature on applications of isothermal titration calorimetry. *J. Mol. Recognit*. **21**: 1–19.
17. Bjelic S, Jelesarov I. 2008. A survey of the year 2007 literature on applications of isothermal titration calorimetry. *J. Mol. Recognit*. **21**: 289–312.
18. Falconer RJ, Penkova A, Jelesarov I, Collins BM. 2010. Survey of the year 2008: applications of isothermal titration calorimetry. *J. Mol. Recognit*. **23**: 395–413.
19. Falconer RJ, Collins BM. 2011. Survey of the year 2009: applications of isothermal titration calorimetry. *J. Mol. Recognit*. **24**: 1–16.
20. Ghai R, Falconer RJ, Collins BM. 2012. Applications of isothermal titration calorimetry in pure and applied research—survey of the literature from 2010. *J. Mol. Recognit*. **25**: 32-52.

(ii) Reviews and perspective articles

21. Eggers DK, Le JM, Pham DN, Nham NT, Contreras FA. 2015. Desolvation energy: A rationale for changes in binding affinity as measured by ITC. *Biophys. J.*108: 114A-114A.
22. Ferenczy GG, Keseru GM. 2012. Thermodynamics of fragment binding. *J. Chem. Inf. Model*. **52**: 1039-1045.
23. Garbett NC, Chaires JB. 2012. Thermodynamic studies for drug design and screening. *Expert Opin. Drug Disc*. **7**: 299-314.
24. Klebe G. 2015. Applying thermodynamic profiling in lead finding and optimization. *Nat. Rev. Drug Disc.* **14**: 95-110.
25. Martin SF, Clements JH. 2013. Correlating structure and energetics in protein-ligand Interactions: Paradigms and paradoxes. *Annu. Rev. Biochem*. **82**:267-293.
26. Pethica BA. 2015. Misuse of thermodynamics in the interpretation of isothermal titration calorimetry data for ligand binding to proteins. *Anal. Biochem*. **472**: 21-29.
27. Rajarathnam K, Roesgen J. 2014. Isothermal titration calorimetry of membrane proteins - Progress and challenges. *BBA-Rev. Biomembranes* **1838**: 69-77.
28. Scott DE, Ehebauer MT, Pukala T, Marsh M, Blundell TL, Venkitaraman AR, Abell C, Hyvoenen M. 2013. Using a fragment-based approach to target protein-protein interactions. *Chembiochem* **14**: 332-342.
29. Willerich I, Groehn F. 2011. Molecular Structure Encodes Nanoscale Assemblies: Understanding driving forces in electrostatic self-assembly. *J. Am. Chem. Soc*. **133**: 20341-20356.

(iii) Methods papers

30. Boudker O, Oh S. 2015. Isothermal titration calorimetry of ion-coupled membrane transporters. *Methods* 76: 171-182.
31. Brautigam CA. 2015. Fitting two- and three-site binding models to isothermal titration calorimetry data. *Methods* **76**: 124-136.
32. Chiappisi L, Li D, Wagner NJ, Gradzielski M. 2014. An improved method for analyzing isothermal titration calorimetry data from oppositely charged surfactant polyelectrolyte mixtures. *J. Chem. Thermodyn*. **68**: 48-52.
33. Dias DM, Van Molle I, Baud MGJ, Galdeano C, Geraldes CFGC, Ciulli A. 2014. Is NMR fragment screening fine-tuned to assess druggability of protein-protein interactions? *ACS Med. Chem. Lett*. **5**: 23-28.
34. Freiburger L, Auclair K, Mittermaier A. 2015. Global ITC fitting methods in studies of protein allostery. *Methods* **76**: 149-161.
35. Gruener S, Neeb M, Barandun LJ, Sielaff F, Hohn C, Kojima S, Steinmetzer T, Diederich F, Klebe G. 2014. Impact of protein and ligand impurities on ITC-derived protein-ligand thermodynamics. *Biochim. Biophys. Acta* **1840**: 2843-2850.
36. Hansen LD, Fellingham GW, Russell DJ. 2011. Simultaneous determination of equilibrium constants and enthalpy changes by titration calorimetry: Methods, instruments, and uncertainties. *Anal. Biochem*. **409**: 220-229.

37. Henzl MT, Markus LA, Davis ME, McMillan AT. 2013. Simultaneous addition of two ligands: A potential strategy for estimating divalent ion affinities in EF-hand proteins by isothermal titration calorimetry. *Methods* **59**: 336-348.
38. Herrera I, Winnik MA. 2013. Differential binding models for isothermal titration calorimetry: Moving beyond the Wiseman isotherm. *J. Phys. Chem. B* **117**: 8659-8672.
39. Keller S, Vargas C, Zhao H, Piszczek G, Brautigam, Schuck P. 2012. High-precision isothermal titration calorimetry with automated peak-shape analysis. *Anal. Chem*. **84**: 5066-5073.
40. Krimmer SG, Klebe G. 2015. Thermodynamics of protein-ligand interactions as a reference for computational analysis: how to assess accuracy, reliability and relevance of experimental data. *J. Comput. Aided Mol. Des*. **29**: 867-883.
41. Le VH, Buscaglia R, Chaires JB, Lewis EA. 2013. Modeling complex equilibria in isothermal titration calorimetry experiments: Thermodynamic parameters estimation for a three-binding-site model. *Anal. Biochem.* **434**: 233-241.
42. Mashalidis EH, Sledz P, Lang S, Abell C. 2013. A three-stage biophysical screening cascade for fragment-based drug discovery. *Nat. Protocols* 8: 2309-2324.
43. Rocklin GJ, Boyce SE, Fischer M, Fish I, Mobley DL, Shoichet BK, Dill KA. 2013. Blind prediction of charged ligand binding affinities in a model binding site. *J. Mol. Biol*. **425**: 4569-4583.
44. Ruehmann E, Betz M, Fricke M, Heine A, Schafer M, Klebe G.2015. Thermodynamic signatures of fragment binding: Validation of direct versus displacement ITC titrations. *Biochim. Biophys. Acta* **1850**: 647-656.
45. Scheuermann TH, Braitigam CA. 2015. High-precision, automated integration of multiple isothermal titration calorimetric thermograms: New features of NITPIC. *Methods* **76**: 87-98.
46. Sgarlata C, Zito V, Arena G. 2013. Conditions for calibration of an isothermal titration calorimeter using chemical reactions. *Anal. Bioanal. Chem*. **405**: 1085-1094.
47. Tellinghuisen J, Chodera JD. 2011. Systematic errors in isothermal titration calorimetry: Concentrations and baselines. *Anal. Biochem.* **414**: 297-299.
48. Tellinghuisen J. 2012. Designing isothermal titration calorimetry experiments for the study of 1:1 binding: Problems with the "standard protocol". *Anal. Biochem*. **424**: 211-220.
49. Transtrum MK, Hansen LD, Quinn C. 2015. Enzyme kinetics determined by single-injection isothermal titration calorimetry. *Methods* **76**: 194-200.
50. Vega S, Abian O, Velazquez-Campoy A. A unified framework based on the binding polynomial for characterizing biological systems by isothermal titration calorimetry. *Methods* **76**: 99-115.
51. Velazquez-Campoy A. 2015. Geometric features of the Wiseman isotherm in isothermal titration calorimetry. *J. Therm. Anal. Calorim.* **122**: 1477-1483.
52. Zhao H, Piszczek G, Schuck P. 2015. SEDPHAT – A platform for global ITC analysis and global multi-method analysis of molecular interactions. *Methods* **76**: 137-148.

