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Fast and accurate: probing the homogeneous
biochemical stage of radiation damage with the new
TRAX-CHEMxt code

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Fast and accurate: probing the homogeneous biochemical stage of radiation damage with the new TRAX-CHEMxt code

In the last years, new interest has grown in exploring the biological effects of free radicals produced by ionising radiation (IR), since this “indirect action” plays a prominent role in damage induction. From the modelling perspective, Monte Carlo (MC) codes can be helpful in this regard. However, to extend the time range of predictability, a computationally lighter approach is needed in combination with the accurate initial description of MC simulations, limited typically to water targets and to μs temporal scales. To this end, TRAX-CHEMxt has been developed.

In what follows, this novel code is presented. The calculation is based on the coordinates of the chemical species produced around one particle track, and solves the set of reaction-diffusion equations numerically with a computationally light approach based on concentration distributions. Its computational structure and abilities in investigating both water and biological environments, following the radicals’ and molecules’ dynamics to longer time regimes (μs – s), are showcased. The robustness of the algorithm has been tested, by comparing its predictions with MC counterparts around the μs . Deviations less than 6 % have been registered, together with an improvement in the computational speed by more than three orders of magnitude. TRAX-CHEMxt results have also been contrasted with those from other codes and experimental data, still showing good agreements. Dependencies of chemical yields on the primary particles’ radiation quality, energy, linear energy transfer (LET) and target oxygenation have been studied as well.

Due to its reliability and accuracy, TRAX-CHEMxt can be applied to investigate the impact of radiation-induced radicals on biological targets at longer times, under different radiation and environmental conditions.

Schnell und genau: Untersuchung des homogenen biochemischen Stadiums der Strahlenschädigung mit dem neuen TRAX-CHEMxt-Code

In den letzten Jahren ist das Interesse an der Erforschung der biologischen Auswirkungen von freien Radikalen, die durch ionisierende Strahlung (IR) erzeugt werden, gewachsen, da diese "indirekte Wirkung" eine wichtige Rolle bei der Schadensinduktion spielen kann. Aus Sicht der Modellierung können Monte-Carlo Codes (MC) in dieser Hinsicht hilfreich sein. Um den zeitlichen Bereich der Vorhersagbarkeit zu erweitern, ist jedoch ein komputatorisch schlankerer Ansatz in Kombination mit der genauen Beschreibung von MC-Simulationen zu Beginn nötig, die auf Wasser-Zielvolumina und zeitliche Skalen im Bereich von μs beschränkt sind. Zu diesem Zweck wurde TRAX-CHEMxt entwickelt.

Im Folgenden wird dieser neuartige Code vorgestellt. Die Berechnungen basieren auf den Koordinaten der chemischen Spezies, die um eine Teilchenspur produziert werden, und lösen das System von Reaktions-Diffusions-Gleichungen numerisch mit einer rechnerisch effizienten Methode, die Konzentrationsverteilungen nutzt. Seine Berechnungsstruktur und Fähigkeiten bei der Untersuchung sowohl von Wasser als auch von biologischen Umgebungen, wobei die Dynamik der Radikale und Moleküle über längere Zeiträume (μs – s) untersucht werden, werden vorgestellt. Die Robustheit des Algorithmus wurde getestet, indem seine Vorhersagen mit MC-Pendants im Bereich um μs verglichen wurden. Es wurden Abweichungen von weniger als 6 % festgestellt, zusammen mit einer Verbesserung der Berechnungsgeschwindigkeit um mehr als drei Größenordnungen. Die Ergebnisse von TRAX-CHEMxt wurden auch mit denen anderer Codes und mit experimentellen Daten verglichen, wobei sich immer noch gute Übereinstimmungen zeigten. Die Abhängigkeit der chemischen Ausbeuten von der Strahlungsqualität der Primärteilchen, der Energie, dem linearen Energietransfer (LET) und der Sauerstoffsättigung des bestrahlten Mediums wurden ebenfalls untersucht.

Aufgrund seiner Zuverlässigkeit und Genauigkeit kann TRAX-CHEMxt eingesetzt werden, um die Auswirkungen von strahleninduzierten Radikalen auf biologische Medien über längere Zeiträume und unter verschiedenen Strahlungs- und Umweltbedingungen zu untersuchen.

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List of Acronyms

e-	Electron
H	Proton
¹²C	Carbon Ion
⁴He	Helium Ion
IR	Ionising Radiation
LET	Linear Energy Transfer
UHDR	Ultra-High Dose Rate
MC	Monte Carlo
SBS	Step-By-Step
RBE	Relative Biological Effectiveness
SSB	Single Strand Break
DSB	Double Strand Break
RN	Random Number
IRT	Independent Reaction Time
ROD	Radiolytic Oxygen Depletion
RBD	Radiolytic Biomolecule Depletion
RAD	Radiolytic Antioxidant Depletion
CG	Computational Gain

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1. Introduction

Radiotherapy is a medical procedure applied to control and/or cure malignant tumours localised in different parts of the human body. Together with surgery and chemotherapy, it represents one of the major treatment modalities exploited in hospitals [1]. As far as external beam irradiations are concerned, it is possible to distinguish between conventional therapy, which refers to photons (both X-rays and γ -rays) and electrons (e-), and ion beam therapy, that utilises in turn positively charged particles, typically protons (H) and carbon ions (^{12}C) [2], yet since recently helium ions (^4He) as well [3].

The physical interactions induced by different ionising radiation (IR) types elicit localised damage in biological targets, which may ultimately lead to cell death. Two general pathways to induce harm can be distinguished, namely the “direct action” against target molecules, and the “indirect action” featured by the intermediate production of reactive chemical species. These originate from the radiolysis of water, and lead to chemical reactions with organic molecules in the medium, such as proteins, amino acids, nucleotides and lipids. Concerning the indirect pathway, similarly to the physical dose deposition pattern, the quantity and localisation of clustered radicals and molecules produced represent a major difference between the effects of low and high linear energy transfer (LET) radiations [4].

Since the reactive species can play a prominent role in damage induction, particularly with regards to late effects, it is important to have a deeper understanding of their dynamics and features. A related aspect is the relevance of comparing various radiation qualities. By studying how the chemical yields change with LET, a better understanding of the major channels responsible for the indirect damage related to one specific beam quality can be obtained. This will contribute to predicting under which conditions normal tissues are subject to severe toxicities, and to improve treatments via new modalities such as ultra-high dose rate (UHDR) irradiations. In this context, it is noteworthy to mention the FLASH therapy. The procedure consists in the delivery of considerable radiation doses, mostly 10 Gy or higher in one fraction (with often pulse values in the order of 1 Gy), and in a very short time, with a duration of 100 ms or less, resulting in a rate ≥ 40 Gy/s. These UHDR beams, as observed for both low [5, 6] and high [7, 8] LET radiations, induce an intriguing

biological phenomenon, for its normal tissue-sparing feature and the simultaneously unchanged efficacy in tumour control. Nevertheless, the underlying mechanisms responsible for this effect remain poorly understood [9, 10]. Since several hypothesised mechanisms imply a relevant role of the radiation-induced radicals [9], to explore in detail the onset of the aforementioned effect, as well as to assess its possible variation as a function of different radiation qualities [11], a detailed description of the reaction kinetics featuring the produced species seems necessary.

Simulations can be very useful tools in this regard, considering they can provide temporal information regarding several chemical species that are arduous to determine experimentally [12]. Nowadays, various codes are available to study radical and molecular yields, mostly focusing on oxygenated water environments, studying especially the initial heterogeneous dynamics of the substances obtained. In fact, one of the greatest computational burdens is the time required by the algorithm to produce the results, which strictly depends on both the time scales considered and the initial conditions – radiation, environment and volume – simulated.

The scope of this work is to develop TRAX-CHEMxt [13], new extension of TRAX-CHEM [14], chemical integration of the Monte Carlo (MC) track structure code TRAX [15, 16]. After the initial physical interactions elicited by particle tracks, all the events featuring the chemical species produced in the target volume, namely diffusion and reactions with other substances in the environment, are followed up by TRAX-CHEM. This was recently updated to address medium oxygenation [17], which made it possible to gain some mechanistic insights into the role of oxygen in the FLASH effect [18]. Nonetheless, a more realistic analysis was limited by the restricted time scale accessible and the simple simulation environment (oxygenated water). The TRAX-CHEMxt code developed in this thesis improves on these limitations. It employs a novel approach that utilises, for the first time, a simplified continuous representation of the different molecules' and radicals' positions as concentration distributions. These are assigned one per each chemical species, and are derived by converting all species spatial positions produced around the particle track. This method differs from the previous approach of considering discrete point clouds holding the individual chemical reactants' localisations. The tedious step-by-step (SBS) MC procedure testing for possible reactions between all the combinations of individual reactants, together with their displacement in space, can now be replaced by a much faster set of differential equations describing the diffusion (of each species) and reactions (of all combinations of species) based on the respective one-dimensional, radial concentration distributions. This efficient mathematical shortcut preserves the relevant local information provided by the MC code, and demonstrates a stable transition between the two models providing excellent data consistency in the overlapping time frame. Furthermore, it predicts the evolution of the various species concentrations at longer time scales, due to a simple resolution of the aforementioned diffusion

and reaction equations, achieved by computing these events in an alternating manner up to an user-specified final point. TRAX-CHEMxt offers enhanced computational efficiency while preserving TRAX-CHEM accuracy, with the additional capability of treating a more complex environment, including biomolecules.

In Chapter 2 a general description of the major theoretical aspects essential to understand the analysis is given, whereas in Chapter 3 the technical details of the newly developed code are described. Initial tests and validations of the novel algorithm are reported in Chapter 4, while the more advanced results are presented in Chapter 5. Two scenarios are considered here, depending on the environment simulated, namely oxygenated water 5.1 or a biological 5.2 target. For both, analyses of the effects on the final chemical yields as a function of particle type and (initial) energy, LET and target oxygenation have been performed. A general overview and discussion of the major results can be found in Chapter 6, and some final reflections together with possible future applications of TRAX-CHEMxt are given in Chapter 7.

2. Theoretical Background

In this Chapter the main theoretical aspects necessary to comprehend the study conducted are presented. An introductory description of the ionising radiations exploited in cancer therapy is given, together with a distinction between the two major pathways of damage induction. Subsequently, attention is given to the stages of the radiobiological events, and to how these can be simulated computationally. Finally, the motivation to extend the calculations to longer time domains is briefly touched upon. The topics addressed below are only outlined in a propaedeutic manner, and do not aim at giving any exhaustive description of the underlying subjects, especially with respect to the therapy with charged particles. For an in-depth description of the physical and biological characteristics, benefits and challenges of cancer treatments with ion beams, refer to [19, 20, 21].

2.1 Cancer therapy with ionising radiations

In the context of cancer care, radiation therapy denotes the medical use of ionising radiations with the final purpose of curing malignant tissues. Nowadays it has achieved a fundamental role as primary treatment modality for many types of cancers. More than 50 % of all patients with localised tumours are treated with radiations [22], for curative or palliative (relief) purposes. The aim is indeed to preserve the surrounding normal tissues, while at the same time achieving the best possible local tumour control (i.e., induction of cancer cells death without regeneration). Noteworthy is the fact that radiotherapy can be used also in combination with other forms of treatment, like surgery and systemic therapies (chemotherapy, hormone therapy, immunotherapy), to further enhance the effect of the overall procedure. The precise intervention modality will depend on several aspects, such as tumour histology, localisation, staging, and other patient-specific parameters based on clinical experience. Nevertheless, the specific fundamental mechanisms leading to the biological radiation effects in tumour and healthy tissues, such as radiosensitisation and radioprotection, are not always fully understood. It is also important to consider that the resulting outcomes are particle-specific. Consequently, in order to enhance their comprehension, the in-

vestigation of these aspects, both from the experimental and theoretical perspectives, is crucial, since cancer cases are rising also among younger individuals [23].

Whereas photons and electrons are referred to as conventional treatment modalities, positively charged particle beams currently used in clinics, spanning from protons to helium and carbon ions, constitute the so-called hadron therapy. Of paramount importance are their differences in the depth-dose profile [19, 20], dominated by the inelastic electromagnetic interactions with target molecular electrons, that induce a slowdown of the primary projectiles mainly through ionisations and molecular excitations. Towards the end of the trajectory (at low energies), nuclear interactions become relevant as well. Independently of the type of radiation, the main consequence of these events is the possibility of irreversibly damaging the irradiated cells, leading to their death as they are unable to repair and duplicate. A fundamental quantity in this regard is the concept of dose, representing the energy deposited in a mass element and expressed in units of Gray, with $1 \text{ Gy} = 1 \text{ J/kg}$. The depth-dose profiles are strictly dependent on the radiation (and initial energy) employed. For photons and electrons, produced usually in linear accelerators with energies up to few MeV, the highest amount of dose is released at the very shallow surface, making these radiations especially useful for superficial tumours. As a consequence, in order to treat deep-seated malignancies, the deposited dose in healthy tissues will be, in principle, higher than the one in the actual target. For this reason, normally, multiple beams or arcs at different angles are delivered, thus reducing the effects on normal tissues. From the physical point of view, whereas photons follow the Beer-Lambert absorption law, the stopping power of charged particles can be described by the Bethe-Bloch formula, which features heavier ions as well. Differently, protons, helium and carbon ions are accelerated by cyclotrons or synchrotrons, up to few hundreds MeV u^{-1} . This implies that these particles have to be sped up to very high velocities, and that their rate of energy loss per path length will result in dose distribution curves considerably different than the photons/electrons ones. For instance, in order to reach tumours located as far as 30 cm in depth, energies up to 250 MeV u^{-1} for protons and helium ions, and 450 MeV u^{-1} for carbon ions, are required [19]. From the energy deposition perspective, at the entrance surface they exhibit a low dose level, a “plateau”, due to the initial high speed of the primary cations. Subsequently, because of the slowdown by target electrons, the local interactions become slightly more likely, with a fall-off of the projectile’s energy, and an increase of the deposited dose up to an energy and particle dependent depth, the so-called “Bragg peak”, is registered. After that, at the distal edge, the projectile comes to rest, resulting in a final drop in the curve. For ions heavier than protons, a residual dose contribution persists in this area, due to the production of secondary elements from nuclear reactions along the stopping path of the initial beam, creating a “fragmentation tail”. In most cases, anyway, this tail is located still within the target region [21]. This peculiar profile represents one of

the key advantages these particles have with respect to conventional therapy, as first pointed out by Robert Rathbun Wilson in 1946 [24]. It elicits improved sparing of normal tissues at the entrance, and a more precise and high dose deposition in the target area. In order to cover the entire tumour volume, the Bragg peak is spread in depth, either passively by introducing different absorbers along the beam path, or actively by energy modulation. On the other hand, the lateral beam movement is achieved by magnetic scanning, or (if passive) by means of scattering foils. Similarly to the photon case, to reduce the dose to healthy tissues while at the same time maintaining the planned one to the target, the treatment can be delivered by using several beams at different angles. Additionally, to further enhance the probability of normal tissue recovery, since tumours are less efficient in repairing, the total dose is delivered in fractions, fx. Administered over the course of approximately one and a half months, during weekdays, 2 Gy/fx are typically employed in conventional radiotherapy plans, with a trend towards higher doses per fraction when particles are used instead.

2.2 Parameters influencing radiation damage

During their interaction process with traversed matter, ionising radiations transfer energies between few tens and few hundreds of eV to the target electrons, sometimes involving other secondary particles, such as photons, as intermediates. Since these energies are larger than the binding ones of outer shell electrons, i.e., few eV, secondary electrons are released, with the ability to induce further ionisation and excitation events. In addition to dose, giving the total amount of energy deposited per mass, an important concept used to describe the localisation characteristics of different radiation qualities is LET. It quantifies the energy loss per unit track length, and is expressed in keV/ μm . Table 2.1 reports examples of LET values associated with specific particle types and energies, since different ionisation densities can be induced around the primary track depending on whether the energy is released in a localised or isotropic/uniform way. It should be noted that the values presented in the Table are related to sufficiently small target thicknesses, to ensure that the primary energy of the projectile remains unaltered. Of course, if the entire dose deposition curve is taken into account, then the LET gradually changes along it.

In order to accurately estimate the biological effectiveness towards a specified endpoint (e.g., cell killing) of a particular particle quality-cell combination, it is necessary to introduce the concept of relative biological effectiveness (RBE). This parameter is defined as the ratio between the dose of a reference radiation, normally X-rays, and the dose of any other radiation quality being tested, resulting in the same biological effect. The RBE of a specific particle quality is dependent upon a number of factors, including the dose itself, the primary particle type, the energy, the biological end-

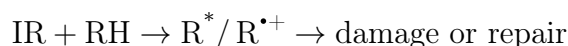
point, and the tissue under consideration. The RBE concept permits the transfer of knowledge accumulated in the field of conventional radiotherapy to be applied to ion beams, thereby quantifying the potential overall enhancement action that could be achieved through the use of particles. In certain scenarios, photon doses up to three times larger than those associated with particles are required to achieve the same effect in the target area.

Table 2.1: LET values associated to different radiation types and energies.

Particle type	Energy	LET [keV/ μm]
Electrons	500 keV	0.2
Protons	65 MeV	1
Carbon ions	150 MeV u^{-1}	20
Carbon ions	10 MeV u^{-1}	160
Argon ions	10 MeV u^{-1}	1300

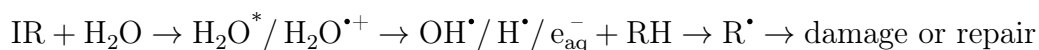
As anticipated in the introductory Chapter 1, there are two major pathways for radiation to elicit damage, which always occur together but in different proportions depending on the characteristics of the particle in question: high LET radiations induce a considerable amount of harm via the “direct pathway”, whereas low LET ones damage biomolecules mostly by means of intermediate water radicals (“indirect damage”). Direct action occurs when the incident radiation interacts directly with biomolecules through physical collision processes. The most sensible target is DNA, since it contains the genetic information that can be altered, eventually leading to cell death if the resulting instabilities can not be repaired by the system. Depending on the physical ionisation pattern, single or double strand breaks (SSBs/DSBs) can be induced in varying proportions. The principal danger of SSBs is the alteration of the DNA sequence (due to base exchanges) and the resulting malfunctioning of the cell is generally due to faulty proteins, deriving from problems during their synthesis. Several SSBs can combine to DSBs if they come close enough to each other in the sequence (approximately within 25 base pairs [25]). On the other hand, DSBs break the DNA molecule into two fragments, and the repair will then depend on the efficacy of the subsequently intervening repair machinery in joining the two ends together, and particularly on whether a second template chromosome is available (depending on the cell cycle phase). Sometimes the repair works completely well, without inducing any future changes within the biological environment (homologous recombination). On the other hand, non-homologous end-joining is error-prone and can fails to connect the correct ends, resulting in a new and erroneous DNA sequence, without a meaningful gene expression, or in a damaged region with some bases completely different from the original ones. In this case there might be an induction of faulty proteins, but

the cell could still be viable for a time, although perhaps without reproducing itself. The higher the amount of DSBs, the more difficult the repair will be for the system, since it becomes increasingly likely that wrong ends of the double helix are joined, resulting in the final death of the cell. This harm modality can contribute up to more than 50 % of the total damage induction for high LET radiations, due to their typical track center extension and high dose deposition within few nm, where a large amount of close DNA damages at both sides of the double helix is registered [19]. Schematically, with IR being the ionising radiation and RH the target site, this pathway can be represented as

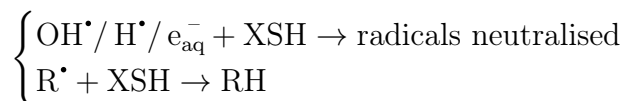


where $\text{R}^* / \text{R}^{\bullet+}$ describe either the damaged excited or ionised states of the various affected molecules.

The “indirect action”, instead, acts via the intermediate production of chemical species called radicals¹. Above all there are $\text{OH}^\bullet$, $\text{H}^\bullet$ and e_{aq}^- , generated primarily from the radiolysis of water, that constitutes about 80 % by weight of living cells [1, 26, 27, 28]. They are known as, respectively, hydroxyl radical, hydrogen radical and hydrated electron (cf. Table 2.2 later in this Chapter for a complete nomenclature summary). This damage induction pathway is responsible for the majority of the final harm elicited by low LET radiations. Given the more sparse ionisation densities observed around the primary track, the crossing of a DNA molecule predominantly results in the creation of SSBs, which are easier to repair. In contrast, when radiation interacts with water molecules, the respective radicals are localised at greater distances from each other, thereby avoiding the stronger recombination observed for higher LET particles. As a consequence, these radicals will be able to diffuse and react with the surrounding biological environment, possibly resulting in damage induction. In this case, the reaction pathway can be schematised as

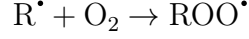


Generally denoted as XSH, enzymatic antioxidants such as glutathione (GSH) or ascorbate (i.e., vitamin C, Asch^-) [27], are responsible for preventing the damage to occur, or for intervening and restoring the biomolecules through hydrogen donation

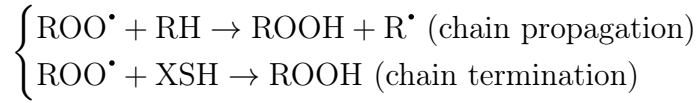


¹Atoms, molecules or ions featured by an unpaired electron in an open shell configuration. Due to this peculiarity, these species are chemically highly reactive. They are usually denoted with a dot next to the substance, such as $\text{OH}^\bullet$.

Conversely other molecules, such as oxygen, can hamper the repairing route by “fixing” the damage and making it permanent, as its attachment to the damaged site will “screen” the intervention of the antioxidants



The resulting peroxy radicals, $ROO^\bullet$, “non-restorable” forms of the original target molecules [22], can in turn induce a peroxidation chain reaction, where these free species “steal” hydrogen atoms from other RH in a recursive way. This phenomenon is also known as oxidative degradation, since oxygen plays a prominent role in disintegrating the macromolecules. Only the intervention of another antioxidant will block this loop, resulting in the formation of an organic hydroperoxide, ROOH



Malignancies featured by low oxygen levels, also called hypoxic tumours – partial oxygen pressure, pO_2 , $< 2\%$ [29] – are more resistant to radiations [21], as the damages will be easier to restore, with respect to those induced in more oxygenated targets. Lastly, the primary chemical species produced by the interaction between IR and H_2O can also undergo reactions between themselves. For instance, two $OH^\bullet$ can recombine in order to produce a hydrogen peroxide molecule, H_2O_2 . It is of interest to note that a single radical can have several possible routes, just like $OH^\bullet$ which can either react with itself/other species, can extract an electron from a target biomolecule, or can be scavenged by an antioxidant.

The preferred undertaken reaction channel by each chemical species, and consequently ultimately also the relative contribution of the indirect with respect to the total effective damage, are strongly related to the physical properties of the radiation type under consideration [30]. From a 150 MeV proton beam, the indirect action to plasmid DNA is higher than 90 % [31], while from a 150 MeV u^{-1} helium irradiation, the indirect contribution to cell killing under hypoxic conditions approaches roughly 50 %, as reported by [32]. A similar result was also found for a 10 MeV u^{-1} carbon beam [33]. Given the relevant contribution of the indirect action, it is important to have a deeper understanding of its features, also for heavy ions that are characterised by high amounts of energy released in the target. Along the path to the final deposition of the desired dose in the tumour, the energy decreases, with a corresponding rise of LET. Therefore, due as well to energy straggling and nuclear fragmentation, a broad distribution of LETs, rather than a single one, should be considered [34], the greater the cancer depth. Additionally, in order to get the spread Bragg peak, the target sees contributions from different energy slices, widening the LET range even more. On top, different LET strongly affect the spatial distribution and heterogeneity of the chemical species produced around the track center [17, 35]. By changing

then this parameter, it is possible to witness how the indirect damage is reflected into the biological endpoint under analysis (e.g., cell death), and to examine if the preferred reaction channels of the various radicals and molecules change or remain the same. Hence, the study of these events from a computational/theoretical perspective could be instructive as well. Simulations can indeed help to figure out the mechanisms behind these phenomena, suggesting also when specific models for describing the chemical reaction network may be applied, and in which circumstances they become limited or insufficient (for a better understanding of this concept, refer to the outcome of the research performed in Section 4.2.3). Such an investigation assumes a relevant connotation for high LET particles, wherein the denser track generated by the physical events results in a higher probability of reactions between the primary species. In turn, this will reflect in a slower transition to the so-called “classical” homogeneous reaction regime, wherein the chemical substances are supposed to be fully homogeneously distributed in the target, and their dynamics can be entirely described by simple reactions, without including the diffusion component (cf. later in this Chapter).

2.3 Track structure simulations of the indirect radiation damage

From the research perspective and within the context of interactions between radiation and matter, simulations are very helpful tools, since they can accompany and support experimental programmes by testing beforehand the set-up, suggesting some possible adjustments in the initial conditions, and giving some preliminary information about what should be expected to happen. They can compare different scenarios, provide new insights, test mechanisms, confirm and discard ideas, all before the actual practical study. However, in turn, it is necessary to prove they function correctly, by performing benchmarks and validations with experimental results.

To handle the stochastic nature of radiation interactions, Monte Carlo codes have been widely used for several decades [36]. These are computational algorithms exploited to predict the outcomes of events, whose description can not be achieved through deterministic/exact solutions. Here a key role is played by random numbers (RNs). The scenario describing the system is indeed calculated repeatedly, using always different RNs to define the stochastic variables, which are sampled by specific probability distributions. Examples of quantities of this kind in the context of radiation interactions are the interaction type, the energy deposited, the direction of the outgoing particles and the distance to the next collision point. The final averaged result obtained will approximate the expected outcome of the event under examination. Here the “law of large numbers” comes into play, meaning that the higher the

number of runs, the more the prediction will be robust and converge to the actual (most probable) value.

Generally, two different types of MC codes for simulating radiation interactions exist, which require the same initial ingredients: particle source, target properties (geometry and material), and set of cross sections describing the probability of each interaction type between the specific projectile and the volume under analysis. “Radiation transport codes”, from one side, are computational approaches where the interest is aimed towards macroscopic quantities, such as the dose distribution in a given target or the residual energy and angular distribution of the outgoing radiation. These are also known as “condensed history” approaches, since a sequence of interactions is condensed into one single event, and just the overall net effect is recorded. Additionally, symmetry and other considerations are usually exploited in order to facilitate re-using particle histories, fully or partially. Applications of this type of simulations in the medical field are studies on radiation protection via shielding materials, and treatment planning systems, i.e., codes dedicated to the planning of the dose that has to be delivered to the patient. It is absolutely mandatory to have not only a forward simulation, but a precise and careful iterative optimisation of all aspects related to a plan (coverage as well as organ sparing, based on clearly determined target values). Differently, “track structure codes” focus on an “event-by-event” description of all the single interactions happening in the target. For the high degree of precision and level of detail provided by these simulations, the computing time increases drastically, limiting their applications to small volumes and to the description of track segments of the primary radiation, following all the secondary electrons released from the target molecules down to very low energies – few meV – when they thermalise with the surrounding environment. In this context, an application of these models is the study of chemical dynamics, which features the diffusion and reactions of radicals and molecules that originate from the initial physical interactions between radiation and the water target (indirect action). Regarding this scenario, two different approaches can, in turn, be distinguished. Step-by-step algorithms [37, 38], from one side, follow every single chemical species in the environment, knowing at any time point their positions. They are very precise, but as a downside they are featured by long computing times. To tackle this aspect, independent reaction time (IRT) methods can be used [39, 40], which can be up to few hundred times faster than the SBS counterparts [14, 41]. In this case, for each potentially reactive chemical pair, a reaction time is computed from specific time-dependent survival probabilities (with each pair treated as if they were isolated [39]), listing then all these times in ascending order. Note that only the initial species distributions, together with their respective exact spatial locations, are known. The reactions and diffusion with respect to each other are then computed starting from the minimum of these values, and the process is carried on until either none of the remaining species can react, or

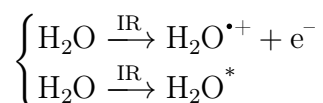
a specified time limit is reached. However, the information on the spatial position of each species at all times is lost, and only probability density distributions of relative distances are retained.

