PREFACE

Reports using modern biological calorimetry began to appear in the literature in the late 1960s and early 1970s. The first generation of microcalorimeters were home built, required large samples, and had sensitivity issues such that large heats of reaction were required for analysis to be successful. As calorimeters evolved and became more sophisticated; however, their utility grew tremendously. In the 1990s, the instruments crossed a significant threshold. They became sufficiently sensitive to be broadly applicable in biology. Shortly thereafter, modest-priced commercial instruments became available such that individual investigators and core facilities could afford to own a microcalorimeter. This led to a rapid rise in their usage. A simple search of PubMed shows that in 1990, approximately 500 cited calorimetry as a key word (Fig. 1). By 2010, 20 years later, this number had increased five-fold, with more that 2500 papers listing calorimetry as a key word. Calorimetry has become a standard method for the biophysical characterization of biological molecules and reactions.

Since 1972, the *Methods in Enzymology* Series has published 26 reviews on the topic of biological calorimetry, starting with a groundbreaking review by one of the founders of the field John Sturtevant (Sturtevant, 1972). Many of these chapters were in volumes focused on specific biological macromolecule (e.g., RNA, GPCRs) or on specific applications of calorimetry to a molecule or structure central to that volume (e.g., membranes). Here in Volume 567, we provide for the first time an entire volume dedicated to applications of microcalorimetry across biology. While not every permutation of biocalorimetry is represented, the collected methods provide a sampling of the ways in which calorimeters are used to understand biological systems and how they are sometimes combined with other types of analysis to dissect the complex behavior of biomolecules.

The calorimetry literature features two dominant classes of experiments: isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC). The first section of this volume looks at ITC experiments. Here, small increments of a titrant are injected into the titrand, while feedback circuitry maintains a constant temperature between a sample cell and a reference cell. This experiment is exquisitely sensitive for the measurement of binding, allowing complete thermodynamic characterization of a reaction (ΔH, ΔS, ΔG and K_d, and n) from a single titration if measured under the

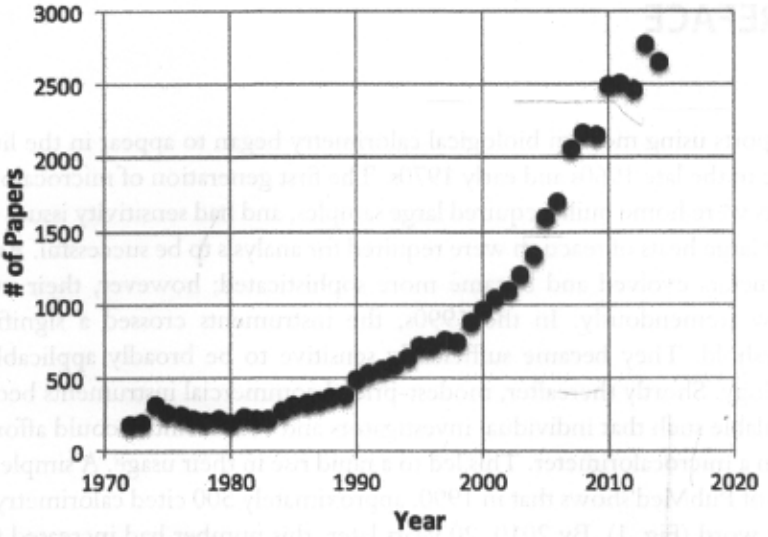

Figure 1 Number of papers indexed per year in PubMed for which "Calorimetry" is listed as a key word.

proper conditions. The experiment measures all heat sources during the reaction, however, and that can cause some practical problems. For instance, if the buffer of the titrant and titrand do not match exactly, then a heat of dilution is observed superimposed upon the binding data. In addition, since most biological reactions are carried out in buffered solutions, biochemists often choose to ignore the protons gained or lost as part of a reaction. However, buffer protonation can contribute significantly to the heats of reaction obscuring the fundamental result of an experiment unless proper corrections are applied. Buffers, even Good's buffers (Good, 1966), are also not always innocent. As a result of their metal binding behavior, buffers sometimes act as enzyme inhibitors or interact with the metal ions critical for activity. These behaviors can be assessed through carefully designed ITC analysis.

Most scientists view ITC as a method to measure the thermodynamics of binding reactions, but as the reviews in this volume show, ITC today is being used for a much wider set of applications. Heat is a very sensitive readout on reactions and is a superb way to measure the rates of enzymatic reactions using natural substrates without resorting to coupled-enzyme assays. ITC allows the user to incrementally increase the substrate concentration to measure Michaelis–Menten kinetics or raise inhibitor concentrations to assess enzyme inactivation. By using enthalpy arrays or high-throughput ITC instruments, the method is even suitable to screening compound libraries for bioactive molecules.

The second section of this volume is devoted to scanning calorimetry. In the DSC instrument, sample and reference cells are gradually heated maintaining the same temperature in each. If molecules within the sample cell undergo phase transitions or unfold, the endo- or exothermic processes can be measured. The heat capacity of the solution changes during the transition, resulting in a deflection in the power function. When measured relative to a comparable sample lacking the macromolecule, one can obtain the enthalpy associated (ΔH) with the transition as well as the transition temperature. Through modeling of the thermogram, a full thermodynamic description of the transition energetics (including ΔG, ΔH, ΔS, and ΔC_P) can be obtained as long as the transition is reversible.

DSC is used for biophysical characterization of many macromolecules. It can be used alone or in conjunction with other methods, including ITC. The reviews collected in this volume describe a few of these applications to illustrate the importance of DSC toward understanding the physical properties of proteins, nucleic acids, and lipids. One particularly enlightening result comes from the use of DSC in pharmaceutical formulation work. Dogma has been that the most thermally stable molecules make for the best pharmaceutical preparations. An illustrative example presents work on therapeutic antibodies and how DSC together with other biophysical methods dissects the multidomain unfolding behavior that impacts their biological activity.

Finally, a somewhat unconventional but hopefully highly useful article describes a biophysical instrumentation facility within an academic department. Because so many investigators want access to calorimetry but do not necessarily need an instrument of their own, the shared-instrument model is quite attractive. This paper articulates some of the best practices that allow shared calorimetry facilities to be successful scientific resources while keeping costs reasonable for the users. It describes the economics required to acquire and maintain the instruments as well as issues related to training new users and the critical mass of investigators necessary to make the investment pay off. This article should be useful for departments or institutions interested in establishing shared facilities or updating the business practices of existing ones.

ANDREW L. FEIG

REFERENCES

Good, N. (1966). Hydrogen ion buffers for biological research. *Biochemistry, 5*, 467–477.
Sturtevant, J. M. (1972). Calorimetry. *Methods in Enzymology, 26*, 227–253.