

Ultrafast sub-nanometric spatial accuracy of a fleeting quantum probe interaction with a biomolecule: innovating concept for spatio-temporal radiation biomedicine

Yann A. Gauduel*, Victor Malka

Laboratoire d'Optique Appliquée, CNRS UMR 7639, Ecole Polytechnique,
ENS Techniques Avancées, 91762 Palaiseau cedex, FRANCE

ABSTRACT

During cancer radiotherapy protocols, the early profile of energy deposition is decisive for the prediction and control of radiation-induced biomolecular and sub-cellular damage. A major challenge of spatio-temporal radiation biomedicine, a newly emerging interdisciplinary domain, concerns the complete understanding of biophysical events triggered by an initial energy deposition inside confined ionization clusters (tracks) and evolving over several orders of magnitude, typically from femtosecond ($1 \text{ fs} = 10^{-15} \text{ s}$) and sub-nanometer scales. The innovating advent of femtosecond laser sources providing ultra-short photon beam and relativistic electron bunches, in the eV and MeV domain respectively, open exciting opportunities for a real-time imaging of radiation-induced biomolecular alterations in nanoscopic tracks. Using a very short-lived quantum probe (2p-like excited electron) and high-time resolved laser spectroscopic methods in the near IR and the temporal window 500 – 5000 fs, we demonstrate that short-range coherent interactions between the quantum probe and a small biosensor of 20 atoms (disulfide molecule) are characterized by an effective reaction radius of 9.6 ± 0.2 angströms. For the first time, femtobioradical investigations performed with aqueous environments give correlated information on spatial and temporal biomolecular damages triggered by a very short lived quantum scalpel whose the gyration radius is around 6 angströms. This innovating approach would be applied to more complex biological architectures such as nucleosomes, healthy and tumour cells. In the framework of high-quality ultra-short penetrating radiation beams devoted to pulsed radiotherapy of cancers, this concept would foreshadow the development of real-time nanobiosimetry combined to highly-selective targeted pro-drug activation.

Keywords: femtosecond laser, relativistic electron bunch, near-infrared spectroscopy, quantum probe, biomolecular target, nanodosimetry, spatio-temporal radiation biomedicine, pulsed cancer radiotherapy

1. INTRODUCTION

In living matter, the early spatial distribution of radical entities may be crucial for the triggering and amplification of primary biomolecular architectures damage induced by ionizing radiations (X- and γ -rays, UV photons, energetic particles in the relativistic regime, accelerated ions)^{1,2}. One important challenge concerns the complete understanding of multiscale events evolving over several orders of magnitude, typically from femtosecond ($1 \text{ fs} = 10^{-15} \text{ s}$) and sub-micrometric scales. The real-time investigation of elementary radical processes in condensed phase can be carried out in synergy with the most recent developments of ultra-short laser sources, combining the complementary concepts of low or high energy radiation femtochemistry³. Indeed, nanoscale insight into early radical processes and native ionisation tracks represents a prerequisite for the complete knowledge of radiation-induced bio-effects in the confined environments of biomolecular targets.

Deeply understanding the multi-scale mechanism of radiation damage in living matter, starting from the early radical and molecular processes to mutagenic DNA lesions, cell signalling, genomic instability, apoptosis, microenvironment and bystander effects, would have, in the near future, many practical consequences like the customization of more predictive and selective radiotherapy protocols. The complex links that exist between the physical aspects of early radiation events and the delayed evolution of biological endpoints, carcinogenesis or cell survivals need the development of advanced *spatio-temporal radiation biomedicine*⁴.

* yann.gauduel@ensta-paristech.fr phone (+33)1 69 31 97 26; fax (+33)1 69 31 97 00

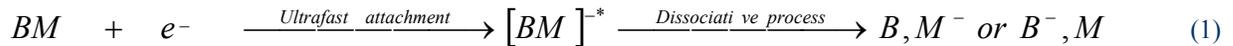
This contribution deals with an interdisciplinary approach that concerns cutting-edge advances in real-time sub-nanometric spatial accuracy of a fleeting quantum probe interaction with a biomolecule, considering the potentialities of innovating strategies based on ultrafast laser science, from femtosecond photon sources to advanced techniques of ultrafast TW laser-plasma accelerator^{3,5-10}. Such research developments are crucial to understand, at the nanometric scale, the early consequences of ultra-short-pulsed radiation on biomolecular environments and to estimate, at cell and tissue levels, the time-dependence of radiation risks for pulsed radiotherapy applications¹¹⁻¹⁵.

2. ULTRAFAST SPECTROSCOPY OF PRETHERMAL RADICAL EVENTS

In condensed phase, radical processes contribute to significant molecular damages and take place by the rearrangement of initial reactants and energy redistribution to provide transient states and final products. Within a very broad temporal window, they can implicate the quantum control of elementary steps such as intra and intermolecular solvation phenomena and caging effects, dielectric relaxation, ions-molecules reactions, charge transfers, bond making-bond breaking, and finally diffusion controlled reactions. The microscopic understanding of reaction dynamics requires the real-time probing of elementary events that assist or impede transient reaction events. Elementary radical processes involve angstroms or sub-angstroms atomic displacements and transient non equilibrium states whose the lifetime is typically in the sub-picosecond regime. With the intensive development of ultra-short laser technologies and advanced high-time resolved spectroscopic methods, the course of short lived nonequilibrium radical trajectories become observable on the molecular motion scale.

2.1 Choice of a well-defined transient quantum probe

One of the most fundamental aspects of a biomolecule (BM) damage induced by ionising radiations concerns the dissociative electron attachment processes occurring in confined ionization spaces (Equation 1).



Such molecular damage involve generally a hierarchy of secondary electron populations for which the energy varies from the thermal value ($kT \sim 0.025$ eV) to the sub-excitation and relativistic levels i.e. few eV and MeV respectively. In this way, the prethermal processes and early spatial distribution of transient couplings between secondary low energy electron and biomolecules may play a crucial role in radiation induced damage at early time. The nano-scale insight of primary radical processes triggered by ionizing radiation requires the real-time probing of non-equilibrium states for which the lifetimes are typically in the sub-picosecond regime.