Regardless of the simulation method chosen, the species yields can be expressed either as G-values, usually referred to as the amount of radicals or molecules produced in a sample per 100 eV of energy deposited in it², or as concentrations, representing the amount of substance present per unit volume of solution, denoted by molar units $M = \text{mol/dm}^3$.

2.3.1 Stages of radiobiological events

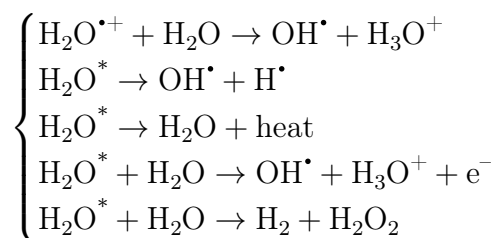
Remaining within the context of the indirect damage evolution at the track structure level, various stages can be discerned, each characterised by different physical, chemical or biological activities. These are schematically summarised in Figure 2.1.

Ionising radiation transfers energy to the water target rapidly, in the order of attoseconds to femtoseconds (physical stage). This phenomenon induces ionisation and electronic excitation events, depending on the energy transferred to the target electrons: $> 10 \text{ eV}$ for the former, approximately between 7 and 10 eV for the main channels of the latter (electronic excitation). These events will lead to the production of ionised and excited water molecules, respectively $\text{H}_2\text{O}^{\bullet+}$ and H_2O^* , together with a substantial high amount of secondary electrons, e^-



In the case of incident particle radiation, the great majority of these secondaries will be featured by low energies, but some of them, named δ -electrons, will be so energetic to form tracks of their own, inducing additional ionisations and excitations along their path, radially developed with respect to the primary projectile trajectory.

The resulting species are highly unstable, and will then dissociate and thermalise with the surrounding environment, reaching thermodynamic equilibrium. More in detail, with H_3O^+ – hydronium ion – being the resulting product after the reaction of H^+ with a water molecule, several channels can be distinguished



²Another common unit to describe G-values is $\mu\text{M/Gy}$, where $1 \mu\text{M/Gy} = 9.68 \text{ molecules/100 eV}$.

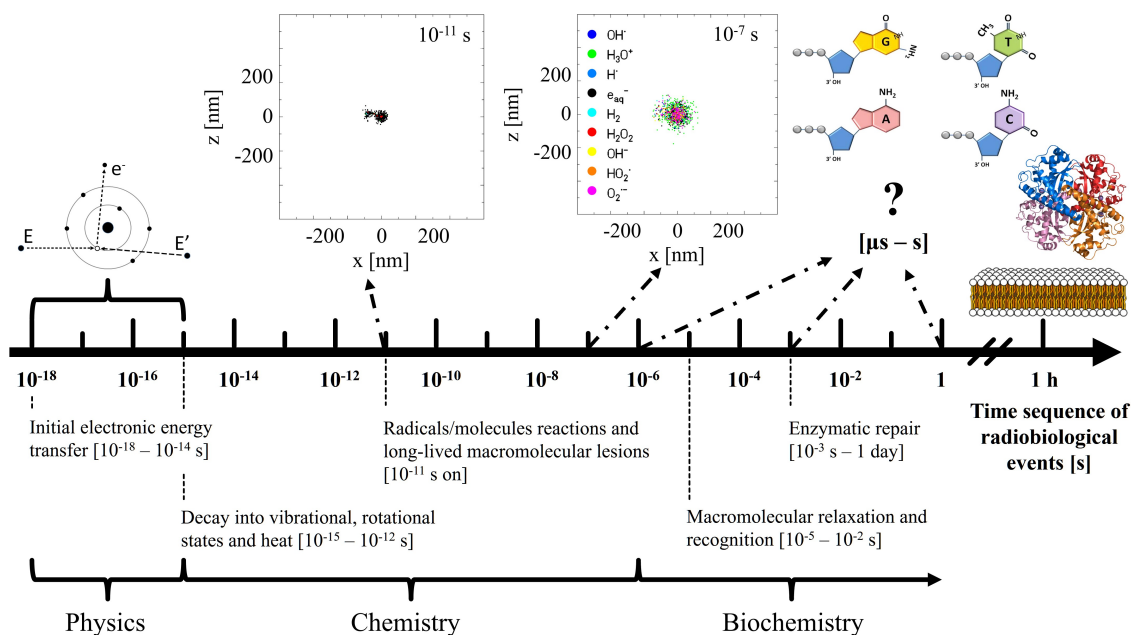


Figure 2.1: Illustration of the time scales for the different radiobiological events featuring the indirect radiation damage pathway. In the upper part, examples of chemical track evolution simulations already available are shown (generated by TRAX-CHEM [14, 17]). At the same time, some ingredients that have to be included in order to investigate the later biochemical stage (e.g., DNA nucleotides, enzymes such as superoxide dismutase, and lipids) are also depicted.

Regarding the secondary electrons e^- , after further interactions with other H_2O molecules, they will be referred to as “subexcitation electrons”³. These will then release their excess energy by repeated subelectronic excitation of target molecules, reaching eventually thermal equilibrium with the liquid, and will either be captured and bounded by the water solution through dipolar interactions, becoming a hydrated electron, $\text{e}^- + \text{H}_2\text{O} \rightarrow \text{e}_{\text{aq}}^-$, or react with a free proton to create a hydrogen radical, $\text{e}^- + \text{H}^+ \rightarrow \text{H}^\bullet$. This represents the end of the so-called intermediate physico-chemical stage (10^{-15} – 10^{-12} s).

At around 1 ps ⁴, the radiolytic products start to behave as chemical species, diffusing away from the track core and reacting with themselves. Different MC codes use various dissociation branching ratios to determine the initial amount of substances. Taking for instance the excited water molecule H_2O^* , several combinations between

³Electrons with sufficiently low energies not to be able to excite other water molecules, but larger than the standard 25 meV, representing thermal equilibrium with the environment.

⁴Approximate time required for the relaxation of a water molecule, and for the conversion of one e^- to its respective chemical counterpart e_{aq}^- .

the various output channels exist, in order to reproduce yields comparable with the few experimental data available [14]. Therefore, in turn, the total production yield of a specific chemical species is related to the interaction cross section tables used to model the physical stage. Interestingly, the starting dissociation of water in radicals and molecules is independent on the primary radiation type [42, 43]. On the other hand, the location of the species determine their following evolution [14]. During this “heterogeneous” chemical stage, the track structure dynamics, related to the type and energy of the incident radiation, rules the development of the radicals and molecules, causing a non-uniform distribution of the species. It is possible to identify “spurs”, approximately spherical areas within which high concentrations of reactive species following absorption of energy from ionising radiation are located. The denser is the track (directly proportional to the projectile LET), the closer these spurs are, and so also the produced radicals, and the more frequent the recombination will be [44].

Two specific events feature the chemical kinematics. From one side, the diffusion, representing the random motion of the species within the environment. Each radical and molecule is featured by a specific parameter, the diffusion coefficient D , that describes the degree of mobility of that particular species. This can be seen as the net flux of radicals/molecules from a region with higher concentration to one of lower concentration (in terms of surface over time), and it is strictly dependent on the environment considered. Fick’s laws describe the diffusion of the species. For instance, for a species A characterised by a diffusion coefficient D_A , its diffusion equation can be expressed as $\partial_t[A] - D_A \Delta[A] = 0$, with $[A]$ being the species concentration, both time and space dependent. On the other hand, the species can also react with each other, sharing electrons or exchanging atoms in order to reach a more stable condition. The rate of a reaction is a measure of its speed, and provides the information of the change in concentration for that species with respect to time. Reaction rate laws are equations providing the rate of a reaction, as a function of the reactant amounts. A general reaction going into completion can be expressed as $aA + bB \rightarrow pP + qQ$, where the uppercase letters refer to the actual species involved in the process, and the lowercase ones represent their respective coefficients (i.e., amount of each substance required for the reaction to take place). Its rate equation can be written as $\nu = \kappa_{AB}(T)[A]^n[B]^m$, with $\kappa_{AB}(T)$ being the reaction rate coefficient, temperature dependent following Arrhenius law. Here, n and m are the partial orders of the reaction, for A and B respectively, with the overall reaction order given by the sum of these two. For elementary, or single-step, reactions, these orders correspond to the specific stoichiometric coefficients, i.e., $n = a$, $m = b$. It is important to note that, for a reaction to occur frequently, both the reactants’ concentrations and the respective reaction rate coefficients have to be high enough.

At later times, $\approx 1 \mu\text{s}$, the species have diffused away from the track core, reaching a state wherein their concentrations change much more slowly and the actual track

structure dependence, together with the spurs, is lost. It is at this point – “homogeneous” biochemical stage – that their kinetics, due to the almost uniform distributions in the target, are featured just by reactions, both with other radicals and with additional molecules in the environment. These include biological targets, such as DNA, proteins, amino acids, and nucleotides, and antioxidants, as glutathione, ascorbate, superoxide dismutase, and so on. A possible indicator of the amount of harmed biological species may be the above-mentioned ROOH molecule, stable and non-restorable form of the initial biomolecular target RH. As will be demonstrated subsequently, this “homogeneous” stage is, in fact, not so homogeneous in reality, and it rather exhibits a more continuous nanoscale structure with fewer localised clusters.

Lastly, from approximately 1 s onwards, there is the biological stage, where a larger scale response to the chemical damages occurs. This is too complex to be simulated in the context of a track structure analysis, and its description goes beyond the scope of this project. As a nomenclature summary, Table 2.2 reports a list of the major radicals and molecules produced in an oxygenated water environment.

Table 2.2: Nomenclature summary of the major radicals and molecules produced by ionising radiation in an oxygenated water environment.

Species	Name
$\text{OH}^\bullet$	Hydroxyl radical
H_3O^+	Hydronium ion
$\text{H}^\bullet$	Hydrogen radical
H_2O	Water
e_{aq}^-	Hydrated/Solvated/Acqueous electron
H_2	Hydrogen
H_2O_2	Hydrogen peroxide
OH^-	Hydroxide ion
O_2	Oxygen
$\text{HO}_2^\bullet$	Hydroperoxyl radical
$\text{O}_2^{\bullet -}$	Superoxide anion
HO_2^-	Hydrogen peroxide anion

2.3.2 Extension to longer time scales

From the modelling and simulation points of view, different state-of-the-art algorithms for the physical and chemical stages (fs – μs), based on Monte Carlo approaches, exist: IONLYS-IRT [1], TRAX-CHEM [14, 17], PARTRAC [28], Geant4-DNA [45], TOPAS-nBio [46], DBREAK [47], RITRACKS [48, 49], NASIC [50, 51]

and gMicroMC [52, 53], the latter two being implemented for running on GPUs (for an extensive review, cf. [54]).

On the other hand, as far as the biochemical stage ($\mu\text{s} - \text{s}$) is concerned, dedicated models have been only recently developed, most of them focusing on implementing a few specific reaction partners [27, 55, 56]. Extending and improving these simulations will allow for a better comprehension of the biological effects of radiation, by linking the radiation track structure events with later biomolecular damages, and by studying how the final yields change with respect to the initial conditions simulated, both in terms of radiation and environment. Nevertheless, simulating the chemical effects induced by radiation in a biological target is, in fact, complicated. The introduction of biomolecular solutes themselves is not trivial and requires information such as biomolecule concentrations, diffusion constants and reaction rate coefficients, which are not always easily accessible. Moreover, regardless of the algorithm used to simulate the chemical stage, be it SBS or IRT, and although being very precise, the computational effort required by MC algorithms prevents them to reach longer time scales. The introduction of additional species, together with the need to extend the time domain by several orders of magnitude ($\mu\text{s} - \text{s}$), may require to tackle the resolution of the reaction-diffusion equations in a different way, for reasons of computational efficiency and sheer feasibility. In this regard, particular attention must be given to the time point at which the information is handed over from the initial algorithm, designed for heterogeneous modelling, to the new one adapted to a situation that becomes more and more uniform. It must be considered also that the chemical distributions change quite considerably depending on the LET simulated. Figure 2.2 shows a comparison of the radical yields produced by two different particle tracks depositing the same energy in the irradiated anoxic ($\text{pO}_2 = 0\%$) water target, respectively, 1 MeV electrons (with $\text{LET} < 1 \text{ keV}/\mu\text{m}$) and a 50 MeV u^{-1} carbon ion ($\text{LET} > 40 \text{ keV}/\mu\text{m}$). While for the electrons the different chemical tracks at 1 ns have already started to diffuse into each other, the carbon ion stays clearly more compact, with the species close to the track center, still 1 μs after the delivery of the radiation. Therefore, an ideal algorithm developed to properly carry on the information from its MC counterpart has to keep this aspect into account as well, in order to predict the final correct yields regardless of the primary projectile simulated.

In conclusion, it is important to make a few final remarks. Firstly, LET is not the only parameter describing the track dynamics and the related chemical yields, even though it is the most useful one. Indeed, as reported also by [12, 42], for two projectiles featured by the same LET, after initial recombination the radicals' amount will be greater, and the respective molecular one will be lower, for the radiation with a higher nuclear charge. In fact, the larger the charge, the greater the velocity, and consequently the larger the energy transferred to the secondary electrons. Therefore, although depositing the same energy per unit path length, the particle with a higher

charge will lose it in a larger volume (from the lateral perspective). This will induce the formation of chemical species further apart, preventing the recombination of radicals. Secondly, it is also crucial to distinguish between “track average” and “track segment” scenarios, as their respective elicited chemical yields may considerably differ. The former refers to a condition in which the primary ion is completely stopped within the target. As a consequence, the chemistry induced will be a result of all projectile energies, from the initial one to zero, and therefore the final species yields will represent an average outcome of such situation. Conversely, the latter refers to volume settings in which LET, energy and all the other parameters of the projectile do not vary. Analyses conducted under this condition will provide a one-on-one correlation between the chemical (and biological) effects registered with the initial physical properties of the radiation simulated.

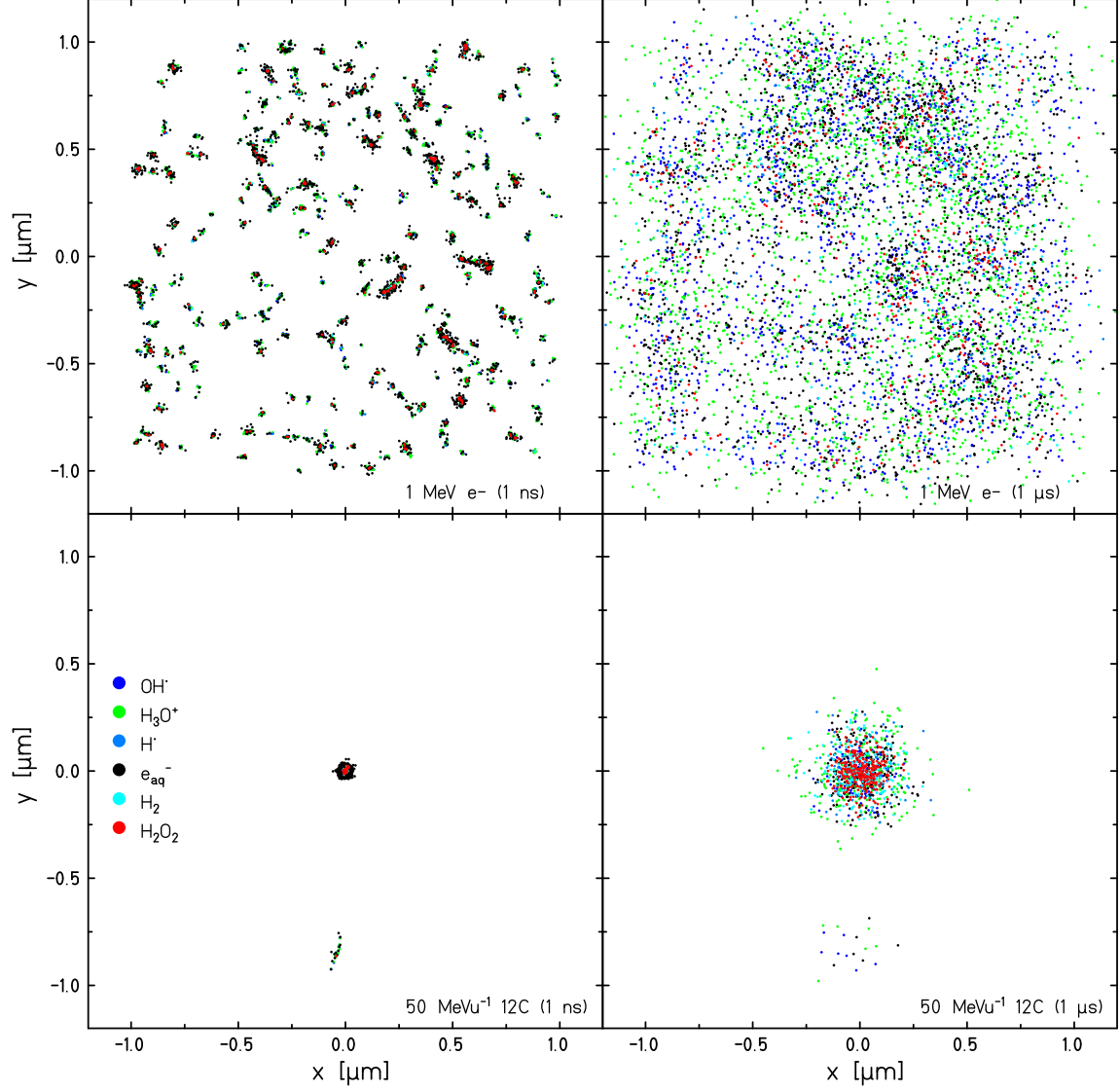


Figure 2.2: Chemical yields produced by two different radiation qualities in an anoxic water target, respectively, 1 MeV electrons (top) and a 50 MeV u^{-1} carbon ion (bottom), depositing the same dose in it. Displayed are snapshots taken at 1 ns (left) and at 1 μs (right). Generated by TRAX-CHEM.

3. Materials and Methods

This Chapter centers on the implementation of TRAX-CHEMxt [13], extension of the previously developed track structure Monte Carlo codes TRAX [15, 16] and TRAX-CHEM [14, 17], property of the GSI Helmholtz Center for Heavy Ion Research (Darmstadt, Germany). For a better understanding of the general picture, the overall simulation can be divided in different phases: on one side the MC part, that provides the description of the chemical track development up to the heterogeneous chemical stage, whereas TRAX-CHEMxt on the other takes these information and extends them into the later biochemical events. In what follows, the various aspects featuring this novel algorithm, from pure code properties to general settings implemented in order to produce the results reported in Chapters 4 and 5 are analysed in details. The final goals were to extend the simulations to longer time scales, testing the smoothness in the transition between the computation of reactions and diffusion events within the two different algorithms, and to introduce the possibility to deal with biomolecules, in order to understand how the chemical endpoints vary by changing the initial radiation and target conditions. None of the six major DNA repair pathways were simulated, since they occur in a longer time scale than the one under analysis (> 1 s). On the other hand, the intervention of antioxidant species present in a typical biological environment was considered. As for TRAX and TRAX-CHEM, the TRAX-CHEMxt code was implemented using the C programming language, while the visual representations of the results were executed using GD (data plotting program developed at GSI) and MATLAB, version 9.14.0.2206163 (R2023a) developed by The Mathworks Inc. (Natick, Massachusetts, USA).

3.1 Components of the overall chemical simulation

The analysis of the chemical effects of radiation on a track structure level can provide insightful information concerning the indirect action pathways with which different particle qualities can harm the system under analysis, possibly describing also mechanisms which are currently unexplained. Fundamental, in this sense, is the capability of the exploited algorithm to cover time scales up to 1 s, since the chemical dynamics

is very particle, energy and LET dependent, and the reactions can still happen even when the track structure is lost. In order to comprise the overall chemical simulation, this Section is divided in two parts. The first refers to the already developed MC codes that take care of reproducing the physical, physico-chemical, and heterogeneous chemical stages, i.e., TRAX [15, 16] and TRAX-CHEM [14, 17]. Vice versa, the second one focuses on the newly developed TRAX-CHEMxt algorithm [13], that covers the subsequent biochemical events. Regarding Sections 3.1.1 and 3.1.2, only the details needed in the extension described later are provided. For a more detailed description of these codes, refer to the respective references given.

3.1.1 TRAX

TRAX [15] is a track structure Monte Carlo algorithm that simulates in a step-by-step modality all the initial physical interactions of radiation with a specific volume. Fundamental input parameters are projectile and target libraries, containing information related to their chemical composition, density, atomic/molecular properties, and the sets of cross section tables for shell specific excitation and ionisation states and elastic scattering events [14]. The accuracy, as well as the spatial resolution of the computed simulations, are strictly related to these data. The actual material and geometry descriptions of the volume of interest have to be given by the user, together with the properties and position of the incident radiation projectile. Instructions on random numbers and amount of particle track repetitions (or histories) to be simulated, decided on the basis of statistical grounds, have to be provided as well.

Despite the fact that TRAX is featured by a great plethora of tested and validated data on both projectile and target composition, the entire analysis was performed by taking water as the material for the volume of interest. On top, focus was given to the ionising radiations exploited in therapy scenarios, meaning to ions with energies lower than few hundreds of MeV u^{-1} , and to electrons below 1 MeV. During the development of TRAX-CHEMxt, an extension of the cross section tables for the computation of electron projectiles up to 15 MeV was performed. Whereas for the ionisation part – 5 different channels – this was done automatically by exploiting a built-in function within the code (based on the binary encounter Bethe model), for the excitation values a different approach was applied, briefly explained in what follows. For each excitation shell (9 in total, i.e., 5 molecular excitations, 3 dissociative excitations, and 1 additional excitation channel for subexcitation electrons), a Fano plot representation¹ of the available cross sections was made. In this way, due to a

¹Graphical technique for studying the interactions between high energy projectiles and atoms/molecules of the target material. It consists of expressing the x-axis as the natural logarithm of the energy, and the y-axis as the product between the energy of the primary projectile and the respective cross section, resulting in a linear correlation between the variables, that can then be extended to higher energies through a fit.

linear dependence registered at high energies, a data extrapolation up to 15 MeV was performed, by applying a linear fit. Only for the shell representing OH^* , one of the eight major excitation states of H_2O [14], a power fit was used instead. To prove the validity of the extended tables, the resulting stopping power $S(E)$ was computed, as a function of the primary projectile energy E , by exploiting equation (3.1) as reported in [57]. The results were then compared with the data from ESTAR [58], as shown in Figure 3.1, demonstrating good agreement

$$S(E) = \sum_i \Delta E_i \cdot \sigma_i(E) + \sum_j \int (T + I_j) \cdot \frac{d\sigma_j(E, T)}{dT} dT \quad (3.1)$$

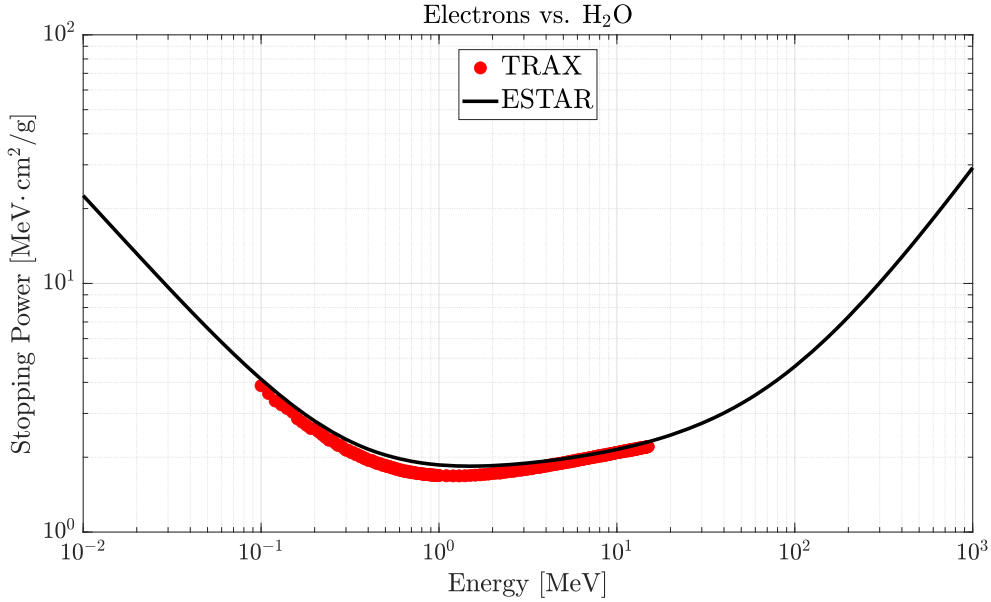


Figure 3.1: Stopping power comparison between TRAX and ESTAR [58] in H_2O . The electron energy range selected in TRAX spans from 100 keV to 15 MeV.

In equation (3.1), $\sigma_i(E)$ represents the cross section for the i^{th} excitation shell, whereas ΔE_i the corresponding energy loss. Regarding the ionisation part, $\frac{d\sigma_j(E, T)}{dT}$ denotes the differential ionisation cross section for the j^{th} shell, function of both E and the kinetic energy of the secondary ejected electron T . I_j is the ionisation potential for the corresponding shell j .

The accuracy of the cross sections is fundamental, since reliable values guarantee to simulate in the most appropriate and close to reality way all the possible interactions happening between the particle beam and the target. These span from elastic to inelastic scattering, with nuclear events as well. The present implementation of

TRAX does not follow the physics, nor permits the simulation, of photon particles. Considering that the focus of this analysis is on energies employed in electron and ion therapy scenarios, together with the simple water target with which they interact, photons contribute a negligible amount to the overall dose deposition events. Furthermore, a dedicated study was conducted to estimate the contributions from this radiation stemming from the interaction between a proton track and high Z-materials, such as platinum and gold. The results predicted yields less than 0.1 %, justifying their neglect [57]. Different output data can be produced by TRAX, such as the energy deposited within the volume of interest, the amount of each interaction type, and the positions of all the secondary electrons produced, together with the event that originated them, as shown in Figure 3.2. These locations are particularly critical for the determination of the primary species distributions (LET dependent) at the beginning of the heterogeneous chemical phase. Additional information can be requested, such as axial/radial dose profiles and electron energy spectra.

