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Chemistry and biology in the new age

Ahmed Zewail won the 1999 Nobel Prize in chemistry for his visionary work in probing the motions of atoms at the femtosecond level. This pioneering research, a decade earlier, opened up a new frontier of scientific knowledge. Zewail and his multidisciplinary team at Caltech are now pushing further into the realm of molecular complexity, with the ultimate aim of exploring the global dynamics of biological systems at atomic resolution. This requires a new method of ‘watching’ reactions – ultrafast electron diffraction.

Ahmed Zewail and Linus Pauling on the Caltech campus

Courtesy of the California Institute of Technology

LEAPS IN SCIENTIFIC PROGRESS are often the result of a series of fortuitous technical developments combined with brilliant insight and ingenious application. Often, it is not immediately clear where a piece of research will lead. These were certainly the circumstances that surrounded the birth of femtochemistry in the 1980s.

When Ahmed Zewail, now Linus Pauling Professor of Chemistry and also Professor of Physics, arrived in at the California Institute of Technology as an assistant professor in the 1970s, he had no thoughts of the femtosecond laser experiments for which he is now well known. His interest lay in studying coherence in optically excited complex systems – in other words, pursuing fundamental ideas about the nature of ground and excited quantum states in matter in terms of their phase relationships. The ultra-short pulse lasers needed for real-time investigations of the phenomena were just starting to be developed, and Zewail's team could start thinking about investigating coherence at picosecond levels.

Zewail recalls that while physicists were impressed with the experiments, many of his chemical colleagues thought that coherence was irrelevant to chemistry. How wrong they were! This became clear when Zewail decided to look at coherence in molecular systems isolated in a supersonic jet, or molecular beam – another technology then only recently developed. He was interested in what happened to the phase coherence in a large molecule like anthracene when energy deposited from a laser pulse redistributes itself via intramolecular vibrations. He found that there was a dynamic cycle of behaviour that was phase-coherent – all the molecules did the same thing at the same time.

Zewail soon realised that the phase coherence induced by the marriage of molecular beams and laser technology would allow him to study transformations that evolve with time – chemical reactions. Atoms in molecules move with a speed of about 1 kilometre per second, and at this speed an atom moves about 1 picometre (one-thousandth of a nanometre) in about 1 femtosecond. So to probe atomic trajectories over 1 nanometre of motion during a reaction would need a laser delivering a femtosecond pulse in order to take 100 frames of the 1-picosecond journey. Eventually by 1986, Zewail was able to install the first femtosecond laser set-up in the lab that had housed Linus Pauling's X-ray machines – later dubbed FEMTOLAND 1, and subsequently succeeded by FEMTOLANDs 1 to 6.

What followed were the ground-breaking experiments that led to the Nobel Prize. The first reaction to be resolved in time was the break-up of iodine cyanide. For the first time, a now classical ‘pump-and probe’ configuration (using a time-delayed laser pulse to interrogate the reaction) revealed that the transition state and products could be monitored with atomic spatial and temporal resolution. Then came the beautiful experiment on the bond breaking and bond re-making in the molecule NaI – “the *Drosophila* of femtosecond chemistry”, as Zewail has called it, which showed there was resonant motion between the ionic and covalent structures of the bond as the sodium and iodine atoms moved away from each other.

This experiment put femtochemistry on a firm foundational base by establishing the credentials of underlying quantum picture that the changing molecular system with its cohort of quantum-state superpositions was coherent and localised. It could thus be followed in time as though the constituents were classical balls rolling on a potential energy surface.

The experiment, and related others, also put to rest a concern that was raised by some experts – the issue of the Uncertainty Principle. Because the pulses were in the femtosecond range in duration, it was thought that the energy uncertainty would be huge and would limit the utility of the femtosecond time resolution. What was overlooked is the coherence of the individual molecules and that of the entire ensemble.[1] Zewail was confident of his insight and went on to elucidate the fundamental nature of the concept which is central to observing atoms in motion and to achieving atomic-scale resolution of chemical and biological transformations. The Nobel citation makes a specific reference to the development of these concepts in femtochemistry: “His contributions have brought about a revolution in chemistry and adjacent sciences. We can now study the actual movements of atoms in molecules. We can speak of them in time and space in the same way that we imagine them. They are no longer invisible.”[2]

These initial experiments triggered an explosion in the field. The Caltech group and many others went on to apply the methodology to a very wide variety of dynamic interactions, from bimolecular reactions, to reactions at surfaces and the behaviour of clusters.

