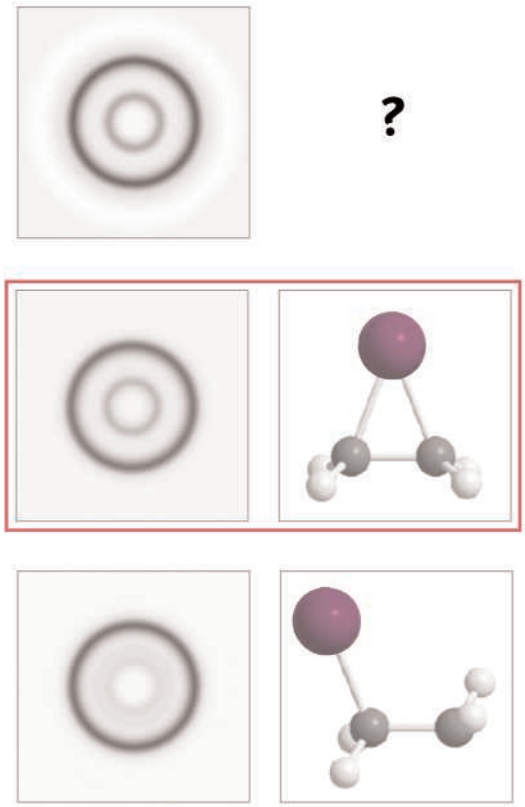
CHEMISTRY

X-ray Fingerprinting of Chemical Intermediates in Solution

Philip Anfinrud and Friedrich Schotte

In the early 1800s, the English scientist John Dalton postulated that matter is made up of indestructible atoms whose identities are unchanged by chemical reactions. These atoms have a definite size and mass, are countable, and through chemical reactions can combine to produce molecules as simple as diatomic oxygen (O_2), the vital component in the air we breathe, or macromolecules far more complex than myoglobin ($C_{822}H_{1035}FeN_{222}O_{220}S_3$), an oxygen-storage protein found in our muscle tissue. It is the controlled structural rearrangement of atoms and molecules that adds value to industrial chemical feedstock, and gives life to biological organisms. The selectivity of the transformation from reactant to product depends on the reaction mechanism, and the structures of intermediates along the reaction pathway are often hotly debated. Because the atoms are so small (~10^{-10} m), and the time it takes for them to slip past their neighbors is so short (~10^{-13} s), the direct observation of these intermediates has proven quite elusive. Recently, researchers working at the European Synchrotron Radiation Facility (ESRF) rose to this experimental challenge and pursued structural studies of photochemically generated, short-lived (<10^{-6} s), iodo radicals. Davidson *et al*. studied diiodomethane (*1*), whereas Ihee and coworkers studied diiodoethane (as reported on page 1223 in this issue) (*2*). In particular, Ihee *et al*. identified the structure of a radical that purportedly plays a crucial role in certain stereoselective chemical reactions.

The old adage, "seeing is believing," implies that disputes are often settled with a picture, or in the case of chemistry, with a molecular structure. Methods capable of extracting structural details at or near atomic resolution include x-ray diffraction (crystals and solutions), electron diffraction (gas phase, thin films, and surfaces), scanning tunneling microscopy (surfaces), nuclear magnetic resonance spectroscopy (solids and solutions), and microwave spectroscopy (gas phase). The diffraction methods make use of photons or particles that can be produced in pulses shorter than 10^{-12} s, and are therefore uniquely suited for structural determinations with ultrafast time resolution. This feat has generally been accomplished with the pump-probe method, where a "pump" laser triggers a reaction and a delayed "probe" pulse captures a snapshot of the transient species. In recent years, structural changes in protein crystals have been determined with ~5-ns (*3*–*5*) and 150-ps (*6*, *7*) time resolution, the structures of short-lived intermediates in the gas phase have been elucidated with 1-ps time resolution (*8*), and photothermally induced structural changes have been monitored in solid-state materials with ~120- to 600-fs time resolution (*9*, *10*) and on surfaces with 1-ps time resolution (*11*).



Matching fingerprints. Time-resolved x-ray diffraction can reveal changes in the pair-distribution function (histogram of interatomic distances) during the course of a chemical reaction in solution. Snapshot of $C_2H_4I_2$ in methanol taken 100 ps after photolysis (**top**). The calculated pair distribution function for the bridged C_2H_4I radical (**middle**) is a better match than that for the anti radical (**bottom**).

Notably absent from the recent literature are time-resolved structural determinations of molecules in solutions, the environment most relevant to biology and industrially important chemical synthesis. And for good reason. Reagents are typically dissolved at relatively low concentration in an ocean of solvent whose scattering is orders of magnitude stronger than that from the reagents themselves. The pump pulse deposits energy into the probed volume, and the resulting jump in the solvent temperature and pressure triggers a time-dependent change in the solvent structure and its scattering signature. The pump pulse rarely transforms 100% of the reagent to intermediates of interest, and the photoexcited sample volume contains a time-evolving mixture of several species. The scattering from the solvation shell surrounding each reacting molecule can be as strong as that from the molecule itself, and its scattering signature changes as the solvent shell adapts to the transforming molecule. Consequently, a time-resolved liquid diffraction experiment will produce a small time-dependent signal on a large time-dependent solvent background, and the weak signal of interest will generally arise from a mixture of species with overlapping features. To have any hope for success, it is crucial that the time-dependent scattering signal be recorded with very high precision. Because the signal-to-noise ratio for this type of experiment is limited by photon-counting statistics, high-precision measurements require a very high flux source. The ID09B time-resolved x-ray beamline at the ESRF, developed by Wulff *et al*. (*12*), produces a flux well suited for these experiments.