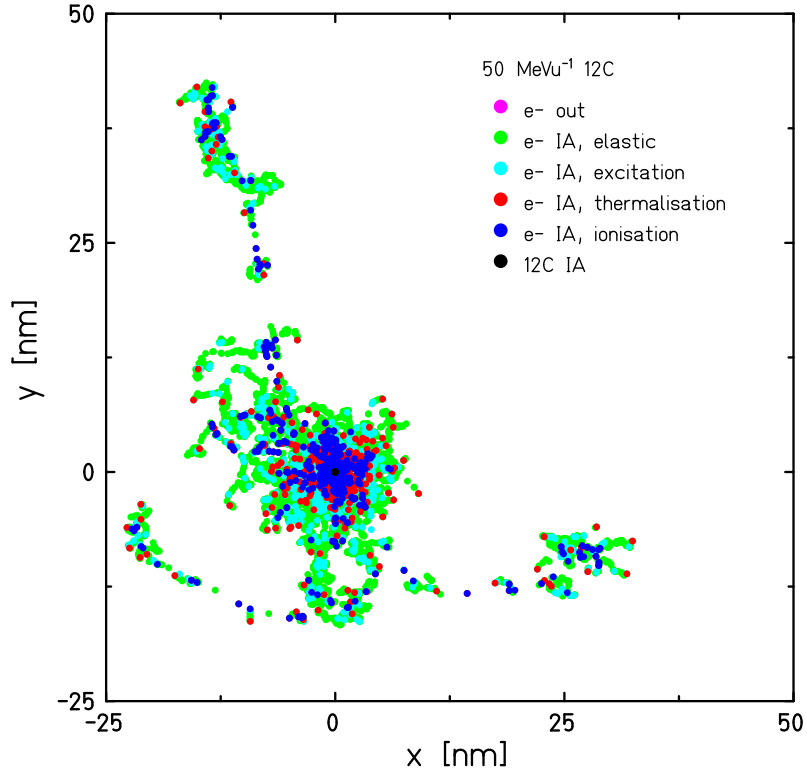


Figure 3.2: Track example generated by TRAX. Represented are the positions of all the interactions (IA) produced by a carbon ion (50 MeV u^{-1}) in a water volume. Each colour represents one interaction type, for both the ^{12}C projectile and secondary electrons, with “e- out” meaning that the electrons have exited the target.

3.1.2 TRAX-CHEM

The physico-chemical and heterogeneous chemical stages of radiation damage were simulated by TRAX-CHEM. For a detailed description of the code features, together with the approximations made, refer to [14]. This Monte Carlo approach was designed to provide as well an event-by-event description of all the diffusion and reaction events happening within the water target, storing this time the positions of all the single radicals and molecules released in it. As input parameters, both the subexcitation electrons and the (shell-specific) ionised and excited molecules' positions are required. Moreover, a reaction-diffusion table is read in. This contains information on the dissociation channel probabilities featuring the various shells characterising H_2O^* and $\text{H}_2\text{O}^{\bullet+}$, the thermalisation scheme for the subexcitation electrons, the diffusion coefficients, and the reaction rate constants. All these data are needed to simulate the dynamics between the species and their diffusion from the track center or point of creation. In more detail, firstly, a conversion of the physical stage products into the actual chemical species is performed. The decision of the dissociation probabilities affects the initial species yields, whereas their 3D distributions and the thermalisation mechanism influence the following track kinetics [14]. Afterwards, the radicals and molecules are allowed to diffuse and react. The diffusion steps are sampled from 3D Gaussian distributions, with displacements determined by the Einstein-Smoluchowski equation $\lambda = \sqrt{6D\Delta t}$. At the same time, reactions are defined through proximity parameters, the "reaction radii". Given two species A and B , featured by diffusion coefficients D_A and D_B and reaction rate constant κ_{AB} , their reaction radius is defined as $R_r = \frac{\kappa_{AB}}{4\pi(D_A+D_B)}$. They will react if their distance is smaller than R_r . With a recent update [17], it is also possible to include molecular oxygen as an additional reaction partner in the water environment (cf. Sections 3.2.1 and 3.3 for more details on its implementation). The outputs that can be obtained are species track snapshots, G-value curves as a function of time, and radial distributions, both in a species-by-species list or in a histogram form. For the sake of clarity, examples of the latter two can be found in Figure 3.3. The histogram output, which exploits the full potential of the MC step-by-step approach with respect to IRT counterparts [48], i.e., storing at each simulation time step all the exact radicals' and molecules' positions generated along the primary irradiation, is the key ingredient for the development of the TRAX-CHEMxt extension to probe longer time scales.

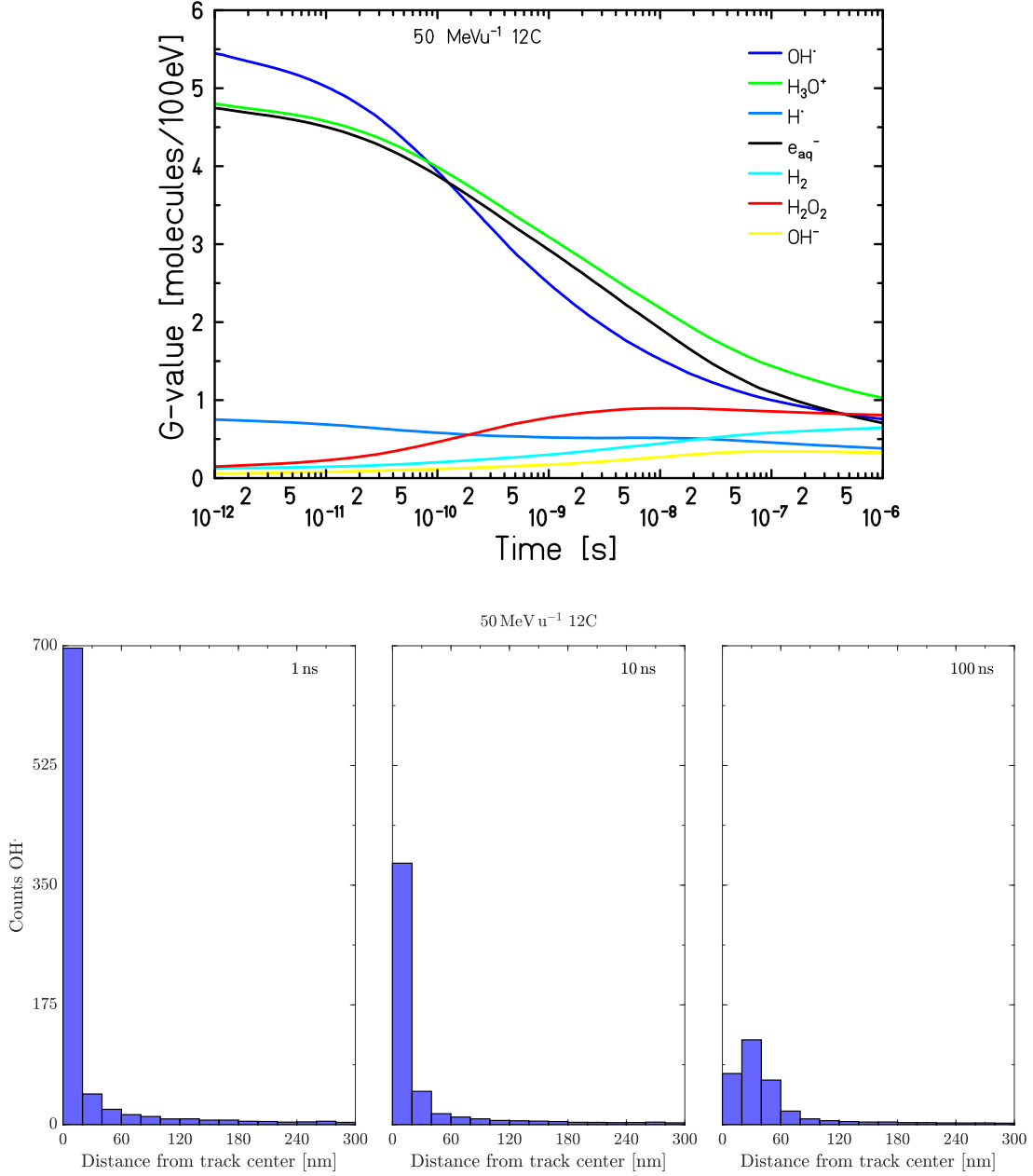


Figure 3.3: Examples of G-values (top) and histograms (bottom) generated by TRAX-CHEM. For the G-values, the yields of the chemical species are represented as a function of time. Concerning the histograms, the counts of the OH[•] radical at three different temporal points, respectively, 1 ns, 10 ns, and 100 ns, are reported. Both figures are shown solely for the sake of clarity, and were obtained by simulating a carbon track (50 MeV u⁻¹) interacting with a water volume.

3.1.3 TRAX-CHEMxt

Main aim of this project was the development of a novel algorithm capable of taking advantage of the information provided by TRAX-CHEM, and extending it to longer time scales in order to probe the biochemical stage of radiation damage. Not only this new code has to carry on the MC data within a reasonable computing time, but it has also to show a good consistency with its predecessor, regardless of the initial conditions simulated, without compromising the overall accuracy.

The events stemming from the initial energy deposition in the target, together with the following physico-chemical and heterogeneous chemical stages, are very stochastic and highly track-structure dependent. Therefore, Monte Carlo codes are irreplaceable tools for the reproduction of these phenomena. TRAX-CHEM, as described in Section 3.1.2, offers the possibility to score all single radicals' and molecules' positions produced around the primary particle track, at all times spanning from 1 ps to 1 μ s, reaching even 10 μ s if necessary, although requiring a higher calculation cost. Only at later stages, when a higher degree of homogeneity is reached by the system, it is possible to "switch" the information to a simplified and less computationally demanding procedure for simulating the chemical processes within the target. This is accomplished by the TRAX-CHEMxt code developed in this thesis through the utilisation of a novel approach employing a simplified continuous representation of the disparate molecules' and radicals' positions. It is the first time species-specific concentration distributions have been introduced instead of discrete point clouds representing the localisations of individual chemical reactants. The time-consuming, SBS Monte Carlo procedure, which tests for potential reactions between all possible combinations of individual reactants, can now be replaced by a set of differential equations that describe the diffusion of each species and the reactions between all combinations of species. These equations are based on the respective one-dimensional, radial concentration distributions. However, dedicated studies are required to understand the method to monitor chemical reactants, the computation of reaction and diffusion events in the lighter approach (core of the analysis), and the optimal conditions for transferring data from one model to the other.

Figure 3.3 shows an example of species radial distributions as a function of the distance from the track center. The first study performed was the proper conversion of these histograms, averaged over several parallel calculations to achieve good statistics (cf. Section 3.2.1), to their respective concentrations, since the latter happen to be good candidates to store the system's information and to extend it into the biochemical stage. If the initial data were excessively fluctuating, or too "peaked" towards the center of the projectile trajectory, assuming good statistical conditions, that meant the species were still too correlated to the track dynamics, and consequently longer times needed to be considered for the information transfer. Taking a cylindrical shaped water target with height h , and supposing the system to be

3. Materials and Methods

developed into a point such that a cylindrical symmetry could be considered around the longitudinal axis, the amount of species n_i localised within the ring $A_i - i^{th}$ bin in the histogram representation – can be converted to a concentration value via

$$C_i [\text{mol/dm}^3] = \frac{n_i}{N_A \cdot h \cdot A_i} \quad (3.2)$$

where $N_A = 6.022 \cdot 10^{23} \text{ mol}^{-1}$ is the Avogadro's number, and the surface A_i is defined as

$$A_i = \pi \cdot (r_{i+1}^2 - r_i^2)$$

with r_i and r_{i+1} being the radii delimiting the ring A_i within which each n_i is contained. By iterating this process for all histogram bins, it is possible then to obtain a concentration distribution profile for each radical and molecule produced in the target. Figure 3.4 is proposed as an example of concentration distributions, obtained from the conversion of the histograms in Figure 3.3, at three different time points by applying equation (3.2). Note that, as time passes by, bins further away from the track center start to get populated, while at the same time the overall $\text{OH}^\bullet$ population decreases due to the reactions with other species in the environment.

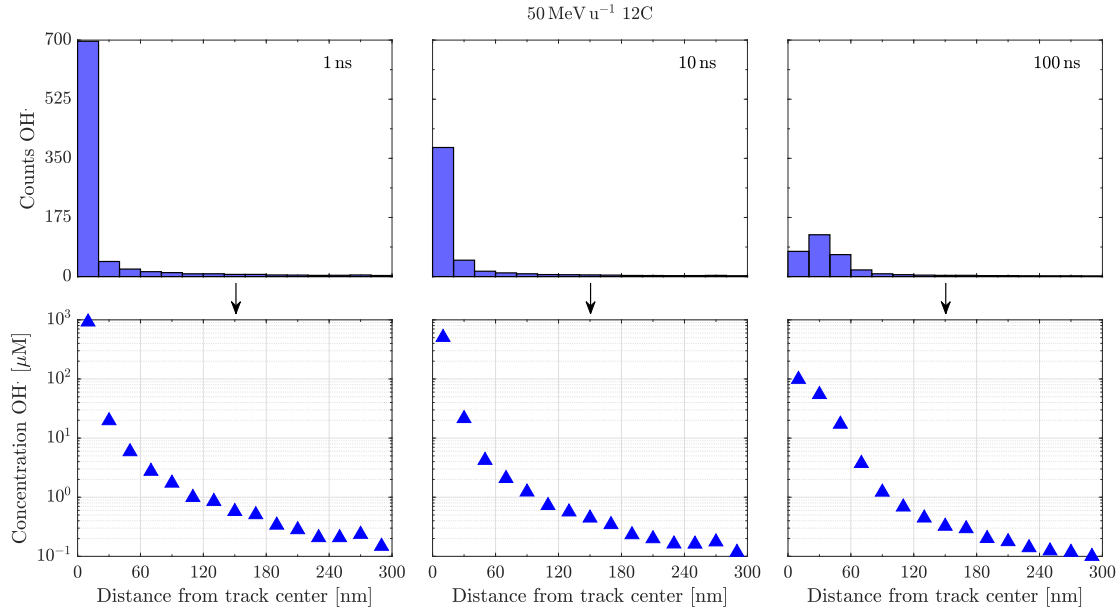


Figure 3.4: Concentration distributions example, obtained by converting the data generated by TRAX-CHEM displayed in Figure 3.3. Represented are the concentration values of the $\text{OH}^\bullet$ radical at three different time points, respectively, 1 ns, 10 ns, and 100 ns, produced by a carbon track (50 MeV u^{-1}) in a water volume.

This preliminary study allowed to get some insights also on the proper time at which the transition of the information from one algorithm to the other has to be performed. For a detailed analysis, refer to Section 4.1 in the validation Chapter 4.

Analytical model – first approach for solving the diffusion events

The following critical point was to understand how to deal with the diffusion and reaction events featuring the species concentration distributions. An analytical approach was first investigated, and attention was given to the diffusion computation. The idea was to perform a fit on the various concentration distributions, in order to represent them with a specific function. Four distinct models were subjected to analysis, in order to identify the most optimal representation of the species displacement as a function of the radius:

- i. Single Gaussian

$$f_{SG}(r) = \alpha_1 \cdot e^{-\left(\frac{r}{\beta_1}\right)^2}$$

- ii. Double Gaussian

$$f_{DG}(r) = \alpha_1 \cdot e^{-\left(\frac{r}{\beta_1}\right)^2} + \alpha_2 \cdot e^{-\left(\frac{r}{\beta_2}\right)^2}$$

- iii. Exponential

$$f_E(r) = \alpha_1 \cdot e^{\beta_1 \cdot r}$$

- iv. Lorentzian

$$f_L(r) = \alpha_1 \cdot \frac{1}{1 + \left(\frac{r}{\beta_1}\right)^2}$$

with $\alpha_{1,2}$ and $\beta_{1,2}$ being the various fit parameters. Interestingly, different behaviours were registered while considering negatively (electrons) and positively (protons and carbon ions) charged projectiles. Whereas for the former, the Lorentzian function was found to be the one better describing all the chemical concentration distributions, for the latter, the single Gaussian distribution (preferred over the double Gaussian due to the lower number of parameters) resulted to be the most optimal one. The behaviour at the fall-off of the concentration profile was the discriminating factor to determine (visually) the validity of one or the other function. Examples referred to the distributions of $\text{OH}^\bullet$ radicals at 600 ns produced from an electron and a carbon ion track, respectively 500 keV and 150 MeV u^{-1} , are presented in Figures 3.5 and 3.6. An initial appreciation of the effects of different particle types and energies on the final chemical species distributions can also be gained. The higher the energy deposited (LET) within the target, the larger the amount of substances released in it (nM for e- against μM for 12C).

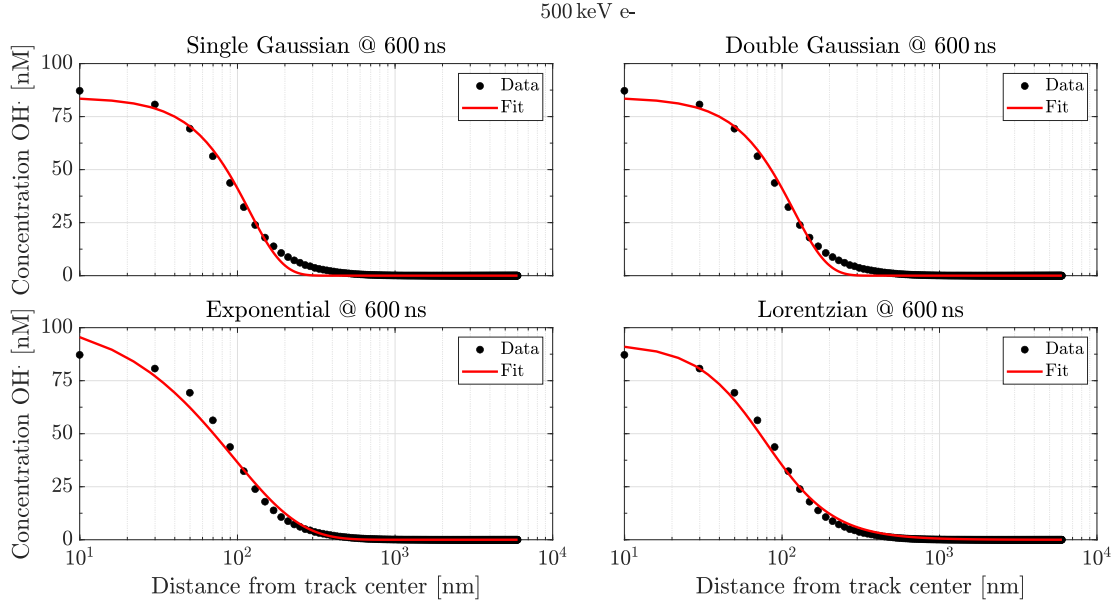


Figure 3.5: Research of the best function representing the distribution of the chemical species (here OH[•] as an example) produced by a 500 keV electron track. The concentration data were taken at 600 ns.

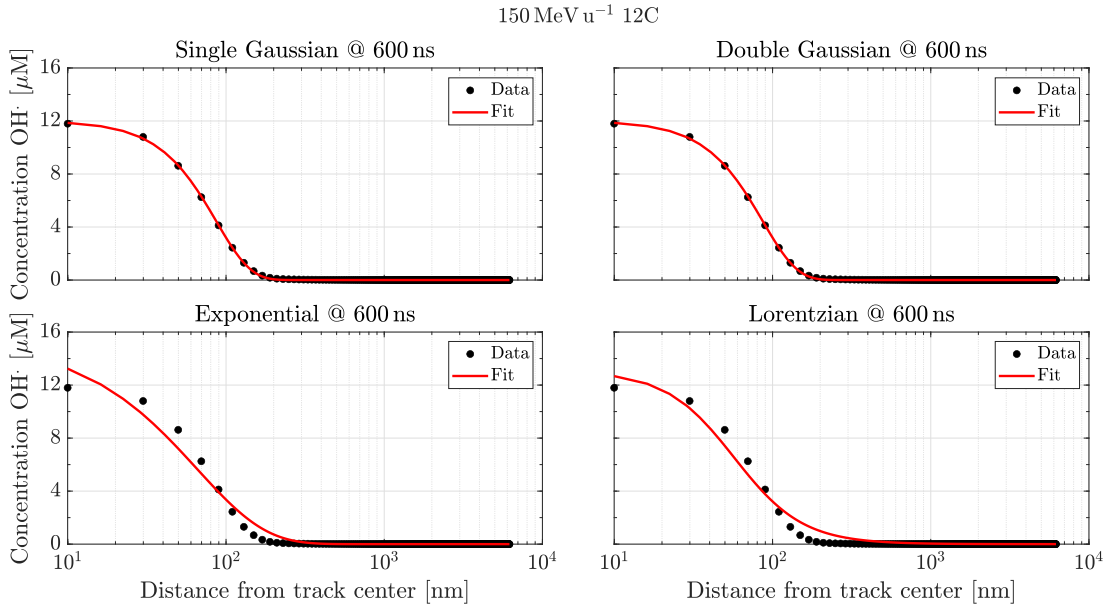


Figure 3.6: Research of the best function representing the distribution of the chemical species (here OH[•] as an example) produced by a 150 MeV u⁻¹ carbon ion track. The concentration data were taken at 600 ns.

As a result, depending on the particle simulated, an appropriate set of analytical functions could be determined, one for each radical and molecule. From these data, the diffusion could be computed by starting from Fick’s second law and exploiting a convolution approach, where the concentration distribution at the current time step $[A_n](r)$ is “smeared out” by the so-called “heat/diffusion kernel”, that in turn represents the solution of the diffusion equation, in cylindrical coordinates, into which a localised δ -function has diffused. The concentration at the following time step, $[A_{n+1}](r)$ can therefore be expressed as

$$[A_{n+1}](r) = \frac{1}{\sigma^2} \int_0^\infty r' [A_n](r') \exp\left(-\frac{(r'^2 + r^2)}{2\sigma^2}\right) I_0\left(\frac{rr'}{\sigma^2}\right) dr' \quad (3.3)$$

with $\sigma^2 = 2Ddt$ (Einstein-Smoluchowski equation in 1D), and I_0 being the 0th order modified Bessel function of the first type. This expression was proposed also in [59], within the context of a radial dose distribution calculation. Nonetheless, it was found that values of time steps dt lower than 1 μ s, although being ideal for the computation of the entire system dynamics, produced unreliable results for the diffusion component, as the species were increasing after the step, and the curves were more “peaked” towards the track center. This happened since the Gaussian function in equation (3.3) was not sampled with enough resolution, it was too narrow and did not guarantee a proper diffusion computation, because the convolution with the concentration distribution was not properly resolved. In other terms, supposing that all radial values within the vector r were equally spaced with bin width bw , only those points with $r = r'$ were represented, whereas the others, situated at distances exceeding the bw , fell directly within the null tail of the Gaussian. Indeed, the assignment of time steps around the ns elicited values of the σ (and consequently of the full width at half maximum, $\text{FWHM} = 2\sqrt{2\ln 2} \cdot \sigma$) around few nm, too small considering typical bin widths in the order of 10 to 20 nm. This implies that the Gaussian itself was approximated with a low number of points. However, an excessive increase of the spatial resolution would result in an overly refined sampling, both in terms of histogram and concentrations. This would lead the occurrence of several possible empty values, which would in turn compromise the fitting procedure. Consequently, a numerical approach was subsequently investigated, and ultimately deemed the most appropriate methodology for estimating the system kinetics.

Numerical model – final approaches for solving the diffusion and reaction events

The final numerical model employed, enabled the simplification and reduction of the amount of information that had to be carried on during the calculation. Nevertheless, similarly to the aforementioned analytical approach, yet differently from other mathematical shortcuts such as the one exploited in [60], the radicals and molecules are still recorded with respect to the track center, but in a less localised fashion. In order to gain a more comprehensive understanding of the data conversion and handling process, it is possible to discern three distinct “phases”, which also serve as the foundation for the extended simulation (TRAX-CHEMxt). These are illustrated in Figure 3.7.

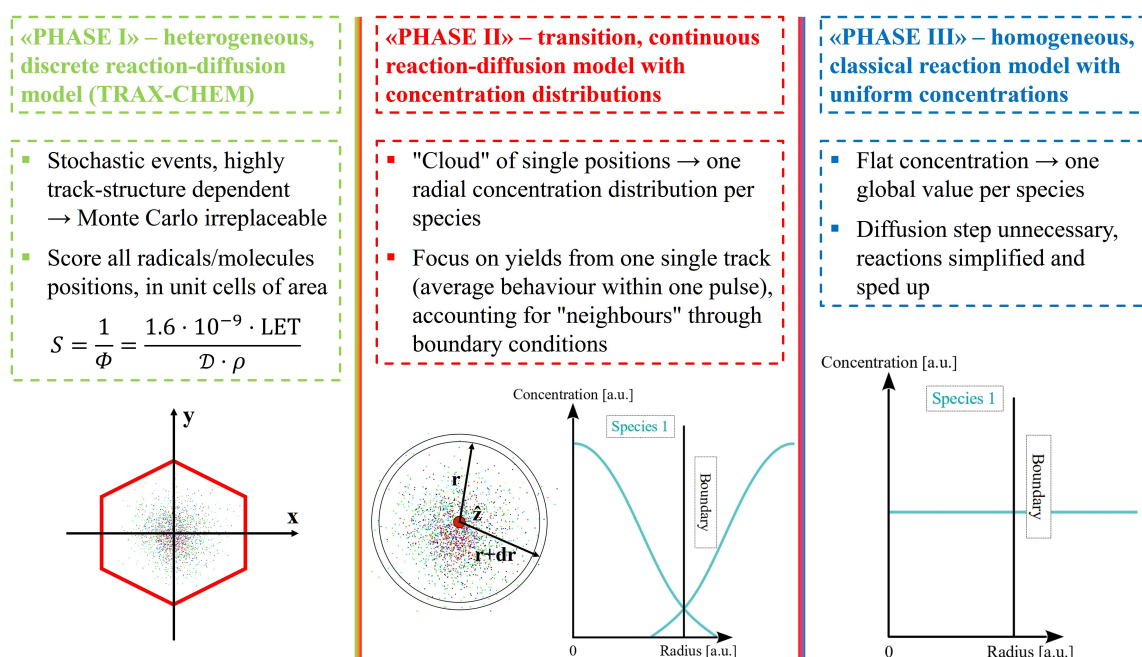


Figure 3.7: Scheme of the different “phases” featuring data conversion. After following every single radical/molecule produced around the track center (TRAX-CHEM), the information is converted into one radial concentration distribution per species (TRAX-CHEMxt – phase II). In the end, once these concentrations reach a uniform value, the classical homogeneous reaction model is applied (TRAX-CHEMxt – phase III). Both concentration and radial values are given in arbitrary units (a.u.).