Zewail's work heralded “a new age for chemistry” in which the emphasis is on exploring structural changes far from equilibrium. He points out that in the 20th century, the main objective was solving three-dimensional static structures – culminating in those of complex biological

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assemblies. However, to obtain a complete picture, the dynamics also need to be elucidated. “Complex systems such as proteins are now occupying our efforts at Caltech,” says Zewail. “The aim is to observe the atoms of an entire molecular structure changing at once. We should like to be able to look at the mechanisms and dynamics of function in biological systems on an ultrashort timescale.”

Ultrafast electron diffraction

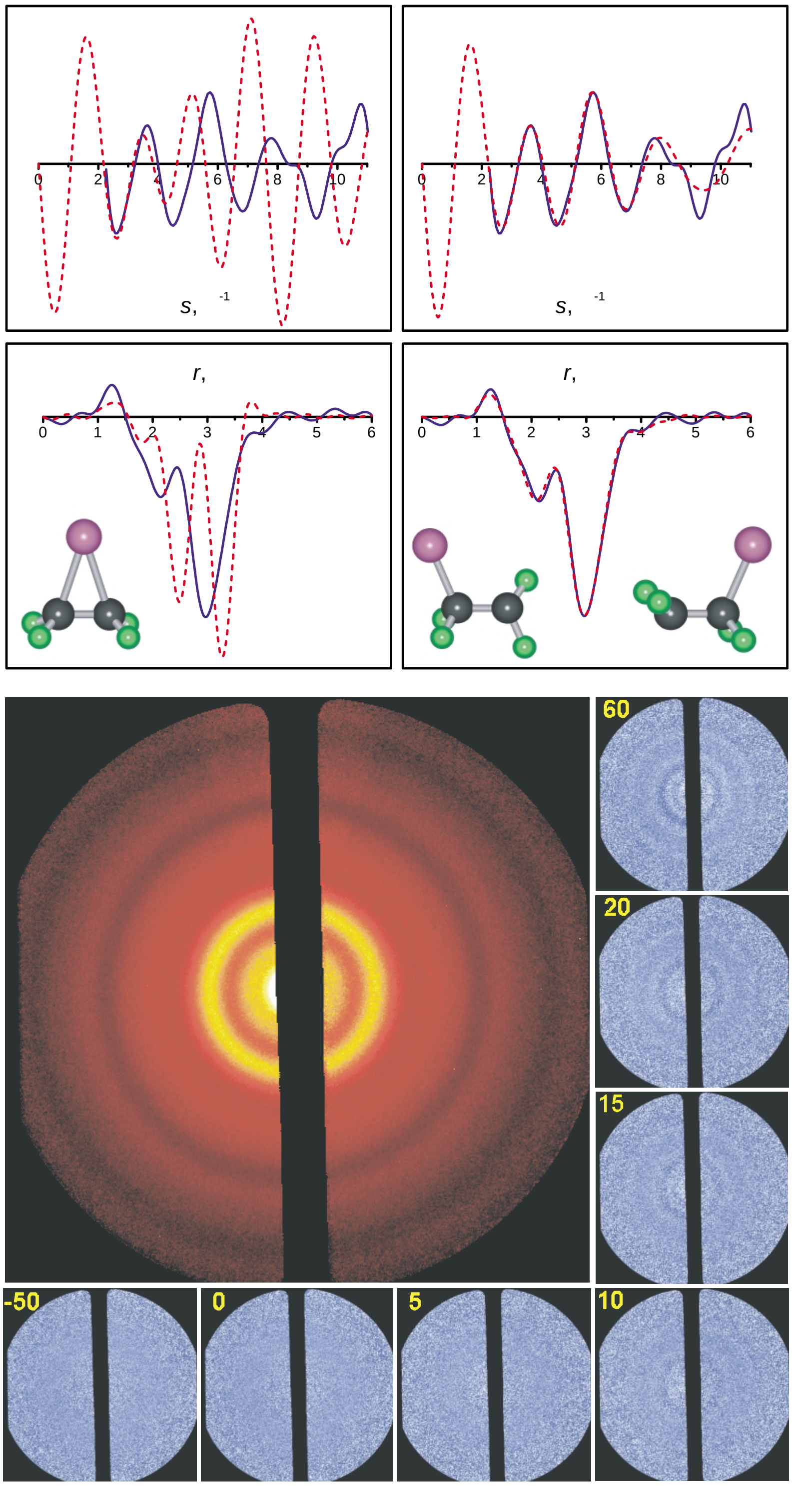
Various probe techniques have been employed in ultrafast chemistry such as laser-induced fluorescence, but to monitor complex structures far from equilibrium requires new approaches. For the past 10 years Zewail and his colleagues have been developing ultrafast electron diffraction (UED), which he feels holds great promise for the future. Conventional electron diffraction, without time resolution, has been successfully used to elucidate structure in the gas phase and at surfaces for many years, and is an ideal tool for mapping all the internuclear coordinates in a complex structure.