(iv) Protein : protein interactions

53. Batchelor JD, Malpede BM, Omattage NS, DeKoster GT, Henzler-Wildman KA, Tolia NH. 2014. Red blood cell invasion by Plasmodium vivax: Structural basis for DBP engagement of DARC. *Plos Pathog.* **10**: e1003869.
54. Bulatov E, Martin EM, Chatterjee S, Knebel A, Shimamura S, Konijnenberg A, Johnson C, Zinn N, Grandi P, Sobott F, Ciulli A. 2015. Biophysical studies on interactions and assembly of full-

size E3 ubiquitin ligase suppressor of cytokine signalling 2 (SOCS2)-elongin BC-cullin 5-ring box protein 2 (RBX2). *J. Biol. Chem.***290**: 4178-4191.

55. Chabot PR, Raiola L, Lussier-Price M, Morse T, Arseneault G, Archambault J, Omichinski JG. 2014. Structural and Functional Characterization of a Complex between the Acidic Transactivation Domain of EBNA2 and the Tfb1/p62 Subunit of TFIIH. *Plos Pathog*. **10**: e1004042.
56. Erales J, Beltrandi M, Roche J, Mate M, Longhi S. 2015. Insights into the Hendra virus N-TAIL-XD complex: Evidence for a parallel organization of the helical MoRE at the XD surface stabilized by a combination of hydrophobic and polar interactions. *BBA-Proteins Proteom*.**1854**: 1038-1053.
57. Francis DM, Rozycki B, Koveal D, Hummer G, Page R, Peti W. 2011. Structural basis of p38 alpha regulation by hematopoietic tyrosine phosphatase. *Nat. Chem. Biol*. **7**: 916-924.
58. Haslbeck V, Eckl JM, Kaiser CJO, Papsdorf K, Hessling M, Richter K. 2013. Chaperone-Interacting TPR Proteins in *Caenorhabditis elegans*. *J. Mol. Biol*. **425**: 2922-2939.
59. Ikenoue T, Lee Y-H, Kardos J, Yagi H, Ikegami T, Naiki H, Goto Y. 2014. Heat of supersaturation-limited amyloid burst directly monitored by isothermal titration calorimetry. *Proc. Natl. Acad. Sci. USA* **111**: 6654-6659.
60. Kozin SA, Kulikova AA, Istrate AN, Tsvetkov PO, Zhokhov SS, Mezentsev YV, Kechko OI, Ivanov AS, Polshakov VI, Makarov AA. 2015. The English (H6R) familial Alzheimer's disease mutation facilitates zinc-induced dimerization of the amyloid-beta metal-binding domain. *Metallomics* **7**: 422-425.
61. Kulikova AA, Tsvetkov PO, Indeykina MI, Popov IA, Zhokhov SS, Golovin AV, Polshakov VI, Kozin SA, Nudler E, Makarov AA. 2014. Phosphorylation of Ser8 promotes zinc-induced dimerization of the amyloid-beta metal-binding domain. *Mol. Biosyst*. **10**: 2590-2596.
62. Matsunaga R, Abe R, Ishii D, Watanabe S-i, Kiyoshi M, Noecker B, Tsuchiya M, Tsumoto K. 2013. Bidirectional binding property of high glycine-tyrosine keratin-associated protein contributes to the mechanical strength and shape of hair. *J. Struct. Biol.* **183**: 484-494.
63. Muratcioglu S, Chavan TS, Freed BC, Jang H, Khavrutskii L, Freed RN, Dyba MA, Stefanisko K, Tarasov SG, Gursoy A, Keskin O, Tarasova NI, Gaponenko V, Nussinov R. 2015. GTP-dependent K-Ras dimerization. *Structure* **23**: 1325-1335.
64. Norwood SJ, Shaw DJ, Cowieson NP, Owen DJ, Teasdale RD, Collins BM. 2011. Assembly and solution structure of the core retromer protein complex. *Traffic* **12**: 56-71.
65. Reille-Seroussi M, Gaucher J-F, Desole C, Gagey-Eilstein N, Brachet F, Broutin I, Vidal M, Broussy S. 2015. Vascular endothelial growth factor peptide ligands explored by competition sssay and isothermal titration calorimetry. *Biochemistry* **54**: 5147-5156.
66. Winstone TML, Tran VA, Turner RJ. 2013. The hydrophobic region of the DmsA twin-arginine leader peptide determines specificity with chaperone DmsD. *Biochemistry* **52**: 7532-7541.
67. Winzen S, Schoettler S, Baier G, Rosenauer C, Mailaender V, Landfester K, Mohr K. 2015. Complementary analysis of the hard and soft protein corona: sample preparation critically effects corona composition. *Nanoscale* **7**: 2992-3001.
68. Wrench AP, Gardner CL, Siegel SD, Pagliai FA, Malekiha M, Gonzalez CF, Lorca GL. 2013. MglA/SspA complex interactions are modulated by inorganic polyphosphate. *Plos One* **8**: e76428.
69. Xu J, Brewer KD, Perez-Castillejos R, Rizo J. 2013. Subtle interplay between synaptotagmin and complexin binding to the SNARE complex. *J. Mol. Biol*.**425**: 3461-3475.

70. Xu Y, Oruganti V, Gopalan V, Foster MP. 2012. Thermodynamics of coupled folding in the interaction of archaeal RNase P proteins RPP21 and RPP29. *Biochemistry* **51**: 926-935.

(v) Protein interactions with other ligands (including metals, nucleic acids, nanoparticles, co-factors and drugs).

71. Anglin JL, Deng L, Yao Y, Cai G, Liu Z, Jiang H, Cheng G, Chen P, Dong S, Song Y. 2012. Synthesis and structure-activity relationship investigation of adenosine-containing inhibitors of histone methyltransferase DOT1L. *J. Med. Chem*. **55**: 8066-8074.

72. Baier G, Costa C, Zeller A, Baumann D, Sayer C, Araujo PHH, Mailaender V, Musyanovych A, Landfester K. 2011. BSA adsorption on differently charged polystyrene nanoparticles using isothermal titration calorimetry and the influence on cellular uptake. *Macromol. Biosci*. **11**: 628-638.

73. Bec G, Meyer B, Gerard M-M, Steger J, Fauster K, Wolff P, Burnouf D, Micura R, Dumas P, Ennifar E. 2013. Thermodynamics of HIV-1 reverse transcriptase in action elucidates the mechanism of action of non-nucleoside inhibitors. *J. Am. Chem. Soc.* **135**: 9743-9752.

74. Becker AL, Welsch N, Schneider C, Ballauff M. 2011. Adsorption of RNase A on cationic polyelectrolyte brushes: A study by isothermal titration calorimetry. *Biomacromolecules* **12**: 3936-3944.

75. Biedermann F, Uzunova VD, Scherman OA, Nau WM, De Simone A. 2012. Release of high-energy water as an essential driving force for the high-affinity binding of cucurbit[n]urils. *J. Am. Chem. Soc*. **134**: 15318-15323.

76. Biela A, Betz M, Heine A, Klebe G. 2012. Water makes the difference: rearrangement of water solvation layer triggers non-additivity of functional group contributions in protein-ligand binding. *ChemMedChem*. **7**: 1423–1434.

77. Biela A, Nasief NN, Betz M, Heine A, Hangauer D, Klebe G. 2013. Dissecting the hydrophobic effect on the molecular level: The role of water, enthalpy, and entropy in ligand binding to thermolysin. *Angew. Chem. Int. Ed*. **52**: 1-8.

78. Biela A, Sielaff F, Terwesten F, Heine A, Steinmetzer T, Klebe G. 2012. Ligand binding stepwise disrupts water network in thrombin: Enthalpic and entropic changes reveal classical hydrophobic effect. *J. Med. Chem*. **55**: 6094-6110.

