ABSTRACT

In this paper we consider the extension of the recent quantitative studies of hyperquenched glassformers to include (1) systems that exhibit first order liquid-liquid phase transitions, and (2) systems which contain molecules that, during normal cooling, undergo internal structural changes above the glass temperature. The general aim of these studies is to trap in a high enthalpy, high entropy, state of the system and then observe it evolving in time at low temperatures during a controlled annealing procedure. In this manner events that normally occur during change of temperature may be observed occurring during passage of time, at much lower temperatures. At such low temperatures the smearing effects of vibrations are greatly reduced. The case of most interest, in the second class, is the refolding of thermally denatured protein molecules, but any reconstructive molecular or chemical exchange process is a potential subject for investigation. Processes that occur in stages can be studied in greater detail, and any stage of interest can be frozen when desired, by drop of temperature, for more detailed spectroscopic examination.

We review an electrospray method for hyperquenching liquids at ca $10^5$ K/s, and discuss some results of such methods in order to illustrate a calorimetric approach to exploiting the hyperquenching-and- cold-equilibration strategy. To apply the idea to the study of proteins, the following protein solvent problems must first be solved (i) the solvents must not crystallize on cooling or heating, yet must not denature the proteins (ii) the solvents must support the denatured molecules without permitting aggregation. We describe two solvent systems, the first of which solves the first problem, but the second only partially. The second solvent system apparently solves both. Preliminary results, only at the proof of concept stage, are reported for cold refolding of lysozyme which, it seems, can be trapped our solvent in the unfolded but refoldable state, with only moderate (ca. 5K/s) quenching rates. Some structure appears in the refolding endotherms, suggestive of stages in the “cold” process, though lysozyme is thought of as a two-state folder.