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INTRODUCTION*

The twentieth century has been witness to major advances in our ability to peer into the microscopic world of molecules, thereby giving us unparalleled insights into their static and temporal behavior.¹ Beginning with X-rays at the turn of the 20th century, diffraction techniques have allowed determination of *equilibrium* three-dimensional structures with atomic resolution, in systems ranging from diatoms (NaCl) to DNA, proteins, and complex assemblies such as viruses.² For dynamics, the time resolution has similarly reached the fundamental atomic-scale of motion. With the

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advent of femtosecond time resolution nearly two decades ago, it has become possible to study—in real time—the dynamics of *non-equilibrium* molecular systems, also from the very small (NaI) to the very large (DNA, proteins, and their complexes).³

Armed with this ability to capture both the static architecture as well as the temporal behavior of the chemical bond, a tantalizing goal that now stimulates researchers the world over is the potential to map out, in real time, the coordinates of all individual atoms in a reaction, as, for example, when a molecule unfolds to form selective conformations, or when a protein docks onto the cell surface. These transient structures provide important insights into the function of chemical and biological molecules. As function is intimately associated with intrinsic conformational dynamics, knowing a molecule's static structure is often only the first step toward unraveling how the molecule functions, especially in the world of biology. Thus, elucidating the real-time 'structural dynamics' of *far-from-equilibrium* conformations at atomic scale resolution is vital to understanding the fundamental mechanisms of complex chemical and biological systems.

Time-resolved experiments with femtosecond time resolution have been performed in the past with probe wavelengths ranging from the ultraviolet to the infrared and far-infrared. On this time scale, one is able to freeze localized structures in space (wave packets) and observe their evolution in time—thus elucidating the elementary processes of bond transformation via transition states, in chemistry and biology.³⁻⁹ Recent advances have been made in multidimensional spectroscopy to correlate frequencies of optical transitions with temporal evolution, thereby probing structural changes in different relaxation processes.^{10, 11} For complex molecular structures, however, the positions of all atoms at a given time can only be obtained if the probe is able to 'see' interferences of all atoms. Diffraction methods using X-rays or electrons have the unique ability of revealing all internuclear coordinates with very high spatial resolution, thus providing a global picture of structural change on the ultrafast time scale with atomic level detail.

Electron or X-ray pulses can, in principle, be used to obtain timevarying molecular structures. These pulses must be short enough to freeze the atomic motions, yet bright enough to provide a discernible diffraction pattern. In the case of X-rays, photons are scattered by electrons in the molecular sample, so the diffracted intensity depends directly on the electronic density. Because most electrons are centered on atoms, these electron densities reflect the positions of nuclei, especially for heavy atoms. At present, ultrafast pulsed X-ray sources¹² include third-generation synchrotron radiation, laser-produced plasma sources, high-order harmonics production in gases and on solid surfaces, and free-electron lasers. While high-flux X-ray pulses from synchrotron sources are relatively long (tens of picoseconds; dictated by the duration of electron bunches in a storage ring), the sub-picosecond X-ray pulses from other generation schemes suffer from rather low fluxes.¹³ As a result, ultrafast X-ray diffraction studies have primarily focused on solid samples¹⁴ where the intrinsic long-range order enhances the signal-to-noise ratio of the interference patterns. X-ray absorption spectroscopy (XAS) techniques such as extended X-ray absorption fine structure (EXAFS) and X-ray absorption near-edge structure (XANES) spectroscopies have been used to obtain local structural information in solutions on the nanosecond timescale,^{15, 16} and on the ultrafast timescale, in gases¹⁷ and liquids.¹⁸

The method of choice in our laboratory has been ultrafast electron diffraction (UED), which has unique advantages. First, unlike X-ray photons, which are scattered by the electron distribution (Thompson scattering), electrons are scattered by both the atomic nuclei and the electron distribution (Fig. 1–1). Because of Coulomb scattering, electron-scattering cross-section is some six orders of magnitude stronger than X-ray scattering from molecules.¹⁹ It was this feature of electron–matter interaction that prompted Mark and Wierl in 1930 to use electrons (instead of X-rays) to study gasphase molecular structures; they produced a diffraction pattern²⁰ from CCl₄ that was more distinct than similar X-ray scattering exposures obtained earlier by Debye and co-workers²¹ and required a fraction of the exposure time (1 s compared to 20 h for the X-ray pattern). Second, UED experiments are 'tabletop' scale and can be implemented with ultrafast laser sources. Third, electrons are less damaging to specimens per useful scattering event. For example, using electrons in microscopy²² has shown²³ that the ratio of inelastic/elastic scattering events for 80–500 keV electrons is 3, and that for 1.5-Å X-rays is 10. The energy deposited per inelastic scattering event for 1.5-Å X-rays is 400 times that of electrons, thus implying that the energy deposited per useful (elastic) scattering event is 1000 times smaller for 80– 500 keV electrons. Fourth, electrons, because of their short penetration depth arising from strong interaction with matter, are well-suited for surface characterization, gases, and thin samples.