79. Bohin MC, Vincken J-P, van der Hijden HTWM, Gruppen H. 2012. Efficacy of food proteins as carriers for flavonoids. *J. Agric. Food Chem.* **60**: 4136-4143.

80. Borisova AS, Isaksen T, Dimarogona M, Kognole AA, Mathiesen G, Varnai A, Rohr AK, Payne CM, Sorlie M, Sandgren M, Eijsink VGH. 2015. Structural and functional characterization of a lytic polysaccharide monooxygenase with broad substrate specificity. *J. Biol. Chem*. **290**: 22955-22969.

81. Breukels V, Konijnenberg A, Nabuurs SM, Touw WG, Vuister GW. 2011. The second Ca2+-binding domain of NCX1 binds Mg2+ with high affinity. *Biochemistry* **50**: 8804-8812.

82. Capkauskaite E, Zubriene A, Smirnov A, Torresan J, Kisonaite M, Kazokaite J, Gylyte J, Michailoviene V, Jogaite V, Manakova E, Grazulis S, Tumevicius S, Matulis D. 2013. Benzenesulfonamides with pyrimidine moiety as inhibitors of human carbonic anhydrases I, II, VI, VII, XII, and XIII. *Bioorgan. Med. Chem.* **21**: 6937-6947.

83. Cecioni S, Faure S, Darbost U, Bonnamour I, Parrot-Lopez H, Roy O, Taillefumier C, Wimmerova M, Praly J-P, Imberty A, Vidal S. 2011. Selectivity among two lectins: Probing the

effect of topology, multivalency and flexibility of "clicked" multivalent glycoclusters. *Chem. Eur. J.* **17**: 2146-2159.

84. Cecioni S, Praly J-P, Matthews SE, Wimmerova M, Imberty A, Vidal S. 2012. Rational design and synthesis of optimized glycoclusters for multivalent lectin-carbohydrate interactions: influence of the linker arm. *Chem. Eur. J.* **18**: 6250-6263.
85. Chabre YM, Giguere D, Blanchard B, Rodrigue J, Rocheleau S, Neault M, Rauthu S, Papadopoulos A, Arnold AA, Imberty A, Roy P.2011. Combining glycomimetic and multivalent strategies toward designing potent bacterial lectin inhibitors. *Chem. Eur. J.* **17**: 6545-6562.
86. Chakraborti S, Joshi P, Chakravarty D, Shanker V, Ansari ZA, Singh SP, Chakrabarti P. 2012. Interaction of polyethyleneimine-functionalized ZnO nanoparticles with bovine serum albumin. *Langmuir* **28**: 11142-11152.
87. Chakraborty S, Joshi P, Shanker V, Ansari ZA, Singh SP, Chakrabarti P. 2011. Contrasting effect of gold nanoparticles and nanorods with different surface modifications on the structure and activity of bovine serum albumin. *Langmu*ir **27**: 7722-7731.
88. Chang Y, McLandsborough L, McClements DJ. 2011. Interactions of a cationic antimicrobial (epsilon-polylysine) with an anionic biopolymer (pectin): An isothermal titration calorimetry, microelectrophoresis, and turbidity study. *J. Agric. Food Chem.* **59**: 5579-5588.
89. Colussi F, Sorensen TH, Alasepp K, Kari J, Cruys-Bagger N, Windahl MS, Olsen JP, Borch K, Westh P. 2015. Probing substrate interactions in the active tunnel of a catalytically deficient cellobiohydrolase (Cel7*). J. Biol. Chem*. **290**: 2444-2454.
90. Cummaro A, Fotticchia I, Franceschin M, Giancola C, Petraccone L. 2011. Binding properties of human telomeric quadruplex multimers: A new route for drug design. *Biochimie* **93**: 1392-1400.
91. Dahms SO, Koennig I, Roeser D, Guehrs K-H, Mayer MC, Kaden D, Multhaup G, Than ME. 2012. Metal binding dictates conformation and function of the amyloid precursor protein (APP) E2 domain. *J. Mol. Biol*. **416**: 438-452.
92. Dobreva MA, Frazier RA, Mueller-Harvey I, Clifton LA, Gea A, Green RJ. 2011. Binding of pentagalloyl glucose to two globular proteins occurs via multiple surface sites. *Biomacromolecules* **12**: 710-715.
93. Du X, Dubin PL, Hoagland DA, Sun L. 2014. Protein-selective coacervation with hyaluronic acid. *Biomacromolecules* **15**: 726-734.
94. Edink E, Rucktooa P, Retra K, Akdemir A, Nahar T, Zuiderveld O, van Elk R, Janssen E, van Nierop P, van Muijlwijk-Koezen J, Smit AB, Sixma TK, Leurs R, de Esch IJP. 2011. Fragment growing induces conformational changes in acetylcholine-binding protein: A structural and thermodynamic analysis. *J. Am. Chem. Soc*. **133**: 5363-5371.
95. Fox JM, Kang K, Sherman W, Heroux A, Sastry GM, Baghbanzadeh, Lockett MR, Whiteside GM. 2015. Interactions between Hofmeister anions and the binding pocket of a protein. *J. Am. Chem. Soc*. **137**, 3859-3866.
96. Freiburger LA, Baettig OM, Sprules T, Berghuis AM, Auclair K, Mittermaier AK. 2011. Competing allosteric mechanisms modulate substrate binding in a dimeric enzyme. *Nat. Str. Mol. Biol.* **18**: 288-294.
97. Gallegos KM, Conrady DG, Karve SS, Gunasekera TS, Herr AB, Weiss AA. 2012. Shiga Toxin Binding to Glycolipids and Glycans. *Plos One* **7**: e30368.
98. Ghosh N, Mondal R, Mukherjee S. 2015. Hydrophobicity is the governing factor in the interaction of human serum albumin with bile salts. *Langmuir* **31**: 1095-1104.

