New and Notable

Temperature Cycles Unravel the Dynamics of Single Biomolecules

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Biomolecules are finely tuned to perform their function in a precise range of temperatures. Variation of temperature outside this range, although not necessarily relevant to function, is a powerful way to explore structure and dynamics of these biomolecules. Well-known examples are the cooling of protein crystals to liquid nitrogen temperatures to investigate their x-ray diffraction patterns (1), and the physical trapping of short-lived reaction intermediates, e.g., by freeze-quenching methods (2) in mixed flows (3). Temperature changes can initiate or terminate biomolecular reactions by promoting or inhibiting energy barrier crossing, so that potential energy landscapes can be explored with adapted time-temperature protocols (4–7). The temperature cycle method consists in applying consecutive cycles with short heating and cooling steps (8,9), so that a dynamical process is decomposed into a series of snapshots, much as early movies decomposed the fast movements of galloping horses.

By recording complete series of structural snapshots of a biochemical process, its whole dynamics can, in principle, be reconstructed. The temperature transitions should be as short as possible to limit unwanted transformations during heating and cooling periods. Because a structural variable can be measured at a low temperature over an arbitrarily long period, it can be determined more accurately than in short measurements at ambient temperature. Moreover, the method provides control of time resolution by tuning the duration and number of the high-temperature steps, and full control of barrier crossing rates by variation of the maximum and minimum temperatures in the cycle. However, the accurate and fast temperature changes required by this method cannot be achieved by conventional heating and cooling stages.

Optical beams can induce highly localized and fast temperature gradients through heating of absorbing materials (8,10,11). Absorption of a laser pulse in a bulk material, for example water, leads to homogeneous heat release in the beam focus within picoseconds or nanoseconds. The cooling rate, however, is limited by heat diffusion out of the heated volume. This volume should therefore be reduced as much as possible to achieve short cooling times. Conventional ensemble spectroscopic methods cannot deal with very small sample volumes because of limitations in signal/noise. Single-molecule optics, however, are not liable to such volume limitations because they address only one molecule, independently of the surrounding sample volume. Single-molecule spectroscopy thus ideally complements temperature cycle techniques, as pointed out some years ago by Zondervan et al. (8). Those authors immerged their sample in a cold cryostat and focused an infrared laser onto a diffraction-limited spot of a metal film, thus demonstrating a laser-driven heating system with cycling times of some microseconds. Generalizing this method to aqueous environments is challenging because of phase transitions of water. Alternatively, raising the sample temperature brings other problems. For example, commonly used links for immobilization, such as the biotin-streptavidin couple, lose strength and can dissociate at elevated temperatures. Another problem is that many fluorophores may not withstand elevated temperatures, and properties such as fluorescence lifetime and quantum yield may be altered irreversibly.

In this issue of the *Biophysical Journal*, Holmstrom et al. (12) describe a pulsed laser heating system that works in an aqueous environment by using water absorption for the fast heating process. Heat diffusion to the ambient environment remains responsible for the slower cooling step. The authors perform a detailed characterization of their temperature cycling system by monitoring the temperature-dependent fluorescence lifetime of Rhodamine B as a function of time and spatial position. They find that the temperature transitions between ambient (20°C) and maximum temperature (100°C) take milliseconds for picoLiter volumes. Because the heating temperature and duration can be precisely measured and controlled, they can minimize the dissociation of biotin-streptavidin by applying a proper temperature program.

The authors also illustrate the power of temperature cycles by applying them to the reaction dynamics of DNA duplex dissociation (12). DNA duplex constructs include a reporter strand hybridized with a splint strand. Upon heating, the splint strand dissociates. When cooling again, the remaining reporter chain folds and hybridizes as a hairpin, bringing fluorescence donor and acceptor together and producing a FRET signal. DNA constructs that have reacted can be counted directly on the room-temperature image of the heated area. By monitoring the fraction of dissociated duplexes as a function of the duration and maximal temperature of the cycle, the authors propose a kinetic model for the dissociation reaction and deduce the energy barrier for duplex dissociation. The nonexponential dissociation kinetics is compatible with a multistep reaction requiring the unzipping of 5–6 basepairs before

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complete dissociation. From combinatorial arguments, the authors conclude that duplex fraying must take place simultaneously at both ends.

Holmstrom et al. (12) demonstrate the combination of temperature cycles and single-molecule microscopy and apply it for, what we believe to be, the first time to provide detailed mechanistic information on a biomolecular reaction. To progress toward the dream of a complete mapping of potential energy landscapes for protein folding or for complex enzymatic reactions, shorter times and broader temperature ranges will be needed. This can be achieved with smaller sample volumes, stronger absorbers than water's vibrational overtone, and lower environment temperatures. This considerable experimental effort promises to be highly rewarding. Extending the application range of fast temperature cycles will open investigations of microsecond timescales, the stabilization of rare and short-lived intermediates, and eventually the elucidation of reaction pathways. Such a general and powerful method could tackle the dynamics and reactions of virtually all biomolecules, including enzymes (13), proteins (14,15), RNA (16), and their many complexes (17), at the single-molecule level.

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