DSC XXVII - Reviews

Beezer A. E., Gaisford S., Hills A. K., Willson R. J., and Mitchell J. C. (1999) Pharmaceutical microcalorimetry: applications to long-term stability studies. *Int J Pharm* **179,** 159-165.
Abstract: Calorimetry has been a mainstay of stability analyses for some time in the form of differential scanning microcalorimetry (DSC). This technique exploits high (relatively) temperature studies of pure materials and of formulations to accelerate any degradation or interactions. The behaviour of the material at storage or ambient conditions is then estimated via extrapolation from the Arrhenius equation. Recent developments in isothermal microcalorimetry allow the direct determination of both kinetic and thermodynamic parameters for long, slow reactions from studies conducted at appropriate temperatures and under designated environmental control (pH, pO2, RH etc.). This review introduces the kinetic analysis of microcalorimetric data and, through selected examples, shows applications of the method.Copyright.

Brandau D. T., Jones L. S., Wiethoff C. M., Rexroad J., and Middaugh C. R. (2003) Thermal stability of vaccines. *J Pharm Sci* **92,** 218-231.
Abstract: Worldwide vaccination programs against infectious diseases and toxins are estimated to save approximately 3 million lives yearly. Tragically, however, another 3 million individuals (primarily children) die of vaccine-preventable diseases. A significant portion of this problem results from the thermal instability of many of the currently used vaccines. This review argues that modern methods of physical and chemical analysis permit for the first time characterization of the degradative pathways of thermally labile vaccines. A rigorous description of these pathways permit a more rational and systematic approach to the stabilization of vaccines. A direct result of the replacement of currently employed, primarily empirical, approaches to vaccine stabilization with a more molecular-based methodology should be the development of more universally available vaccinations against life-threatening diseases. This has the potential to have a dramatic impact on world health.

Breslauer K. J. (1994) Extracting thermodynamic data from equilibrium melting curves for oligonucleotide order-disorder transitions. *Methods Mol Biol* **26,** 347-372.

Bruylants G., Wouters J., and Michaux C. (2005) Differential scanning calorimetry in life science: thermodynamics, stability, molecular recognition and application in drug design. *Curr Med Chem* **12,** 2011-2020.
Abstract: All biological phenomena depend on molecular recognition, which is either intermolecular like in ligand binding to a macromolecule or intramolecular like in protein folding. As a result, understanding the relationship between the structure of proteins and the energetics of their stability and binding with others (bio)molecules is a very interesting point in biochemistry and biotechnology. It is essential to the engineering of stable proteins and to the structure-based design of pharmaceutical ligands. The parameter generally used to characterize the stability of a system (the folded and unfolded state of the protein for example) is the equilibrium constant (K) or the free energy (deltaG(o)), which is the sum of enthalpic (deltaH(o)) and entropic (deltaS(o)) terms. These parameters are temperature dependent through the heat capacity change (deltaCp). The thermodynamic parameters deltaH(o) and deltaCp can be derived from spectroscopic experiments, using the van't Hoff method, or measured directly using calorimetry. Along with isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) is a powerful method, less described than ITC, for measuring directly the thermodynamic parameters which characterize biomolecules. In this article, we summarize the principal thermodynamics parameters, describe the DSC approach and review some systems to which it has been applied. DSC is much used for the study of the stability and the folding of biomolecules, but it can also be applied in order to understand biomolecular interactions and can thus be an interesting technique in the process of drug design.

Chan H. S., Shimizu S., and Kaya H. (2004) Cooperativity principles in protein folding. *Methods Enzymol* **380,** 350-379.

Cooper A. and Johnson C. M. (1994) Introduction to microcalorimetry and biomolecular energetics. *Methods Mol Biol* **22,** 109-124.