99. Gundlach J, Dickmanns A, Schroeder-Tittmann K, Neumann P, Kaesler J, Kampf J, Herzberg C, Hammer E, Schwede F, Kaever V, Tittmann K, Stulke J, Ficner R. 2015. Identification, characterization, and structure analysis of the cyclic di-AMP-binding P-II-like signal transduction protein DarA. *J. Biol. Chem*. **290**: 3069-3080.
100. Guo J, Catchmark JM. 2013. Binding specificity and thermodynamics of cellulose-binding modules from *Trichoderma reesei* Cel7A and Cel6A. *Biomacromolecules* **14**: 1268-1277.
101. Hamre AG, Jana S, Holen MM, Mathiesen G, Vaeljamaee P, Payne CM, Sorlie M. 2015. Thermodynamic relationships with processivity in *Serratia marcescens* family 18 chitinases. *J. Phys. Chem.B* **119**: 9601-9613.
102. Henry G, Deleu M, Jourdan E, Thonart P, Ongena M. 2011. The bacterial lipopeptide surfactin targets the lipid fraction of the plant plasma membrane to trigger immune-related defence responses. *Cell. Microbiol*. **13**: 1824-1837.
103. Herold JM, Wigle TJ, Norris JL, Lam R, Korboukh VK, Gao C, Ingerman LA, Kireev DB, Senisterra G, Vedadi M, Tripathy A, Brown PJ, Arrowsmith CH, Jin JA, Janzen WP, Frye SV. 2011. Small-molecule ligands of methyl-lysine binding proteins. *J. Med. Chem*. **54**: 2504-2511.
104. Hoernke M, Schwieger C, Kerth A, Blume A. 2012. Binding of cationic pentapeptides with modified side chain lengths to negatively charged lipid membranes: Complex interplay of electrostatic and hydrophobic interactions. *BBA-Rev Biomembanes* **1818**: 1663-1672.
105. Huang R, Carney RR, Ikuma K, Stellacci F, Lau BLT. 2014. Effects of surface compositional and structural heterogeneity on nanoparticle-protein interactions: Different protein configurations. *Acs Nano* **8**: 5402-5412.
106. Jha NS, Kishore N. 2011. Thermodynamic studies on the interaction of folic acid with bovine serum albumin. *J. Chem. Thermodyn*. **43**: 814-821.
107. Karonen M, Oraviita M, Mueller-Harvey I, Salminen J-P, Green RJ. 2015. Binding of an oligomeric ellagitannin series to bovine serum albumin (BSA): Analysis by isothermal titration calorimetry (ITC). *J. Agric. Food Chem*. **63**: 10647-10654.
108. Krimmer SG, Betz M, Heine A, Klebe G. 2014. Methyl, ethyl, propyl, butyl: Futile but not for water, as the correlation of structure and thermodynamic signature shows in a congeneric series of thermolysin inhibitors. *ChemMedChem* **9**: 833-846.
109. Li X, Wang G, Chen D, Lu Y. 2014. Interaction of procyanidin B3 with bovine serum albumin. *Rsc Advances* **4**: 7301-7312.
110. Li X, Wang S. 2015. Study on the interaction of (+)-catechin with human serum albumin using isothermal titration calorimetry and spectroscopic techniques. *New J. Chem*. **39**: 386-395.
111. Lin H, Kitova EN, Klassen JS. 2014. Measuring positive cooperativity using the direct ESI-MS assay. Cholera toxin B subunit homopentamer binding to GM1 pentasaccharide. *J. Am. Soc. Mass Spectr*. **25**: 104-110.
112. Lin P-H, Chen R-H, Lee C-H, Chang Y, Chen C-S, Chen W-Y. 2011. Studies of the binding mechanism between aptamers and thrombin by circular dichroism, surface plasmon resonance and isothermal titration calorimetry. *Colloid. Surface. B* **88**: 552-558.
113. Loch JI, Bonarek P, Polit A, Ries D, Dziedzicka-Wasylewska M, Lewinski K. 2013. Binding of 18-carbon unsaturated fatty acids to bovine beta-lactoglobulin-Structural and thermodynamic studies. *Int. J. Biol. Macromol*. **57**: 226-231.
114. Macias AT, Williamson DS, Allen N, Borgognoni J, Clay A, Daniels Z, Dokurno P, Drysdale MJ, Francis GL, Graham CJ, Howes R, Matassova N, Murray JB, Parsons R, Shaw T, Surgenor AE, Terry L, Wang YK, Wood M, Massek AJ. 2011. Adenosine-derived inhibitors of 78 kDa glucose

regulated protein (Grp78) ATPase: Insights into isoform selectivity. *J. Med. Chem*. **54**: 4034-4041.

115. Madrona Y, Hollingsworth SA, Khan B, Poulos TL. 2013. P450cin active site water: Implications for substrate binding and solvent accessibility. *Biochemistry* **52**: 5039-5050.
116. Martinez A, Suarez J, Shand T, Magliozzo RS, Sanchez-Delgado RA. 2011. Interactions of arene-Ru(II)-chloroquine complexes of known antimalarial and antitumor activity with human serum albumin (HSA) and transferrin. *J. Inorg. Biochem*. **105** :39-45.
117. McRae JM, Ziora ZM, Kassara S, Cooper MA, Smith PA. 2015. Ethanol concentration influences the mechanisms of wine tannin interactions with poly(L-proline) in model wine. *J. Agric. Food Chem*. **63**: 4345-4352.
118. Mecinovic J, Snyder PW, Mirica KA, Bai S, Mack ET, Kwant RL, Moustakas DT, Heroux A, Whitesides GM. 2011. Fluoroalkyl and alkyl chains have similar hydrophobicities in binding to the "hydrophobic wall" of carbonic anhydrase. *J. Am. Chem. Soc*. **133**: 14017-14026.
119. Neeb M, Czodrowski P, Heine A, Barandun LJ, Hohn C, Diederich F, Klebe G. 2014. Chasing protons: How isothermal titration calorimetry, mutagenesis, and pKa calculations trace the locus of charge in ligand binding to a tRNA-binding enzyme. *J. Med. Chem*. **57**: 5554-5565.
120. Olsson TSG, Ladbury JE, Pitt WR, Williams MA. 2011. Extent of enthalpy-entropy compensation in protein-ligand interactions. *Protein Sci*. **20**: 1607-1618.
121. Palde PB, Carroll KS. 2015. A universal entropy-driven mechanism for thioredoxin-target recognition. *Proc. Natl. Acad. Sci. USA* **112**: 7960-7965.
122. Perspicace S, Rufer AC, Thoma R, Mueller F, Hennig M, Ceccarelli S, Schulz-Gasch T, Seelig J. 2013. Isothermal titration calorimetry with micelles: Thermodynamics of inhibitor binding to carnitine palmitoyltransferase 2 membrane protein. *Febs Open Bio*. **3**: 204-211.
123. Qin SB, Shimamoto S, Maruno T, Kobayashi Y, Kawahara K, Yoshida T, Ohkubo T. 2015. *Biochem. Bioph. Res. Co.* **468**: 234-239.
124. Ren J, He Y, Chen W, Chen T, Wang G, Wang Z, Xu Z, Luo X, Zhu W, Jiang H ,Shen JS, Xu YC. 2014. Thermodynamic and structural characterization of halogen bonding in protein-ligand interactions: A case study of PDE5 and its inhibitors. *J. Med. Chem*. **57**: 3588-3593.
125. Reynolds M, Marradi M, Imberty A, Penades S, Perez S. 2012. Multivalent gold glycoclusters: High affinity molecular recognition by bacterial lectin PA-IL. *Chem. Eur. J*. **18**: 4264-4273.
126. Rich AM, Bombarda E, Schenk AD, Lee PE, Cox EH, Spuches AM, Hudson LD, Kieffer B, Wilcox DE. 2012. Thermodynamics of Zn2+ binding to Cys(2)His(2) and Cys(2)HisCys zinc fingers and a Cys(4) transcription factor site. *J. Am. Chem. Soc*. **134**: 10405-10418.
127. Rogez-Florent T, Duhamel L, Goossens L, Six P, Drucbert A-S, Depreux P, Danze P-M, Landy D, Goossens J-F, Foulon C. 2014. Label-free characterization of carbonic anhydrase-novel inhibitor interactions using surface plasmon resonance, isothermal titration calorimetry and fluorescence-based thermal shift assays. *J. Mol. Recognit*. **27**: 46-56.
128. Sacco C, Skowronsky RA, Gade S, Kenney JM, Spuches AM. 2012. Calorimetric investigation of copper(II) binding to A beta peptides: thermodynamics of coordination plasticity. *J. Biol. Inorg. Chem*. **17**: 531-541.
129. Sack JS, Thieffine S, Bandiera T, Fasolini M, Duke GJ, Jayaraman L, Kish KF, Klei HE, Purandare AV, Rosettani P, Trioani S, Xie DL, Bertrand JA. 2011. Structural basis for CARM1 inhibition by indole and pyrazole inhibitors. *Biochem. J*. **436**: 331-339.
130. Safaee N, Kozlov G, Noronha AM, Wilds CJ, Gehring K. 2012. Interdomain allostery promotes assembly of the poly(A) mRNA complex with PABP and eIF4G. *Mol. Cell* **48**: 375-386.