The principle of UED is similar to ultrafast spectroscopies in that a femtosecond laser pulse initiates the change, and then a time-delayed second pulse is directed onto a photocathode to generate electron pulses via the photoelectric effect (*ie.* instead of laser pulses). After interaction with the molecules, the diffracted electron beam is recorded with a CCD camera capable of detecting single electrons. To obtain the ground-state or starting structure of the molecule, an electron pulse is timed so as to arrive before the initiating laser pulse (in ‘negative’ time); the two-dimensional diffraction pattern obtained can then be differenced from subsequent electron images to reveal the transient species as they appear at ‘positive’ times.

The Caltech team has continuously improved the performance of the apparatus, which is now in its third generation offering spatial and temporal resolution about 0.001 nm and 1 ps. A time-of-flight mass spectrometer is also part of the apparatus to aid species identification.

One of the first successful experiments was to investigate the elimination of iodine from 1,2-diiodotetrafluoroethane ($C_2F_4I_2$).[3] UED data were collected from time zero and over a total period of 500 ps and the changes in the difference-diffraction pattern monitored. The diffraction patterns of the transient states showed that the reaction is nonconcerted, with the breakage of the first C-I bond in about 200 fs and the second C-I bond breaking more slowly in about 17 ps. Surprisingly, however, the structure of the intermediate haloethyl radical (C_2F_4I) was shown to have a classical structure, with the iodine attached to just one of the carbons rather than forming a bridged structure between the two carbons – as suggested by the stereoselectivity seen in reactions involving haloethyl radicals.

More recently, Zewail’s group has looked at radiationless transitions (where energy is dissipated as heat rather than light) in aromatic molecules, in particular, pyridine.[4] Such transitions are common in biological and other systems but difficult to study because they are so fast. UED was able to plot changes in the



Ultrafast electron diffraction (UED)
The determination of the molecular structure for the transient intermediate formed by iodine elimination in $C_2F_4I_2$. The structure of the transient intermediate, at a reaction time of 1 picosecond, is evident in the change of UED with time. Comparison with theoretical curves (red) clearly shows that the intermediate structure is classical, not bridged. (Bottom) UED images of ring-opening in pyridine. The red image is the diffraction from ground-state structure. The diffraction signals in the time-resolved difference images (blue) – observed to grow in with a time constant of 17 ps – directly correspond to structural changes during the unexpected ring-opening process.

internuclear distances over a period of about 300 ps, from -9 to +185 ps, and indicated how the internal energy is dissipated. A variety of channels are open to ‘hot’ pyridine – conformational changes, fragmentation and ring opening. Unexpectedly, the UED data revealed that the dominant intermediate species was a previously unknown ring-opened diradical structure resulting from the breakage of the C-N bond.

Two other cyclic hydrocarbons successfully studied with UED are transient structures of 1,3,5-cycloheptatriene and 1,3-cyclo-hexadiene formed far from equilibrium.[5] The former compound forms a vibrationally excited structure with bond distances similar to those in the ground state but with a nonequilibrium population of vibrational levels; while the latter undergoes ring-opening to form structures with C-C bond distances greater than the expected equilibrium value. The technique has also been successful in studies of the reactive intermediates of organometallics, and it is clear that it is now opening up new vistas for further research in different phases.

Biological dynamics

Building on these results, Zewail now plans to extend UED studies into the biological area. “Electron diffraction is highly suitable for biological dynamics,” he says. At the Laboratory for Molecular Sciences (LMS), which he directs, Zewail and his group have already been investigating ultrafast phenomena in large biomolecules. In collaboration with Jacqueline Barton, also at Caltech, they observed charge transport in DNA covalently tethered to electron donor and acceptor molecules.[6] Using the femtosecond experimental set-up they characterised the transport process and showed that it was in fact quite slow – on the picosecond timescale, indicating that DNA is not an efficient molecular wire. With Fred Anson’s group at Caltech, they also studied picket-fence cobalt porphyrins which are model systems for haemoglobin and myoglobin in that they form reversible complexes with oxygen at room temperature.[7] The Zewail group was able to dissect the steps that led to the rapid release of dioxygen in less than 2 ps and the rebinding of oxygen on the microsecond time-scale.