Cooper A. (2005) Heat capacity effects in protein folding and ligand binding: a re-evaluation of the role of water in biomolecular thermodynamics. *Biophys Chem* **115,** 89-97.
Abstract: Large "anomalous" heat capacity (DeltaC(p)) effects are a common feature of the thermodynamics of biomolecular interactions in aqueous solution and, as a result of the improved facility for direct calorimetric measurements, there is a growing body of experimental data for such effects in protein folding, protein-protein and protein-ligand interactions. Conventionally such heat capacity effects have been ascribed to hydrophobic interactions, and there are some remarkably convincing demonstrations of the usefulness of this concept. Nonetheless, there is also increasing evidence that hydrophobic interactions are not the only possible source of such effects. Here we re-evaluate the possible contributions of other interactions to the heat capacity changes to be expected for cooperative biomolecular folding and binding processes, with particular reference to the role of hydrogen bonding and solvent water interactions. Simple models based on the hydrogen-bonding propensity of water as a function of temperature give quantitative estimates of DeltaC(p) that compare well with experimental observations for both protein folding and ligand binding. The thermodynamic contribution of bound waters in protein complexes is also estimated. The prediction from simple lattice models is that trapping of water in a complex should give more exothermic binding (DeltaDeltaH-6 to -12 kJ mol(-1)) with lower entropy (DeltaDeltaS(0) approximately -11 J mol(-1) K(-1)) and more negative DeltaC(p) (by about -75 J mol(-1) K(-1)) per water molecule. More generally, it is clear that significant DeltaC(p) effects are to be expected for any macromolecular process involving a multiplicity of cooperative weak interactions of whatever kind.

Cooper A. and Johnson C. M. (1994) Differential scanning calorimetry. *Methods Mol Biol* **22,** 125-136.

Cooper A., Johnson C. M., Lakey J. H., and Nollmann M. (2001) Heat does not come in different colours: entropy-enthalpy compensation, free energy windows, quantum confinement, pressure perturbation calorimetry, solvation and the multiple causes of heat capacity effects in biomolecular interactions. *Biophys Chem* **93,** 215-230.
Abstract: Modern techniques in microcalorimetry allow us to measure directly the heat changes and associated thermodynamics for biomolecular processes in aqueous solution at reasonable concentrations. All these processes involve changes in solvation/hydration, and it is natural to assume that the heats for these processes should reflect, in some way, such changes in solvation. However, the interpretation of data is still somewhat ambiguous, since different non-covalent interactions may have similar thermodynamic signatures, and analysis is frustrated by large entropy-enthalpy compensation effects. Changes in heat capacity (Δ Cp) have been related to changes in hydrophobic hydration and non-polar accessible surface areas, but more recent empirical and theoretical work has shown how this need not always be the case. Entropy-enthalpy compensation is a natural consequence of finite Δ Cp values and, more generally, can arise as a result of quantum confinement effects, multiple weak interactions, and limited free energy windows, giving rise to thermodynamic homeostasis that may be of evolutionary and functional advantage. The new technique of pressure perturbation calorimetry (PPC) has enormous potential here as a means of probing solvation-related volumetric changes in biomolecules at modest pressures, as illustrated with preliminary data for a simple protein-inhibitor complex.

Cooper A., Nutley M.A., and Wadood A. (2001) Differential Scanning Calorimetry Protein-Ligand Interactions: Hydrodynamics and Calorimetry. Harding, S.E., Chowdhry, B.Z., eds., Oxford University Press, Oxford UK, pp. 287-318.

Cooper M. A. (2004) Advances in membrane receptor screening and analysis. *J Mol Recognit* **17,** 286-315.
Abstract: During the last decade there has been significant progress in the development of analytical techniques for the screening of ligand binding to membranes and membrane receptors. This review focuses on developments using label-free assays that facilitate ligand-membrane-receptor screening without the need for chemical-, biological- or radiological-labelled reagents. These assays include acoustic, optical surface plasmon resonance biosensing, sedimentation (analytical ultracentrifugation), chromatographic assays, isothermal titration calorimetry and differential scanning calorimetry. The merits and applications of cell-based screening systems and of different model membrane systems, including planar supported lipid layers, bead-supported membranes and lipid micro-arrays, are discussed. Recent advances involving more established techniques including intrinsic fluorescence, FRET spectroscopy, scintillation proximity assays and automated patch clamping are presented along with applications to peripheral membrane proteins, ion

channels and G protein-coupled receptors. Novel high-throughput assays for determination of drug- and protein-partitioning in membranes are also highlighted. To aid the experimenter, a brief synopsis of the techniques commonly employed to purify and reconstitute membranes and membrane receptors is included.