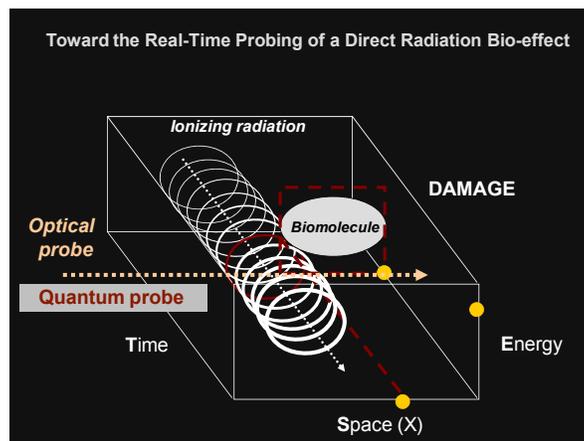


Figure 1. Ultrafast spectroscopic strategy used for the real time observation of an efficient coupling between a very short-lived quantum probe and a biomolecule in a liquid water bath. The spatio-temporal coherence linking a quantum probe and a biomolecular sensor is investigated at the femtosecond time scale and nanometric level.

Ultrafast radical events occurring in less than 1ps (10^{-12} s) after an energy deposition induced by a ionizing radiation represent a specific domain for which the quantum character of short-lived events becomes preeminent. This is particularly important when biomolecular damage take place in the prethermal regime of secondary electrons³. Consequently, one important challenge concerns the spatio-temporal description of events induced by the initial energy deposition of an electromagnetic ray or energetic particle in the medium and the subsequent effects on a solvated biomolecule. For that we are developing a multi-parametric approach that includes some energetic, spatial and temporal considerations (Figure 1). Previous Gauduel's experimental researches devoted to low and high energy radiation effects on water molecules have suggested the crucial role of ultra-fast quantum effects for which short-lived configuration of very low energy quasi-delocalized secondary electrons would be involved^{16,17}. For instance, in the framework of a nonlinear two-photon excitation process of water molecules by femtosecond UV pulses (pulse duration ~ 50 fs, $E_{\text{Excit}} = 2 \times 4$ eV), multiple non-equilibrium configurations of delocalized electron are generated in less than 5×10^{-13} s at 294K. One of these short-lived configurations can be analyzed in term of eigenstate and used as quantum probe for the development of preliminary investigations on prethermal chemistry and biomolecular damage induced by the interaction of ionizing radiations (photons or accelerated particles) with a water bath¹⁸⁻²⁵. The specific spatio-temporal coherence which is required to get an efficient coupling between a very short-lived quantum probe and a biomolecule needs more complete studies which can be performed by ultrafast near-infrared spectroscopy, using different pump-probe configurations. The multi-parametric data extracted from ultrafast spectroscopic experiments are used for advanced physico-chemical understandings at early time. Some of these parameters will be presented in the next section.

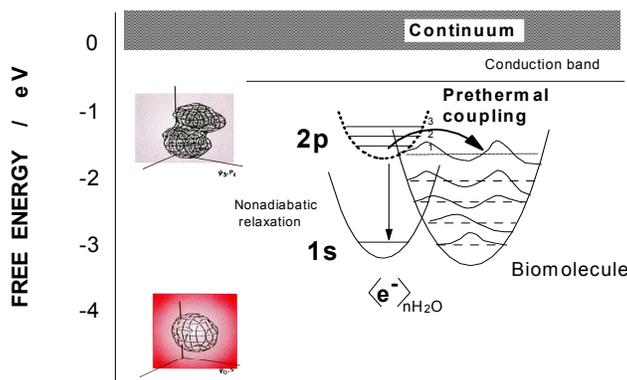


Figure 2. Energy level diagram of a prethermal coupling between the quantum p-state of a localized electron and a disulfide biomolecule. Within an aqueous environment, the short-lived coupling between this quantum configuration of a trapped electron and a biomolecule competes with an ultrafast nonadiabatic p-s transition leading to the formation of a fully relaxed electron in water bath (1s configuration)¹⁸. The left part of the figure gives a representation of 2p and 1s state wavefunctions calculated by semi-quantum molecular dynamic simulations²⁶.

For this multi-parametric approach, the transient quantum probe which is taken into consideration corresponds to a well-defined p-state of trapped electron (Figure 2). This eigenstate is generated in less than 150fs and represents a localized electron for which the energy is about -1.0 eV below the bottom of water conduction band (excited prehydrated electron). This state exhibits an absorption spectral signature in the near-infrared and its lifetime (~ 250 fs in pure water at 294K) is directly governed by a non-adiabatic $2p(e_{\text{prehyd}}^-) \rightarrow 1s(e_{\text{hyd}}^-)$ transition¹⁸. Considering semi-quantum Molecular Dynamics simulations, the predicted gyration radius of p-state electron is around 6 Å in water bath²⁶.

2.2 Temporal and spatial coherence for an efficient coupling between a quantum probe and a biomolecular target

In the framework of an energy/space/time profile, the temporal and spatial coherences that are required to obtain an efficient coupling between the quantum probe 2p-state electron and a biomolecular target (cystamine molecule) are investigated by using Ti:Sa laser amplifier system generating amplified femtosecond pulses in the mJ energy domain and a pump-probe configuration: a femtosecond two-photons excitation in the ultraviolet (pump beam of 2×4 eV, pump beam power density $\sim 10^{11}$ Wcm⁻² at 0.31 μm) and an infrared probe centered at 0.99 eV (1.25 μm). The transient

quantum probe (2p-state excited electron) is triggered by a two-photon excitation of chloride ion in water bath, via an ultrashort lived excited CTTS state (CTTS**) ^{27,28}. The resulting formation of a quantum p-state electron can be investigated in real time, considering its very short-lived signature in the near infrared (Figure 3). The choice of a disulfide biomolecular target (chlorhydrate of cystamine) permits to use the chloride counterion to initiate the two-photon excitation process and compare spectroscopic data with those obtained with a control sample (aqueous sodium or magnesium chloride solution for instance). The biomolecular target (cystamine (NH₃-(CH₂)₂-SS-(CH₂)₂-NH₃)) represents the reactive center of oxidized glutathione (GSSG).

The direct coupling which takes place between the quantum probe (p state electron) and a disulfide biomolecule can be investigated from femtosecond infrared signal dynamics, considering the real-time contribution of quantum probe formation and the ultrafast collapse of this probe on the molecular target (ultrafast infrared p-state electron attachment, Equation 1). This collapse induces an early partition between a reactive trajectory ($\{e^-_{IR}\}_{Reac}$) and a nonreactive trajectory that corresponds to a nonadiabatic relaxation of the quantum probe ($\{e^-_{IR}\}_{p-s}$). The branching ratio between these two ultrafast electronic trajectories has been expressed in term of probabilities, as reported in the equation of Figure 3.