The starting “phase I” represents the heterogeneous chemical stage, where the species distributions are not homogeneous, not even locally, and the Monte Carlo approach is irreplaceable to describe the reaction-diffusion kinetics [27, 47, 61]. In other terms, this discrete phase represents the output from TRAX-CHEM. After this

stage, all the single positions are scored as histograms, within an area determined through the dose-LET relation – cf. equation (3.7). “Phase II” is a transitional phase, where a local homogeneity for the various species can be supposed. The information is therefore converted from a “cloud” of discrete positions to continuous radial concentration distributions, describing each radical and molecule. Reaction and diffusion processes, in a geometry wherein the track structure dependence is still present, are carried on under the assumption that the species concentration within each histogram bin is uniform. In other terms, the system is in a quasi-continuous regime, where species have diffused away from the track center, and where it is possible to consider an “average” effect along the longitudinal direction, without anymore the presence of localised spurs. This method can be seen as a chemical extension of the concept of the amorphous track model for the physical radial dose. For a better visualisation of how the information is converted before being handed over to the new resolutions of the reaction and diffusion events, refer to Figure 3.8.

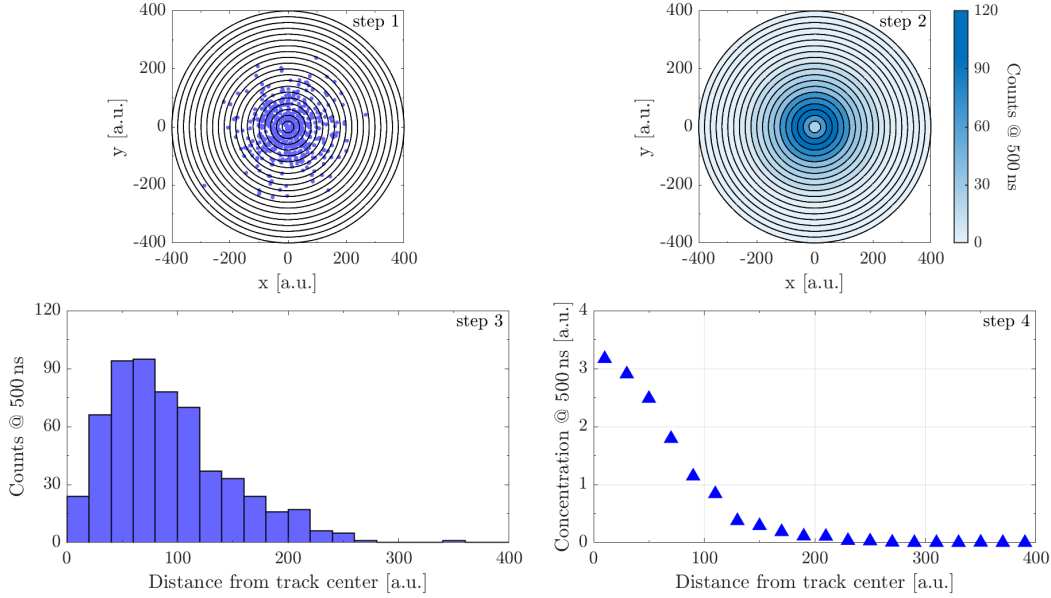


Figure 3.8: Steps constituting the conversion of the information from histograms to concentration distributions. After collecting the positions of a specific radical or molecule around the track center (step 1) and counting its amount within each radial bin (step 2), the respective histogram is created (step 3). Subsequently, the various counts are converted into concentration values via equation (3.2) – step 4 – supposing thus a uniform concentration within each bin. The concentrations are assigned to the respective bin centers. In the proposed example, a transitional time (from TRAX-CHEM to TRAX-CHEMxt) of 500 ns is considered, whereas both concentration and radial values are given in arbitrary units.

In principle, the handover time should depend on the radiation quality simulated, which in turn affects both the time point at which the positions around the track center have to be considered, together with the specific spatial and temporal parameters used to describe the distributions and their kinetics. Thorough analyses were conducted, in order to assign, for all particles simulated, general values for the bin sizes and the computational time steps, with also a general transitional time point from TRAX-CHEM to TRAX-CHEMxt (Section 4.1). Effects of possible neighbouring tracks could be treated as well, via the boundary conditions imposed on the reaction-diffusion problem, cf. equation (3.8). However, throughout the entire analysis, for both TRAX-CHEM and TRAX-CHEMxt, the chemical effects of the species produced by one single track were studied (or, in other terms, conventional dose rates were considered). In this way, possible intertrack interactions, both spatially and temporally, were disregarded. Due to this transitional second phase, provided the information accuracy attained remain the same, longer time scales could be probed with a quite significant improvement of the computational time. Finally, there is the third phase, “phase III”, wherein the various species distributions become flat. One global concentration value can therefore be assigned to each species, and the classical homogeneous reaction model can be applied without the necessity of computing the diffusion, further accelerating the computation.

Due to the novel representation of the chemical species distributions, a new approach is required to numerically solve the partial differential equations featuring the reaction and diffusion events within the system. The problem is divided into a pure diffusion part and a pure reaction part, resolved pairwise and alternatively for each time step. This is due to two main reasons. First, the reaction part is non-linear and highly coupled (e.g., reactions between two chemical species of the same type), and thus it does not allow for a proper treatment in finite difference schemes. Second, in order to conserve the particle concentrations, it is necessary to perform the reactions accurately and at the same time step. Therefore, it is not feasible to linearise non-linear reactions in order to incorporate them into the linear diffusion solution strategy. Concerning the diffusion part, this is described by applying the method proposed in [62]. The initial-boundary value problem associated with Fick’s second law on a cylindrical domain “*Dom*” of radius r_{max} , for the species *A* characterised by a diffusion coefficient *D* is

$$\begin{cases} \partial_t[A](t, \vec{x}) - D\Delta[A](t, \vec{x}) = 0 \\ [A](t = 0, \vec{x}) = [A_0](\vec{x}) \text{ Initial condition} \\ \text{Boundary conditions at the boundary of } Dom \end{cases} \quad (3.4)$$

Assuming a cylindrical symmetry of the solution, with $[A](t, r)$ symbolising the concentration distribution as a function of time *t* and of the distance from the track

center r , the initial-boundary value problem becomes

$$\begin{cases} \partial_t[A](t, r) - D\partial_r^2[A](t, r) - \frac{D}{r}\partial_r[A](t, r) = 0 \\ [A](t = 0, r) = [A_0](r) \text{ Initial condition} \\ \text{Boundary conditions at } r = 0 \text{ and } r = r_{max} \end{cases} \quad (3.5)$$

The initial condition in (3.5), that is, the concentration distributions given at the initial time $t = 0$, is provided by the TRAX-CHEM code. On the other hand, concerning the boundary conditions at the extremes, at $r = 0$ a consistency requirement is imposed. As the solution of equation (3.4) is known to be smooth on the interval $[\epsilon, \infty) \times Dom$ for every $\epsilon > 0$ [63], a consistency requirement is needed in the cylindrical coordinate formulation under the assumption of angular symmetry, to ensure smoothness at the origin. This requirement is $\partial_r[A](t, r)|_{r=0} = 0$. On the other hand, at $r = r_{max}$, a Neumann boundary condition is introduced, that is, the value of the derivative of the function at the boundary of the domain is specified. Radicals and molecules are free to diffuse and react. However, due to symmetry considerations in a parallel, uniform radiation field, the number of chemical species leaving the surface delimited by r_{max} is equal to the amount produced by other neighbouring tracks entering into it. Therefore, a net null flux can be supposed at the border, i.e., $\partial_r[A](t, r)|_{r=r_{max}} = 0$. For all the cases studied in this analysis, r_{max} is set large enough, i.e., 5 μm , to avoid any of these effects at the border since the study of possible intertrack interactions is beyond the scope of this project. Furthermore, a too low r_{max} would have introduced complications in terms of comparison with TRAX-CHEM predictions. It is evident that, despite the species exiting the main target are tracked by the MC code, their probability of reacting with other substances is lower than that of the same species “reflected” (bouncing back) into the volume of interest, where the overall amount is greater. Generally, r_{max} can be computed by the dose-LET relation. The initial expression is

$$\mathfrak{D} [\text{Gy}] = 1.6 \cdot 10^{-9} \cdot \text{LET} [\text{keV}/\mu\text{m}] \cdot \Phi [\text{particles}/\text{cm}^2] \cdot \frac{1}{\rho} [\text{cm}^3/\text{g}] \quad (3.6)$$

where $\mathfrak{D}$ is the dose, Φ is the particle fluence and ρ is the water density. It is then possible, by inverting (3.6), to obtain the (average) surface around one particle track

$$S = \frac{1}{\Phi} = \frac{1.6 \cdot 10^{-9} \cdot \text{LET}}{\mathfrak{D} \cdot \rho} \quad (3.7)$$

and the corresponding value of r_{max} . By definition, this parameter stands for the representative average distance between the particle tracks and it is, as a consequence, dose rate dependent. Returning to the initial-boundary value problem, it acquires

the final form

$$\begin{cases} \partial_t[A](t, r) - D\partial_r^2[A](t, r) - \frac{D}{r}\partial_r[A](t, r) = 0 \\ [A](t = 0, r) = [A_0](r) \text{ Initial condition} \\ \partial_r[A](t, r)|_{r=0} = \partial_r[A](t, r)|_{r=r_{max}} = 0 \text{ Boundary conditions} \end{cases} \quad (3.8)$$

A finite difference approximation of the derivatives in a Crank-Nicolson scheme is applied. This is a numerical, implicit method used to compute values of approximate solutions of partial differential equations, where both the current, $[A](t_n, r)$, and consecutive, $[A](t_{n+1}, r)$, states of the system are used. The method was found to be unconditionally stable through a von Neumann stability analysis. Explicitly, considering a time step dt , discrete times $t_{n+1} = t_n + dt$, and using the notation $[A_n](r) = [A](t_n, r)$, the differential equation (3.8) becomes

$$\frac{[A_{n+1}](r) - [A_n](r)}{dt} = \frac{1}{2}D\partial_r^2[A_{n+1}](r) + \frac{1}{2}\frac{D}{r}\partial_r[A_{n+1}](r) + \frac{1}{2}D\partial_r^2[A_n](r) + \frac{1}{2}\frac{D}{r}\partial_r[A_n](r) \quad (3.9)$$

endowed with the same initial and boundary conditions as (3.8). By approximating the radial derivatives in a finite-difference scheme, using the centers of the bins as a partition of the spatial domain $[0, r_{max}]$, it is possible to express eq. (3.9) by collecting the terms depending on $[A_{n+1}](r)$ on the left side of the equation, whereas those depending on $[A_n](r)$ are moved to the right side. Two “virtual” radial points are additionally introduced in order to ensure the validity of the partial differential equation at the boundary and to implement the boundary conditions. These are mathematical tools without physical relevance, and are used to implement the numerical method. By defining $\vec{a}_{n+1}$ and $\vec{a}_n$ as two vectors whose components are the concentration functions $[A_{n+1}](r)$ and $[A_n](r)$ evaluated at the bin centers, and due to the linearity of equation (3.9), it is possible to recast the rewritten equation in a matrix (M, N) representation, in order to obtain the values of the distribution $[A_{n+1}](r)$ at the bin centers after the time step dt

$$M\vec{a}_{n+1} = N\vec{a}_n \rightarrow \vec{a}_{n+1} = M^{-1}N\vec{a}_n \quad (3.10)$$

with matrices N and M depending on the derivative approximations of (3.9). At this point, due to its tridiagonal form (i.e., with non-zero elements only in the main diagonal and in the first diagonals above and below it), M can be inverted with the algorithm proposed by [64], which exploits the principal minors of the matrix in a recursive way. The resulting product $M^{-1}N$ has been validated with MATLAB’s internal routine for matrix inversion, as shown in Figure 3.9. The matrix definition is a useful tool, as after defining the set of matrices for all chemical species, only the simple product with $\vec{a}_n$ remains to be performed in order to determine the diffused distributions, without the need to redefine the matrices within each computing loop.

Concerning the determination of the time step (dt) and the bin widths (bw , i.e., distances between bin centers) for the distributions, homogeneous time and space partitions were opted for, with respectively $dt = 0.5$ ns and a bw of approximately 20 nm (Section 4.1). These parameters have been chosen small enough to guarantee the correct approximation of the derivatives, and to avoid missing any reaction, as suggested by [52]. Moreover, in accordance with what mentioned in [65], due to the different dynamics featuring the various events, small step sizes are needed.

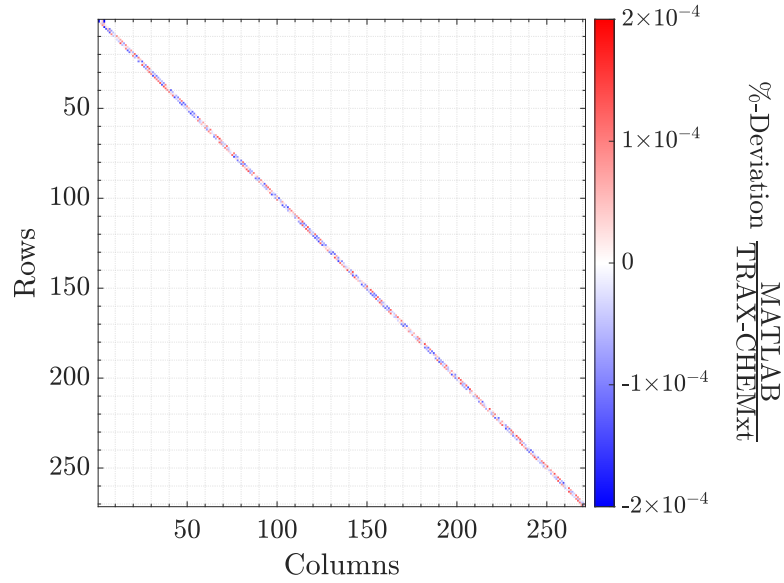


Figure 3.9: Deviations between the matrix values for the product $M^{-1}N$, computed by MATLAB and the implemented algorithm in TRAX-CHEMxt following [64], for the diffusion of the hydroxyl radical. The set of parameters exploited is: time step $dt = 0.5$ ns, diffusion coefficient $D = 2.8 \cdot 10^9$ nm² s⁻¹, bin width $bw \approx 20$ nm. The error is on the order of 10^{-6} .

As for the reactions resolution, an explicit algorithm (forward Euler method) was applied, by approximating the derivatives through a Taylor expansion. Given a simple reaction for species A , B , and C



with rate

$$\frac{d[A](t, r)}{dt} = -\kappa_{AB}[A](t, r)[B](t, r)$$

the increment $\Delta A_n(r)$ for the species A at time t_n can be defined as

$$\Delta A_n(r) = -\kappa_{AB}[A_n](r)[B_n](r)dt$$

with a negative sign since A is consumed. The final distribution after the time step dt can be obtained by simply evaluating at the bin centers equation (3.11)

$$[A_{n+1}](r) = [A_n](r) + \Delta A_n(r) \quad (3.11)$$

Firstly, the increments are defined, accounting for all the reactions both consuming and producing the species. These are specific for each locally homogeneous bin concentration (note the dependence on r). Subsequently, the distributions are updated. The reactions were as well simulated with a Runge-Kutta algorithm, and the results demonstrated minimal fluctuations. In summary, whereas the reactions were computed between the same bins, the diffusion process was calculated across the bins of one single species, in a similar fashion as [60]. After the algorithms description, it is also easier to understand why it was decided to keep separated the resolution of the two events. Due to the presence of self-recombination reactions, such as $A + A \rightarrow B$, the respective contribution for the reduction of the concentration of A , i.e., $-\kappa_{AA}[A_n](r)[A_n](r)dt$, can not be easily merged in the diffusion matrix.

To further improve the computational speed, especially at longer times, a control on the flattening of the radial concentrations has been introduced. Once the relative standard deviation featuring one distribution becomes lower than a user-specified threshold (e.g., 5 %), the entire concentration is recast into one constant (flat) value. More in details, this single value is computed from a weighted integral mean of the distribution, accounting also for the geometry of the problem, in order to properly redistribute the total amount of the specific radical or molecule in the entire volume. The diffusion step will then become unnecessary, and the overall calculation will be sped up (“phase IIP”). Thanks to this shortcut, with the introduction as well of a proper larger dt for very long time scales, times as far as 1000 s (more than 15 minutes) could be simulated.

As a concluding remark, it is essential to reiterate the primary approximation employed in the implementation of the aforementioned resolution. The hypothesis of local homogeneity for each bin, associated with the condensed information along the longitudinal direction in one dimension, is the foundation upon which the concentration distributions are constructed. However, this assumption can lead to results in possible discordance with those from the Monte Carlo counterpart, for two reasons. TRAX-CHEM is capable of tracking chemical species that exit the volume of interest, both laterally and longitudinally. However, this is distinct from considering their bouncing off the walls and reflection within the target (a form of “chemical equilibrium”), since the environment they encounter is markedly different, with more chances to react for the latter scenario, particularly along the direction of the primary track. As a consequence of the approximation employed in TRAX-CHEMxt, the balance of species along the longitudinal direction is always maintained. Moreover, in the case of the MC, the reaction radii between species act as a limiting factor for

reactions to occur. Once a species has reacted, it is removed from the milieu. On the other hand, this sort of binary behaviour is not present when a concentration representation is considered. In other words, discrepancies may arise between the “quantised” approach of TRAX-CHEM, and the “continuous” approach of the distributions in the extension. Nevertheless, as presented in the validation Sections 4.2 and 4.3, only small differences were registered in the end.

3.2 Computational details of TRAX-CHEMxt

From the pure computational point of view, the core of the algorithm is focused on simulating the chemical kinetics of the environment, by looping between reaction and diffusion events, including the periodic monitoring of flatness convergence. The number of loops is determined by the initial and final times, together with the respective sampling time steps. The three major functions are therefore:

- i. Reactions. In this context, it is possible to distinguish between an anoxic, oxic, and full biological (Section 3.4) milieu.
- ii. Diffusion. Updates of the matrices are admissible, provided that the time step dt is not excessively long (cf. Table 4.1).
- iii. Flatness concentration check. This control is conducted on a periodic basis. If one species distribution becomes flat, further diffusion events are prevented from occurring.

A small test was performed, in order to assess the robustness of the computed loop, employing a Strang splitting method. The system was solved by decomposing the reactions with respect to the time step. This entailed “dividing” the contribution of dt into two parts, such that the resulting new loop was: reactions (time step, $dt/2$), diffusion (time step, dt), possible flatness concentration check, reactions again (time step, $dt/2$). The outcomes yielded by this alternative approach were found to be largely comparable to those obtained by the previous one, confirming its validity.

In order to facilitate the management of the principal variables, “structures” were created in the C code for each property of the species. By selecting, then, the n^{th} element of each “struct”, the overall picture for that specific radical/molecule is provided. This refers to the index of the species (a unique number associated with each distinct chemical product), the flatness of the distribution (in terms of “true”/“false” flags), the diffusion coefficient, the matrix exploited for the diffusion computation (as illustrated in Figure 3.9), and the (possibly flat) concentration distribution. This approach has proven to be highly beneficial for the management of variables, both in terms of initial conditions and subsequent development of the simulation.

On the other hand, TRAX-CHEMxt provides several output parameters, regardless of the environment considered:

- i. Concentration distributions, both at the initial, intermediate (user-specified), and final times of the simulation.
- ii. G-values as a function of time.
- iii. Parameters describing the radiolytic depletion of oxygen (ROD), biomolecule (RBD), and antioxidant (RAD), at the final simulation time. The possibility of computing the overall reaction activity of the different scavengers in the milieu has been introduced, since the requisite information can be easily extracted from the MC output files, and carried on within the extension. These values are typically expressed in terms of $\mu\text{M}/\text{Gy}$, and consequently their computation follows the same process applied for computing the G-values.
- iv. Singular reactions contributions to the overall increase/decrease of a chemical species. These outputs need to be specified by the user, and have been introduced to study the preferred reaction channels featuring the desired species at specific time points. In turn, this provide information also on how, when and to which extent different reactions compete with one another [66], determining, for instance, the proportion of neutralisation versus damaging contributions for a specific initial radical (cf. Section 5.2.4).
- v. Information on fully consumed species already in TRAX-CHEM.
- vi. Time point at which each specific species reaches a constant concentration, with on top the overall time required for the computation.

3.2.1 Simulation target settings

It is necessary to provide some commentary on the simulation settings. The studies conducted were always performed under track segment condition. This implies that the sample thickness must be sufficiently small, so that no significant variations in the characteristics of the primary radiation, such as LET and energy, are observed within the computed volume of interest [42]. Regarding the target dimensions, a cylindrical geometry with a radius of $5\text{ }\mu\text{m}$ was selected. Consequently, at the transition of the information from TRAX-CHEM to the extension, any chemical species located outside the target volume will no longer be tracked. Target heights of $\approx 10\text{ }\mu\text{m}$ were employed for simulations with electron and proton projectiles, whereas smaller values of $(1 - 2)\text{ }\mu\text{m}$ were preferred for higher LETs. Concurrently, thin water phantoms in front and behind the main one were introduced, to prevent “edge effects” and to enable secondary electron build-up and back-scattering, so that the radiation chemistry

could be simulated in a sort of secondary species (physical) equilibrium [35]. These were still cylindrically shaped, with a 5 μm radius and a 0.5 μm height.

For oxygenated environments, the presence of the scavenger was processed as in the previous implementation [17]. The molecular oxygen was therefore treated as a continuum, i.e., as one uniform concentration value, homogeneously distributed regardless of the distance from the track center. However, whereas in TRAX-CHEMxt its fluctuations are actively controlled, due to the method exploited for computing the reactions, in the Monte Carlo counterpart variations in the global O_2 level within the target are not accounted for. This hypothesis is valid under low radiation-induced consumption with respect to the total O_2 dissolved, and was proven both by [67] under conventional dose rates, and by [35] within the framework of an irradiation featured by a large ionisation density in the track core. In the context of a biological tissue, this corresponds to a situation wherein blood oxygen resupply is faster than its removal by radicals. Concomitantly, O_2 concentrations must not be excessive, as this might result in direct interactions with the primary radiation [35, 56]. Notwithstanding this, the amount of molecules, both consumed and produced, was tracked during the SBS computation, to provide information on the final ROD. For gas-phase reactants, it is possible to utilise Henry's law to convert their partial pressure, p , to the concentration, c , which represents the amount of dissolved gas in a liquid: $c = K_H \cdot p$. The Henry's constant $K_H = 1.3 \cdot 10^{-3} \text{ mol}/(\text{dm}^3 \text{ atm})$, temperature dependent, describes the gas solubility in the considered fluid. During the course of some validation tests, it became evident that the approach employed to address the problem of considering a scavenger in the water milieu was not as straightforward as initially conceived. This is discussed in greater detail in the following Section 3.3.

To conclude, the reactions were supposed to be in a diffusion-controlled regime, with rates determined under normal conditions, i.e., neutral pH and 25 $^\circ\text{C}$. In TRAX-CHEMxt, both rate constants and diffusion coefficients were taken in water, and were the same ones used in TRAX-CHEM [17], Tables 3.1 and 3.2. Moreover, the pH was fixed at 7 and its effects on the acid-base pairs were not explicitly simulated. This is because non-neutral pH conditions and pH variations within the computed time are not yet implemented in the MC code. In order to reduce the statistical uncertainties and fluctuations of the species concentration distributions, parallel calculations were performed. Depending on the LET, several hundred to several thousand (300 – 5000) independent tracks were simulated, each impinging at the center of the cylinder and parallel to its main axis. The result was then averaged to obtain the general behaviour for that specific particle quality. Fewer runs were needed for higher LET radiations as compared to low LET, due to the increased amount of radicals produced.

Table 3.1: List of reactions and respective reaction rate constants implemented in TRAX-CHEMxt. The κ values are determined under normal conditions, i.e., neutral pH and 25 °C. Adapted from [17].

	Reaction	κ [$10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$]
(i)	$\text{OH}^\bullet + \text{OH}^\bullet \rightarrow \text{H}_2\text{O}_2$	0.6
(ii)	$\text{OH}^\bullet + \text{e}_{\text{aq}}^- \rightarrow \text{OH}^-$	2.2
(iii)	$\text{OH}^\bullet + \text{H}^\bullet \rightarrow \text{H}_2\text{O}$	2.0
(iv)	$\text{OH}^\bullet + \text{H}_2 \rightarrow \text{H}^\bullet + \text{H}_2\text{O}$	0.0045
(v)	$\text{OH}^\bullet + \text{H}_2\text{O}_2 \rightarrow \text{HO}_2^\bullet + \text{H}_2\text{O}$	0.0023
(vi)	$\text{e}_{\text{aq}}^- + \text{e}_{\text{aq}}^- + \text{H}_2\text{O} + \text{H}_2\text{O} \rightarrow \text{H}_2 + \text{OH}^- + \text{OH}^-$	0.55
(vii)	$\text{e}_{\text{aq}}^- + \text{H}^\bullet + \text{H}_2\text{O} \rightarrow \text{H}_2 + \text{OH}^-$	2.5
(viii)	$\text{e}_{\text{aq}}^- + \text{H}_3\text{O}^+ \rightarrow \text{H}^\bullet + \text{H}_2\text{O}$	1.7
(ix)	$\text{e}_{\text{aq}}^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^\bullet + \text{OH}^-$	1.0
(x)	$\text{H}^\bullet + \text{H}^\bullet \rightarrow \text{H}_2$	1.0
(xi)	$\text{H}^\bullet + \text{H}_2\text{O}_2 \rightarrow \text{OH}^\bullet + \text{H}_2\text{O}$	0.01
(xii)	$\text{H}^\bullet + \text{OH}^- \rightarrow \text{e}_{\text{aq}}^- + \text{H}_2\text{O}$	0.002
(xiii)	$\text{H}_3\text{O}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O} + \text{H}_2\text{O}$	10.0
(xiv)	$\text{e}_{\text{aq}}^- + \text{O}_2 \rightarrow \text{O}_2^{\bullet -}$	1.9
(xv)	$\text{H}^\bullet + \text{O}_2 \rightarrow \text{HO}_2^\bullet$	2.0
(xvi)	$\text{OH}^\bullet + \text{HO}_2^\bullet \rightarrow \text{O}_2 + \text{H}_2\text{O}$	1.0
(xvii)	$\text{OH}^\bullet + \text{O}_2^{\bullet -} \rightarrow \text{O}_2 + \text{OH}^-$	0.9
(xviii)	$\text{OH}^\bullet + \text{HO}_2^- \rightarrow \text{HO}_2^\bullet + \text{OH}^-$	0.5
(xix)	$\text{e}_{\text{aq}}^- + \text{HO}_2^\bullet \rightarrow \text{HO}_2^-$	2.0
(xx)	$\text{e}_{\text{aq}}^- + \text{O}_2^{\bullet -} + \text{H}_2\text{O} \rightarrow \text{OH}^- + \text{HO}_2^-$	1.3
(xxi)	$\text{H}^\bullet + \text{HO}_2^\bullet \rightarrow \text{H}_2\text{O}_2$	2.0
(xxii)	$\text{H}^\bullet + \text{O}_2^{\bullet -} \rightarrow \text{HO}_2^-$	2.0
(xxiii)	$\text{H}_3\text{O}^+ + \text{O}_2^{\bullet -} \rightarrow \text{HO}_2^\bullet + \text{H}_2\text{O}$	3.0
(xxiv)	$\text{H}_3\text{O}^+ + \text{HO}_2^- \rightarrow \text{H}_2\text{O}_2 + \text{H}_2\text{O}$	2.0
(xxv)	$\text{HO}_2^\bullet + \text{HO}_2^\bullet \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	0.000076
(xxvi)	$\text{HO}_2^\bullet + \text{O}_2^{\bullet -} \rightarrow \text{O}_2 + \text{HO}_2^-$	0.0085

Table 3.2: List of diffusion coefficients implemented in TRAX-CHEMxt. Adapted from [17].

