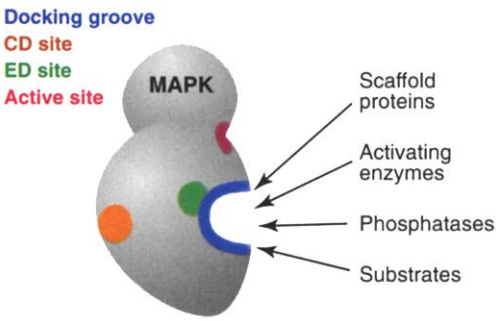

MAP kinase interactions with scaffold molecules, MAPKKs, phosphatases, and substrates.

natively, the CD site interaction may occur only with activated MAPK or full-length proteins; the structures reported by Chang and colleagues include nonactivated p38 MAPK and isolated D domains. A second MAPK site implicated in the interaction of MAPKs with MAPK-activated protein kinases (MAPKAPKs), the ED site (*7*), also did not participate in the interaction of p38 MAPK with MEF2A or MKK3b in the crystal structures (*3*). The ED site, however, is located close to the docking groove in the crystal structures (see the first figure). Thus, the ED site may aid the docking of some interacting proteins, although the mutational analysis used to identify the ED site may have indirectly affected the docking groove identified by crystallography.

Chang and colleagues unexpectedly observed that the binding of proteins to D domains induces conformational changes in p38 MAPK. The largest change occurs in the loop between αd and αe, which narrows the binding groove between these helices and the β7-β8 reverse turn. The binding and conformational changes that MEF2A and MKK3b induce differ and may contribute to p38 regulation. For example, alterations in the p38 activation loop caused by docking to MKK3b could aid p38 phosphorylation, and the conformational changes caused by docking MEF2A may activate p38.

Disease research may benefit from understanding the mechanisms that regulate MAPK signaling specificity. For example, lethal factor (LF), a protease that binds to and proteolytically cleaves the NH_2-terminal region of MAPKK, blocks MAPK function during anthrax infection (*9*, *10*). The proteolysis separates the NH_2-terminal D domain of MAPKK from the catalytic kinase domain that phosphorylates and activates MAPK (*9*). Loss of the D domain prevents recognition of the cognate MAPK by the cleaved MAPKK. This observation could lead to the use of small molecules (that is, drugs) to disrupt MAPK interactions and to block selectively individual MAPK pathways.

The structural insights of Chang and colleagues will provide a foundation for future studies of the molecular basis of MAPK signaling specificity. These studies will further our understanding of MAPK signaling networks in health and disease.

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PERSPECTIVES: MOLECULAR DYNAMICS

Biomolecules See the Light

David W. Pratt

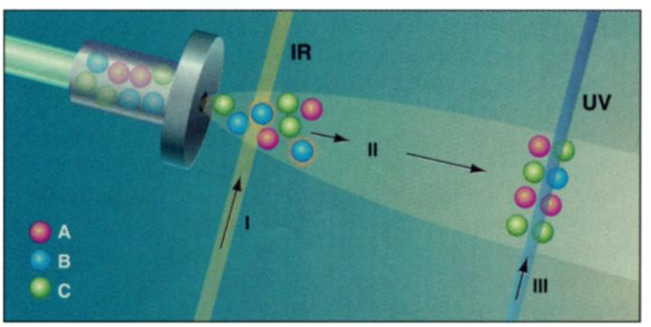
Molecular-level understanding of the complex dynamics of biological processes such as protein folding will greatly advance the treatment of human disease. Rapid progress toward this goal has been made in the past few years, owing to advances in experimental and theoretical techniques (*1*–*3*). For example, multidimensional nuclear magnetic resonance techniques have helped to determine the solution structures of large proteins and probe their dynamical behavior (*4*).

But existing approaches cannot fully elucidate the heterogeneity of biological systems, their intricate energy landscapes, and the possible role of solvent in the dynamics. On page 2369 of this issue, Dian *et al.* report the development of a new approach that overcomes some of these limitations (*5*). The method provides surprisingly detailed insights into the dynamics of a small biomolecule.

Using a supersonic jet, infrared (IR) and ultraviolet (UV) lasers, and ab initio quantum chemical calculations, the authors isolated a biomolecule in the gas phase, and identified and determined the relative populations of its energetically accessible "conformational substates" under collision-free conditions. They then manipulated these populations with tunable laser light, using collisions to relax the molecules back into their lowest energy conformations after they had visited less favorable regions of the energy landscape. Surprisingly, they found that the resulting population distributions depend upon the precise nature of the excitation, demonstrating for the first time that there are distinguishable pathways for conformational change.

The authors studied a methyl-capped dipeptide called NATMA (*N*-acetyl-tryptophan methylamide). The 36-atom molecule is small by biomolecule standards but nonetheless complex in its dynamics. The energy landscape of the molecule is calculated to have 164 minima connected by 714 transition states, with 65 minima lying within 40 kJ/mol of the global minimum (*6*). Different minima correspond to geometries with extended or partially folded peptide chains. Comparison of the relative abundances of the different conformations before and after IR excitation provides information about how a given structure evolves along a particular pathway.

The experiment of Dian *et al.* (*5*) is illustrated schematically in the figure. NATMA was heated to 150°C, entrained in helium, and passed through a ~1-mm orifice into a vacuum chamber, creating a supersonic expansion that cools each molecule into one of



Exploring the energy landscape with light. NATMA molecules expanded in a supersonic jet of helium are observed by UV spectroscopy (III) to exist in three different conformational substates (A, B, and C), corresponding to geometries with extended or partially folded side chains. Irradiating the ensemble with IR light (I) and relaxing it by collisions (II) changes the population ratio in a conformation-specific way, demonstrating that unique pathways govern the folding dynamics of this dipeptide.