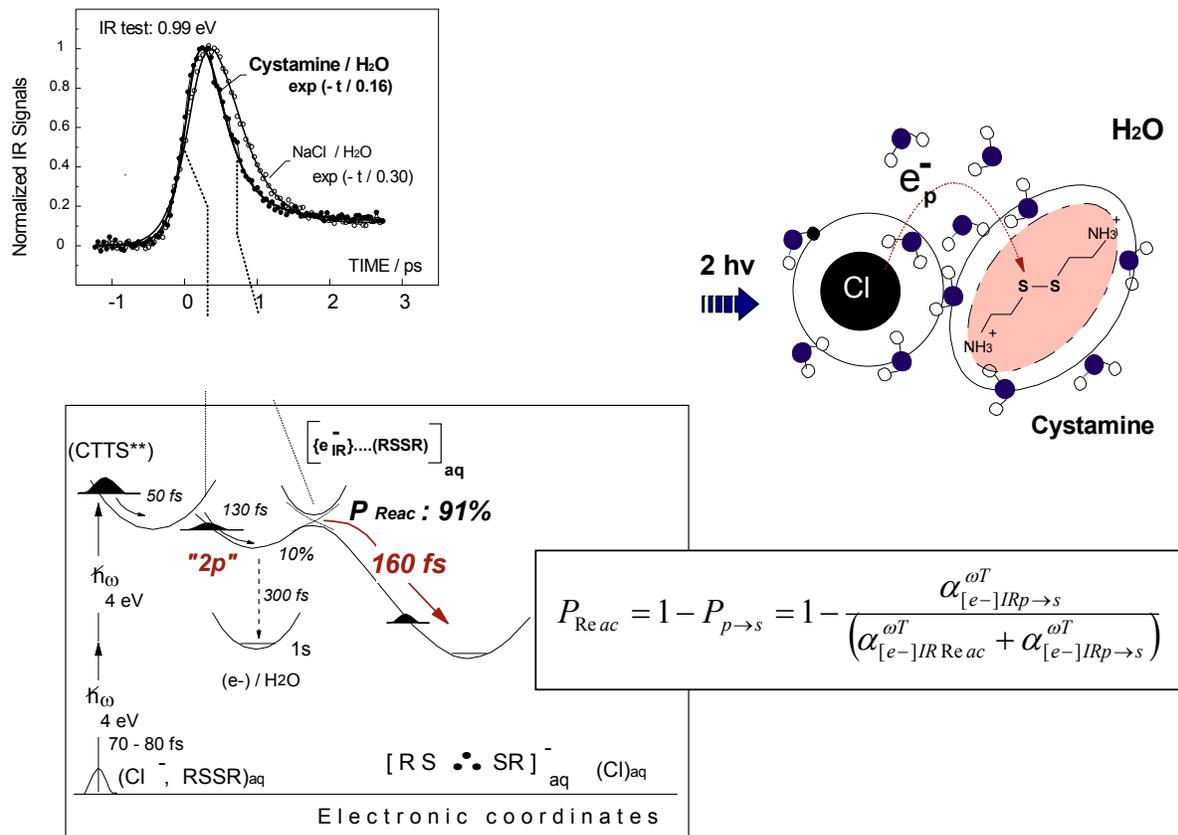


Figure 3. Ultrafast infrared spectroscopy of an efficient coupling between the quantum 2p state of an excited electron and a biomolecular target (chlorhydrate of cystamine) in water bath (see also Figure 2). Femtosecond infrared absorption signals are investigated at 0.99eV (1.25 μm). The molecular ratio of aqueous ionic solution (NaCl) and biomolecular target (Cystamine) equal 110. The short-lived quantum probe (p-state electron) is generated by a two photon UV excitation of an halide ion (Cl⁻), via a transient CTTS state (Charge Transfer To Solvent).

In the equation of Figure 3, P_{Reac} , $P_{p \rightarrow s}$ represent respectively the probability of an ultrafast collapse of a 2p-like excited electron on a cystamine molecule and an electron solvation process via a nonadiabatic relaxation process (2p→1s

transition). In this equation, the adjusted parameter $\alpha_i^{\omega T}$ represents the spectral contribution of different electronic state "i" trajectories probed at 0.99eV. The subpicosecond competition involving the ultrafast electron attachment on cystamine and the energy gap relaxation from the first excited state to the ground state of trapped electron is characterized by a high branching ratio ($P_{\text{Reac}} = 1 - P_{p \rightarrow s} \sim 0.91 \pm 0.02$). This ultrafast radical process would involve some distortions of p-like orbitals of prehydrated electron in the vicinity of a sulfur-sulfur bond of cystamine. The subpicosecond lifetime of the infrared quantum p state electron (e_p state) represents a temporal limit for that an early SS bond energy rearrangement facilitates an ultrafast electron tunnelling in the prethermal regime, through a potential barrier separating the infrared p-state electron of an unpaired electron. In this way, the effective radius r_{eff} of an ultrafast coupling between a non-equilibrium p-like excited electron and a cystamine molecule (RSSR) is defined by Equation 2. This effective radius r_{eff} equals 9.6 ± 0.2 angstroms for a molecular concentration of cystamine of 0.5 M in water bath (molecular ratio = 110).

$$r_{\text{eff}} = \sqrt[3]{\frac{\text{Ln}\left(\frac{1}{P_{p \rightarrow s}}\right) \cdot 3 \times 10^3}{4\pi [RSSR]}} = \sqrt[3]{\frac{\text{Ln}\left(\frac{\alpha_{[e-]IR \text{ Reac}}^{\omega T} + \alpha_{[e-]IR p \rightarrow s}^{\omega T}}{\alpha_{[e-]IR p \rightarrow s}^{\omega T}}\right) \cdot 3 \times 10^3}{4\pi [RSSR]}} \quad (2)$$

The effect of this efficient coupling between a quantum probe and the disulfide biomolecule leads to the formation of a native $\text{RS}\cdot\text{SR}^-$ radical anion²⁹. It can be underlined that the additional antibonding electron contributes to the formation of an anionic radical characterized by a 2center-3 electron bond in less than 650 fs (Figure 4).