Species	D [$10^{-9} \text{ m}^2 \text{ s}^{-1}$]
$\text{OH}^\bullet$	2.8
H_3O^+	9.0
$\text{H}^\bullet$	7.0
e_{aq}^-	4.5
H_2	4.8
H_2O_2	2.3
OH^-	5.0
O_2	2.1
$\text{HO}_2^\bullet$	2.0
$\text{O}_2^{\bullet-}$	2.1
HO_2^-	2.0

3.3 Simulate a uniform background scavenger in a Monte Carlo step-by-step code

The explicit introduction of a scavenger in a water milieu (alike radiolytic product species) will result in excessive simulation times for computing both reaction and diffusion events, as evidenced by [35, 39]. It is therefore necessary to employ an alternative method for the handling of the scavenger kinetics. The pivotal point, upon which the subsequent algorithm is based, is the observation that typical scavengers are characterised by concentration values that are orders of magnitude larger than those of the radiolytic species. In what follows, O_2 will be considered as an example of a possible scavenger.

Supposing a homogeneous distribution, and due to its high initial concentration, the dynamics featuring oxygen will be reduced to simple reaction events, as its diffusion will be irrelevant, at least in the context of conventional dose rates. The expression exploited to simulate the reactions with O_2 can be derived from the definition of the survival probability of a chemical species at time t , $\Omega(t)$, supposing it is still present at the time $t_0 < t$

$$\Omega(t) = \exp \left[-c_s \int_{t_0}^t k(t') dt' \right]$$

with c_s being the oxygen concentration. Here, the time-dependent reaction rate

$k(t)$ can be defined as

$$k(t) = 4\pi D' R_r \left(1 + \frac{R_r}{\sqrt{\pi D' t}} \right) \quad (3.12)$$

where D' is the sum of the diffusion coefficients of the two species in play, and R_r is the corresponding reaction radius. The probability $W(t)$ for a species reacting with O_2 during a period of time t , supposing it is still present at $t_0 < t$, can therefore be defined as

$$W(t) = 1 - \Omega(t) = 1 - \exp \left[-4\pi D' R_r c_s \int_{t_0}^t \left(1 + \frac{R_r}{\sqrt{\pi D' t'}} \right) dt' \right] \quad (3.13)$$

For the mathematical details concerning the derivation of the expression (3.12), together with the first definitions of the reaction probability (3.13), refer to [68, 69, 70, 71, 72]. Of particular interest is the concept proposed by Noyes [69], main reference for the majority of models dealing with a scavenger present in the milieu. The aforementioned expressions were derived by the author after the introduction and description of a chemical kinetics theory featuring changes occurring in a system under rapid reactions conditions, in a solution between A and B molecules. The objective was to describe the distribution of unreacted molecules as a function of time. Noyes highlighted that, if the reactivity between two species is greater than their diffusion rates, persistent reactions result in a depletion of the concentration of a particular species (for example, the B molecules) in the vicinity of another species (for example, the A molecule). This effectively slows down the reaction dynamics. By considering a hypothetical system, wherein randomly distributed A and B molecules can not react, but where it is possible to denote as “reacted” any species that have been able to react in the real system [69], as the time proceeds there would be a flux of “reacted” B moving away from “reacted” A . In other terms, close to an unreacted A , the probable concentration of unreacted B would be lower with respect to the total average concentration of unreacted B in solution. This relates to the real system, where the increasing lack of species around the real A , as time passes by, is sufficient to maintain the flux corresponding to the reaction rate of A . This phenomenon can be described by diffusion equations due to a concentration gradient. As a consequence, when a newly created reactant is introduced, initially its reactivity is very high, but then it falls rapidly into a smaller value, corresponding to the steady-state one (i.e., when the reactivity temporal derivative is null), representing the relaxation of the density distribution of one species around another.

After performing some robustness tests related to the comparison between the reaction algorithms exploited in TRAX-CHEM and TRAX-CHEMxt, it was noticed that the use of the reaction probability (3.13) is not so straightforward, at least in the framework of a Monte Carlo step-by-step code. More in details, the time integral is not as trivial as it may appear, since approximations of the extremes of integration

warrant careful consideration. For a better understanding of the problem, consider two different scenarios: integrand in equation (3.13) constant, such as 1, or function of t , such as $1 + \frac{R_r}{\sqrt{\pi D' t}}$. Regarding the first case, if $t = t_0 + dt$, with dt being the time step, the integral will result in

$$\int_{t_0}^{t_0+dt} (1) dt' = (t_0 + dt) - (t_0) = dt$$

and equation (3.13) turns into

$$W(t_0 + dt) = 1 - \exp[-4\pi D' R_r c_s dt]$$

which corresponds to the probability after the first time step, $W(dt)$, with $t_0 = 0$. In this situation, the probability is said to be time independent, and regardless of the actual state of the system, i.e., independently on the time t , $W(t)$ can always be traced back to $W(dt)$. The integral can therefore be iteratively resolved within the time domain $[0, dt]$.

On the other hand, for the second case, the integral will be

$$\int_{t_0}^{t_0+dt} \left(1 + \frac{R_r}{\sqrt{\pi D' t'}}\right) dt' = dt + \frac{2R_r}{\sqrt{\pi D'}} \left(\sqrt{t_0 + dt} - \sqrt{t_0}\right)$$

and equation (3.13) becomes

$$W(t_0 + dt) = 1 - \exp \left[-4\pi D' R_r c_s \left(dt + \frac{2R_r}{\sqrt{\pi D'}} \left(\sqrt{t_0 + dt} - \sqrt{t_0} \right) \right) \right] \quad (3.14)$$

that is substantially different than the counterpart

$$W(dt) = 1 - \exp \left[-4\pi D' R_r c_s \left(dt + \frac{2R_r}{\sqrt{\pi D'}} \sqrt{dt} \right) \right]$$

It is possible to conclude that a reaction rate expressed with the additional squared-root temporal term induces a probability which is no longer time independent. Consequently, continuously integrating between the interval $[0, dt]$ will result in an overestimation of the reactions with the scavenger. As reported by [35], it is crucial not to ignore the correlation between the states of the molecules in the milieu within the series of time steps, since otherwise the outcome will depend greatly on dt and lead to incorrect predictions [35]. It is therefore fundamental, for a step-by-step algorithm, to keep track of the “age” of the chemical species, such that the final correct probability for a radical to react with oxygen within the time domain $[t_1, t_2]$ is computed only if the radical itself has survived until time t_1 (i.e., age since creation). Different ways have been proposed to implement computationally the reaction probability (3.13) within the general time interval $[t_1, t_2]$ [35, 39, 41, 56, 61, 73]. Coliaux

and co-authors [35], for instance, have derived it through a method based on the definition of the conditional probability that the species survives a reaction during the “actual” time interval $[t_1, t_2]$, with $t_2 = t_1 + dt$, given that it has already survived until t_1 , with t_1 being the age of that species. The expression for the correct generalised reaction probability can then be written in a formal way, by stressing the actual time domain under consideration

$$W(t_1, t_2) = 1 - \exp \left[-4\pi D' R_r c_s \left(dt + \frac{2R_r}{\sqrt{\pi D'}} (\sqrt{t_2} - \sqrt{t_1}) \right) \right] \quad (3.15)$$

Interestingly, since oxygen in the milieu starts to play a role within the reaction scheme only at ≈ 1 ns [17, 35], the additional contribution coming from the squared-root term can be neglected. Indeed, the chemical yields obtained by exploiting the full probability (3.15) resembles those recorded by utilising a constant reaction rate coefficient $k = 4\pi D' R_r$. On the other hand, the G-values coming from the wrong application of the time independence hypothesis on equation (3.13) greatly overestimate the reactions with the scavenger. Therefore, although having implemented the correct probability in TRAX-CHEM, to reduce the computational burden of recording the age of each species in the Monte Carlo code, the command for the exclusion of the squared-root temporal term present in equation (3.12) was kept active. Concurrently, this facilitates greater consistency with TRAX-CHEMxt, wherein the O_2 concentration is employed in a more explicit manner than within a probability function, and the reactions are computed with a distinct algorithm (Section 3.1.3). In conclusion, for completeness, the impact of incorporating an additional temporal term is expected to be less pronounced in the context of IRT algorithms, where the time allotted for each potential reactive couple is inherently starting from the species formation stage.

3.4 Biological environment implementation

Before stating with the description of the simulated biological milieu, it seems prudent to make an initial clarification. The principal objectives of this project were, as anticipated, twofold: firstly, to ascertain the quality of transition between two distinct computational algorithms, with special attention in assessing the precision with which TRAX-CHEMxt carries on the information from the Monte Carlo counterpart to longer time scales. Secondly, to improve the computation itself by introducing a biological environment. Regarding the new reaction network featuring the latter point, approximations were moved, as it will be discussed later, both in terms of the species included and the parameters considered. As a result, the findings presented should be interpreted as indicative trends rather than precise numerical values, given that they do not represent a specific endpoint. The determination of all parameters

was indeed a challenging process, due to the lack of consistency in reported values from the literature, which made it difficult to ascertain the correct numbers. It was therefore decided that, for almost all the cases, an “order of magnitude” would be preferable to a specific value, given that high fluctuations have been reported even within the same “category”, such as for the protein class. It is important to note that the data presented should not be regarded as definitive, and that some adjustments may be necessary in the future.

The initial focus was on the concentration values, which have been derived from a series of references: [65, 74, 75, 76, 77, 78, 79, 80, 81]. Attention was given to free-floating components, conscious of the possibility that radicals may also react with immobile macromolecules. The objective was indeed to examine species that are ubiquitous within the cell. More in detail, no specific biomolecules were targeted (e.g., a specific protein type), as the existing literature does not provide a substantial amount of data in this direction. Similarly, also the attack on the DNA was not considered. This is due to the fact that DNA is highly site-specific, necessitating the division of the implemented environment into a “nucleus” and a “cytosol”, with lipid membranes situated in between. The actual simulation of this conformation is a highly complex undertaking, and the crucial approximation of concentration distributions would have been challenged around the lipid bilayers, necessitating a different approach for computing radicals’ diffusion. The creation of a more variegated (heterogeneous) milieu would therefore have required a very careful tuning and definition of the simulation settings, geometry, and assumptions. It was consequently decided to consider two additional species for the computation, namely a “generic” biomolecule, RH, and a “generic” antioxidant, XSH. From one side, a concentration of 43 mM was assigned to the former, representing a cumulative effect of three major categories. Firstly, the proteins (10 mM), constituting roughly the 70 % of the dry mass of the organic material within the cell [75, 82, 83]. These were studied as monomer units, since it was not possible to simulate, even at the MC level, their complete 3D structure. At the same time, they are also deemed to be the main biological target of oxidation by the primary water radicals, for their abundance and high reaction rate coefficients [65, 74, 82, 84, 85]. The other two categories were, respectively, the amino acids (30 mM) and the nucleotides (3 mM). Interestingly, the total RH concentration value is way larger than the maximum value associated to a fully-oxygenated water environment (21 % or 273 μ M). This suggests that a time shift should be expected in the chemical kinetics featuring O_2 . Conversely, to describe the “generic” antioxidant scavenger, the contributions from glutathione (3 mM) and ascorbate (1 mM) were included, as performed in other studies [56, 78, 82]. For completeness, it has to be mentioned that the concentrations attributed to GSH and AscH⁻ vary significantly depending on the tissue considered, as reported by [82]. The overall concentration was therefore 4 mM. This is also a first approx, considering that other enzymes play a role in the

chemical reaction network. However, these are featured by concentration values at least 3 orders of magnitude smaller than GSH/AscH^- . As reported by [82], the most prominent small antioxidant molecules in a cellular system are indeed glutathione and ascorbate. The scenario described above is a simplified representation of a more complex reality, since specific compartments with relative dynamics were omitted, and as a consequence the resulting outcomes are constrained by the limitations of the environment under consideration. To offset this aspect to some extent, different reaction channels for the same species were considered [65], in order to guarantee at least a small degree of competition.

In regard to the determination of reaction rate constants, the process proved particularly challenging due to the unavailability of certain values, necessitating the use of crude approximations or empirically derived numbers, as in [86]. The list of consulted peer-reviewed papers, that allowed to create Table 3.3, is the following: [26, 35, 55, 56, 65, 74, 75, 78, 82, 85, 86, 87, 88, 89, 90, 91, 92].

Table 3.3: List of reactions and respective reaction rate constants featuring the biological environment and implemented in both TRAX-CHEM and TRAX-CHEMxt. The κ values are determined under normal conditions, i.e., neutral pH and 25 °C. The reaction numbering sequence is continued from that presented in Table 3.1.

	Reaction	κ [$10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$]
(xxvii)	$\text{OH}^\bullet + \text{RH} \rightarrow \text{R}^\bullet + \text{H}_2\text{O}$	0.1
(xxviii)	$\text{R}^\bullet + \text{O}_2 \rightarrow \text{ROO}^\bullet$	0.1
(xxix)	$\text{R}^\bullet + \text{R}^\bullet \rightarrow \text{R}_2$	0.01
(xxx)	$\text{R}^\bullet + \text{ROO}^\bullet \rightarrow \text{ROOR}$	0.01
(xxxi)	$\text{ROO}^\bullet + \text{RH} \rightarrow \text{ROOH} + \text{R}^\bullet$	5e-5
(xxxii)	$\text{ROO}^\bullet + \text{ROO}^\bullet \rightarrow \text{ROOR} + \text{O}_2$	0.01
(xxxiii)	$\text{e}_{\text{aq}}^- + \text{RH} + \text{H}_2\text{O} \rightarrow \text{H}_2 + \text{R}^\bullet + \text{OH}^-$	0.05
(xxxiv)	$\text{H}^\bullet + \text{RH} \rightarrow \text{H}_2 + \text{R}^\bullet$	0.001
(xxxv)	$\text{OH}^\bullet + \text{XSH} \rightarrow \text{XS}^\bullet + \text{H}_2\text{O}$	1
(xxxvi)	$\text{e}_{\text{aq}}^- + \text{XSH} \rightarrow \text{HS}^- + \text{X}^\bullet$	0.3
(xxxvii)	$\text{H}^\bullet + \text{XSH} \rightarrow \text{H}_2 + \text{XS}^\bullet$	0.7
(xxxviii)	$\text{R}^\bullet + \text{XSH} \rightarrow \text{RH} + \text{XS}^\bullet$	5e-4
(xxxix)	$\text{ROO}^\bullet + \text{XSH} \rightarrow \text{ROOH} + \text{XS}^\bullet$	5e-5

As this is a preliminary study on the effects of new species in a water target, the data were deemed suitable for the analysis, given that the precise nature of the biomolecules is not a primary concern at this stage. In fact, rate constants for, e.g., dimerisation processes like $\text{R}^\bullet + \text{R}^\bullet \rightarrow \text{R}_2$, are very molecule-dependent [75], so it is complicated to determine a representative value for κ . Related to this aspect, it was

decided to stop at the products of reactions between water radicals and biological species, without considering “higher-orders”/“later generations”. Indeed, as reported by [82] in the context of protein peroxidation, it is challenging to ascertain which specific (protein) site reacts with the primary water radicals, and to understand the subsequent dynamics, through experimental means. Consequently, only global damages, such as ROOH yields, can be quantified. Similarly, the products of the reactions with XSH, i.e., $\text{XS}^\bullet$, HS^- , and $\text{X}^\bullet$, were not followed, since attention was given just to the “first-order” neutralisation mechanisms, without considering secondary dynamics. For a better description of the system, these solutes should be included [56, 83]. Concurrently, certain approximations were made with regard to the reaction products. Indeed, some species from Table 3.3, such as H_2 and OH^- in reaction (xxxiii), have been derived and introduced in accordance with stoichiometric principles. In other words, while the biological products, such as $\text{R}^\bullet$, are consistently generated, the vast plethora of possible byproducts is contingent upon the specific biomolecule under examination [83]. It is therefore necessary to omit from the results discussion the yields of both H_2 , OH^- , as well as its most frequent reaction partner H_3O^+ , in order to avoid any potential misinterpretations.

As far as the diffusion coefficients are concerned, which were assigned to both reactants and all the respective products, they were derived from the following references: [93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105], and are summarised in Table 3.4.

Table 3.4: List of diffusion coefficients featuring the biological environment implemented in both TRAX-CHEM and TRAX-CHEMxt.

Species	D [$10^{-9} \text{ m}^2 \text{ s}^{-1}$]
RH & products	0.6
XSH & products	1.0

Concerning the final value for RH, it was derived from a weighted mean (where, for proteins, smaller values were given greater weight due to considerations moved previously for the concentrations part), accounting also for the migration of damaged sites along the molecule [82] – intramolecularity effect, resulting in an increased mobility. This latter consideration was introduced to partially offset the fact that the Monte Carlo method treats only point-like radicals, lacking any three-dimensional information. Furthermore, the values were derived from water, as this was the basis for all other species and the entire calibration and validation process for the TRAX-CHEM code. The introduction of a viscous environment would have necessitated significant modifications, already at the physico-chemical stage. Conversely, the diffusion coefficients for both GSH and AscH⁻ were found to be quite similar.

From the computational point of view, the two new scavengers were treated in the same fashion as oxygen, therefore, spatially homogeneously distributed [26, 55, 65, 106]. This also means that direct interactions between the primary ionising radiation and RH/XSH were not considered, since their concentrations are way lower than that of water [41, 56, 107]. For TRAX-CHEMxt, this resulted in a simple updating and modification of the reaction network scheme and the pool of simulated species. On the other hand, from the Monte Carlo side, the new scavengers introduction increased the level of complication for the algorithm in terms of computational effort, although not accounting for any structure nor geometry, nor for considering concentration variations. The reaction probabilities were defined in the same way as for O_2 . However, a selection procedure needed to be introduced. The probability of a reaction between a radical and a specific scavenger (if applicable) can be expressed as P_{O_2} , P_{XSH} , and P_{RH} . All the probabilities are way less than 1, such that their sum is lower as well. Concurrently, the radical can also interact with other species surrounding it. Therefore, for each time step, only one reaction pathway can occur, in a sort of “competition” between the other reactants, and it is not granted that the radical will react at all. Hence, for each simulation loop, a random number is extracted, x , in order to assign to the radical its possible reaction partner:

- i. If $0 < x < P_{O_2}$, then the radical reacts with O_2 .
- ii. If $P_{O_2} < x < (P_{O_2} + P_{XSH})$, then the radical reacts with XSH.
- iii. If $(P_{O_2} + P_{XSH}) < x < (P_{O_2} + P_{XSH} + P_{RH})$, then the radical reacts with RH.
- iv. Otherwise, it may react with another radical, depending on their distance and the respective reaction radius.

During the implementation, dedicated variables were introduced to account for the precise numbers of RH/XSH molecules that have reacted and were produced, in order to determine their overall total depletion, respectively RBD and RAD. It should be noted, however, that in the case of the antioxidant, the depletion parameter RAD should be regarded as an “intervention” of XSH rather than an overall depletion, given that several higher-order reactions were not considered, and even signalling reactions (to maintain equilibrium) were excluded. In conclusion, despite the scenario described above is a highly approximate biologically-equivalent medium, it represents the initial step towards a more realistic yet complex situation, with an environment that differs from the simple oxygenated water case. Furthermore, it allows for the investigation of long time scales, ranging from the μs to the s. Even if the choices of biological and antioxidant molecules have a considerable impact on the results [108], some preliminary track structure effects of ionising radiation on the final chemical yields can be appreciated. But first, a series of validation tests have to be performed.

4. Settings and Code Validations

As the code itself is the primary result of this project, an entire Chapter is devoted to the initial settings adjustments, tests, and validations conducted to ascertain its precision in comparison to the MC counterpart, in both oxygenated water and biological environments. The investigation of the chemical yields at extended time scales, with a particular focus on the impact of LET and pO_2 , together with some experimental benchmarks, are presented in Chapter 5.

4.1 Time step, bin width and transitional time point

Firstly, the parameters necessary for the resolution of the reaction-diffusion equations are identified, and the data obtained are applied to both environments, with only one exception as outlined in Section 4.3.

The initial time step for TRAX-CHEMxt is chosen to be 0.5 ns. This dt was originally determined from a G-value analysis, by monitoring a 5 % maximal change in the $OH^\bullet$ curve (one of the species with the highest change rate, due to its high reactivity). However, a built-in function was implemented as well, to increase the time step automatically after some specific “flags”, with a maximum value of 100 ns for times longer than 1 s. The list of all the time steps adopted in the novel algorithm, associated with their respective temporal intervals, is shown in Table 4.1. Every value but the last was empirically proven to provide reliable results by monitoring that the predictions, both in terms of species distributions and G-values, closely match those from TRAX-CHEM, at both 1 and 10 μ s. The validation test was different for the largest dt , since it is introduced in a context where all the species, regardless of the radiation/environment simulated, have uniform concentrations (i.e., are in “phase III”). In this case, it was monitored that the species concentrations and G-values resembled those computed with lower time steps, such as $dt = 50$ ns, avoiding simultaneously the occurrence of negative (and thus unphysical) outcomes. In fact, it was necessary to fix an upper limit on the time step, to prevent error amplifications due to the forward Euler method applied for computing the reactions, while also keeping spurious oscillations under control, guaranteeing the code’s self-sustainability.

Table 4.1: List of all the time steps dt implemented in TRAX-CHEMxt, assigned to the respective time intervals.

Time interval	dt [ns]
$t_{in} - 1 \mu\text{s}$	0.5
$1 \mu\text{s} - 10 \mu\text{s}$	1
$10 \mu\text{s} - 100 \mu\text{s}$	2.5
$100 \mu\text{s} - 1 \text{ ms}$	5
$1 \text{ ms} - 1 \text{ s}$	10
$1 \text{ s} - t_{end}$	100

A comparable empirical investigation was undertaken to identify reliable bin widths, eschewing an undue refinement of the spatial resolution, as this would have resulted in initial concentration values from TRAX-CHEM to exhibit excessive fluctuations between neighbouring bins. Ultimately, a bw of 20 nm was chosen.

The last relevant parameter to fix is the transition time, i.e., the time when the information has to be handed over from the full Monte Carlo code to TRAX-CHEMxt, t_{in} . Its determination is intricate, since it depends on the energy deposition associated with the particle quality simulated. This, in turn, gives rise to intrinsic differences in chemical dynamics. Hence, depending on the initial LET, the time at which the local homogeneity is reached within the histogram bins fluctuates significantly [60]. Transitions at too early stages of the chemical evolution will result in unphysical outcomes, as the system is still highly track structure dependent. On the other hand, a change between the MC and the concentration-based methods at advanced stages will make the entire simulation less efficient, due to increasing computational costs, especially for high LET irradiations [60]. The optimal time point for a smooth transition has been found to lie around 500 ns, for all the projectiles considered. Examples of various t_{in} effects on the number of species produced at 1 μs , for a 500 keV electron track and a 150 MeV u^{-1} carbon ion in oxygenated water (respectively at 1 % and 7 % pO_2), are presented in Figure 4.1. Switching times around 100 ns compromise the predictions, especially for the higher LET particle, resulting in deviations approaching 15 % with respect to TRAX-CHEM results. In this case, radicals and molecules are still too close to the track center and to each other, with a rather strong dependence on the track structure dynamics. Therefore, values around 500 ns should be preferred to forward the information, owing to the small deviations registered (almost all within 2 %). From another perspective, this transitional time domain represents a “quasi-continuous” regime, where species have diffused away from the track core, and a reasonable homogeneity along the track direction, and locally within each radial bin has been reached, without the influence of localised spurs.

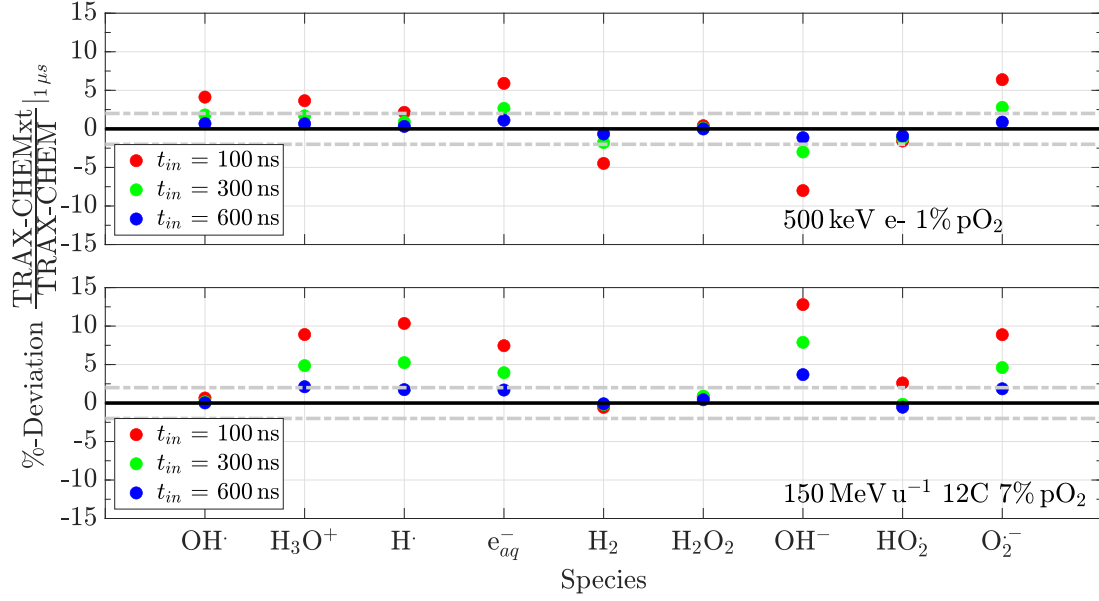


Figure 4.1: Deviations at 1 μ s as a function of the time chosen to transfer the species localisation information from TRAX-CHEM to TRAX-CHEMxt. The deviations are determined by the difference between the total number of every radical and molecule predicted by TRAX-CHEMxt and the respective quantity produced by TRAX-CHEM, divided by the latter. Tests on two radiation qualities are shown, namely a 500 keV electron track (top) and a 150 MeV u^{-1} carbon track (bottom), in a water target under pO_2 of, respectively, 1 % and 7 %. Grey dash-dotted lines are introduced to mark deviations of $\pm 2\%$.