131. Safi S, Creff G, Jeanson A, Qi L, Basset C, Roques J, Solari PL, Simoni E, Vidaud C, Den Auwer C. 2013. Osteopontin: A uranium phosphorylated binding-site characterization. *Chem. Eur. J.* **19**: 11261-11269.
132. Sharma S, Maris C, Allain FHT, Black DL. 2011. U1 snRNA directly interacts with polypyrimidine tract-binding protein during splicing repression. *Mol. Cell* **41**: 579-588.
133. Smith E, Vekaria R, Brown KA, Longstaff C. 2013. Kinetic regulation of the binding of prothrombin to phospholipid membranes. *Mol. Cell. Biochem*. **382**: 193-201.
134. Tavares GM, Croguennec T, Le S, Lerideau O, Hamon P, Carvaho AF, Bouhallab S. 2015. Binding of folic acid induces specific self-aggregation of lactoferrin: Thermodynamic characterization. *Langmuir* **31**: 12481-12488.
135. Trapaidze A, Hureau C, Bal W, Winterhalter M, Faller P. 2012. Thermodynamic study of Cu2+ binding to the DAHK and GHK peptides by isothermal titration calorimetry (ITC) with the weaker competitor glycine. *J. Biol. Inorg. Chem*. **17**: 37-47.
136. Wang I, Hennig J, Jagtap PKA, Sonntag M, Valcarcel J, Sattler M. 2014. Structure, dynamics and RNA binding of the multi-domain splicing factor TIA-1. *Nucleic Acids Res*. **42**: 5949-5966.
137. Wang S, Chen K, Li L, Guo X. 2013. Binding between Proteins and Cationic Spherical Polyelectrolyte brushes: Effect of pH, ionic strength, and stoichiometry. *Biomacromolecules* **14**: 818-827.
138. Weaver KD, Vrikkis RM, Van Vorst MP, Trullinger J, Vijayaraghavan R, Foureau DM, McKillop IH, MacFarlane DR, Krueger JK, Elliott GD. 2012. Structure and function of proteins in hydrated choline dihydrogen phosphate ionic liquid. *Phys. Chem. Chem. Phys*. **14**: 790-801.
139. Welsch N, Becker AL, Dzubiella J, Ballauff M. 2012. Core-shell microgels as "smart'' carriers for enzymes. *Soft Matter* **8**: 1428-1436.
140. Xu C, Liu K, Tempel W, Demetriades M, Aik W, Schofield CJ, Min J. 2014. Structures of human ALKBH5 demethylase reveal a unique binding mode for specific single-stranded N-6-methyladenosine RNA demethylation. *J. Biol. Chem*. **289**: 17299-17311.
141. Zakharov MN, Bhasin S, Travison TG, Xue R, Ulloor J, Vasan RS, Carter E, Wu F, Jasuja R. 2015. A multi-step, dynamic allosteric model of testosterone's binding to sex hormone binding globulin. *Mol. Cell. Endocrinol*. **399**: 190-200.
142. Zhao T, Chen K, Gu H. 2013. Investigations on the interactions of proteins with polyampholyte-coated magnetite nanoparticles. *J. Phys. Chem.B* **117**: 14129-14135.
143. Zhao Y, Chen L, Han L, Marzinek JK, Mantalaris A, Pistikopoulos EN, Lian G, Bond PJ, Noro MG. 2013. Molecular and thermodynamic basis for EGCG-keratin interaction-part II: Experimental investigation. *Aiche J*. **59**: 4824-4827.

(vi) Lipids, micelles and membranes

144. Brinatti C, Mello LB, Loh W. 2014. Thermodynamic study of the micellization of zwitterionic surfactants and their interaction with polymers in water by isothermal titration calorimetry. *Langmuir* **30**: 6002-6010.
145. Cambon A, Alatorre-Meda M, Juarez J, Topete A, Mistry D, Attwood D, Barbosa S, Taboada P, Mosquera V. 2011. Micellisation of triblock copolymers of ethylene oxide and 1,2-butylene oxide: Effect of B-block length. *J. Colloid Interf. Sci*. **361**: 154-158.

146. Fan Z, Tong W, Zheng Q, Lei Q, Fang W. 2013. Surface activity and micellization parameters of quaternary ammonium surfactants containing a hydroxyethyl group. *J. Chem. Eng. Data* **58**: 334-342.
147. Kamboj R, Bharmoria P, Chauhan V, Singh G, Kumar A, Singh S, Kang TS. 2014. Effect of cationic head group on micellization behavior of new amide-functionalized surface active ionic liquids. *Phys. Chem. Chem. Phys* **16**: 26040-26050.
148. Kroflic A, Sarac B, Bester-Rogac M. 2011. Influence of the alkyl chain length, temperature, and added salt on the thermodynamics of micellization: Alkyltrimethylammonium chlorides in NaCl aqueous solutions. *J. Chem. Thermodyn*. **43**: 1557-1563.
149. Walrant A, Correia I, Jiao C-Y, Lequin O, Bent EH, Goasdoue N, Lacombe C, Chassaing G, Sagan S, Alves ID. 2011. Different membrane behaviour and cellular uptake of three basic arginine-rich peptides. BBA-Rev. *Biomembranes* **1808**: 382-393.
150. Yokoyama H, Ikeda K, Wakabayashi M, Ishihama Y, Nakano M. 2013. Effects of Lipid Membrane Curvature on Lipid Packing State Evaluated by Isothermal Titration Calorimetry. *Langmuir* **29**: 857-860.
151. Zhang H, Zeeb B, Salminen H, Weiss J. 2015. Isothermal titration calorimetric analysis on solubilization of an octane oil-in-water emulsion in surfactant micelles and surfactant-anionic polymer complexes. *J. Colloid Interf. Sci*. **438**: 7-13.

(vii) Polysaccharides

152. Huang Y, Lapitsky Y. 2011. Monovalent salt enhances colloidal stability during the formation of chitosan/tripolyphosphate microgels. *Langmuir* **27**: 10392-10399.
153. Menchicchi B, Fuenzalida JP, Bobbili KB, Hensel A, Swamy MJ, Goycoolea FM. 2014. Structure of chitosan determines its interactions with mucin. *Biomacromolecules* **15**: 3550-3558.
154. Mertins O, Dimova R. 2011. Binding of chitosan to phospholipid vesicles studied with isothermal titration calorimetry. *Langmuir* **27**: 5506-5515.
155. Sheng G-P, Xu J, Luo H-W, Li W-W, Li W-H, Yu H-Q, Xie Z, Wei S-Q, Hu F-C. 2013. Thermodynamic analysis on the binding of heavy metals onto extracellular polymeric substances (EPS) of activated sludge. *Water Res*. **47**: 607-614.

(viii) Nucleic acids

156. Alatorre-Meda M, Taboada P, Hardl F, Wagner T, Freis M, Rodriguez JR. 2011. The influence of chitosan valence on the complexation and transfection of DNA The weaker the DNA-chitosan binding the higher the transfection efficiency. *Colloid. Surface. B* **82**: 54-62.
157. Boncina M, Lah J, Prislan I, Vesnaver G. 2012. Energetic basis of human telomeric DNA folding into G-quadruplex structures. *J. Am. Chem. Soc*. **134**: 9657-9663.
158. Haris P, Varughese M, Haridas M, Sudarsanakumar C. 2015. Energetics, thermodynamics, and molecular recognition of piperine with DNA. *J. Chem. Inf. Model*. **55**: 2644-2656.
159. Khakshoor O, Wheeler SE, Houk KN, Kool ET. 2012. Measurement and theory of hydrogen bonding contribution to isosteric DNA base pairs. *J. Am. Chem. Soc*. **134**: 3154-3163.
160. Kumar S, Xue L, Arya DP. 2011. Neomycin-neomycin dimer: An all-carbohydrate scaffold with high affinity for AT-rich DNA duplexes. *J. Am. Chem. Soc*. **133**: 7361-7375.