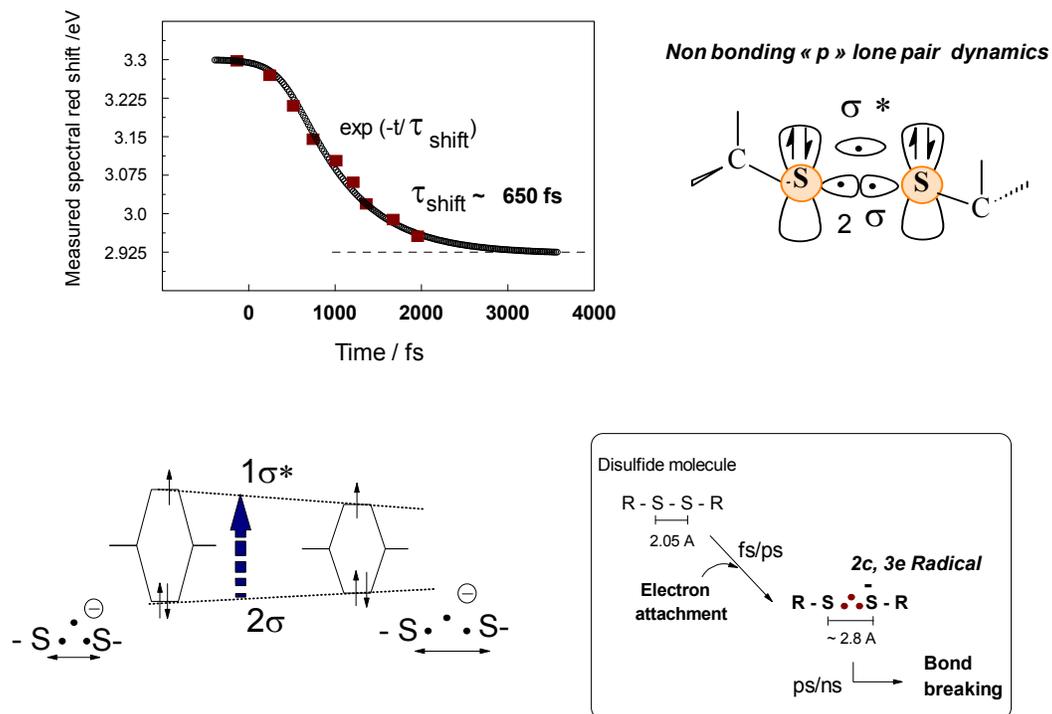


Figure 4. Femtosecond UV spectroscopy of a $(2\sigma/\sigma^*)$ transition inside a nascent 2c, 3e anionic radical following an ultrafast electron attachment on a disulfide molecule. The real time SS bond weakening triggered by an antibonding electron is characterized by a decrease of the gap energy inside the $2\sigma/\sigma^*$ transition and involves a red shift of the transient UV absorption signal. A delayed intramolecular SS bond breaking can take place in the sub-nanosecond window.

The concomitant formation of an anionic radical and a weakening of a nascent $2\sigma / \sigma^*$ bond energy may induce a disulfide bond breaking at longer time (molecular damage). This femtobioradical investigation performed in aqueous environment gives correlated information on spatial and temporal biomolecular damages triggered by a *very short lived quantum scalpel* for which the gyration radius is around 6 angströms. The ultrafast sub-nanometric spatial accuracy obtained by femtosecond infrared spectroscopy permits ⁱ⁾ to precise that the effective radius r_{eff} for getting an ultrafast coupling is greater than this gyration radius, ⁱⁱ⁾ to define an effective interaction cross section (ICS) of $3 \times 10^{-6} \mu\text{m}^2$. This interesting parameter can only be discriminated within a very short-time window ($t \ll 1$ ps). It characterizes an efficient and transient coupling between the polarizable fleeting quantum probe (2p state electron) and a disulfide biomolecule. The potential effects of local environments on this effective interaction cross section at early time require additional investigations which are actually performed.

3. GENERALISATION OF THIS CONCEPT WITH LASER-PLASMA ACCELERATORS

The powerful laser techniques (table-top terawatt Ti:Sa laser amplifier systems) combined to laser plasma interactions provide femtosecond high-energy electrons beams, typically in the 2.5 - 150 MeV range ^{7-10,30,31}, which might conjecture the direct observation of primary radiation events in native ionisation spurs and tracks ^{32,33}. This emerging domain is characterized by the development of high energy radiation femtochemistry (HERF), conducted in synergy with fundamental knowledge of low energy radiation femtochemistry (LERF).

3.1 High energy deposition and early inhomogeneous phenomena induced by relativistic particle

The interactions of relativistic MeV electrons with water molecules induce ultrafast energy scattering processes and fractionated ionization clusters (Figures 2 and 3). These ionization processes involve a hierarchy of electron populations for which the energy varies from relativistic levels to the thermal value (kT ~ 0.025 eV). Typically, in less than 10^{-16} s, different energy quanta in the 200-20 eV range may be delivered in nanometric tracks or spurs. Due to the uncertainty relation between time and energy, the fastest ionising events in confined clusters take place in less than 0.33×10^{-16} s. Consequently, a quantum energy of 20 eV deposited by relativistic particles occurs on 10^{-6} cm (~ 100 Å) and involves nanometric ionization clusters configurations (Figure 5).

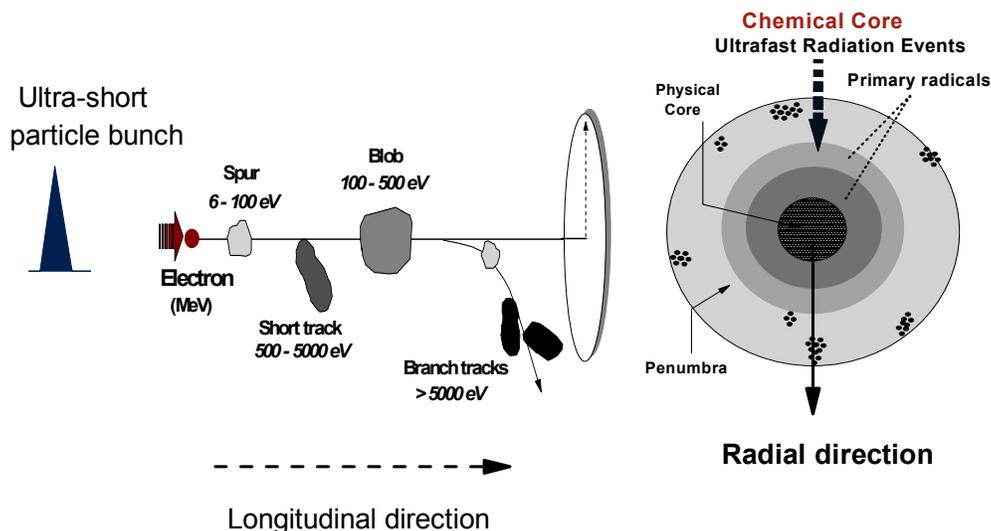


Figure 5. Femtolysis experiments of water molecules in liquid phase with ultrashort high energy electron bunches: schematic representation of a spatial distribution of inhomogeneous energy deposition processes and ionisation tracks in the longitudinal direction of a beam propagation.. In the radial direction of this radiation beam, early radiation events take place within the chemical core i.e. at the interface of the physical core and penumbra zone.