4.2 Water environment – comparison with existing approaches

Once all parameters required for the computation of the reaction and diffusion events in the new TRAX-CHEMxt algorithm have been defined, the next objective is to validate its precision, by comparing its predictions with those from other available sources. In other terms, both approaches implemented to deal with phase II and phase III require confirmation. This Section presents a comprehensive examination of the novel algorithm’s accuracy, considering a wide range of various scenarios. Conversely, the validation presented in Section 4.3, given both the favourable outcomes from this Section and the unavailability of data pertaining to the biological framework, was conducted solely with the results from TRAX-CHEM.

4.2.1 TRAX-CHEM (phase II)

The predictions of TRAX-CHEMxt are compared with those from the standard TRAX-CHEM code, in the overlapping time scale (500 ns – 10 μ s), for different particle qualities, LET, oxygenation conditions, and for all the chemical species produced. It was resolved that a comparison of the two algorithms at longer time scales would be unwarranted, in light of the considerable computational burden imposed by the Monte Carlo beyond the μ s. Furthermore, it is expected that differences will moderately increase over time, as a consequence of the diffusion of the species along the longitudinal direction. Indeed, as already mentioned in Section 3.1.3, even though within the MC all the radicals and molecules are followed when they exit the volume of interest, the situation is completely different than the one where these “rebound” at the target borders and return inwards. Since the extension recasts the information on the species amount in a simple radial distribution, it does not account for the species exiting the target longitudinally. Instead, it resembles more the case of ricochets at the edges (chemical equilibrium), while the diffusion is computed only radially. Therefore, as time progresses, the total number of species predicted might slightly disagree, since the SBS approach follows singularly each one of them, whereas the other keeps them “confined”. This is not regarded as a significant issue, as both the results look promising, and the chemical equilibrium considered by TRAX-CHEMxt is more reflective of authentic experimental conditions.

In Figures 4.2 and 4.3, examples of radial distributions and G-values are shown. A good agreement is found for all the chemical species studied. This proves that TRAX-CHEMxt is able to carry on the information provided by the MC algorithm to longer time scales with a lower computational effort while preserving its accuracy. Additionally, it can be confirmed from another perspective that the choice of the time point for a smooth transition between the two approaches, around 500 ns, is valid.

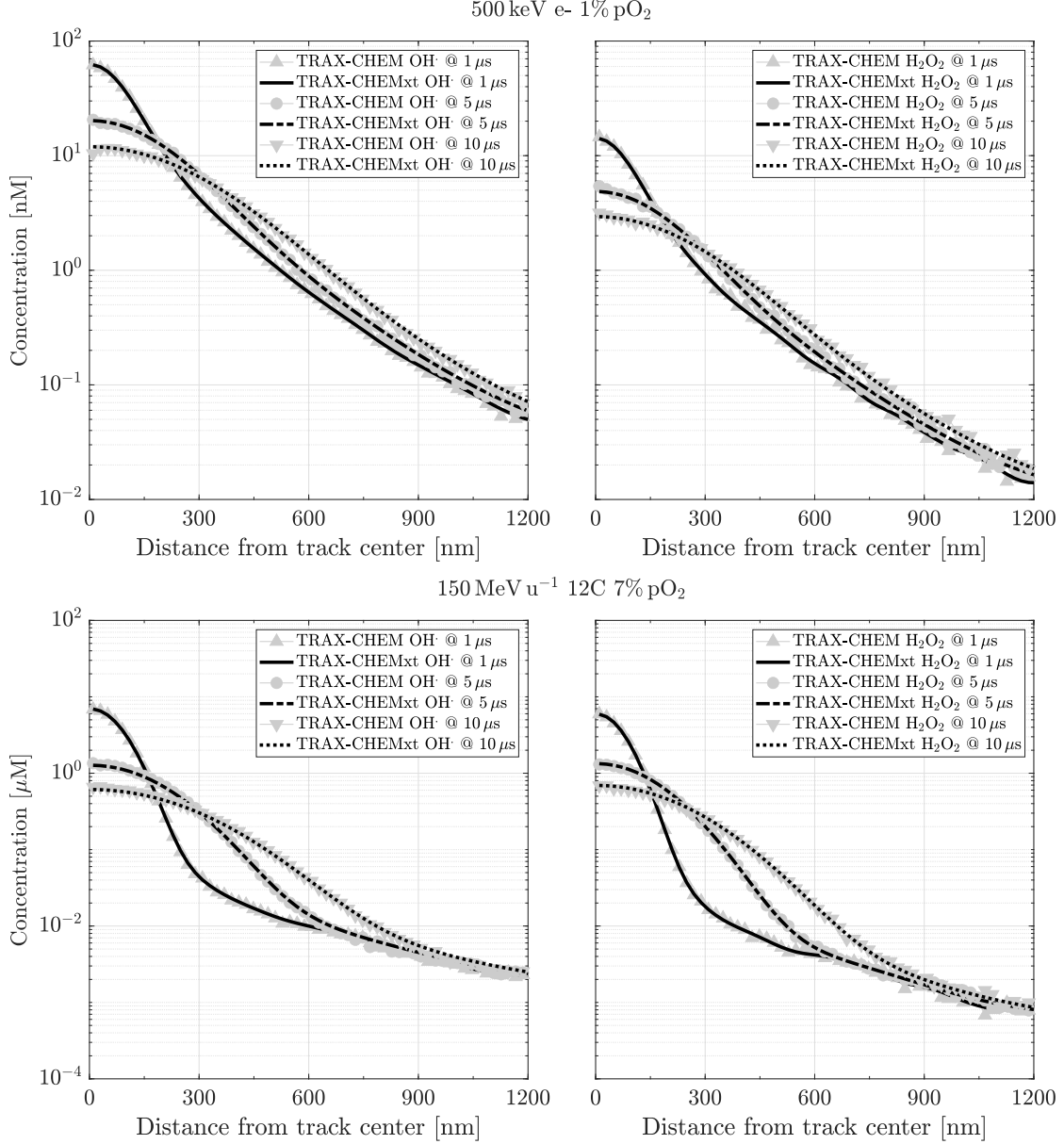


Figure 4.2: Examples of compared radial concentration distributions for OH• (left) and H₂O₂ (right), produced by a 500 keV electron track (top) and a 150 MeV u⁻¹ carbon track (bottom), under oxygenation conditions of, respectively, 1 % and 7%. Initial data taken at $t_{in} = 600$ ns.

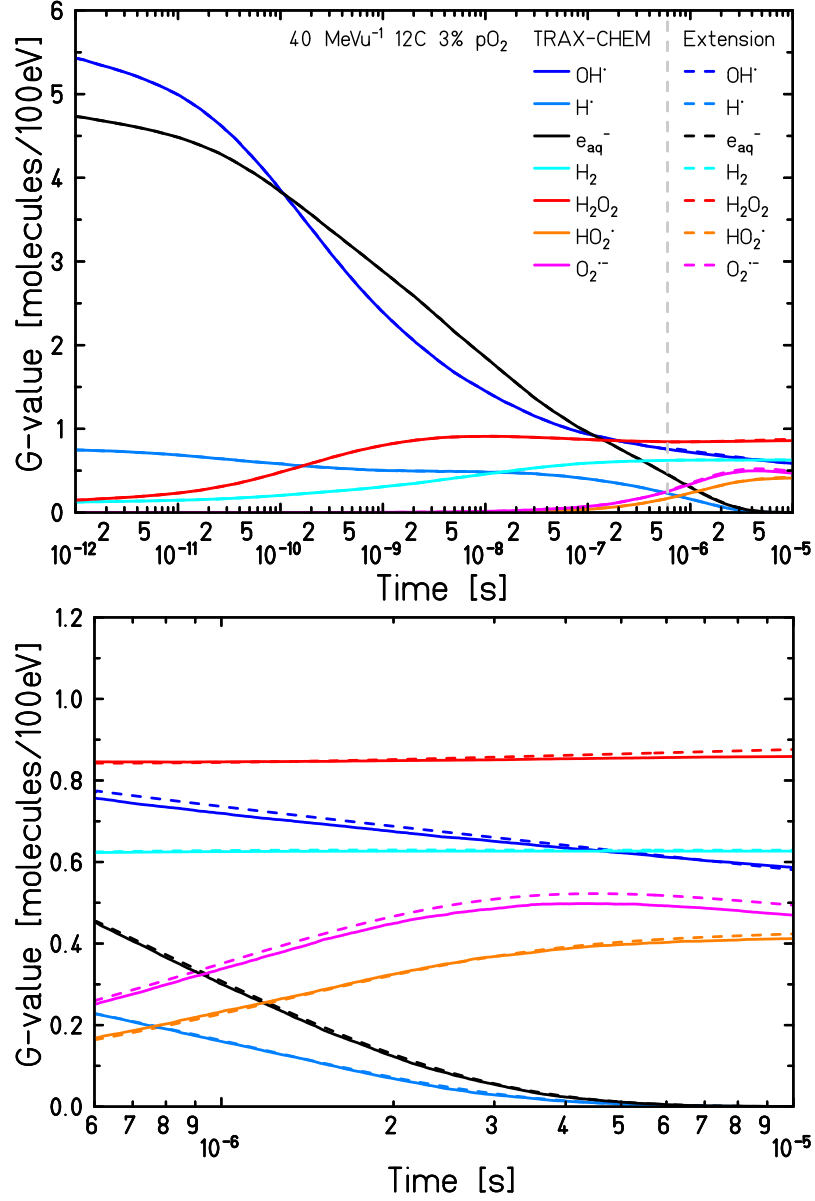


Figure 4.3: Example of compared G-values for some of the species produced by a 40 MeV u⁻¹ carbon track in a water environment with pO₂ = 3 %, up to 10 μs. On the bottom panel, an inset to visualise the differences between the two algorithms within the overlapping time scale 600 ns – 10 μs. Initial data taken at $t_{in} = 600$ ns (grey dashed line).

The TRAX-CHEMxt validity is also tested with a different approach. In Table 4.2, yields at 1 μ s of ($\text{H}^\bullet + \text{e}_{\text{aq}}^-$) in anoxia, and of ($\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$) in 21 % pO_2 are compared, for two low LET radiations: 500 keV electron and 90 MeV proton. Due to the reactions $\text{e}_{\text{aq}}^- + \text{O}_2 \rightarrow \text{O}_2^{\bullet-}$ and $\text{H}^\bullet + \text{O}_2 \rightarrow \text{HO}_2^\bullet$, respectively (xiv) and (xv) in Table 3.1, it is expected that the total number of reactants turns into the respective products when a substantial amount of oxygen is present. The resulting yields agree within 1 %. For completeness, the respective quantities calculated for a higher LET radiation, 90 MeV u^{-1} carbon ion, are also shown. In this case, the discrepancies between the two reaction channels are marginally larger (10 %), due to a change in the kinetics between competing radicals within the track. Indeed, while the conversion rate for $\text{e}_{\text{aq}}^- \rightarrow \text{O}_2^{\bullet-}$ remains constant through all LETs, ≈ 99 %, the result varies for $\text{HO}_2^\bullet$. As far as low LET radiations are concerned, there is at least a 10 % contribution of other reactions in the final hydroperoxyl radical yield, attributed mostly to the channel $\text{H}_3\text{O}^+ + \text{O}_2^{\bullet-} \rightarrow \text{HO}_2^\bullet + \text{H}_2\text{O}$, since conversion rates around 110 % are registered for $\text{H}^\bullet \rightarrow \text{HO}_2^\bullet$. Instead, for the carbon case, a 40 % contribution from other events is recorded (potentially to a minor extent also from $\text{OH}^\bullet$ radicals).

Table 4.2: Yield predictions (molecules/100 eV) of reactants in anoxic conditions, ($\text{H}^\bullet + \text{e}_{\text{aq}}^-$), and the respective products in an oxic environment, ($\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$), for a 500 keV electron track and a 90 MeV proton track at 1 μ s after irradiation. The registered deviations are, respectively, 0.6 % and 1 %. In addition, the quantities for a 90 MeV u^{-1} carbon track are also shown. Initial data taken at $t_{\text{in}} = 500$ ns.

Particle type	pO_2 [atm]	Species	Yield
500 keV e-	0 %	$\text{H}^\bullet + \text{e}_{\text{aq}}^-$	3.19
	21 %	$\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$	3.21
90 MeV H	0 %	$\text{H}^\bullet + \text{e}_{\text{aq}}^-$	3.12
	21 %	$\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$	3.15
90 MeV u^{-1} 12C	0 %	$\text{H}^\bullet + \text{e}_{\text{aq}}^-$	1.40
	21 %	$\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$	1.56

In general, the discrepancies in the total number of species predicted by the two algorithms at 1 μ s never exceed 6 %, as illustrated in Table 4.3, which provides an overview of the differences recorded for all the various scenarios simulated. These represent the uncertainty associated with the novel model. The highest deviations registered, within the same particle quality, relate to high oxygenation levels, with a further increase from low to high LET radiations. This growing disagreement is likely due to different track dynamics featuring the projectiles, originating from the initial time chosen to transfer the information. Furthermore, additional contributions may come from the possible production of secondary electrons (cf. Chapter 6).

Table 4.3: Differences associated with TRAX-CHEMxt, for the various initial conditions simulated. Each deviation is derived by taking the biggest value from the absolute differences between the total number of every radical and molecule predicted by the extension (with initial concentrations taken at 600 ns) and the respective quantities produced by TRAX-CHEM, divided by the latter. To determine these values, contributions from HO_2^- have been disregarded due to their very low yields.

Particle type	Energy	pO ₂ [atm]	Difference at 1 μs
Electrons	500 keV	0 %	1 %
Electrons	500 keV	0.5 %	1 %
Electrons	500 keV	1 %	1 %
Electrons	500 keV	3 %	1 %
Electrons	500 keV	7 %	1 %
Electrons	500 keV	21 %	2 %
Electrons	1 MeV	0 %	1 %
Protons	40 MeV	0 %	1 %
Protons	65 MeV	21 %	2 %
Protons	90 MeV	0 %	1 %
Protons	90 MeV	4 %	1 %
Protons	90 MeV	21 %	2 %
Helium ions	150 MeV u ⁻¹	21 %	2 %
Carbon ions	10 MeV u ⁻¹	21 %	6 %
Carbon ions	20 MeV u ⁻¹	21 %	6 %
Carbon ions	40 MeV u ⁻¹	0 %	4 %
Carbon ions	40 MeV u ⁻¹	3 %	5 %
Carbon ions	40 MeV u ⁻¹	21 %	5 %
Carbon ions	90 MeV u ⁻¹	0 %	4 %
Carbon ions	90 MeV u ⁻¹	0.5 %	4 %
Carbon ions	90 MeV u ⁻¹	2 %	4 %
Carbon ions	90 MeV u ⁻¹	3 %	4 %
Carbon ions	90 MeV u ⁻¹	7 %	5 %
Carbon ions	90 MeV u ⁻¹	21 %	5 %
Carbon ions	150 MeV u ⁻¹	7 %	4 %
Carbon ions	300 MeV u ⁻¹	21 %	3 %

Compared to the pure Monte Carlo SBS approach, with TRAX-CHEMxt a major improvement in the computational effort is registered. A summary of the simulation times (τ) required from both algorithms¹ to obtain the data in Figure 4.2, together with the relative computational gains (CG, more than 3 orders of magnitude), is presented in Table 4.4. The computational gain refers to the physical computing time saved while using TRAX-CHEMxt, in order to reach the same final conditions, due to the spatial concentration distribution simplification. Even if the difference is evident, it must be pointed out that, whereas TRAX-CHEM is a full Monte Carlo code, which on top simulates many primary events, TRAX-CHEMxt is based on a numerical approach and deals with the final averaged result between the different histories computed. The two models are indeed designed to work within two different spatiotemporal regimes. To conclude, regardless of the initial conditions simulated, the extension requires around 30 min to reach 1 ms.

Table 4.4: Computational time required by TRAX-CHEM ($\tau_{\text{TRAX-CHEM}}$) and TRAX-CHEMxt ($\tau_{\text{TRAX-CHEMxt}}$) to obtain the data at 10 μs , from different particle qualities and oxygenation conditions (simulation settings). Furthermore, a computational gain at 10 μs for each scenario is also displayed.

Simulation settings	$\tau_{\text{TRAX-CHEM}}$	$\tau_{\text{TRAX-CHEMxt}}$	CG
500 keV e-, 1 % pO ₂	≈ 12 h	75 s	1369
150 MeV u ⁻¹ 12C, 7 % pO ₂	> 2 d	80 s	2189

ROD validation

TRAX-CHEM does not compute implicitly the oxygen consumed within the different reaction events, as the fluctuations in its initial concentration value are deemed to be irrelevant. However, it does provide all the data (molecules removed and produced) to compute the oxygen depletion, ROD, at the end of the computation. In contrast, the concentration approach implemented in TRAX-CHEMxt enables the calculation of this parameter internally. A comparison of the predicted values from both algorithms can provide additional insights into the robustness of TRAX-CHEMxt. It should be noted that the extension only calculates the ROD where it is defined. Consequently, the depletion value up to the switching time point between the two codes remains

¹About TRAX-CHEM: τ refers to CPU time, averaged over parallel calculations, to compute 500 and 20 events for, respectively, 500 keV electron and 150 MeV u⁻¹ carbon ion tracks. Multithreading was not used, whereas instead trivial parallelisations were performed on a cluster featured by multiple machines with processors from IBM Power8 and above. The RAM exploited was between 2 GB and 8 GB.

to be determined. Examples of the differences registered at $1\ \mu\text{s}$ between the ROD computed from the MC alone and the combination of MC with the extension are shown in Table 4.5. In order to cover a proper range of initial conditions, two very different radiation qualities were considered, i.e., electrons and carbon ions, with as well three distinct oxygenation values representing, respectively, hypoxic (0.5 %), physoxic (5 %), and normoxic (21 %) states.

Table 4.5: ROD differences associated with TRAX-CHEMxt, for various initial conditions simulated. The Monte Carlo concentrations were taken at 600 ns, and the output values were compared at $1\ \mu\text{s}$. Each deviation is derived by taking the absolute difference between the total ROD from the combination of TRAX-CHEM (at 600 ns) and the extension (from 600 ns to $1\ \mu\text{s}$), and the respective one from the MC alone (at $1\ \mu\text{s}$), divided by the latter.

Particle type	Energy	pO ₂ [atm]	Difference at $1\ \mu\text{s}$
Electrons	500 keV	0.5 %	< 1 %
Electrons	500 keV	5 %	< 1 %
Electrons	500 keV	21 %	< 1 %
Carbon ions	90 MeV u ⁻¹	0.5 %	< 1 %
Carbon ions	90 MeV u ⁻¹	5 %	< 1 %
Carbon ions	90 MeV u ⁻¹	21 %	< 1 %

It has to be pointed out that the observed differences are so low because the majority of radiolytic oxygen depletion, particularly at high pO₂, occurs still within TRAX-CHEM. In hypoxic and physoxic conditions, more than 60 % of the total ROD is derived from the MC, regardless of the primary radiation. At extreme oxygenation levels, this contribution reaches upwards of 90 %. Nevertheless, it can be concluded that the extension does not significantly underestimate or overestimate the reactions. It is noteworthy that, even under the most extreme conditions, the oxygen depletion never exceeds 1 % of the initial concentration value. Therefore, the hypothesis assumed in the Monte Carlo computational side, which posited that the changes are insufficient to produce significant effects on the total O₂, is confirmed, ascertaining that there is no need to treat the actual updates at this level, at least under conventional (low) dose rates.

4.2.2 TRACIRT and experiment (phase II)

The TRAX-CHEMxt outcomes have also been validated with the experimental data presented in [109]. For this purpose, yields of $\text{OH}^\bullet$, $(\text{HO}_2^\bullet + \text{O}_2^{\bullet-})$, and H_2O_2 produced by a 500 keV electron track – since the current version of the TRAX code does not allow for simulations of photon radiation – are computed. Electrons are used due to their similar LET (0.2 keV/ μm) with the $\gamma\text{-Co}^{60}$ source. Moreover, as shown by several authors [49, 106], these two radiations result in similar G-values, especially on short time scales, given the former to be under track segment condition, i.e., constant LET within the simulated volume. The chemical yields obtained for a pO_2 of 21 % are summarised in Table 4.6. TRAX-CHEMxt predictions agree well with both the experimental data and another Monte Carlo code exploiting an IRT algorithm, TRACIRT [35].

Table 4.6: Chemical yields (molecules/100 eV) determined at 10 μs in a 21 % oxygenated water environment. “Experiment”: experimental data from [109]; “TRACIRT”: simulation with $\gamma\text{-Co}^{60}$ from [35]; “This work”: values from TRAX-CHEMxt, with 500 keV electrons and initial radial distributions taken from the Monte Carlo at 600 ns.

Species	Experiment [109]	TRACIRT [35]	This work
$\text{OH}^\bullet$	2.7	2.8	2.7
$\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$	3.3	3.4	3.2
H_2O_2	0.67	0.61	0.61

Additionally, some G-value curves have also been compared with those obtained by TRACIRT [35]. Results are shown in Figure 4.4 for a 65 MeV proton track in a 21 % oxygenated water environment. Some discrepancies are registered, already at 1 ps, mainly due to different physical and physico-chemical models adopted by the two codes, as detailed in [14]. These result in distinct spatial distributions of the species produced around the primary radiation, and thus in diverse developments of the reaction network. In addition, substantial differences are present between the resolution algorithms applied to compute the track evolution during the heterogeneous chemical stage. On one side an IRT, and on the other a combination of a SBS and a diffusion-reaction approach with concentration distributions. Overall, the biggest deviations are just on the order of 0.3 molecules/100 eV in the cases of both $\text{OH}^\bullet$ and $\text{HO}_2^\bullet$. Interestingly, when comparing the outcomes from Geant4-DNA [45] and PARTRAC [28], differences in the radical yields were found also by [52], with an even larger discrepancy for the $\text{OH}^\bullet$ species.

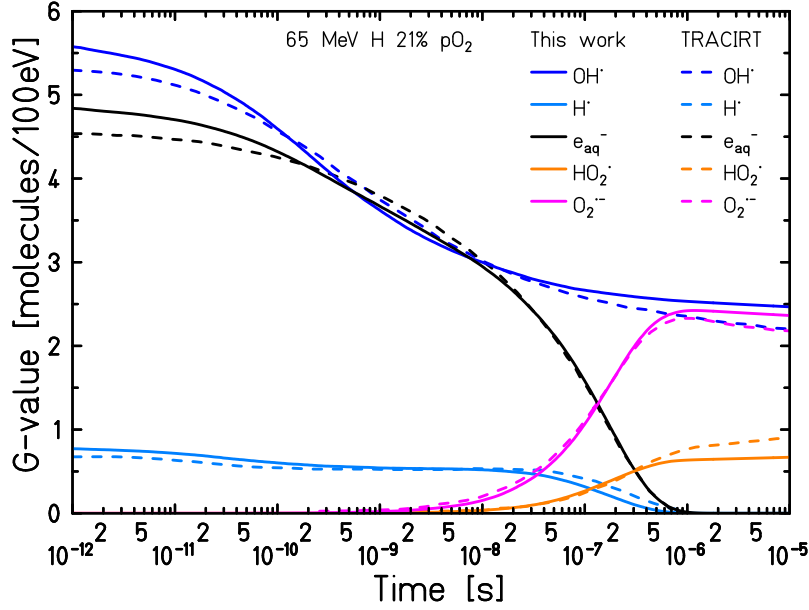


Figure 4.4: G-values comparison of some species produced by a 65 MeV proton track in a water environment with $pO_2 = 21\%$. Solid lines correspond to the results from the combination of TRAX-CHEM and TRAX-CHEMxt, whereas the dashed ones to those from TRACIRT [35]. The extension took over the simulation at 500 ns.

4.2.3 Kinetiscope (phase III)

In this Section, the hypothesis of supposing a fully homogeneous approach from $1\ \mu\text{s}$ onwards to simulate the reaction network, regardless of the initial particle quality, is investigated. To this aim, the predictions of TRAX-CHEMxt are compared with those from Kinetiscope (Version 1.1.1136) [110], an online software based on a fully homogeneous stochastic model to compute the reactions. The kinematics scheme featuring the evolution of the system is determined by a random selection of probability-weighted reaction channels, whereas diffusion events are not considered. Each species is assigned one constant concentration, in order to have a homogeneous composition featuring the reactants in the domain.

To perform the comparison, data from TRAX-CHEM at $1\ \mu\text{s}$ are handed over to both software. For Kinetiscope, the uniform concentrations required are obtained by dividing the number of radicals and molecules by A , the surface around the track, with $A = \frac{1}{\Phi}$ and Φ being the particle fluence (particles/cm²). A conventional dose rate $\dot{\mathcal{D}}$ of $\approx 1\ \text{Gy/min}$ is supposed, and different radiation qualities are tested, with fluences typically applied in standard treatment plans, namely 10^7 particles/cm², 10^9 particles/cm² and 10^{10} particles/cm² for carbon ions, protons and electrons respectively. Starting from these values, fluences at the μs time scale are computed

and then converted into the respective surfaces. At final times, 10 μ s or 100 μ s, the differences between the two algorithms are determined from the number of species predicted. The results of this comparison are shown in Figure 4.5.

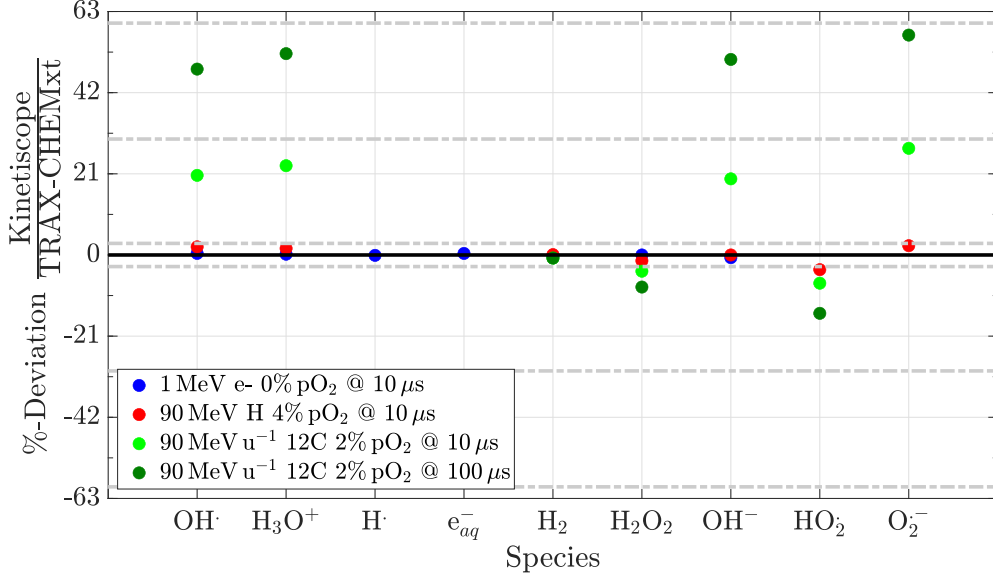


Figure 4.5: Deviations between Kinetiscope and TRAX-CHEMxt, registered for different particle qualities, oxygenation conditions, and final time points. Marked with grey dash-dotted lines are discrepancies of $\pm 3\%$, $\pm 30\%$ and $\pm 60\%$. Initial data handed over to both codes at 1 μ s.