One of Zewail’s current interests is the role of water in biological systems. The interaction of water molecules with proteins is known to play a crucial part in their conformational stability and function. Earlier this year, Zewail and colleagues reported on prototype experiments investigating the hydration dynamics at the surface of tryptophan, Trp, (a protein residue used as a fluorescent probe) in bulk water, also in the iron-sulfur protein rubredoxin (which has a hydrophobic core containing two Trp residues) and in the denatured protein. They found that in water Trp solvation is ultrafast – between 160 fs and 1.1 ps. In a series of papers studying the proteins *Subtilisin Carlsberg* (SC) and *Monellin*, where tryptophan resides at the surface, they have shown the existence of a dynamical water layer of thickness less than 0.7 nm, and that in this layer water has residence times of a bimodal nature – femtosecond times four bulk water and tens of picoseconds for the ordered water layer.[8,9]

Zewail and his team are now building a fourth-generation UED apparatus suitable for femtobiology. He is not intimidated by the challenge of unravelling complex behaviour. “I believe that once we design the right experiment, it will be simple and we will see the beauty in complexity of structures and the concepts behind their behaviour,” he says. Looking ahead to the possibilities his approach is opening up, Zewail is currently making extensions to the condensed phase and to biological assemblies. His ultimate goal is to decipher their dynamics – their coherent single-molecule trajectories in time and space. The new age of chemistry, and now biology, has truly arrived.

Nina Hall

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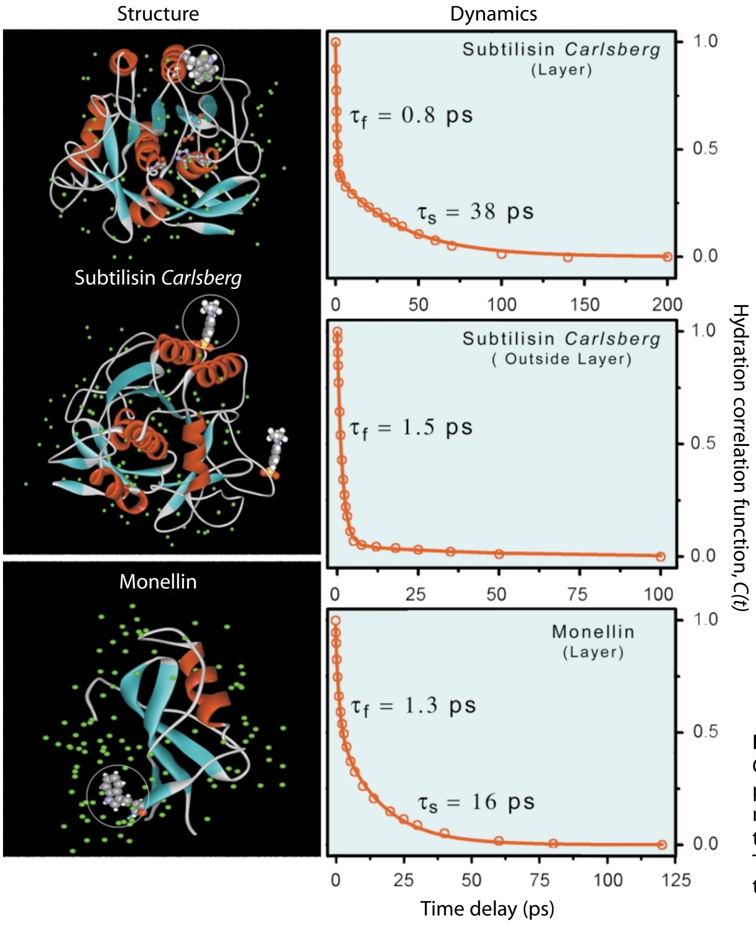
REFERENCES

1. A.H. Zewail, *Nature (London)*, 2001, **412**, 279.
2. A.H. Zewail, Angew. *Chem., Int. Ed.*, 2000, **39**, 2586.
3. H. Ihee, V. A. Lobastov, U. M. Gomez, B. M. Goodson, R. Srinvasan, C.-Y. Ruan and A. H. Zewail, *Science (Washington, D.C.)*, 2001, **281**, 458.
4. V. A. Lobastov, R. Srinvasan, B. M. Goodson, C.-Y. Ruan, J. S. Feenstra and A. H. Zewail, *J. Phys. Chem. A*, 2001, **105**, 11159.
5. C.-Y. Ruan, V. A. Lobastov, R. Srinvasan, B. M. Goodson, H. Ihee and A. H. Zewail, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 7117.
6. C. Wan, T. Fiebig, O. Schiemann, J. K. Baron and A. H. Zewail, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 14052.
7. B. Steiger, J. S. Baskin, F. C. Anson and A. H. Zewail, *Angew. Chem., Int. Ed.*, 2000, **39**, 257.
8. D. Zhong, S. K. Pal, D. Zhang, S. I. Chan and A. H. Zewail, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 13.
9. S. K. Pal, J. Peon and A. H. Zewail, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 1763.



Biological water
On the left is the molecular structures of two types of proteins. On the right are the corresponding experimental molecular dynamics of hydration. The time-scales for the two proteins are vastly different from that of bulk water. They are all studied utilising one native amino acid, tryptophan, at the surface of the protein.