As early radiation damages can be highly dependent on ⁱ⁾ the survival probability of very low-energy secondary electrons and ⁱⁱ⁾ the spatial distribution of primary radicals produced from water molecules, a thorough knowledge of native tracks requires the real-time probing of radiation events in the $10^{-15} - 10^{-11}$ s range. In aqueous environments, this temporal domain concerns mainly prethermal events for which the quantum states of short-lived excited electrons involve femtosecond nonadiabatic processes.

3.2 Ultrashort relativistic electron bunches and generation of native ionization tracks

From the technical point of view, a 820 nm TW titanium-doped sapphire laser beam with an on-target energy of 960 mJ in 30 fs FWHM pulses can be focused onto the sharp edge of a 2 mm diameter supersonic helium gas jet. With intensities on the order of 2.7×10^{19} W/cm² in vacuum, the femtosecond laser pulse ionises the gas and excites relativistic plasma waves in the underdense plasma. The electrons of the plasma are accelerated by the intense electric field in the forward direction producing a well collimated electron beam with an initial duration of the same order of the TW laser pulse. Typically, a total charge of the electron beam determined by an integrating current transformer equals 2.5 ± 0.2 nC and corresponds to a mean number of $(1.55 \pm 0.15) \times 10^{10}$ electrons. The specific qualities and properties of ultra-short relativistic particle bunches foreshadow the development of advanced research to deepen the biophysical understanding of ultrafast radiation events in native ionization tracks. Recent femtosecond ^{radio}lysis experiments (Femto_{second} radio_{lysis}) of aqueous targets performed with pulsed electron bunches of 2.5-15 MeV were devoted to the investigation of early radical processes in nascent ionisation tracks. For an effective temperature of the femtosecond electron beam of about 4.5 MeV, the integrated energy flux in aqueous samples equals 5.8×10^{10} MeV cm⁻². Using a highly-time-resolved pump (femtosecond electron bunch) – optical probe (femtosecond photon pulse) orthogonal configuration, the real-time investigation of primary radiation processes can be performed in the radial direction of femtosecond electron bunches (Figure 5). Using a highly-time-resolved pump (femtosecond electron bunch) – optical probe (femtosecond photon pulse) orthogonal configuration and a detection system based on cooled 16-bit CCD camera (Andor Technology), the real-time investigation of primary radiation processes is performed in the radial direction of femtosecond electron bunches. In the vacuum chamber, the femtosecond high-energy electron pulse passes through a 10 mm/10 mm suprasil cell containing liquid samples. The synchronization of the two laser pulses within a 30 fs precision was performed by shadowgraphy of the plasma created by the pump laser. This arrangement permitted to give a very accurate synchronization and was free of jitter. Absorption spectroscopic measurements were performed at L = 5-6 mm downstream from the helium gas jet and 1mm depth path in the suprasil cell containing liquid water at 294K. With these conditions, the electron beam was visualized with a radiochromic film and the electron beam divergence measured in function of the energy.

3.3 Towards a nanoscale approach of early radiation events in nascent ionization tracks

Laser-plasma accelerator based high energy radiation femtochemistry gives new insights into the time dependence of early events occurring in native ionisation clusters (Figure 6). Indeed, as expressed in Equation 3, the short-time dynamics of the ubiquitous e^-_{aq} radical (1s ground state) is mainly governed by its transient recombination with surrounding water products (hydronium ion H_3O^+ and radical hydroxyl OH).

$$e^-_{aq}(t) = \int_{-\infty}^t \frac{dn_{e^-_{aq}}(t')}{dt} \left\{ 1 - \gamma + \gamma \operatorname{erf} \left(\frac{T_J}{t-t'} \right)^{1/2} \right\} dt' \quad (3)$$

$$\text{with } \operatorname{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-u^2} du$$

In this analytical expression, two adjustable parameters are considered: 1D geminate recombination time which is equivalent to a jump time (T_j) and a recombination probability γ . This random 1D walking electrons satisfies both the experimental absorption signal dynamics in the 0-75 ps range and the time-dependence $1/(t)^{1/2}$ of dispersive recombination processes inside non-homogeneous ionization clusters (multi-scales phenomena). The least-squares fit of 820 nm signal dynamics emphasize that dispersive geminate recombinations between e^-_{aq} and neoformed OH radical or H_3O^+ ion take place immediately after a femtosecond energy deposition in the water bath. Such non synchronized events are mainly observed between 0 and 20 ps (upper part of Figure 6).