The homogeneous approximation at 1 μ s proves to be valid for low LET radiations, demonstrating at the same time the consistency and reliability of the present code when appropriate conditions are fulfilled. In these cases, the deviations between the two algorithms at 10 μ s do not reach 1 % for 1 MeV electrons, whereas a maximum of 4 % is registered for a 90 MeV proton track. However, as both the LET and the final recording time increase, so does the disagreement. For a 90 MeV u⁻¹ carbon track, a maximum discrepancy of $\approx 30\%$ is registered at 10 μ s, approaching almost 60 % at 100 μ s. This is a strong indication that, to reliably simulate a wider range of initial conditions, the species track structure dependence needs to be explicitly accounted for, still at the μ s time scale but in a less “localised” fashion. TRAX-CHEMxt does so through the use of radial concentration distributions, while fully homogeneous codes can not manage the description under these conditions. This holds especially for intermediate and high LET radiations, where the (local) steady states for the radicals and molecules are still not fully reached at the end of the heterogeneous chemical stage [43]. Lastly, to ensure that both software yield identical results in a reaction-limited domain (i.e., also TRAX-CHEMxt under phase III conditions), a “dummy”

case with uniform concentrations randomly assigned to each chemical species, in a water environment with $pO_2 = 1\%$, is presented in Figure 4.6. Within this homogeneous assumption for the radicals and molecules, all the deviations registered are below 1%, as expected.

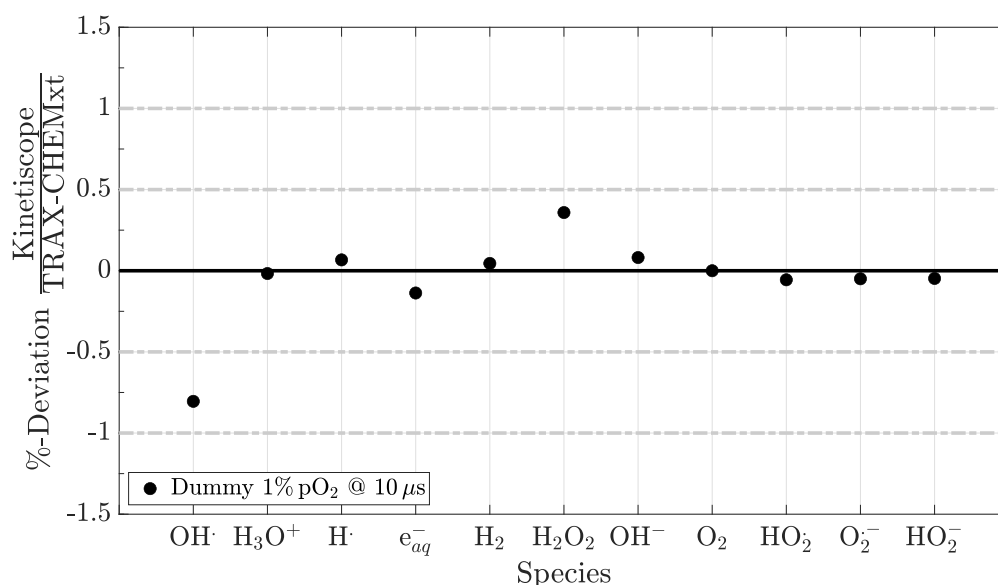


Figure 4.6: Deviations between Kinetiscope and TRAX-CHEMxt, registered for a “dummy” case with uniform concentrations assigned randomly to each chemical species, in a water environment with $pO_2 = 1\%$. Marked with grey dash-dotted lines are discrepancies of $\pm 0.5\%$ and $\pm 1\%$. Initial data handed over to both codes at $1\ \mu s$, and the chemical network in this reaction-limited domain was simulated up to $10\ \mu s$. Values for O_2 and HO_2^- have been included for the sake of completeness.

4.3 Biological environment – comparison with TRAX-CHEM

Following the exhaustive examination performed in a water environment, the subsequent phase of the investigation entails the validation of the proposed extension within the context of a biological framework. The objective is still to demonstrate the reliability of the method in conveying information regarding the diverse chemical species, regardless of the primary particle qualities, LETs, and oxygenation levels. This is accomplished by monitoring the fluctuations in concentration distributions, G-values, and radiolytic depletion of all three scavengers. Due to the more complex milieu simulated, to the relative change in its kinetics, and to leave the system some more time to achieve the local homogeneity within each bin of the concentration distributions, it was decided that the information transition time between the algorithms, t_{in} , should be increased to 1 μ s.

Before presenting the results, it is worth highlighting one point. Differences arose in the comparison with TRAX-CHEM for both R_2 and ROOR. This is probably attributed to the “condensing” of the species distribution along the longitudinal direction, resulting, as the time passes by, in a higher impact in the TRAX-CHEMxt resolution. This effect is more pronounced for those species present in a very low amount, since the differences between the “quantised/discrete” and “continuous” approaches become more evident. In fact, while from the MC side once a species reacts it is removed, for the extension, due to the concentrations representation, small contributions (from a “fraction” of a radical/molecule) can still occur after the species has reacted. Anything which is much below a count of 1 can be reflected in TRAX-CHEM only with great difficulty, being it a discrete code. On top, also for a continuous numerical code small numbers which arise from an improbable reaction out of a large reactant pool has a considerably higher uncertainty. In the end, it is similar to looking at some tiny flakes of a molecule in a pixelated volume. While monitoring the respective distributions, the highest differences arose for the bins closer to the track center. Since in TRAX-CHEM there is no “equilibrium” at the borders of the target along the longitudinal direction (meaning, from the chemical point of view, that the species going out are not balanced by others coming in), the track density and the related reactions will be affected, altering the dynamics of the entire network. It is clear that a species has a higher chance of reacting with the others produced by the track if it is kept within the target, rather than if it is let go outside of it, due to different local densities encountered. However, this difference became evident only when dealing with R_2 and ROOR, which are featured by very low numbers: G-values $\leq 10^{-3}$ molecules/100 eV, representing less than one actual radical/molecule, that has practically no meaning, it is essentially 0. Therefore, their deviations were not accounted for in the final validation assessment. Even if TRAX-CHEM underestimates

the concentration of R_2 , whereas the extension manages to keep track of the $R^\bullet$ reacting with themselves, the final total amount of species actually produced, in both scenarios, are too small to address possible bugs, if any, in the codes. On the other hand, the yields related to the most important final species, ROOH, that actually derives from a series of reactions: $R^\bullet \rightarrow ROO^\bullet \rightarrow ROOH$, always resemble the MC ones, with fluctuations $< 4\%$.

To gain insights into the maximum fluctuations deriving from the implementation of the new biological environment, a 500 keV electron track and a 90 MeV u^{-1} carbon track were examined, irradiating a water target at two extreme oxygenation levels, respectively 0.5 % and 21 %. All final G-values show differences below 4 % with respect to the Monte Carlo counterparts, as documented in Table 4.7.

Table 4.7: Differences associated with TRAX-CHEMxt, for various initial conditions simulated. Each deviation is derived by taking the biggest value from the absolute differences between the total number of every radical and molecule predicted by the extension (with initial concentrations taken at 1 μs) and the respective quantities produced by TRAX-CHEM, divided by the latter. To determine these values, contributions from HO_2^- , R_2 and ROOR have been disregarded due to their very low yields.

Particle type	Energy	pO ₂ [atm]	Difference at 1.5 μs
Electrons	500 keV	0.5 %	3 %
Electrons	500 keV	21 %	1 %
Carbon ions	90 MeV u^{-1}	0.5 %	4 %
Carbon ions	90 MeV u^{-1}	21 %	4 %

In Figure 4.7 concentration distributions from TRAX-CHEMxt saved at 1.5 μs and obtained under the same four cases analysed in Table 4.7 are compared with the respective TRAX-CHEM ones. Good agreements are registered for all chemical species, regardless of the initial conditions simulated. Note that, in principle, high concentrations are produced also for $XS^\bullet$, but since no further reactions are considered for it, no volume effects are recorded, unlike R_2 and ROOR that derive from $R^\bullet$.

Lastly, the radiolytic depletions of the three different scavengers were also monitored. As observed in the water environment, all three showed excellent accordance, as demonstrated in Table 4.8. Since both O_2 , RH and XSH are present everywhere in TRAX-CHEM, they are not affected by volume issues.

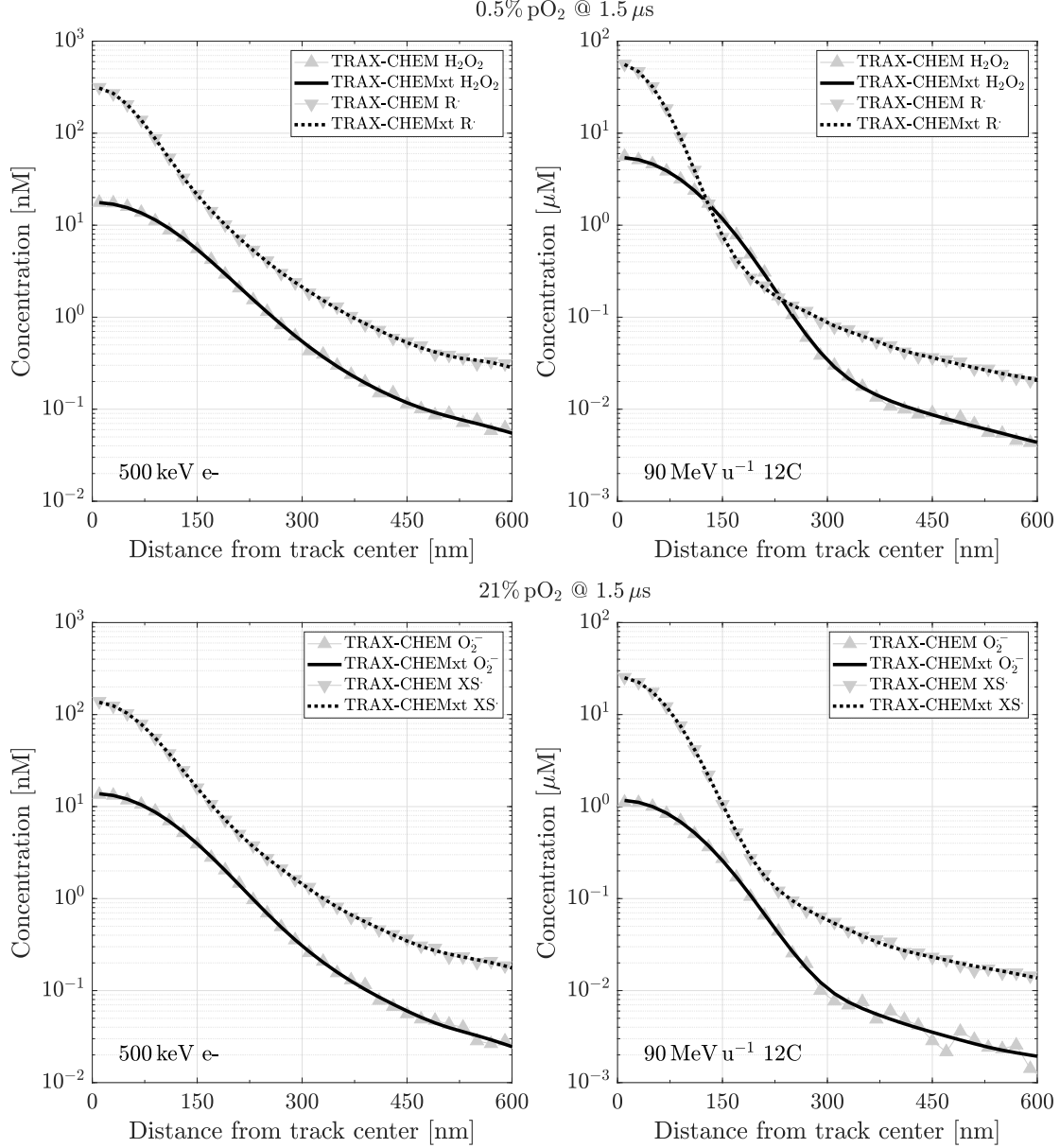


Figure 4.7: Examples of compared radial concentration distributions for H₂O₂, R[•], O₂^{•-}, and XS[•], produced by a 500 keV electron track (left) and a 90 MeV u⁻¹ carbon track (right), under oxygenation conditions of, respectively, 0.5 % (top) and 21 % (bottom). Initial data taken at $t_{in} = 1 \mu s$, and final distributions exported at 1.5 μs.

Table 4.8: Summary of the maximum radiolytic depletions differences associated with TRAX-CHEMxt, for the three scavengers present in the environment, i.e., O₂, RH, and XSH. The Monte Carlo concentrations were taken at 1 μ s, and the output values were compared at 1.5 μ s. Each deviation is derived by taking the absolute difference between the total computed scavenger’s depletion from the combination of TRAX-CHEM (at 1 μ s) and the extension (from 1 μ s to 1.5 μ s), and the respective one from the MC alone (at 1.5 μ s), divided by the latter.

Scavenger	Difference at 1.5 μ s
O ₂	< 1 %
RH	< 1 %
XSH	< 1 %

It can therefore be concluded that both TRAX-CHEM and TRAX-CHEMxt can simulate a greater number of scavengers. Furthermore, there is evidence of an overall good data transition between the codes, with a preserved level of accuracy by the extension. Once again, the intermediate phase (phase II) plays a pivotal role in ensuring that information regarding concentration dependencies on the distance from the track center are maintained, before all species become uniformly distributed. For instance, considering the electron cases, by assigning a threshold value of 5 % (cf. Section 3.1.3), the registered time at which all distributions got uniform was around 10 ms. The possibility to keep monitored the track structure dependence, in turns, enables the formulation of a comprehensive description of the chemical dynamics, encompassing a wide range of radiation and environmental conditions. It should be noted that, if the approach applied by the novel algorithm yields the reported uncertainties, the application of a fully homogeneous algorithm from 1 μ s onwards would be featured by even larger fluctuations. At this point, longer time scales can be investigated, to study how the chemical yields develop during the biochemical stage of radiation damage.

5. Results

This Chapter presents the results obtained by exploiting the full capabilities of the novel TRAX-CHEMxt algorithm, which enables both probing longer computational time scales, and simulating a more complex biological environment. Furthermore, comparisons of some predictions with experimental data are presented as well, to prove the algorithm's reliability also at later times. The uniqueness and full potential of the code are demonstrated, through its ability to deal with intermediate and high LET radiation chemistry. Similarly to Chapter 4, two distinct Sections are delineated, depending on whether a water environment, in Section 5.1, or a biological milieu, in Section 5.2, is under investigation.

5.1 Water environment

5.1.1 Chemical track evolution at the ms time scale

Longer time scales are probed, to investigate the yields development of those radicals and molecules that escape the (strong recombination dynamics of the) heterogeneous chemical stage. For simplicity, the species are here grouped into three different categories, subdivided as follows. The first one (i) represents the typical direct products of water radiolysis ($\text{OH}^\bullet$, H_3O^+ , $\text{H}^\bullet$ and e_{aq}^-). The second (ii) refers to the intratrack recombination molecules (H_2 , H_2O_2 and OH^-), whereas the last category (iii) accounts for the products of the reactions with oxygen dissolved in the target medium ($\text{HO}_2^\bullet$, $\text{O}_2^{\bullet-}$ and HO_2^-). Figure 5.1 shows the radial distributions of these quantities, produced by a 40 MeV u^{-1} carbon track up to 1 ms and under two different pO_2 values, 0 % and 3 %. The fluctuations at 600 ns are due to the intrinsic statistical fluttering of the species when imported from TRAX-CHEM in a histogram format. In both oxygenation conditions, the concentrations approach a flat value only at later stages, approximately around 1 ms, whereas at 100 μs they still exhibit a radial dependence. As time passes, the concentrations of the first category radicals (i) decrease, in favour of an increase in the products from intratrack (ii) and oxygen (iii) reactions.

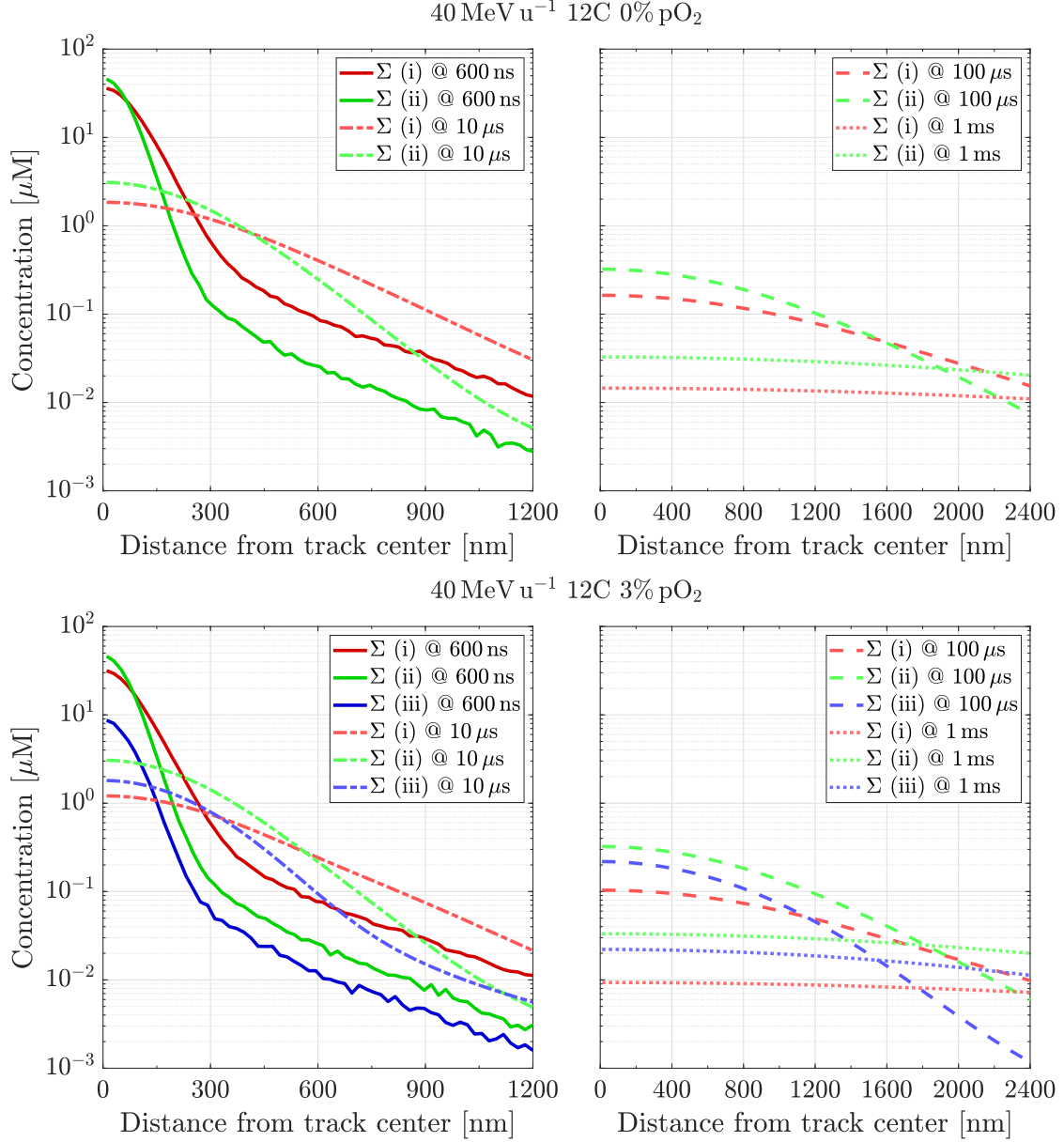


Figure 5.1: Sum distributions, at different time points, of the three species categories produced by a 40 MeV u⁻¹ carbon track in 0% pO₂ (top) and 3% pO₂ (bottom) water targets. Category (i): OH[•], H₃O⁺, H[•] and e_{aq}⁻; category (ii): H₂, H₂O₂ and OH⁻; category (iii): HO₂[•], O₂^{•-} and HO₂⁻. Initial data taken at $t_{in} = 600$ ns.

The G-values of the species produced from the aforementioned irradiation and environmental conditions are displayed in Figure 5.2. The number of intratrack products increases as time passes. Moreover, in oxygenated conditions, $\text{H}^\bullet$ and e_{aq}^- are fully consumed at earlier times, as already observed in [17, 35]. While at 0% pO_2 hydrogen peroxide seems to have reached a steady state, at 3% its G-value rises with time. A slight increase could be caused by the rearrangement of the information from TRAX-CHEMxt itself (cf. Chapter 6). However, a similar trend was observed with the standard version of the TRAX-CHEM code when running up to 10 μs , suggesting a direct involvement of the reaction network (Table 3.1). The main channel responsible for the H_2O_2 production is $\text{OH}^\bullet + \text{OH}^\bullet \rightarrow \text{H}_2\text{O}_2$. In anoxic conditions, this competes with $\text{OH}^\bullet + \text{e}_{\text{aq}}^- \rightarrow \text{OH}^-$. When oxygen is present in the environment, the majority of hydrated electrons react with O_2 to produce $\text{O}_2^{\bullet-}$, leaving more hydroxyl radicals the chance to recombine and generate H_2O_2 . The other reactions involved in the production of hydrogen peroxide, namely $\text{H}^\bullet + \text{HO}_2^\bullet$, $\text{H}_3\text{O}^+ + \text{HO}_2^-$ and $\text{HO}_2^\bullet + \text{HO}_2^\bullet$, have been shown to have a negligible effect, at least up to 1 ms (cf. Section 5.1.4). Overall, the species that were thought to have reached a “saturation” level at 1 μs , are instead still registering some changes in their yields around this time scale.

5.1.2 LET impact on chemical yields

Figure 5.3 compares the impact of two very different LET values, respectively a low one, 1 keV/ μm , and a high one, 160 keV/ μm , on the radial distributions of the three different categories introduced previously, in a water target at 21% oxygenation. The chemical species concentrations increase with LET, due to the larger energy deposited within the volume of interest. In particular, for the higher LET radiation, a larger production of intratrack molecules (ii) is observed at all time points, suggesting a more frequent recombination of radicals during the early stages of the chemical evolution. For the lower LET one, instead, a predominance of primary radicals (i) and products from reactions with oxygen (iii) is registered. At 100 μs , the average molecules' concentration (ii) seems to scale with LET, passing from a value of approximately 4 nM for $\text{LET} = 1 \text{ keV}/\mu\text{m}$, to $\approx 700 \text{ nM}$ for $\text{LET} = 160 \text{ keV}/\mu\text{m}$. At the same time, whereas for the higher LET radiation, the distance from the track center at which these species (ii) exceed the radicals (i) is quite low ($< 200 \text{ nm}$), this crossing point is absent for the lower LET case. Whilst similar trends, during the heterogeneous chemical stage, were already pointed out in previous works [11, 17], the present findings are showing the significant persistence of the LET impact at longer times.

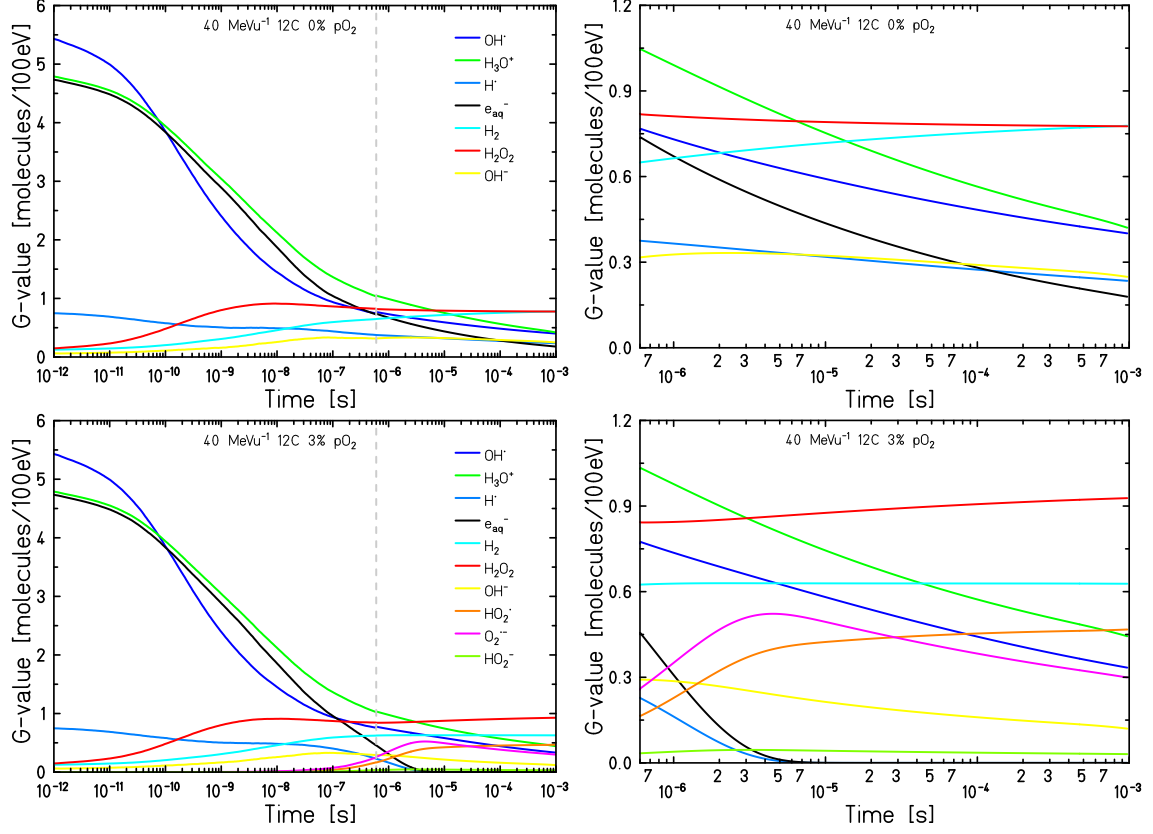


Figure 5.2: G-values up to 1 ms of the species produced by a 40 MeV u^{-1} carbon track in 0 % pO_2 (top) and 3 % pO_2 (bottom) water targets. On the right side, details between 600 ns and 1 ms of the curves presented on the left side are reported. Initial data taken at $t_{in} = 600$ ns (grey dashed lines).