Edgcomb S. P. and Murphy K. P. (2000) Structural energetics of protein folding and binding. *Curr Opin Biotechnol* **11,** 62-66.
Abstract: Structural energetics is a method for calculating the energetics of protein folding and binding reactions as a function of temperature. This approach allows measured energetics to be interpreted with regards to the protein structure and the prediction of energetics from known structures. Recent advances include improvements in the parameterization of enthalpy, entropy and heat capacity terms and new applications, especially with regards to understanding dynamic properties of proteins and how these are affected by ligand binding.

Freire E. (1994) Statistical thermodynamic analysis of differential scanning calorimetry data: structural deconvolution of heat capacity function of proteins. *Methods Enzymol* **240,** 502-530.

Freire E. (1995) Thermal denaturation methods in the study of protein folding. *Methods Enzymol* **259,** 144-168.

Freire E. (1995) Differential scanning calorimetry. *Methods Mol Biol* **40,** 191-218.

Freire E. (1995) Thermodynamics of partly folded intermediates in proteins. *Annu Rev Biophys Biomol Struct* **24,** 141-165.
Abstract: Until recently, the energetics of protein-folding intermediates eluded direct measurement by high-sensitivity microcalorimetric techniques. But during the past year, the direct measurement of thermodynamic parameters for folding intermediates of alpha-lactalbumin, apomyoglobin, cytochrome c, and staphylococcal nuclease has provided new insights on the nature of the forces involved in the stabilization of nascent protein structures. In this review, I summarize those results and discuss the structural implications of the observed thermodynamic behavior.

Freire E. (1995) Differential scanning calorimetry. *Methods Mol Biol* **40,** 191-218.

Freire E. (2001) The thermodynamic linkage between protein structure, stability, and function. *Methods Mol Biol* **168,** 37-68.

Gromiha M. M., An J., Kono H., Oobatake M., Uedaira H., and Sarai A. (1999) ProTherm: Thermodynamic Database for Proteins and Mutants. *Nucleic Acids Res* **27,** 286-288.
Abstract: The first release of the Thermodynamic Database for Proteins and Mutants (ProTherm) contains more than 3300 data of several thermodynamic parameters for wild type and mutant proteins. Each entry includes numerical data for unfolding Gibbs free energy change, enthalpy change, heat capacity change, transition temperature, activity etc., which are important for understanding the mechanism of protein stability. ProTherm also includes structural information such as secondary structure and solvent accessibility of wild type residues, and experimental methods and other conditions. A WWW interface enables users to search data based on various conditions with different sorting options for outputs. Further, ProTherm is cross-linked with NCBI PUBMED literature database, Protein Mutant Database, Enzyme Code and Protein Data Bank structural database. Moreover, all the mutation sites associated with each PDB structure are automatically mapped and can be directly viewed through 3DinSight developed in our laboratory. The database is available at the URL, http://www.rtc.riken.go.jp/protherm.htm l.

Jelesarov I. and Bosshard H. R. (1999) Isothermal titration calorimetry and differential scanning calorimetry as complementary tools to investigate the energetics of biomolecular recognition. *J Mol Recognit* **12,** 3-18.
Abstract: The principles of isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC) are reviewed together with the basic thermodynamic formalism on which the two techniques are based. Although ITC is particularly suitable to follow the energetics of an association reaction between

biomolecules, the combination of ITC and DSC provides a more comprehensive description of the thermodynamics of an associating system. The reason is that the parameters ΔG, ΔH, ΔS, and ΔCp obtained from ITC are global properties of the system under study. They may be composed to varying degrees of contributions from the binding reaction proper, from conformational changes of the component molecules during association, and from changes in molecule/solvent interactions and in the state of protonation.

