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A novel spectroscopic window on conical intersections in biomolecules

(Watching a wavepacket pass through a conical intersection in real time)

Giulio Cerullo¹ and Marco Garavelli²

¹*IFN-CNR, Dipartimento di Fisica, Politecnico di Milano, Piazza Leonardo da Vinci 32, I-20133 Milano, Italy*

²*Dipartimento di Chimica Industriale, Università degli Studi di Bologna, Viale del Risorgimento 4, I-40136 Bologna, Italy.*

In the current issue of PNAS, Keefer et al. propose a new experimental approach which allows to visualize in detail the dynamics of a molecule as it passes a conical intersection (CI), i.e. a real crossing point, between two electronic states (1). In order to describe the photoinduced dynamics of molecules one needs to solve the time-dependent Schrödinger equation for a complex wavefunction which depends on many electronic and nuclear degrees of freedom. To simplify the problem, one typically adopts the Born-Oppenheimer approximation, which consists in separating the electronic and the nuclear coordinates in the wavefunction. This approximation, which is justified by the much higher speed of the electrons with respect to the nuclei, consists in solving the Schrödinger equation for the electrons at given fixed nuclear positions and then obtaining the electronic energies as a function of those fixed nuclear coordinates, the so-called potential energy surfaces (PES), in what is often referred to as the adiabatic approximation.

In many molecules, however, there are regions of the potential energy landscape, such as CIs, where the electronic and nuclear degrees of freedom become strongly mixed and the Born-Oppenheimer (adiabatic) approximation breaks down. CIs are ubiquitous features in the photophysics and photochemistry of molecules, and can be considered as “doorways” through which the photoexcited wavepacket (WP) is efficiently funneled to a lower energy electronic state (2, 3), thus accounting for efficient non-radiative relaxation (also called internal conversion). CIs are topologies of the PES for which two or more electronic states become iso-energetic, forming a multi-dimensional “seam” (see Fig. 1). Depending on the topography around the seam, CIs can be classified as “sloped” or “peaked”. In a peaked CI (Figure 1, left panel) the WP is guided to the intersection seam regardless of the initial approach direction, resulting in a more efficient conversion and triggering photochemistry. In a sloped CI (Figure 1, right panel), on the other hand, the WP is led to the seam less efficiently, as the WP has to climb uphill, and has the possibility to miss it, often resulting in slower decays that do not involve photochemical processes.

CIs play a dual role in the interaction of bio-molecules with light: either to promote efficient conversion from a reactant to a product state in a photochemical reaction, or to enable efficient dissipation of excess electronic energy, thus preventing a potentially harmful photochemical reaction. A striking example of the first case are opsin proteins, in which the photoexcited retinal chromophore exploits a CI to promote the ultrafast photoisomerization (all-*trans* to 13-*cis* in Type I opsins and 11-*cis* to all-*trans* in Type II or visual opsins) to a spectrally red-shifted ground state photoproduct (4). An important example of the second case are nucleobases, the building blocks of DNA, for which CIs are used to promote rapid dissipation of excited state energy, preventing photoreactions which could damage the genetic code (5).

Given the extreme speed of the processes leading to CIs, ultrafast optical spectroscopy is the elective tool for their observation. However, the direct visualization of a WP passing through a CI is challenging, because the energy bandgap between the interacting levels changes very rapidly over a short time, thus calling for the combination of high temporal and spectral resolution. A pioneering experiment in this direction was performed by Polli *et al.*, who used broadly tunable ≈ 10 -fs visible/near-infrared pulses to track in real time the isomerization of retinal in rhodopsin (6). Transient absorption spectroscopy allowed to follow the rapid shrinking of the energy gap, as the WP moves towards the CI on the excited state reactant PES, and the subsequent widening of the gap, as the WP leaves the CI and evolves on the ground state PES of the photoproduct. However, the very moment of the passage through the CI remained inaccessible to these

experiments, since it would require probing with very long wavelengths a transition with vanishing oscillator strength. Recently, considerable new insight into the nature of CIs has been afforded by the development of novel ultrafast probes, such as element specific ultrafast soft X-ray sources (7, 8) or ultrafast electron diffraction (9, 10).