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its lowest energy structures (A, B, or C). The resulting ensemble was then probed with two lasers: an IR laser irradiating the mixture during the expansion (~1 mm from the nozzle), and a UV laser further downstream (~4 mm from the nozzle). The UV absorption spectrum of the sample gives information about the relative populations of A, B, and C in the mixture. Each structure exhibits a unique spectrum (*7*). When the IR laser was turned on and tuned to a specific NH stretching vibration of a particular conformation (A, B, or C), the relative intensities of the three different bands in the UV spectrum changed, providing evidence for an IR-induced isomerization reaction.

It is not surprising that the relative populations of A, B, and C change when the ensemble is irradiated by IR light. IR-induced isomerization of a polyatomic molecule (nitrous acid, HONO, in a low-temperature matrix) was first observed more than 40 years ago (*8*). Real-time studies of protein folding often use IR pulses to initiate conformational change, either directly or indirectly (*9*), thereby mimicking the well-known behavior of proteins to denature at elevated temperature.

What is surprising about the results of Dian *et al.* (*5*) is that they are conformation specific. The substates (A, B, or C) populated after IR excitation are the same as in the absence of IR excitation. But the population ratios A:B:C before and after IR irradiation are different. Further, the experiments show that the population ratios depend uniquely on which conformation is excited, and on which NH stretching vibration is excited within a given conformation.

According to conventional wisdom, this kind of mode specificity should not exist in such a large molecule, at least not on the few-microsecond time scale of the experiment. NATMA has 102 normal modes of vibration. Some of these have relatively high frequencies, such as the NH stretching modes at ~3400 cm^{-1} (the energy of IR excitation), but most have much lower frequencies of a few hundred cm^{-1} or less. Hence, there is a high density of states at the energy of excitation. It is further expected that some couplings must exist between these modes. The energy deposited in the NH stretching mode of a particular structure should therefore be redistributed rapidly and irreversibly among all or most of the remaining modes, a process known as intramolecular vibrational relaxation (IVR).

As stated by Dian *et al.* (*5*), "given that the three conformers (A, B, and C) have a similar energy (within ~2 kJ/mol) and receive nearly identical amounts of energy in the vibrational excitation, one might have imagined that all transitions out of the three conformers would distribute their excited population similarly . . . this is clearly not the case." Instead, the IR-induced dynamics is nonstatistical, an unprecedented result in such a large molecule (*10*).

Under the conditions of the experiment, collisions must play an important role in the dynamics. Selective collisional cooling of molecules with flexible side-chain conformations has been observed by several groups [see, for example (*11*)]. In their elegant "chemical timing" experiments, Parmenter and co-workers (*12*) arrested IVR in an electronically excited state of *p*-difluorobenzene by using an O_2 buffer gas to relax the molecules back to the ground state on short time scales. The first collisions after IR excitation of NATMA likely occur in less than a nanosecond. They may thus be similarly responsible for the observed nonstatistical behavior. More sophisticated ab initio calculations (*13*) will be required to identify the regions of the energy landscape to which access is blocked in such large molecules.

The results of Dian *et al.* (*5*) clearly show that there are distinguishable pathways on the energy landscape of NATMA, at least on some time scales. These pathways play a critical role in dictating the redistribution of populations under the conditions of the experiment. We do not know the extent to which this will be true in even larger molecules. But if such pathways exist and can be accessed, then we can look forward to obtaining ever more detailed information about biological processes at the molecular level.

IR lasers provide an entry point to previously unexplored regions of the energy landscape. Solvent-induced changes and the role of water and other small molecules in influencing conformational choice can be probed by forming solvent-solute complexes in the supersonic jet (*14*). It may also be possible to determine the extent to which different pathways are connected and to probe the vibrational coordinates along which these connections are made. Such experiments would provide stringent tests of the "new view" that multiple routes dominate the dynamics of protein folding and other complex biological processes (*15, 16*). The future of biomolecules in the gas phase is bright.

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PERSPECTIVES: EVOLUTION

Chaperones as Buffering Agents?

Thomas Mitchell-Olds and Charles A. Knight

Does evolution draw on existing genetic variation in animals and plants or must it wait around for new mutations to arise? Sixty years ago, Waddington argued that cryptic genetic variation is present for many traits, but that expression of these variants under normal environmental conditions is prevented by a process of "genetic buffering" (*1*). As Waddington demonstrated, stressful environmental conditions compromise the genetic buffering system, leading to the breakdown of normal development and enabling the expression of cryptic genetic variation as visible phenotypic changes. Recently, heat shock proteins (HSPs), a type of molecular chaperone, have been implicated in the genetic buffering of the fruit fly *Drosophila*. Now, Queitsch *et al.* (*2*) report in a recent issue of *Nature* that the chaperone HSP90 provides genetic buffering in *Arabidopsis* and may contribute to the evolutionary adaptation of this plant.

HSPs are induced by high-temperature stress in organisms as diverse as bacteria, fungi, plants, and animals. These molecular chaperones prevent irreversible aggregation of denatured proteins after heat or other protein-denaturing stresses. They also bind to a range of client proteins that are crucial for regulating growth and development. The evolutionary conservation of the heat shock response, and

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