

Microcalorimetry applied to the characterization of protein stability, interaction and solvation

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Calorimetry allows universal detection and quantitative characterisation of any reaction, because heat is released or absorbed by every chemical process. Biomolecular interactions usually deal with the formation of non-covalent complexes which are important in most biochemical processes and also in chemistry. Basically, two different techniques are used in biocalorimetry. In differential scanning calorimetry (DSC), a solution of macromolecules is heated at a constant rate of temperature change, allowing the determination of its heat capacity. This technique investigates phase transitions, yielding information on intramolecular interactions within macromolecules. This includes the thermodynamic analysis of structure formation, stability of biomolecules, and the establishment of very tight (basically permanent) complexes. Considerable literature is concerned with the use of this method, e.g. (Bains and Freire, 1991; Privalov et al., 1995; Martinez et al., 1995; Freire, 1995a; Freire, 1995b; Lassalle et al., 1998; Ladbury and Chowdhry, 1998; Kholodenko and Freire, 1999). The major focus of this course is laid on the calorimetric characterization of formation of non-covalent complexes, particularly, in biomolecular systems having K_d values in the nanomolar to micromolar range. Therefore, we will describe in detail the use of the second technique, isothermal titration calorimetry (ITC) which is most suitable for this purpose. In this technique, as implied by the name, the two reaction partners are mixed stepwise and the resulting heat uptake or release is detected under conditions of constant temperature. We will also use this approach in order to address the enthalpy of hydration of the second solvation shell of proteins or cosolute compounds. We will learn about the technical background and experimental strategies in order to produce conclusive data, examples of which are given in the lecture preceding the experimental part of the course.

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