When x-ray photons pass through a liquid sample that is thin compared to its x-ray absorption depth, less than 1% of the photons are scattered. Photons scattered from atoms whose separation is narrowly defined by chemical bonding or molecular packing can interfere constructively or destructively, with the resulting scattering

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pattern appearing as a set of concentric rings. The pump-induced change of the radial intensity distribution is related, through Fourier transformation, to the pair-distribution function, and provides a "fingerprint" of the characteristic interatomic distances (see the figure). The scattering data are one-dimensional, but a molecule's structure is three-dimensional. Consequently, a structure "determination" from liquid scattering data requires much help from theory. First, the solvent contribution to the scattering pattern must be accurately calculated and subtracted from the data. This procedure is far from trivial. Next, the diffraction patterns from three-dimensional models of putative intermediates must be calculated and compared with the solvent-corrected curves. Finding a match between the experimental and theoretical curves should not be confused with a structure determination in the crystallographic sense; nevertheless, a match that exhibits significantly higher fidelity than other proposed structures makes a compelling case for that structural assignment.

Ihee and co-workers chose their system wisely. When comparing the scattering patterns from bridged and anti iodoethane radicals with their solvent-subtracted scattering curves, the bridged form gave a much better match, thereby providing the most direct and compelling case for the structural assignment of this important radical intermediate. This achievement was aided by the fact that iodine atoms scatter x-rays more than 10 times as strongly as methanol, thereby enhancing the signal arising from iodo radical intermediates. Because diiodoethane ($C_2H_4I_2$) has only four heavy atoms (hydrogen scatters very weakly), scattering from it and its photo-generated intermediates is relatively easy to assign and interpret. Moreover, the iodo radical intermediate is sufficiently long-lived to be easily captured with 100-ps time-resolved snapshots. Finally, the radical is produced with relatively high quantum efficiency, so a sizable population could be generated and characterized. Although it should be possible to study molecules that lack heavy-atom substituents, such systems would require that the signal-to-noise ratio of the scattering data be improved. For example, to recover a signal that is 10 times weaker, the data integration time would have to be increased by at least that factor squared, or 100 times longer. One could envision studying more complex molecules as well; however, diffraction rings from disordered solutions are not sharply defined, so the amount of structural information that can be extracted from the radial intensity distribution is limited. Therefore, there is a molecular size beyond which it would prove increasingly difficult to correctly match the scattering pattern to a specific three-dimensional molecular structure.

When seeking a match to a "fingerprint," the correct structure must be included in the lineup. As more candidates are included, the chance for a false-positive becomes greater. As a result, one must exercise sound chemical intuition when selecting candidate structures for comparison, as was done in the study by Ihee and co-workers.

Although it has a few limitations, the technique of picosecond time-resolved liquid diffraction can provide an unprecedented glimpse into the structures of reactive intermediates involved in solution-phase chemistry. Once efforts to generate high-flux x-ray pulses on the few-picosecond (*13*) and the femtosecond (*14*–*16*) time scales are realized, the time resolution of liquid diffraction studies could be extended to the so-called chemical time scale, where a wealth of new insights into chemical reaction pathways awaits discovery.

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EVOLUTION

Is Invariance Across Animal Species Just an Illusion?

Gerdien de Jong

There is obvious variation in the way different animals live their lives—in their life span, in their age and size at maturity, and in their size as full-grown adults, to name a few attributes. But are there fundamental similarities in the life history strategies that different animals use? Charnov (*1*) argued that there are: He proposed fundamental similarities—"life history invariants"—to be a major explanatory ingredient of life history evolution. Life history invariants generalize a life history model over species boundaries and over a wide range of animal sizes, leading to an understanding of universal life history strategies. On page 1236 of this issue, Nee *et al.* (*2*) call into question the principal method to detect life history invariants. The authors have determined that the approach is misleading, throwing the very existence of the concept into doubt.

Life history invariants are dimensionless ratios of two life history traits—for instance, age at maturity and average length of life. Such a ratio is used to answer questions such as "At what relative age do animals first reproduce?" Whether we talk about rabbits or whales, we hope the ratio will enable us to forget about differences in life span, size, environment, and taxonomy. Thus, life history invariants point to common properties of organisms not immediately clear from direct observation. As such, they are potentially very useful for understanding and modeling life history evolution: The models are meant to be general, doing away with the need to model each species separately. The existence of life history invariants is a major argument for one general theory of life history evolution, rather than a theory as a set of recipes for how to make species-specific models.

Life history invariants are canonically identified from a log-log plot of two life history traits involved in a dimensionless ratio. In such a plot, the slope is expected to equal 1. Consider two life history traits, a and b, and ask whether their dimensionless ratio a/b is a life history invariant. If their ratio is constant (c), a log-log plot with $\ln(b)$ on the x axis and $\ln(a)$ on the y axis

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