Kasper M. (2004) G.W.H. Hohne, W.F. Hemminger, H.-J. Flammersheim: Differential scanning calorimetry. *Anal Bioanal Chem* **380,** 366-367.

Koenigbauer M. J. (1994) Pharmaceutical applications of microcalorimetry. *Pharm Res* **11,** 777-783.
Abstract: General principles and applications of microcalorimetry are reviewed. Microcalorimetry is useful in the study of physical, chemical, and biological drug interactions. The sensitivity of the present instrumentation is approximately 0.1 μW. With this high sensitivity, additional applications have been developed, including the interactions of drugs with food, lymphoma cells, microorganisms, blood, excipients, and cyclodextrin. A recent application of microcalorimetry is the measurement of degradation rates of drugs.

Krell, T (2008) Microcalorimetry: a response to challenges in modern biotechnology. *Microbial Biotechnol* **1,** 126-136.
Abstract: Almost any process in life is accompanied by heat changes which can be monitored by isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC). Both techniques are now established tools in fundamental research but over the last decade a clear tendency towards more problem driven applications is noted. This review aims at summarizing these problem-oriented applications of microcalorimetry and the solutions both techniques can provide to problems in biotechnology. The biotechnological issues to which microcalorimetry has been successfully applied are as diverse as rational drug design, overcoming drug resistance, optimization of long-term stability of proteins, estimation of the bioavailability of drugs, control of complex pharmaceutical products or the optimization of gene delivery efficiency. The main limitation of microcalorimetry, which is the relatively large amounts of sample necessary for analysis, is less important in the biotechnology sector which frequently uses large-scale produced bulk products for analysis. The recently developed high-throughput DSC and ITC microcalorimeters will additionally reduce the labour intensity of these techniques. Due to the precision of microcalorimetric analyses and the versatility of processes which can be studied, it is expected that ITC and DSC will soon be key technologies in biotechnological research.

Ladbury J. E. (1995) Counting the calories to stay in the groove. *Structure* **3,** 635-639.
Abstract: High-sensitivity microcalorimetry is beginning to make an impact on the determination of thermodynamic parameters associated with protein-DNA interactions and the understanding of the relationship of these data to structural details of complex formation.

Lopez M. M. and Makhatadze G. I. (2002) Differential scanning calorimetry. *Methods Mol Biol* **173,** 113-119.

Luque I., Leavitt S. A., and Freire E. (2002) The linkage between protein folding and functional cooperativity: two sides of the same coin? *Annu Rev Biophys Biomol Struct* **31,** 235-256.
Abstract: During the course of their biological function, proteins undergo different types of structural rearrangements ranging from local to large-scale conformational changes. These changes are usually triggered by their interactions with small-molecular-weight ligands or other macromolecules. Because binding interactions occur at specific sites and involve only a small number of residues, a chain of cooperative interactions is necessary for the propagation of binding signals to distal locations within the protein structure. This process requires an uneven structural distribution of protein stability and cooperativity as revealed by NMR-detected hydrogen/deuterium exchange experiments under native conditions. The distribution of stabilizing interactions does not only provide the architectural foundation to the three-dimensional structure of a protein, but it also provides the required framework for functional cooperativity. In this review, the statistical thermodynamic linkage between protein stability, functional cooperativity, and ligand binding is discussed.

Makhatadze G. I. and Privalov P. L. (1995) Energetics of protein structure. *Adv Protein Chem* **47,** 307-425.