Imaging transient molecular structures on ultrafast time scales demands not only the marriage of ultrafast probing techniques with those of conventional diffraction, but also the development of new concepts for reaching simultaneously the temporal and spatial resolutions of atomic scale. Following the development of femtochemistry in the mid 1980s, the Zewail group embarked upon the challenge of achieving time-resolved electron diffraction in the sub-picosecond and picosecond regime. In 1991, it was proposed that replacing the 'probe' laser pulse in femtochemistry experiments with an electron pulse (Fig. 1–2) would open up new vistas in our understanding of structural dynamics.^{24, 25} A year later, diffraction patterns with picosecond electron pulses were reported, but without recording the temporal evolution of the reaction.²⁶ Since those first images, technical and theoretical advances in our laboratory^{27.45} have culminated in the thirdgeneration UED apparatus (UED-3) with spatial and temporal resolution of 0.01 Å and 1 ps, respectively. Moreover, we can now detect chemical change as low as 1%. As a result of these advances, a wide variety of phenomena has been studied in our laboratory.^{27.45}

Historically, the first gas-phase electron diffraction (GED) investigation of a molecular structure, that of CCl₄, was reported by Mark and Wierl in 1930,²⁰ only three years after the discovery of electron diffraction by Davisson and Germer for a crystal of nickel,⁴⁶ and by Thomson and Reid for a thin film of celluloid.⁴⁷ The utility of gas-phase electron diffraction was recognized by several research groups, beginning with that of Linus Pauling and his graduate student, Lawrence Brockway,⁴⁸ at Caltech. GED was further refined to elucidate the precise arrangement of atoms in molecules for understanding the *static* nature of the chemical bond.^{19, 49, 50} The original method for analyzing GED data, initiated by Mark and Wierl, and further developed by Pauling and Brockway, was called the 'visual method' because the patterns were analyzed simply by measuring the positions of maxima and minima, and by estimating their relative height and

depth by eye, thanks to the extraordinary ability of the human eye to correct for the steeply falling background. Soon, however, a more direct method of determining bond distances was proposed by Pauling and Brockway⁵¹—the so-called radial distribution method—that invoked the Fourier transform of the estimated intensity data.

A significant advance in the quantitative measurement of the intensity distribution was the introduction of the 'rotating sector' into the diffraction apparatus, proposed by Trendelenbur,⁵² Finbak,⁵³ and Debye⁵⁴ in the 1930s, which obviated the use of visual estimates. This rotating sector (a metallic disk of special shape)-which attenuates the inner, more intense part of the pattern, effectively enhancing the outer, weaker signals—was a crucial step in the development of what came to be known as the 'sector-microphotometer' method. Until the early 1970s, diffraction patterns were recorded exclusively with photographic film. The replacement of these film-based detectors with an electronic detector by Fink and Bonham⁵⁵ was a turning point towards electronic microdensitometry—evolving from scintillator-photomultipliers^{56, 57} to linear array detectors.⁵⁸ The introduction of 2D area detectors—chargecoupled device (CCD) with fiber optic coupling and image intensification—in our laboratory^{26, 30, 35} represents the current state-of-the-art in digital diffraction imaging.

It is not surprising that the earliest attempts at introducing time resolution into electron diffraction mirrored the development of digital detection techniques. Ischenko *et al.*⁵⁹ created microsecond electron pulses by chopping a continuous electron beam with an electromagnetic chopper to study the IR multiphoton dissociation of CF₃I (see ref.⁵⁰ for a critique). Rood and Milledge⁶⁰ conducted diffraction studies on the decomposition of ClO₂ with 100-µs electron pulses, while Bartell and Dibble⁶¹ studied phase change in clusters produced in supersonic jets, with a time-of-flight resolution of *ca*. 1 µs. Ewbank et al.⁶² advanced the temporal resolution to nanoseconds (and later shorter⁶³) by combining a laser-initiated electron source with a linear diode array detector and investigated the photofragmentation of small molecules (e.g., CS_2). Mourou and Williamson⁶⁴ pioneered the use of a modified streak camera to generate 100-ps electron pulses to record diffraction images from thin aluminum films in transmission mode; they subsequently produced 20-ps electron pulses to study the phase transformation in these films before and after irradiation with a laser.⁶⁵ Elsayed-Ali and co-workers succeeded in using 200-ps (and later shorter) electron pulses to investigate surface melting with reflection high-energy electron diffraction (RHEED).66,67

In the field of ultrafast electron diffraction, for the studies of isolated structures *evolving with time*, the leap forward came from the use of digital processing with CCD cameras, generation of ultrashort electron packets using femtosecond lasers and high extraction fields, and *in situ* pulse sequencing and clocking—all of which gave us unprecedented levels of sensitivity and spatiotemporal resolution. Using these developments, we have studied a variety of complex molecular structures and resolved the temporal evolution of different classes of reactions, as discussed in this thesis. More recently, Weber and co-workers have succeeded in obtaining ultrafast diffraction images of cyclohexadiene,⁶⁸ a system we have studied both theoretically and experimentally. Theoretical analysis of the diffraction signatures of individual vibrational modes in polyatomic molecules prepared in a specific vibrational state was also reported.^{69, 70}









Figure 1-2. The UED Experiment. Properly timed sequences of ultrafast pulses are employed–femtosecond laser pulse to initiate the reaction and ultrashort electron pulses to probe the ensuing structural change in the molecular sample. The resulting electron diffraction patterns are then recorded on a CCD camera.

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