161. Lewis EA, Munde M, Wang S, Rettig M, Le V, Machha V, Wilson WD. 2011. Complexity in the binding of minor groove agents: netropsin has two thermodynamically different DNA binding modes at a single site. *Nucleic Acids Res*. **39**: 9649-9658.
162. Lu Y, Wang G, Tang W, Liao X, Xu M, Li X. 2011. Study on the interaction of amino phosphine ester derivatives with DNA by spectroscopy, modeling and calorimetry. *Spectrochim. Acta A* **82**: 247-252.
163. Priftis D, Laugel N, Tirrell M. 2012. Thermodynamic characterization of polypeptide complex coacervation. *Langmuir* **28**: 15947-15957.
164. Subastri A, Ramamurthy CH, Suyavaran A, Mareeswaran R, Rao PL, Harikrishna M, Kumar MS, Sujatha V, Thirunavukkarasu C. 2015. Spectroscopic and molecular docking studies on the interaction of troxerutin with DNA. *Int. J. Biol. Macromol*. **78**: 122-129.
165. Trotta R, De Tito S, Lauri I, La Pietra V, Marinelli L, Cosconati S, Martino L, Conte MR, Mayol L, Novellino E, Randazzo A. 2011. A more detailed picture of the interactions between virtual screening-derived hits and the DNA G-quadruplex: NMR, molecular modelling and ITC studies. *Biochimie* **93**: 1280-1287.
166. Vander Meulen KA, Butcher SE. 2012. Characterization of the kinetic and thermodynamic landscape of RNA folding using a novel application of isothermal titration calorimetry. *Nucleic Acids Res*. **40**: 2140-2151.
167. Wang G, Wu H, Wang D, Yan C, Lu Y. 2013. Exploring the binding mechanism of phosphoramidate derivative with DNA: Spectroscopy, calorimetry and modeling. *Spectrochim. Acta A* **104**: 492-496.
168. Wang G, Yan C, Lu Y. 2013. Exploring DNA binding properties and biological activities of dihydropyrimidinones derivatives. *Colloids Surface. B* **106**: 28-36.
169. Zhang J, Jones CP, Ferre-D'Amare AR. 2014. Global analysis of riboswitches by small-angle X-ray scattering and calorimetry. *BBA-Gene Regul. Mech*. **1839**: 1020-1029.

(ix) Synthetic chemicals, polymers and nanoparticles

170. Adriaenssens L, Gil-Ramírez G, Frontera A, Quiñonero D, Escudero-Adán EC, Ballester P. 2014. Thermodynamic characterization of halide−π interactions in solution using “two-wall” aryl extended calix[4]pyrroles as model system. *J. Am. Chem. Soc*. **136**, 3208-3218.
171. Amendola V, Fabbrizzi L, Mosca L, Schmidtchen F-P. 2011. Urea-, squaramide-, and sulfonamide-based anion receptors: A thermodynamic study. *Chem. Eur. J*. **17**: 5972-5981.
172. Aparicio F, Garcia F, Sanchez L. 2013. Supramolecular polymerization of C3-symmetric organogelators: Cooperativity, solvent, and gelation relationship. *Chem. Eur. J*. **19**: 3239-3248.
173. Arranz-Mascaros P, Bazzicalupi C, Bianchi A, Giorgi C, Godino-Salido M-L, Gutierrez-Valero M-D, Lopez-Garzon R, Savastano M. 2013. Thermodynamics of anion-π interactions in aqueous solutions. *J. Am. Chem. Soc*. **135**: 102-105.
174. Brotin T, Goncalves S, Berthault P, Cavagnat D, Buffeteau T. 2013. Influence of the cavity size of water-soluble cryptophanes on their binding properties for cesium and thallium cations. *J. Phys. Chem.B* **117**: 12593-12601.
175. Castellano BM, Eggers DK. 2013. Experimental support for a desolvation energy term in governing equations for binding equilibria. *J. Phys. Chem. B* **117**: 8180-8188.
176. Cawthray JF, Creagh AL, Haynes CA, Orvig C. 2015. Ion exchange in hydroxyapatite with lanthanides. *Inorg. Chem*. **54**: 1440-1445.

177. Chen S, Yamasaki M, Polen S, Gallucci J, Hadad CM, Badjic JD. 2015. Dual cavity basket promotes encapsulation in water in an allosteric fashion. *J. Am. Chem. Soc*. **137**: 12276-12281.
178. Dale EJ, Vermeulen NA, Thomas AA, Barnes JC, Juricek M, Blackburn AK, Strutt NL, Sarjeant AA, Stern CL, Denmark SE, Stoddart JF. 2014. ExCage. *J. Am. Chem. Soc*. **136**: 10669-10682.
179. De Lisi R, Giammona G, Lazzara G, Milioto S. 2011. Copolymers sensitive to temperature and pH in water and in water plus oil mixtures: A DSC, ITC and volumetric study. *J. Colloid Interface Sci*. **354**: 749-757.
180. Dionisio M, Oliviero G, Menozzi D, Federici S, Yebeutchou RM, Schmidtchen FP, Dalcanale E, Bergese P. 2012. Nanomechanical recognition of N-methylammonium salts. *J. Am. Chem. Soc.* **134**: 2392-2398.
181. Francisco V, Piñeiro A, Nau WM, Garcia-Rio L. 2013. The "true" affinities of metal cations to p-sulfonatocalix[4]arene: a thermodynamic study at neutral pH reveals a pitfall due to salt effects in microcalorimetry. *Chem. Eur. J*. **19**: 17809-17820.
182. Gibb CLD, Oetling EE, Velaga S, Gibb BC. 2015. Thermodynamic profiles of salt effects on a host-guest system: New insight into the hofmeister effect. *J. Phys. Chem. B* **119**: 5624-5638.
183. Hansen A, Bannwarth C, Grimme S, Petrovic P, Werle C, Djukic J-P. 2014. The Thermochemistry of London dispersion-driven transition metal reactions: Getting the 'right answer for the right reason'. *Chemistryopen* **3**: 177-189.
184. Holzerny P, Ajdini B, Heusermann W, Bruno K, Schuleit M, Meinel L, Keller M. 2012. Biophysical properties of chitosan/siRNA polyplexes: Profiling the polymer/siRNA interactions and bioactivity. *J. Control. Release* **157**: 297-304.
185. Hornung J, Fankhauser D, Shirtcliff LD, Praetorius A, Schweizer WB, Diederich F. 2011. Cycloalkane and alicyclic heterocycle complexation by new switchable resorcin[4] arene-based container molecules: NMR and ITC binding studies. *Chem. Eur. J*. **17**: 12362-12371.
186. Huang Y, Lapitsky Y. 2013. Determining the colloidal behavior of ionically cross-linked polyelectrolytes with isothermal titration calorimetry. *J. Phys. Chem.B* **117**: 9548-9557.
187. Jungbauer SH, Schindler S, Herdtweck E, Keller S, Huber SM. 2015. Multiple multidentate halogen bonding in solution, in the solid state, and in the (calculated) gas phase. *Chem. Eur. J*. **21**: 13625-13636.
188. Kabiri M, Bushnak I, McDermot MT, Unsworth LD.2013. Toward a mechanistic understanding of ionic self-complementary peptide self-assembly: Role of water molecules and ions. *Biomacromolecules* **14**: 3943-3950.
189. Le VH, Yanney M, McGuire M, Sygula A, Lewis EA. 2014. Thermodynamics of host–guest interactions between fullerenes and a buckycatcher. *J. Phys. Chem B* **118**: 11956-11964.
190. Lin W, Walter J, Burger A, Maid H, Hirsch A, Peukert W, Segets D. 2015. A general approach to study the thermodynamics of ligand adsorption to colloidal surfaces demonstrated by means of catechols binding to zinc oxide quantum dots. *Chem. Mater*. **27**: 358-369.
191. Lisbjerg M, Nielsen BE, Milhoj BO, Sauer SPA, Pittelkow M.2015. Anion binding by biotin[6]uril in water. *Org. Biomol. Chem*. **13**: 369-373.
192. McCann N, Maeder M, Hasse H. 2011. A calorimetric study of carbamate formation. *J. Chem. Thermodyn.* **43**: 664-669.
193. Moers C, Nuhn L, Wissel M, Stangenberg R, Mondeshki M, Berger-Nicoletti E, Thomas A, Schaeffel D, Koynov K, Klapper M, Zendel M, Frey H. 2013. Supramolecular linear-g-hyperbranched graft polymers: Topology and binding strength of hyperbranched side chains. *Macromolecules* **46**: 9544-9553.