Mason J. T. (1998) Investigation of phase transitions in bilayer membranes. *Methods Enzymol* **295,** 468-494.
Abstract: This article described three techniques used to study phase transitions in phospholipid bilayers. The complementarity of the three techniques in characterizing the thermotropic and structural properties of phospholipid bilayers has been demonstrated by describing their use to characterize a series of mixed-chain-length PCs. It has been shown that an understanding of the energetics that govern the packing of phospholipid chains in the gel phase can be used to construct a model to interpret thermodynamic data of the PCs. This model, in turn, provided a framework for designing and interpreting the Raman spectroscopic and X-ray diffraction experiments on this series of phospholipids. The result was a complete description of the phase transitions and gel phase packing properties of the mixed-chain-length PCs. The phase diagram of Fig. 5B has been expanded to include the mixed-chain-length PC series C18C18PC through C18C0PC. Furthermore, the phase diagram and the chain inequivalence parameter have been shown to describe the behavior of any mixed-chain-length PC, irrespective of the lengths of the hydrocarbon chains or the position of the chains on the glycerol backbone. This is demonstrated by the additional mixed-chain-length PCs plotted in Fig. 5B. With minor modifications, the phase diagram also accurately describes the behavior of mixed-chain-length phosphatidylethanolamines, sphingomyelins, and unsaturated PCs. Finally, it has been demonstrated that a correlation exists between the thermodynamic and the Raman spectroscopic parameters determined for the phase transition of phospholipid bilayers. This correlation is based on the common chain energetics being measured by these two techniques.

Mavromoustakos T. M. (2007) The use of differential scanning calorimetry to study drug-membrane interactions. *Methods Mol Biol* **400,** 587-600.
Abstract: Differential-scanning calorimetry is a thermodynamic technique widely used for studying drug-membrane interactions. This chapter provides practical examples on this topic, highlighting the caution to be taken in analyzing thermal data as well as scientific information that can be derived by the proper use of the technique. An example is given using model bilayers containing high concentration of the anesthetic steroid alphaxalone. It is shown that the breadth of the phase transitions and the maximum of the phase-transition temperature of the bilayer depend on the equilibration conditions before acquiring the thermal scan. In addition, the quality of the thermo-gram depends on its perturbation and incorporation effects; for dissecting these effects, a complementary technique such as solid-state nuclear magnetic resonance spectroscopy is necessary. Differential-scanning calorimetry is a useful technique to study the interdigitation effect of a drug by monitoring DeltaH changes. Cholesterol, a main constituent of membrane bilayers, appears to disrupt the interdigitating effect. In general, the thermal effects of the drug incorporated into a membrane bilayer depends on the drug stereoelectronic properties.

Morikis D. and Lambris J. D. (2004) Physical methods for structure, dynamics and binding in immunological research. *Trends Immunol* **25,** 700-707.
Abstract: We present four experimental physical methods--X-ray and neutron diffraction, nuclear magnetic resonance spectroscopy, mass spectrometry and calorimetry--and two computational methods--molecular dynamics simulations and electrostatics calculations--which are general and widely applicable in the study of protein structure, dynamics and binding. These methods are useful tools for biologists that lead to structure-function, dynamics-function and binding-function correlations, in efforts to understand biomolecular function. Standard and emerging technologies within these methods are discussed and representative examples of applications in immunology are presented, from antigen-antibody, complement and MHC-T-cell receptor research. The examples demonstrate the power of the reviewed methods in immunological studies at the molecular level.

O'Brien R., and Haq I. (2004) Applications of Biocalorimetry: Binding, stability and enzyme kinetics *in* Biocalorimetry 2: Applications of Calorimetry in the Biological Sciences. Ladbury, J.E., Doyle, M.L., eds., John Wiley & Sons Ltd., Chichester UK, pp. 3-34.

Plum G. E. and Breslauer K. J. (1995) Calorimetry of proteins and nucleic acids. *Curr Opin Struct Biol* **5,** 682-690.
Abstract: The availability of sensitive calorimetric instrumentation has led to a considerable increase in

thermodynamic studies of proteins, nucleic acids, and their interactions. This article reviews some of the recent contributions of calorimetry to characterizing the thermodynamic origins of protein and nucleic acid stability and conformational preferences, as well as the interactions of proteins with each other, with small molecules, and with nucleic acids.

Privalov G. P. and Privalov P. L. (2000) Problems and prospects in microcalorimetry of biological macromolecules. *Methods Enzymol* **323,** 31-62.

Privalov P. L. (2007) Reflections on the origins of microcalorimetry of biopolymers. *Biophys Chem* **126,** 13-15.