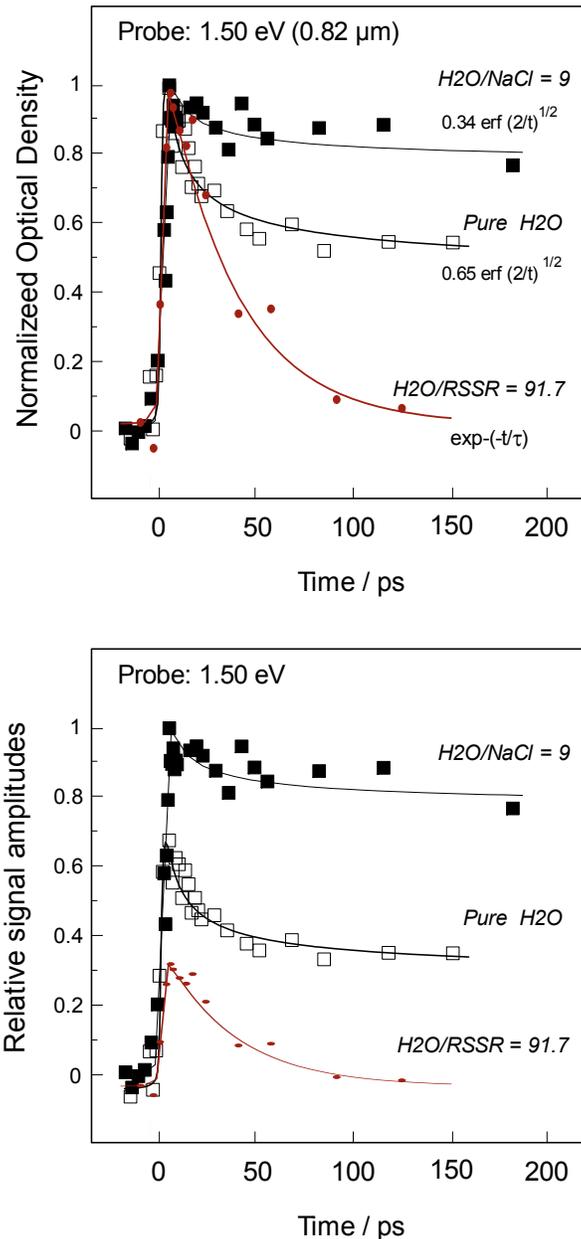


Figure 6. Dynamics of early radiation events occurring inside ionization tracks triggered by femtosecond bunches of relativistic electrons ($E: 2.5 - 15$ MeV). The radiation processes are investigated at 820 nm (1.5 eV). The absorption signal amplitude and time dependence assigned to the 1s ground state hydrated electrons is significantly influenced by the presence of a biomolecule (cystamine) in the aqueous phase. Normalized signal curves are reported in the upper part of the figure and relative amplitudes of near-infrared signals are shown in the lower part.

Femtolytic experiments emphasize that in native ionisation tracks, ultrafast geminate recombinations are dependent on the transient distribution of electron-hole pairs and the spatial configurations of electron-prototropic entities. A limit case corresponds to very short-range electron-proton couplings for which hydronium ion (H_3O^+) undergoes one jump (1D motion by a finite process). In this way, considering a diffusion coefficient of H_3O^+ expressed as $D \sim \lambda d^2/6$ and an experimental jump frequency $\lambda = 1/T_j = 0.5 \times 10^{12} \text{ s}^{-1}$, the initial proton jump distance would be around 2.8 Å. This early electron recombination process dynamic is significantly influenced by the nature of the surrounding environment. Indeed, laser-plasma accelerator based HERF may allow the direct observation of primary radiation events in function of local environments induced by the presence of ionic entities or bio-molecules in aqueous bath (Figure 6).

Indeed, when the high density of hydrogen bonding is significantly broken by a ionic strength of sodium chloride ($\text{H}_2\text{O}/\text{NaCl} = 9$), the contribution of early recombination processes is significantly reduced by specific field effects of anions and cations on the sub-structure of native ionization tracks. HERF studies strongly suggest the preeminence of quantum effects during the sub-picosecond recombination of non-independent radical pairs within a fluctuating hydrogen bonds network. The dynamics of early events can be also modulated significantly by the presence of molecules of biological interest such as cystamine. In the chemical core induced by femtosecond electron bunches, this disulfide compound favors a bimolecular reaction: an electron-biomolecule attachment from 1s-ground state hydrated electrons. For a molecular ratio of 91.7 (0.4 M) in an aqueous environment, this bimolecular reaction exhibits a pseudo-first order dynamics, ($\exp(-t/\tau)$ with $\tau = 45 \text{ ps}$). As previously observed with femtosecond low energy photon pulses, this diffusion-controlled process leads to the formation of sulfur-centered radical anions $\text{RS} \cdot \cdot \text{SR}^-$ characterized by a two-centre-three electron bond. Consequently, small bio-molecules can be used as a sensor of specific environments triggered by ultrashort relativistic electron bunches and characterized by confined ionisation spaces.

Advanced laser accelerator based femtolytic experiments would permit to extend our understanding of very short-time radical events in the prethermal regime of nascent ionization tracks, typically in the 10^{-14} - 10^{-12} s window, and at the nanoscopic level. Following a dose delivery of 15 Gy in less than 500 fs, an ultrafast collapse takes place between low energy electron (precursors of fully hydrated electrons) and cystamine molecules localized in the chemical core i.e. at the interface between the physical core and penumbra zone. The decrease of the early signal amplitude assigned to the population of hydrated electron in native ionization tracks (lower part of Figure 6) argues for a prethermal p state one-electron attachment on a disulfide biomolecule (cystamine) in less than 0.85×10^{-12} s. Consequently, in sub-nanometric ionization tracks, the anionic radical formation ($\text{RS} \cdot \cdot \text{SR}^-$)_{aq} would occur faster than the radiationless relaxation ($2p \rightarrow 1s$ transition) of excited prehydrated electrons (Equation 1 and Figure 2). A major stride of a short-range biomolecular alteration triggered by an ultrafast electron attachment in the prethermal regime of ionization clusters will be the quantitative characterization of two parameters at very short time ($t \ll 1 \text{ ps}$): the effective reaction radius and interaction cross section of the fleeting quantum probe (2p state electron) with a biomolecule. Real-time infrared spectroscopic measurements in the spectral range 1.1-1.6 μm , based on laser plasma accelerator technologies and HERF concepts, are in progress.

4. FUTURE DEVELOPMENTS AND CONCLUDING REMARKS

The real-time investigation of elementary bioradical processes in condensed phase, with a sub-nanometric spatial accuracy, can be carried out in synergy with the most recent developments of ultra-short laser sources and low or high energy radiation femtochemistry concepts. One of the most promising developments of future experimental HERF researches will concern ⁱ⁾ the real-time investigation of early radical events in the prethermal regime of native ionization tracks, considering short-lived quantum probes produced at the frontier of physical core and penumbra zone (2p state electron), ⁱⁱ⁾ the quantification of ultrafast sub-nanometric bio-molecular damages (bond weakening and bond breaking) in the radial direction of relativistic particle beams, using the infrared spectroscopic properties of the very-short lived p-like configuration of excited electron. Its quantum character provides a ubiquitous sub-nanometric probe to explore short-time interactions with bio-molecules and would offer the opportunity to characterize pertinent bio-effect parameters in native ionization tracks, as a function of the radiation quality (energy, time, fluence density and dose delivery rate profiles).

A major aspect of biodosimetry performed at the molecular level concerns the ultrafast damage mediated by the efficient attachment of a very short-lived quantum probe (p-like excited prehydrated electron) on covalent bond and the deleterious formation of 2c-3e bond. The ultrafast probing of low-energy excited electrons eigenstates (quantum states) within native ionization clusters would permit to carefully investigate sub-nanometric molecular alteration at early time.