194. Mokhtari B, Pourabdollah K. 2012. Binding mechanisms of nano-baskets toward alkali metals. *J. Therm. Anal. Calorim*. 110: 1043-1051.
195. Schumacher S, Katterle M, Hettrich C, Paulke B-R, Hall DG, Scheller FW, Gajovic-Eichelmann N. 2011. Label-free detection of enhanced saccharide binding at pH 7.4 to nanoparticulate benzoboroxole based receptor units. *J. Mol. Recognit*. 24: 953-959.
196. Taladriz-Blanco P, Buurma NJ, Rodriguez-Lorenzo L, Perez-Juste J, Liz-Marzan LM, Herves P. 2011. Reversible assembly of metal nanoparticles induced by penicillamine. Dynamic formation of SERS hot spots. *J. Mater. Chem*. **21**: 16880-16887.
197. Turcu I, Mic M. 2013. Size dependence of molecular self-assembling in stacked aggregates. 2. Heat exchange effects. *J. Phys. Chem. B* **117**: 9083-9093.
198. Uchman M, Gradzielski M, Angelov B, Tosner Z, Oh J, Chang T, Stepanek M, Prochazka K.2013. Thermodynamic and kinetic aspects of coassembly of PEO-PMAA block copolymer and DPCl surfactants into ordered nanoparticles in aqueous solutions studied by ITC, NMR, and time-resolved SAXS techniques. *Macromolecules* **46**: 2172-2181.
199. Wang Z, Xu S, Acosta E. 2015. Heat of adsorption of surfactants and its role on nanoparticle stabilization. *J. Chem. Thermodyn*. **91** :256-266.
200. Werber L, Preiss LC, Landfester K, Munoz-Espi R, Mastai Y. 2015. Isothermal titration calorimetry of chiral polymeric nanoparticles. *Chirality* **27**: 613-618.
201. Willerich I, Groehn F. 2011. Thermodynamics of photoresponsive polyelectrolyte-dye assemblies with irradiation wavelength triggered particle size. *Macromolecules* **44**: 4452-4461.
202. Wszelaka-Rylik M, Gierycz P. Isothermal titration calorimetry (ITC) study of natural cyclodextrins inclusion complexes with drugs. *J. Therm. Anal. Calorim*. **111**: 2029-2035.
203. Wyrzykowski D, Pilarski B, Jacewicz D, Chmurzynski L. 2013. Investigation of metal-buffer interactions using isothermal titration calorimetry. *J. Therm. Anal.* Calorim. **111**: 1829-1836.
204. Wyrzykowski D, Zarzeczanska D, Jacewicz D, Chmurzynski L. 2011. Investigation of copper(II) complexation by glycylglycine using isothermal titration calorimetry. *J. Therm. Anal. Calorim*. **105**: 1043-1047.
205. Xu Z, Singh NJ, Kim SK, Spring DR, Kim KS, Yoon J. 2011. Induction-driven stabilization of the anion-pi interaction in electron-rich aromatics as the key to fluoride inclusion in imidazolium-cage receptors. *Chem. Eur. J.* **17**: 1163-1170.
206. Yousefpour P, Atyabi F, Farahani EV, Sakhtianchi R, Dinarvand R. 2011. Polyanionic carbohydrate doxorubicin-dextran nanocomplex as a delivery system for anticancer drugs: in vitro analysis and evaluations. *Int. J. Nanomed*. **6**: 1487-1496.
207. Zeng Z, Patel J, Lee S-H, McCallum M, Tyagi A, Yan M, Shea KJ. 2012. Synthetic polymer nanoparticle-polysaccharide interactions: A systematic study. *J. Am. Chem. Soc.* **134**: 2681-2690.

(x) Enzyme kinetics

208. Aguirre C, Condado-Morales I, Olguin LF, Costas M. 2015. Isothermal titration calorimetry determination of individual rate constants of trypsin catalytic activity. *Anal. Biochem*. **479**: 18-27.
209. Bai W, Shen J, Zhu Y, Men Y, Sun Y, Ma Y. 2015. Characteristics and kinetic properties of L-rhamnose isomerase from *Bacillus subtilis* by isothermal titration calorimetry for the production of D-allose. *Food Sci. Technol. Res*. **21**: 13-22.

210. Demarse NA, Killian MC, Hansen LD, Quinn CF. 2013. Determining enzyme kinetics via isothermal titration calorimetry. Methods in molecular biology (Clifton, N.J.) 978:21-30
211. Ertan H, Siddiqui KS, Muenchhoff J, Charlton T, Cavicchioli R. 2012. Kinetic and thermodynamic characterization of the functional properties of a hybrid versatile peroxidase using isothermal titration calorimetry: Insight into manganese peroxidase activation and lignin peroxidase inhibition. *Biochimie* **94**: 1221-1231.
212. Krauss M, Zebisch M, Strater N. 2014. Development of an ITC based enzyme assay for nucleoside triphosphate diphosphohydrolases (NTPDases). *Purinerg.Signal.* **10**: 751-752.
213. Maximova K, Trylska J. 2015. Kinetics of trypsin-catalyzed hydrolysis determined by isothermal titration calorimetry. *Anal. Biochem*. **486**: 24-34.
214. Mendez-Lorenzo L, Porras-Dominguez JR, Raga-Carbajal E, Olvera C, Rodriguez-Alegria ME, Carrillo-Nava E, Costas M, Lopez Munguia A. 2015. Intrinsic levanase activity of *Bacillus subtilis* 168 levansucrase (SacB). *PloS one* **10**: e0143394.
215. Olsen SN, Bohlin C, Murphy L, Borch K, McFarland KC, Sweeny MD, Westh P. 2011. Effects of non-ionic surfactants on the interactions between cellulases and tannic acid: A model system for cellulase-poly-phenol interactions. *Enzyme and Microb. Tech*. **49**: 353-359
216. Rohatgi N, Gudmundsson S, Rolfsson O. 2015. Kinetic analysis of gluconate phosphorylation by human gluconokinase using isothermal titration calorimetry. *FEBS Lett*. **589**: 3548-55.
217. Volkova N, Ibrahim V, Hatti-Kaul R. 2012. Laccase catalysed oxidation of syringic acid: Calorimetric determination of kinetic parameters. *Enzyme Microb. Tech*. **50**: 233-237.