Mukamel and coworkers (11) have introduced a novel spectroscopic technique, which they called Transient Redistribution of Ultrafast Electronic Coherences in Attosecond Raman Signals (TRUECARS), that allows to directly monitor the passage of a WP through a CI. TRUECARS bears some resemblance to Coherent Anti-Stokes Raman Scattering (CARS) (12), but extends it from vibrational to electronic coherences. In CARS two optical pulses, the pump at frequency ω_{pu} and the Stokes at frequency ω_s , generate a vibrational coherence at frequency $\Omega = \omega_{pu} - \omega_s$, which is read by a further interaction with a probe pulse at frequency ω_{pr} (often one chooses $\omega_{pr} = \omega_{pu}$) to generate the Anti-Stokes signal at $\omega_{as} = \omega_{pr} + \Omega$. In time-domain or impulsive CARS, the probe is an ultrashort pulse whose time delay with respect to the pump/Stokes pulses is varied, so that the CARS signal oscillates as a function of delay with the period(s) of the excited vibrational coherence(s) (13).

TRUECARS aims at probing the electronic coherence between the two states involved in the CI; to this end it uses a hybrid pulse sequence consisting of a visible actinic (pump) pulse followed by two phase-locked X-ray pulses, a broadband one (E_0 , with attosecond duration) and a narrowband one (E_1 , with few-femtosecond duration). The pump pulse promotes the molecule to the electronic excited state, creating a nuclear WP in the Franck-Condon region which starts to evolve on the excited state PES towards the CI, in the vicinity of which (i.e., where the Born-Oppenheimer approximation starts to break down) an electronic coherence between the interacting electronic states is created by the non-adiabatic coupling. This coherence is then probed by the X-ray pulses, chosen to be off-resonant with any electronic transition in the molecule, via a stimulated Raman process, resulting in gain/loss for different spectral components of the frequency-dispersed broadband attosecond E_0 pulse. TRUECARS thus observes electronic coherences in the time domain, analogously to the vibrational coherences measured in CARS. It is important to note that in TRUECARS, differently from attosecond transient absorption spectroscopy (7, 8), the probe pulses are not absorbed by the molecule, but there is simply a redistribution of the energy of the broadband pulse between its different spectral components, according to electronic stimulated Raman gain/loss processes. The use of X-ray probe pulses is justified by the simultaneous requirements of broad bandwidth (a few eV for the E_0 pulse) and high time resolution (a few femtoseconds for the E_1 pulse), which cannot be satisfied with visible pulses.

The seminal TRUECARS paper has applied the method to a minimal “toy” model, consisting of two electronic states coupled to two vibrational degrees of freedom. In their current PNAS paper, Keefer et al. apply TRUECARS to gas-phase uracil, using a realistic *ab-initio* molecular Hamiltonian (1). Uracil is a pyrimidine nucleobase found in RNA which replaces thymine in DNA, differing from it by the lack of a methyl group. Like all nucleobases, uracil displays a strong absorption band in the UV, peaking around 4.75 eV, towards the ($^1\pi\pi^*$) singlet excited state. Below this state there is another excited state, the $^1n\pi^*$ state, which is optically dark. For this reason, the bright excited state is called S_2 and the dark state S_1 . Following photoexcitation of the S_2 state, the WP reaches very quickly, within 100 fs, a CI with the S_1 state, from which in turn it rapidly decays to the ground state S_0 via another CI (14). This extremely fast non-radiative dissipation of electronic energy through CIs is common to all nucleobases and has a great biochemical significance. The excess electronic energy deposited in DNA by UV absorption could, in fact, trigger a series of photochemical reactions resulting in an alteration of the structure of the base sequence (15). One notable example is the covalent binding (dimerization) of two adjacent thymines, resulting in the formation of the cyclobutane dimer, which is linked to carcinogenesis. The extremely fast excited state deactivation of nucleobases through CIs, however, strongly reduces the efficiency of such adverse photochemical

reactions, because it does not give them enough time to happen, and thus acts as a powerful built-in photoprotection mechanism for DNA.