Privalov P. L. and Dragan A. I. (2007) Microcalorimetry of biological macromolecules. *Biophys Chem* **126,** 16-24.
Abstract: The capabilities of contemporary differential scanning and isothermal titration microcalorimetry for studying the thermodynamics of protein unfolding/refolding and their association with partners, particularly target DNA duplexes, are considered. It is shown that the predenaturational changes of proteins must not be ignored in studying the thermodynamics of formation of their native structure and their complexes with partners, particularly their cognate DNA duplexes

Remmele R. L. (2005) Microcalorimetric approaches to biopharmaceutical development in Analytical techniques for biopharmaceutical development, Rodriguez-Diaz, R., Wehr, T. Tuck, S., eds., Marcel Dekker, New York NY, pp. 327-381.

Sanchez-Ruiz J. M. (1995) Differential scanning calorimetry of proteins. *Subcell Biochem* **24,** 133-176.

Spink C. H. (2008) Differential scanning calorimetry. *Methods Cell Biol* **84,** 115-141.
Abstract: Differential scanning calorimetry (DSC) has emerged as a powerful experimental technique for determining thermodynamic properties of biomacromolecules. The ability to monitor unfolding or phase transitions in proteins, polynucleotides, and lipid assemblies has not only provided data on thermodynamic stability for these important molecules, but also made it possible to examine the details of unfolding processes and to analyze the characteristics of intermediate states involved in the melting of biopolymers. The recent improvements in DSC instrumentation and software have generated new opportunities for the study of the effects of structure and changes in environment on the behavior of proteins, nucleic acids, and lipids. This review presents some of the details of application of DSC to the examination of the unfolding of biomolecules. After a brief introduction to DSC instrumentation used for the study of thermal transitions, the methods for obtaining basic thermodynamic information from the DSC curve are presented. Then, using DNA unfolding as an example, methods for the analysis of the melting transition are presented that allow deconvolution of the DSC curves to determine more subtle characteristics of the intermediate states involved in unfolding. Two types of transitions are presented for analysis, the first example being the unfolding of two large synthetic polynucleotides, which display high cooperativity in the melting process. The second example shows the application of DSC for the study of the unfolding of a simple hairpin oligonucleotide. Details of the data analysis are presented in a simple spreadsheet format.

Straume M. (1994) Analysis of two-dimensional differential scanning calorimetry data: elucidation of complex biomolecular energetics. *Methods Enzymol* **240,** 530-568.

Straume M. (1994) Sequential versus simultaneous analysis of data: differences in reliability of derived quantitative conclusions. *Methods Enzymol* **240,** 89-121.

Sturtevant J. M. (1996) Calorimetric studies of biopolymers. *Protein Sci* **5,** 391-394.

Streicher W. W. and Makhatadze G. I. (2007) Advances in the analysis of conformational transitions in peptides using differential scanning calorimetry. *Methods Mol Biol* **350,** 105-113.
Abstract: Differential scanning calorimetry can measure the heat capacity of a protein/peptide solution over a range of temperatures at constant pressure, which is used to determine the enthalpy function of the

system. There are several experimental factors that can have a significant impact on the determined enthalpy and subsequent derived thermodynamic parameters. These factors are discussed in terms of sample and instrument preparation, as well as data collection and analysis.

Weber P. C. and Salemme F. R. (2003) Applications of calorimetric methods to drug discovery and the study of protein interactions. *Curr Opin Struct Biol* **13,** 115-121.
Abstract: Recent studies report the application of isothermal titration calorimetry and differential scanning calorimetry to the study of protein-ligand interactions, allosteric cooperativity and aspects of protein folding. New methods of data analysis compare alternative methods for determining ligand binding enthalpy and analyze potential sources of error in the experimental measurement of other thermodynamic parameters. Several reports examine issues concerning drug design and the correlation of thermodynamic and X-ray structural data. New instruments allow volumetric effects in biochemical systems to be evaluated calorimetrically and to substantially expand the throughput of differential scanning calorimetry measurements in drug discovery and other high-throughput applications.