(xi) Pre-2011 and non-ITC references

218. Wiseman T, Williston S, Brandts JF, Lin LN. 1989. Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Anal. Biochem*. **179**: 131–137.
219. Kuriyan J, Konforti B, Wemmer D. 2013. The Molecules of Life: Physical and Chemical Principles. Garland Science: New York.
220. Atkins P, De Paula J. 2011. Physical Chemistry for the Life Sciences, 2nd Edition. W. H. Freeman and Company: New York.
221. Kozliak EI, Lambert FL. 2005. "Order-to-disorder" for entropy change? Consider the numbers! *Chem. Educator* **10**: 24-25.
222. Frank HS, Evans MW. 1945. Free volume and entropy in condensed systems. 3. Entropy in binary liquid mixtures – partial molal entropy in dilute solutions – structure and thermodynamics in aqueous electrolytes. *J. Chem. Phys*. **13**: 507-532.
223. Head-Gordon T. 1992. Is water structure around hydrophobic groups clathrate-like? *Proc. Natl. Acad. Sci. USA* **92**: 8308-8312.
224. Lambert FL. 2006. A modern view of entropy. *Chemistry* **15**: 13-21.
225. Ebbinghaus S, Kim SJ, Heyden M, Yu X, Heugen U, Gruebele M, Leitner DM, Havenith M. 2007. An extended dynamical hydration shell around proteins. *Proc. Natl. Acad. Sci*. **104**: 20749-20752.
226. Bye JW, Meliga S, Ferachou D, Cinque G, Zeitler JA, Falconer RJ. 2014. Analysis of the hydration water around bovine serum albumin using terahertz coherent synchrotron radiation. *J. Phys. Chem. A* **118**: 83-88.

227. Comez L, Lupi L, Morresi A, Paolantoni M, Sassi P. 2013. More is different: Experimental results on the effect of biomolecules on the dynamics of hydration water. *J. Phys. Chem. Lett*. **4**: 1188−1192.
228. King JT, Arthur EJ, Brooks III CL, Kubarych KJ. 2014. Crowding induced collective hydration of biological macromolecules over extended distances. *J. Am. Chem. Soc*. **136**: 188–194.
229. Wallace VP, Ferachou D, Ke P, Day K, Uddin S, Cacas-Finet J, van der Waal CF, Falconer RJ, Zeitler JA. 2015. Modulation of the hydration water around monoclonal antibodies on addition of excipients detected by terahertz-time domain spectroscopy. *J. Pharma. Sci*. **104**: 4025–4033.
230. Sigurskjold BW. 2000. Exact analysis of competition ligand binding by displacement isothermal titration calorimetry. *Anal. Biochem*. **277**: 260−266.
231. Velazquez-Campoy A, Freire E. 2006. Isothermal titration calorimetry to determine association constants for high-affinity ligands. *Nat. Protoc.* **1**: 186−191.
232. Collins, KD. 1995. Sticky ions in biological systems. *Proc. Natl. Acad .Sci. USA* **92**: 5553-5557.
233. Bye JW, Falconer RJ. 2014. Three-stages of lysozyme thermal stabilization by high and medium charge density anions. *J. Phys. Chem B* **118**: 4282-4286.
234. Platts L, Falconer RJ. 2015. Controlling protein stability: Mechanisms revealed using formulations of arginine, glycine and guanidinium HCl with three globular proteins. *Int. J.Pharm*. **486**: 131-135.

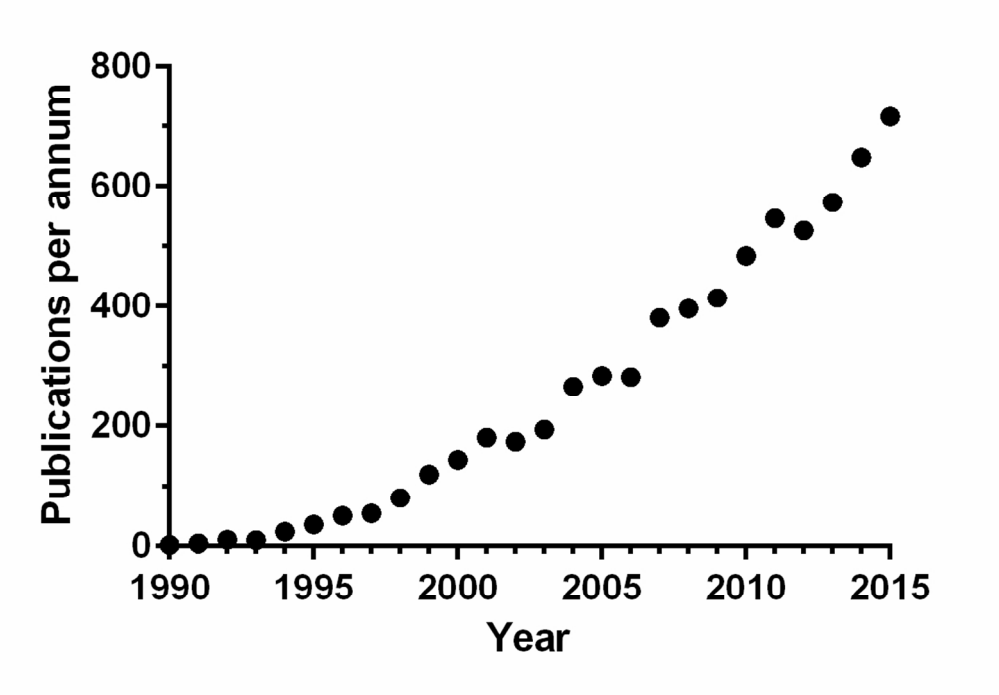
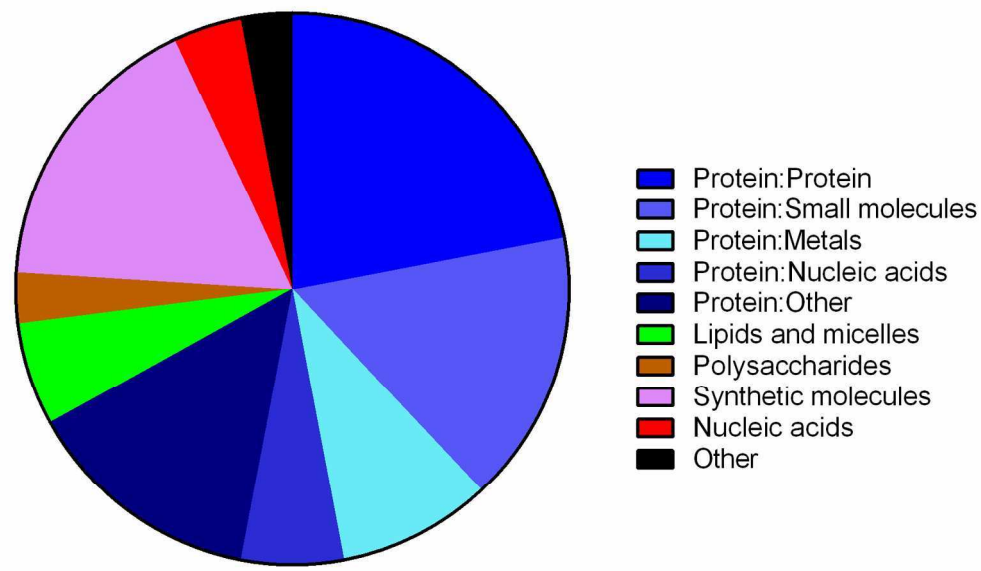


Figure 1
105x73mm (300 x 300 DPI)



154x90mm (300 x 300 DPI)