This new concept of real-time biomolecular nanodosimetry will be developed, considering ⁱ⁾ experimental parameters defined from sub-picosecond infrared measurements of p-like excited prehydrated electron (probability of nonadiabatic p→s transition vs probability of ultrafast electron attachment on the sigma bond of a small biomolecule), ⁱⁱ⁾ the specific aspect of target volumes of mass per area. In this way, for biomolecular targets whose the size is less than 20 Å at a density of 1.0 g cm⁻³, the corresponding target volume of mass per area in size will be less than 1 x 10⁻⁶ g cm⁻². A major stride of a short-range biomolecular alteration triggered by an ultrafast electron attachment and the subsequent cleavage of a covalent-bond at early time will be the quantitative characterization of two parameters: an effective reaction radius (r_{eff}) and an interaction cross section of 2p-like excited electron (ICS). In order to define these two parameters for aqueous non-homogeneous environments of native tracks, high-time resolved infrared spectroscopic experiments will be carried out in presence of small biomolecular architectures.

The emergence of ultrafast high-energy radiation femtochemistry of biological interest would foreshadow the time-dependent and nanometric spatially defined effects in biomolecular architectures, such as aqueous groove of DNA, nucleosomes, protein pockets, and sub-cellular compartments. Establishing an innovating approach of real-time nanodosimetry on the time scale of molecular motions, i.e. angstrom or sub-angstrom displacements, spatio-temporal radiation biology represents a prerequisite for the control of irradiations of living cells at very high dose rates ^{14, 34-36}. From a theoretical and computational point of view, the stochastic modelling of ultra fast bioradical processes and biomolecular alterations in native radiation tracks would be upgraded, taking into account the quantum character of nonequilibrium electronic configurations during the prethermal regime of very low energy electrons.

In the framework of innovative applications of pulsed radiation sources in medical physics and cancer radiotherapy ^{14,15, 36,37}, the spatio-temporal radiation processes must be underpinned by advanced biophysical concepts on the impact of native tracks in biologically relevant environments. In synergy with semi-quantum molecular dynamic simulations and time-dependent ab-initio calculations on primary radiation events ³⁸, these transdisciplinary advances would provide guidance for the conceptualisation of time-dependent molecular RBE (Relative Biological Efficiency and real-time biodosimetry at nanometer scale. The nanoscale insight into native ionisation tracks would have biomedical applications such as the radio-sensitisation of tumour cells with vectorized nanoparticles or pulsed cancer chemoradiation therapies. Compared to classical dose rate delivery generally used for conventional radiotherapies, ~ 1 Gy min⁻¹, the very high dose rate delivered with laser plasma accelerators, ~10¹³ Gy s⁻¹, may challenge our understanding of radiation effects on molecular targets, at the time scale of molecular motions, i.e. angstrom or sub-angstrom displacements. Regarding integrated living systems such as normal and cancerous cells, the effects of high dose rate profiles delivered by laser-plasma accelerators require the developments of bio-dosimetry concept at the nanometric level.

ACKNOWLEDGEMENTS

The authors acknowledge the financial supports of the European Council (PARIS ERC project contract number 226424) and AVIESAN-INCa, France (NANOBIODOS project) . They thank also several scientific members of the *Radiation Biology* MELUSYN Network (France) for very fruitful discussions.

REFERENCES

- [1] Von Sonntag, C., [Free-radical-Induced DNA Damage and its Repair] Springer, Heidelberg (2006).
- [2] Shukla, M. and Leszczynski, J. [Radiation induced molecular phenomena in nucleic acids: a comprehensive theoretical and experimental analysis] Springer (2008).
- [3] Gauduel, Y.A., Femtochemistry: lasers to investigate ultrafast reactions, Lasers in Chemistry, M. Lackner (Ed), VCH, Vol. 2861-898 (2008).
- [4] Gauduel, Y.A., ‘‘Spatio-temporal radiation biology: an emerging transdisciplinary domain,’’ Mut. Res. Rev. 704, 1, (2010).
- [5] Diels, J.C. and Rudolph, W., [Ultrashort laser pulse phenomena] Academic Press, New York (1996).
- [6] Tajima, J. and Davson, J.M., ‘‘Laser electron accelerator,’’ Phys. Rev. Lett. 43, 267-270 (1979).
- [7] Malka, V., Fritzler, S., Lefebvre, E., Aleanard, M.M., Burgy, F., Chambaret, J.P., Chemin, J.F., Krushelnick, K., Malka, G., Mangles, S.P.D., Najmudin, Z., Pittman, M., Rousseau, J.P., Scheurer, J.N., Walton, B. and Dangor, A.E., ‘‘Electron acceleration by a wake field forced by an intense ultrashort laser pulse,’’ Science 298 1596-1600 (2002).