In their study, Keefer *et al.* concentrate on the first CI between the S_2 and the S_1 state of uracil. By launching a nuclear WP on S_2 by a ≈ 30 -fs UV actinic pulse, they observe an almost ballistic motion to the CI with S_1 , which is reached within ≈ 100 fs. At this point of the simulation the hybrid broadband/narrowband X-ray probe pulse is switched on, tuned to a non-resonant 354-eV photon energy, which is intermediate between the Carbon (≈ 291 eV) and the Nitrogen (≈ 405 eV) K edges. The TRUECARS signal for uracil shows several striking features. First of all, when the WP reaches the CI a vibronic coherence is created which lives for hundreds of femtoseconds. This is a surprising observation, given the rapid nature of the crossing of the CI, which can be rationalized by considering the spatially delocalized nature of the WP on the S_2 PES: different portions of the WP reach the CI at different times, thus allowing the coherence to survive for several hundreds of femtoseconds. Further insight into the physics of the CI process is provided by the Wigner spectrogram of the TRUECARS signal, which reveals the contributing vibronic states. In particular, the analysis highlights the difference between a classical description of the non-adiabatic process, where the energy splitting between the PES is well defined and vanishes at the CI, and a quantum description, for which there is a distribution of energy splittings according to the WP frequencies, which needs to be averaged in order to obtain the effective time-dependent splitting.

The TRUECARS approach, here illustrated for the important biomolecule uracil, is generalizable to a broad class of photochemical and photophysical problems. Its experimental implementation will require the combination of visible/UV actinic pulses (easily produced by current femtosecond laser technologies) with femtosecond to attosecond X-ray probe pulses. Nowadays, there are two main approaches to the generation of (sub)-femtosecond X-ray pulses: high harmonic generation (HHG) from gases and X-ray free electron lasers (XFELs). HHG has proven the capability to generate attosecond X-ray pulses in a tabletop setup (16) at energies up to the water window (≈ 300 -500 eV), although currently with limited pulse energies. XFELs, on the other hand, while being large-scale facilities, enable the generation of X-ray pulses with much higher energies and much broader frequency tunability, and have recently proven the capability to generate attosecond pulses (17, 18). Therefore, both HHG and XFELs provide the X-ray pulse parameters required for the technique. There is however an important consideration to be made when designing a TRUECARS experiment: the signal carries a phase factor $e^{i(\varphi_1 - \varphi_0)}$, where φ_i are the phases of the X-ray pulses, so that it will average to zero unless the phase difference of the X-ray pulses is stabilized. Phase-locking between the two X-ray probe pulses is a crucial technical requirement which is not easy to satisfy. The 354 eV X-ray pulses used by Keefer *et al.* have an oscillation period of the carrier wave of only 11.7 attoseconds, so that zeptosecond-level delay control will be required in order to achieve phase control. Very recently, however, an important step in this direction has been made by Wituscheck and coworkers (19), who have demonstrated the generation of a phase-locked pulse pair from a seeded FEL, although still at comparatively low photon energies (up to 47.5 eV).

As a final consideration, we emphasize that, in the still burgeoning field of ultrafast optical spectroscopy, theory and experiments are deeply intertwined (20). On the one hand, theory is required to interpret the complex, and often multidimensional, experimental data, and to relate the physical observables to the underlying molecular dynamics. On the other hand, as beautifully shown by Keefer *et al.*, theory can suggest entirely novel experimental approaches which offer fresh new insight into key molecular processes, thus motivating the continuous technical developments in the field of ultrashort pulse generation and guiding future experiments.

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Figure captions:

Figure 1: simple one-dimensional schematic representation of a peaked (left panel) and a sloped (right panel) conical intersection.

