New and Notable

Expanding Time Scales Usher in a New Era for Kinetic Studies

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INTRODUCTION

In 1985 an article was published in *Review of Scientific Instrumentation* (vol. 56, 283–290), titled "Mixing liquids in microseconds," from the laboratory of Thomas M. Jovin (Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany), authored by Peter Regenfuss, Robert M. Clegg, Mack J. Fulwyler, Francisco J. Barrantes, and Thomas M. Jovin. This pioneering work described the development of a continuous-flow microsecond mixing device. This article was clearly published before its time, and the design languished essentially unused in the scientific community for the next 9 years or so. I think that there are quite a few reasons for this, and I will give you one perspective.

The technique of using stopped-flow mixing to examine fast reactions in the millisecond time scale had reached prominence in the 1960s and 1970s, and by the time the work by Regenfuss et al. (1985) appeared, emphasis in research had radically shifted from "classical biophysics" (e.g., kinetics, reaction dynamics, thermodynamics) to "modern biology" (e.g., protein structure and molecular biology). This shift in emphasis may have "diluted" the impact of the development of this important new kinetic tool. The research "pendulum," however, is beginning to swing back, with renewed interest being placed on obtaining a more detailed understanding of how protein structures "actually work" and are assembled (i.e., folded). To answer mechanistic questions of this type, kinetic studies are required, and renewed interest in rapid kinetic methods is once again surfacing. Pioneering microsecond mixing studies of protein folding from the laboratories of Denis L. Rousseau (Takahashi et al., 1995, 1997) and James Hofrichter (Chan et al., 1997) have recently appeared that utilize a T-mixer version of the original Regenfuss (1985) design.

In this issue of *Biophysical Journal*, the laboratory of Heinreich Roder have an article titled "A continuous-flow capillary mixing method to monitor reactions on the microsecond time scale," authored by M. C. Shastry, Stanley D. Luck, and Heinrich Roder. This article provides a detailed description of the assembly and operation of a modern microsecond mixing, continuous-flow instrument, and is an adaptation of the original Regenfuss (1985) design. Using a combination of both continuous-flow microsecond mixing and conventional stopped-flow mixing, this work reveals how continuous kinetic data from 50 μs to $>$10 s after the initiation of a chemical reaction (in this case, the refolding of cytochrome *c* during a chemical jump from pH 2 to pH 4.5) can be obtained. Kinetic studies of this type should radically enhance our understanding of the mechanism of protein folding, as well as a wide variety of other important biological reactions. Although equilibrium-based methods used to study protein folding are certainly very useful, it should be remembered that Josiah Willard Gibbs (1839–1903) formulated the thermodynamic functions to be state functions, independent of path. Although the concept of a folding "pathway" has evolved into a more realistic concept of "energy landscapes" and "folding funnels" (see, e.g., Dill and Chan, 1997) to resolve these complex processes, detailed kinetic studies over as wide a range of time scales as possible are absolutely crucial.

Do fast kinetic studies of this type make all of the slower millisecond mixing experiments obsolete? Certainly not—for processes such as protein folding, it is clear that important kinetic events are occurring over all of the time scales that have been examined: from nanoseconds (using the newly developed laser-based T-jump methods), to microseconds (microsecond mixing; see Shastry et al., this issue of *Biophysical Journal*), to milliseconds (conventional stopped-flow mixing), to seconds. One must not loose sight of the fact that recovery of enzyme activity almost invariably occurs only in the slowest phase of a refolding experiment. With the availability of kinetic experiments that can now span almost 10 log units in time, the real challenge for the future is not so much how to achieve the time resolution. Instead, what is desperately needed is advanced spectroscopic detection methodologies and experimental designs that can be performed in a kinetic mode and yield *structural* information concerning folding intermediates (e.g., Lillo et al., 1997). Especially lacking is good experimental approaches to quantifying heterogeneous folding ensembles in terms of something other than abstract states on a complex energy landscape. Roder's (and related technologies from other laboratories) microsecond mixing device will greatly help bridge the gap between reaction rate theory (ps/ns) and biological structure/function (ΔÅ per ps-μs-ms-s).

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