- [8] Malka, V., Faure, J., Gauduel, Y., Lefebvre, E., Rouse, A. and Ta Phuoc, K., "Principles and applications of compact laser-plasma accelerators," *Nat. Phys.* 4, 447-453 (2008).
- [9] Erasey, E., Schroeder, C.B. and Leemans, W.P., "Physics of laser-driven plasma-based electron accelerators," *Rev. Mod. Phys.* 81, 1229-1285 (2009).
- [10] Malka, V., *Laser Plasma Accelerators: Towards High Quality Electron Beam, Laser pulse phenomena and applications*, F. J. Duarte (Ed.), Intechweb.org. (2010).
- [11] Tillman, C., Grafström, G., Jonsson, A.C., Mercer, I., Mattsson, S., Stand, S.E. and Svanberg, S., "Survival of mammalian cells exposed to ultrahigh dose rates from a laser-produced plasma X-ray source," *Radiobiol.* 213, 860-865 (1999).
- [12] DesRosiers, C., Moskin, V., and Minsong, C., "Laser-plasma generated very high energy electrons in radiation therapy of the prostate," *Proc. of SPIE* 6881, 688109-1 (2008).
- [13] Yogo, A., Sato, K., Nishikino, M., Mori, M., Teshima, T., Numasaki, K., Murakani, M., "Application of laser-accelerated protons to the demonstration of DNA double-strand breaks in human cancer cells," *Appl. Phys. Lett.* 94, 181502 (2009).
- [14] Rigaud O., Fortunel N.O., Vaigot P., Cadio E., Martin M.T., Lundh O., Faure J., Rechatin C., Malka V. and Gauduel Y.A., "Exploring ultrashort high-energy electron-induced damage in human carcinoma cells," *Cell Death Disease* 1, e73 (2010).
- [15] Malka, V., Faure, J., Gauduel, Y.A., "Ultra-short electron beams based spatio-temporal radiation biology and radiotherapy," *Mut. Res. Rev.* 704, 142-151 (2010).
- [16] Brozek-Pluska, B., Gligler, D., Hallou, A., Malka, V. and Gauduel, Y., "Direct observation of elementary radical events: low and high-energy radiation femtochemistry in solution," *Rad. Phys. Chem.* 72, 149-157 (2005).
- [17] Gauduel, Y.A., Faure, J. and Malka, V., "Ultrashort relativistic electron bunches and spatio-temporal radiation biology," *Proc. SPIE*, 7080, 708002-1 (2008).
- [18] Migus, A., Gauduel, Y., Martin, J.L. and Antonetti, A., "Excess electrons in liquid water: First evidence of a prehydrated state with femtosecond lifetime," *Phys. Rev. Lett.* 58, 1159-1562 (1987).
- [19] Gauduel, Y., Berrod, S., Migus, A., Yamada, N. and Antonetti, A., "Femtosecond charge separation in organized assemblies: free-radical reactions with pyridine nucleotides in micelles", *Biochemistry*, 27, 2509-2518 (1988).
- [20] Gauduel, Y., Pommeret, S., Migus, A., Yamada, N. and Antonetti, A., "Femtosecond investigation of single-electron transfer and radical reactions in aqueous media and bioaggregate-mimetic systems", *J. Opt. Soc. Am.*, B, 7, 1528-1539 (1990).
- [21] Gauduel, Y., Sander, M. and Gelabert, H., "Ultrafast reactivity of IR-excited electron in aqueous ionic solutions", *J. Phys. Chem. A*, 102, 7795-7803 (1998).
- [22] Gauduel, Y., Gelabert, H. and Guilloud, F., "Real-time probing of a three-electron bonded radical: Ultrafast one-electron reduction of a disulfide biomolecule," *J. Am. Chem. Soc.* 122, 5082-5091 (2000).
- [23] Gauduel, Y., Hallou, A. and Charles, B., "Short-time water caging and elementary prehydration redox reactions in ionic environments", *J. Phys. Chem. A*, 107, 2011-2024 (2003).
- [24] Alizadeh, E. and Sanche, L. "Precursors of solvated electrons in radiological physics and chemistry", *Chem. Rev.*, 112, 5578-5602 (2012).
- [25] Nguyen, J., Ma, Y., Luo, T., Bristow, R.G., Jaffray, D.A. and Lu, Q.B., "Direct ultrafast-electron-transfer reaction unravels high effectiveness of reductive DNA damage," *Proc. Nat. Acad. Sci.*, 108, AA778-11783 (2011).
- [26] Turi, L. and Rossky, P.J., "Theoretical studies of spectroscopy and dynamics of hydrated electrons," *Chem. Rev.* 112, 5641-5674 (2012).
- [27] Blandamer, M.J. and Fox, M.F., "Theory and applications of charge-to-transfer-to-solvent spectra," *Chem. Rev.*, 70, 59-93 (1970).
- [28] Sheu, W.S. and Rossky, P.J., "Charge-transfer-to-solvent spectra of an aqueous halide revisited via computer simulation," *J. Am. Chem. Soc.*, 115, 7729-7735 (1993).
- [29] Alfassi, Z.B., *S-centered radicals*, Wiley & Sons, Chichester (1999).
- [30] Faure, J., Rechatin, C., Norlin, A. Lifschitz, A., Glinec Y. and Malka, V., "Controlled injection and acceleration of electrons in plasma wakefields by colliding laser pulses," *Nature* 444, 737-739 (2006).

- [31] Rechatin, C., Faure, J., Ben-Ismaïl, A., Lim, J., Fitour, R., Specka, A., Videau, H., Tafzi, A., Burgy, F. and Malka, V., "Controlling the phase-space volume of injected electrons in a laser-plasma Accelerator," *Phys. Rev. Lett.* 102, 164801 (2009).
- [32] Oulianov, D.A., Crowell, R.A., Gosztola, D.J., Shkrob, I.A., Korovyanko, O.J. and Rey-de-Castro, R.C., "Ultrafast pulse radiolysis using a terawatt laser wakefield accelerator," *J. Appl. Phys.* 101, 053102-1-9 (2007).
- [33] Gauduel, Y.A., Glinec, Y., Rousseau, J.P., Burgy, F. and Malka, V., "High energy radiation femtochemistry of water molecules: early electron-radical pairs processes," *Eur. Phys. J. D* 60, 121-135 (2010).
- [34] Beyreuther, E., Enghardt, W., Kaluza, M., Karsch, L., Laschinsky, L., Lessmann, E., Nicolai, M., Pawelke, J., Richter, C., Sauerbrey, R., Schlenvoigt, H.P. and Baumann, M., "Establishment of technical prerequisites for cell irradiation experiments with laser-accelerated electrons," *Med. Phys.* 37, 1393-1400 (2010).
- [35] Kraft, S.D., Richter, C., Zeil, K., Baumann, M., Beyreuther, E., Bock, S., Bussmann, M., Cowan, T.E., Dammene, Y., Enghardt, W., Helbig, U., Karsch, L., Kluge, T., Laschinsky, L., Lessmann, E., Metzkes, J., Naumburger, D., Sauerbrey, R., Schürer, M., Sobiella, M., Woithe, J., Schramm, U. and Pawelke, J., "Dose-dependent biological damage of tumour cells by laser-accelerated proton beams," *New J. Phys.* 12, 085003 (2010).
- [36] Gauduel, Y.A., Lundh, O., Martin, M.T., Malka, V., "Laser-plasma accelerators based high energy radiation femtochemistry and spatio-temporal radiation biomedicine," *Proc. SPIE*, 8433, 843316 (2012).
- [37] Malka, V., *Laser plasma accelerators, Laser-Plasma Interactions and Applications*, P. McKenna, D. Neely, R. Bingham and D. Jaroszynski (Eds), Springer International Publishing, Switzerland, 231-301 (2013).
- [38] Lopez-Tarifa, P., Gageot, M.P., Vuilleumier, R., Tavernelli, I., Alcamí, M., Martin, F., du Penhoat, M.A.H. and Politis, M.F., "Ultrafast damage following radiation-induced oxidation of uracil in aqueous solution," *Angew Chem. Int. Ed.*, 52, 3160-3163 (2013).