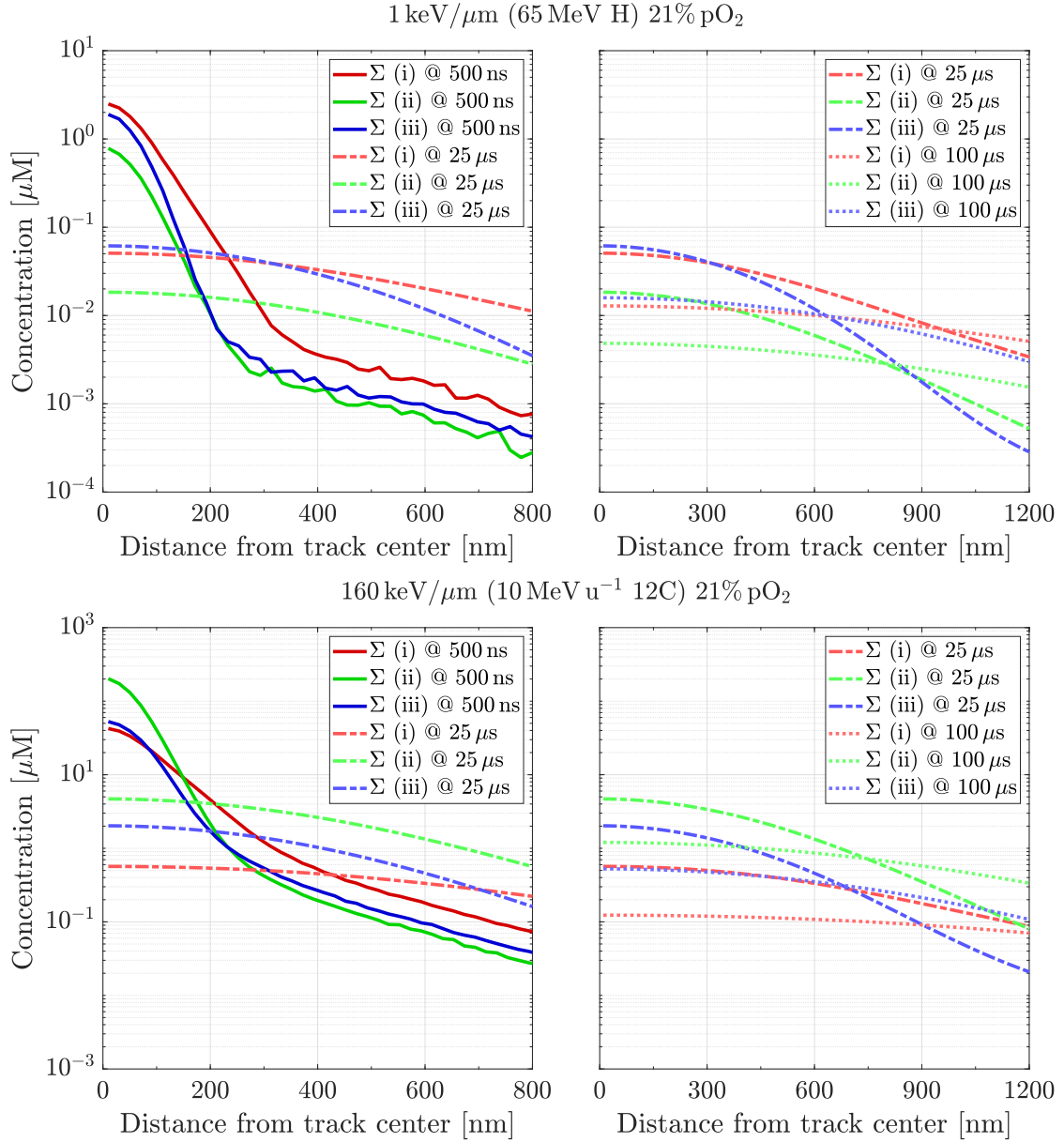


Figure 5.3: Distribution sums, at 500 ns, 25 μs and 100 μs , of the three species categories for two extreme LET values, respectively 1 keV/ μm (from a 65 MeV proton track, top) and 160 keV/ μm (from a 10 MeV u⁻¹ carbon ion track, bottom), under pO₂ = 21 %. Category (i): OH $\cdot$, H₃O⁺, H $\cdot$ and e_{aq}⁻; category (ii): H₂, H₂O₂ and OH⁻; category (iii): HO₂ $\cdot$, O₂ $\cdot$ ⁻ and HO₂⁻. Initial data taken at $t_{in} = 500$ ns.

The cumulative G-values computed at different time points as a function of LET are shown in Figure 5.4. Despite the absolute number of species produced grows with LET, their respective G-values decrease, because the normalisation factor related to the energy deposited in the target increases as well. Besides, recombinations of the chemical species such as $\text{OH}^\bullet + \text{OH}^\bullet \rightarrow \text{H}_2\text{O}_2$ are more pronounced, due to track density effects. The overall G-value for very low LET radiations ($< 1 \text{ keV}/\mu\text{m}$) changes only slightly over time, at least in the frame investigated, with a ratio between the amounts at 25 μs and 500 ns close to 1. On the contrary, as the LET increases, so do the divergences between the two time points, registering a decrease of almost 20 % under high LET domains. This suggests that the time at which the reaction network dynamics stabilises is strongly LET-dependent. It can be also inferred that the choice of t_{in} is indeed relevant for these radiations, with 500 ns being at the acceptability border, by noticing how much the G-values drop between 500 ns and 25 μs .

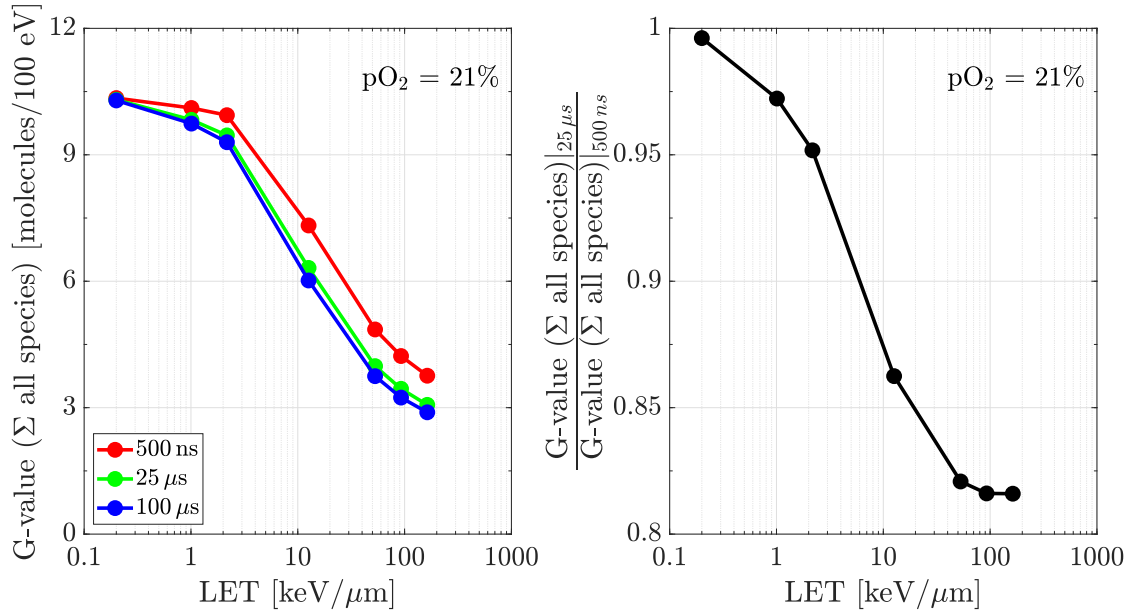


Figure 5.4: (left) G-values of all the species produced by different LET radiations, recorded at 500 ns, 25 μs and 100 μs . (right) Ratios between the G-values at 25 μs and 500 ns. The environment was kept at an oxygen level of 21 %, with initial data taken at $t_{in} = 500 \text{ ns}$.

5.1.3 Oxygenation impact on chemical yields

G-values for the major products of reactions with O_2 , $\text{HO}_2^\bullet$ and $\text{O}_2^{\bullet-}$, are reported in Figure 5.5 for two LET radiations, respectively $0.2 \text{ keV}/\mu\text{m}$ and $28 \text{ keV}/\mu\text{m}$, as a function of environment oxygenation and at three time points: 500 ns, 25 μs and 100 μs .

Two “saturation” levels are registered in the time frame studied, 3.2 molecules/100 eV and 1.4 molecules/100 eV, respectively for the lower and the higher LET projectile. These values are reached at 25 μ s, with the exception of minor deviations for very low oxygenation conditions (saturation attained for $pO_2 > 3\%$). Furthermore, the species amounts increase differently depending on the particle quality. Under the same (small) pO_2 , for the low LET radiation the G-values grow by almost 16 times when moving from 500 ns to 25 μ s, whereas those for the higher LET one increase by a factor 11. As the oxygenation grows, these increments diminish, and the difference between the two particle types attenuates as well.

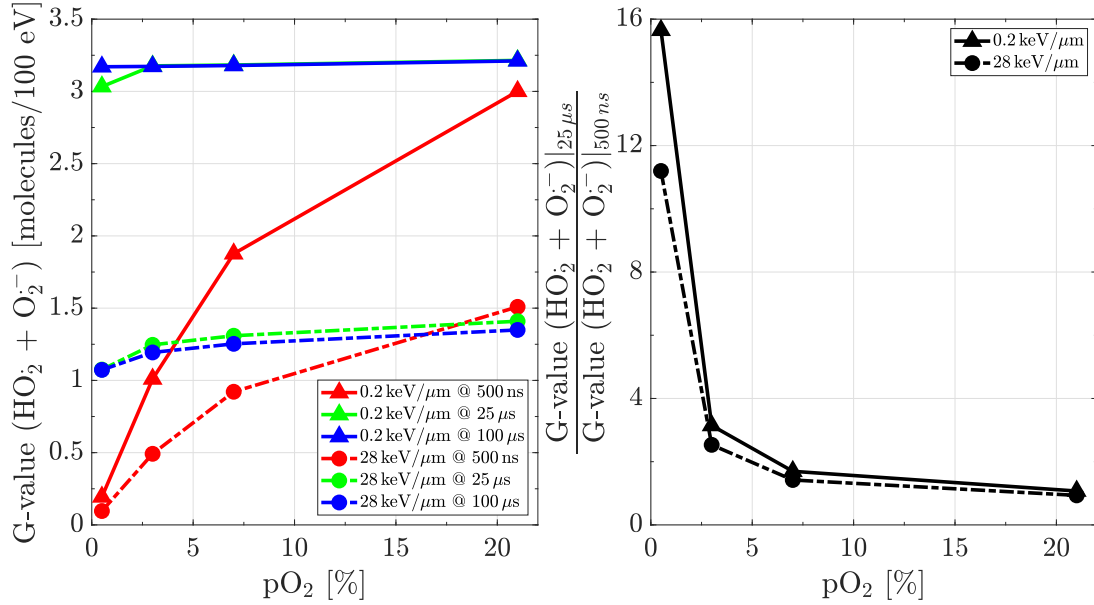


Figure 5.5: (left) G-values of $(HO_2\cdot + O_2\cdot^-)$ produced by radiations with LET of 0.2 keV/ μ m and 28 keV/ μ m, recorded at 500 ns, 25 μ s and 100 μ s, as a function of pO_2 . (right) Ratios between the G-values at 25 μ s and 500 ns. Initial data taken at $t_{in} = 500$ ns.

In Figure 5.6, distributions for the couple $(HO_2\cdot + O_2\cdot^-)$ are reported, for both ionising radiations considered above, at oxygenation levels of 0.5 %, 3 %, 7 %, 21 %, and at three time points: 500 ns, 25 μ s, 100 μ s. As the pO_2 increases, the concentration fluctuations at the initial time (500 ns) decrease, due to the higher number of products present. At 100 μ s and under $pO_2 = 21\%$, the distribution for the low LET radiation tends to become uniform at approximately 1 nM, whereas the one from the higher LET does so at around 100 nM. For both particle types, even if at 500 ns different concentrations are registered depending on the initial oxygenation, at longer times the distributions tend to stabilise to similar values, independently of pO_2 .

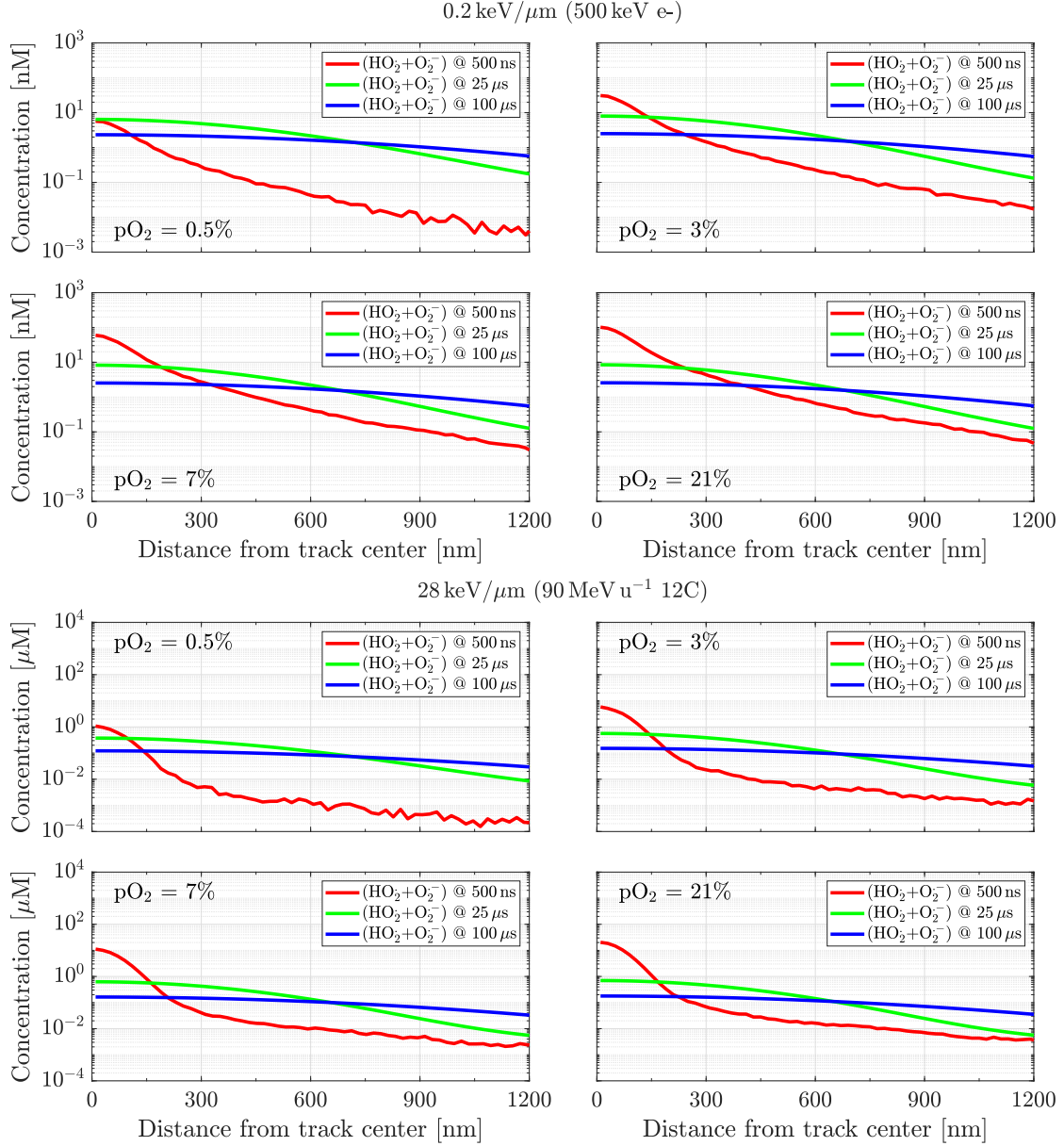


Figure 5.6: Effects of target oxygenation on the concentration distributions of $(\text{HO}_2 + \text{O}_2^-)$, recorded at 500 ns, 25 μs and 100 μs, for two LET values, respectively $0.2 \text{ keV}/\mu\text{m}$ (from a 500 keV electron track, top) and $28 \text{ keV}/\mu\text{m}$ (from a 90 MeV u^{-1} carbon ion track, bottom). Initial data taken at $t_{in} = 500 \text{ ns}$.

5.1.4 Comparison with experiments

In light of the scarcity of experimental data pertaining to long term chemical yields produced in a water target by ionising radiations [43], coupled with the inherent limitations associated with initial conditions than can be simulated, such as treatment of low dose rates and constant LET values, the benchmark of TRAX-CHEMxt with experiments represents a significant challenge. As documented in [42], the discrepancies between yields generated under track average and track segment conditions can be considerable at long times. Only when the initial particle energy approaches zero, or when a specific track averaged yield is energy independent, then a satisfactory resemblance can be achieved. It is generally expected that molecular G-values produced by a constant LET will be larger than those resulting from the same radiation quality and LET, but obtained under a track average condition instead [41]. To understand this concept, consider the proton case that will be presented in details afterwards. In order to achieve the same LET as that reported for the experimental case (under a track average condition, with a beam energy of 5 MeV), the energy assigned to the primary projectile computed under a track segment simulation set-up was 1.5 MeV. In the former scenario, the effects on the various species produced are derived from all different energy layers, with all given equal weight. Consequently, there will invariably be a greater contribution from lower LET components with respect to the track segment one, resulting in a net reduction of the molecular yields. This Section presents a comparative analysis between three experimental data/observations and TRAX-CHEMxt predictions, each of which features a distinct radiation quality simulated under track segment conditions: γ /e-, H, 12C.

Regarding both photon/electron and proton experiments, the experimental results have been extrapolated from Roth and LaVerne [111], with the G-value of H_2O_2 at long time scales as the final endpoint. The recording time at which the species are reported depends on the dose rate employed. Values around few Gy/s were delivered, therefore a time scale around 15 minutes is deemed to be sufficient for saving the computational yields from TRAX-CHEMxt. As far as the simulations are concerned, the approximation moved consisted to deal with projectiles whose energies were sought such that their respective track segment LETs corresponded to the track average ones associated with the relative experimental beams. The uncertainties for the final computed values were derived from a quadratic summation between the novel algorithm ones, extrapolated from Table 4.3, and the MC ones, stemming from statistical considerations (i.e., standard error determined from all the independent tracks simulated). As anticipated, overestimation from theoretical predictions are expected. Apart from differences between the initial conditions considered, a further cause of discrepancy can be due to the few reactions implemented [43], since low reaction rate coefficients featuring other possible channels were disregarded. As will be demonstrated, and as also postulated by [43, 111], higher-order reactions, char-

acterised by relatively low values of κ , may become relevant at longer time scales. Moreover, another difference comes from the acid-base equilibrium in the experimental environment. The set-up is at $\text{pH} = 7$, and since the acid dissociation constant¹ for the pair $\text{HO}_2^\bullet/\text{O}_2^{\bullet-}$ is 4.9 [55], some of the hydroperoxyl radicals actually convert into their respective conjugated base. This mechanic is not yet implemented in TRAX-CHEMxt, therefore a certain overestimation of $\text{HO}_2^\bullet$ is expected, with a consequently larger production of hydrogen peroxide via $\text{HO}_2^\bullet + \text{HO}_2^\bullet \rightarrow \text{H}_2\text{O}_2$.

Starting with the photon case, in the experimental set-up [111] a $\gamma\text{-Co}^{60}$ source was employed for the irradiation of a fully aerated environment. In a similar fashion as in Section 4.2.2, a 500 keV electron track in an oxygenated target ($\text{pO}_2 = 19\%$) was simulated for the chemical yields comparison, due to the similar LET, $0.2 \text{ keV}/\mu\text{m}$. Figure 5.7 summarises both the radicals' and molecules' trends predicted by TRAX-CHEMxt, as well as the experimental data reported by Roth and LaVerne.

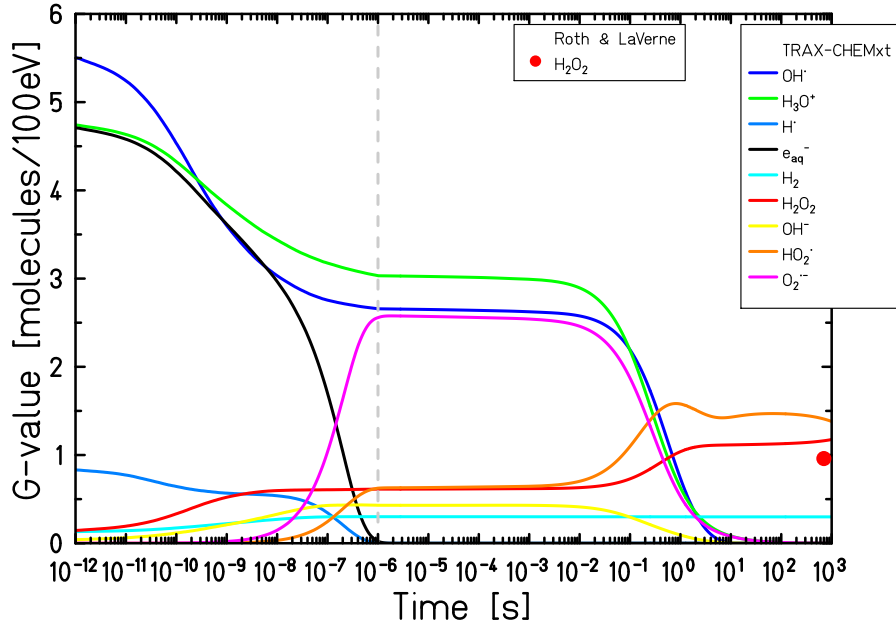


Figure 5.7: H_2O_2 yields comparison between the experimental data from Roth and LaVerne [111], and the theoretical prediction from TRAX-CHEMxt. Whereas the former employed a $\gamma\text{-Co}^{60}$ source, for the computation a 500 keV electron track in a water environment with $\text{pO}_2 = 19\%$ was simulated, due to their similar LET, around $0.2 \text{ keV}/\mu\text{m}$. Initial data taken at $t_{in} = 1 \mu\text{s}$ (grey dashed line). The uncertainty of the experimental value is smaller than the symbol and therefore not visible.

¹Value representing the acidity of the environment at which equilibrium is reached between the acid and its respective conjugated base. A pH below the aforementioned constant favours the production of the acid, whereas larger ones facilitate the decomposition into the base.

$G\text{-value}(\text{H}_2\text{O}_2)_{\text{exp}} = (0.96 \pm 0.05)$ molecules/100 eV is the final experimental yield, whereas $G\text{-value}(\text{H}_2\text{O}_2)_{\text{theo}} = (1.2 \pm 0.2)$ molecules/100 eV is the theoretical one. Differences are mostly due to the two radiation qualities. From the physical perspective, $\gamma\text{-Co}^{60}$ is featured by two sharp energy lines, one at 1.17 MeV and the other at 1.33 MeV. Moreover, even if two photon sources are compared, such as $\gamma\text{-Cs}^{137}$ and $\gamma\text{-Co}^{60}$, their differences in energy, approximately 600 keV for the former and 1.2 MeV for the latter, and LET, respectively 0.4 keV/ μm against 0.2 keV/ μm , cause small changes in final H_2O_2 yields [12]. In general, the two values exhibit a good compatibility.

For the proton case, the system under track segment condition was simulated since the total range to be considered for the track average scenario would have been too computationally demanding: more than 36 μm , against the standard (5 – 10) μm usually treated for this particle quality. The water environment was still fully aerated, with 19 % pO_2 . From the experiment point of view [111], a 5 MeV proton beam, with track average LET = 20.7 keV/ μm (from [112]) was employed. On the other hand, a 1.5 MeV proton track with a constant LET of 19.7 keV/ μm was simulated. The final yields are reported in Figure 5.8.

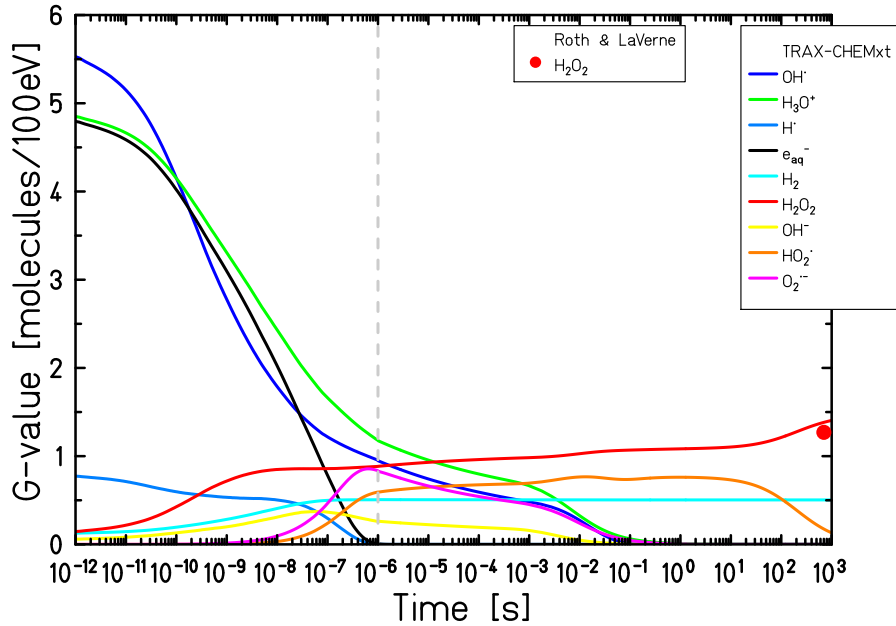


Figure 5.8: H_2O_2 yields comparison between the experimental data from Roth and LaVerne [111], and the theoretical prediction from TRAX-CHEMxt. Whereas the former employed a 5 MeV H beam, for the computation a 1.5 MeV proton track in a water environment with $\text{pO}_2 = 19\%$ was simulated, due to their similar LET, around 20 keV/ μm . Initial data taken at $t_{\text{in}} = 1 \mu\text{s}$ (grey dashed line). The uncertainty of the experimental value is smaller than the symbol and therefore not visible.

In this case the final yields are $G\text{-value}(\text{H}_2\text{O}_2)_{\text{exp}} = (1.27 \pm 0.07)$ molecules/100 eV for the experiment, and $G\text{-value}(\text{H}_2\text{O}_2)_{\text{theo}} = (1.40 \pm 0.04)$ molecules/100 eV for TRAX-CHEMxt. From the simulation curve, it is interesting to notice that the initial increase in hydrogen peroxide is due to hydroxyl radical recombination, whereas the further rise at longer times comes from the reactions between two $\text{HO}_2^\bullet$. This chemical dynamics lead to the full consumption of hydroperoxyl radicals, and at the same time to a saturation plateau for H_2O_2 (long-term chemistry, [111]). Also in this case, the two values exhibit reasonable agreement.

Lastly, for the carbon case the situation was a bit different. Experimental observations [12, 44, 111, 112] report decrease in the G-values of H_2O_2 after an average LET of 100 keV/ μm , due to intratrack reactions. However, these experimental scenarios are featured by too high LET values, resulting in a prohibitive computational time for simulating the heterogeneous chemical phase. Therefore, a qualitative test was performed instead, with a 15 MeV u^{-1} carbon ion track, under track segment conditions ($\approx 115 \text{ keV}/\mu\text{m}$) in a 0% pO_2 water environment. The final yields are shown in Figure 5.9.

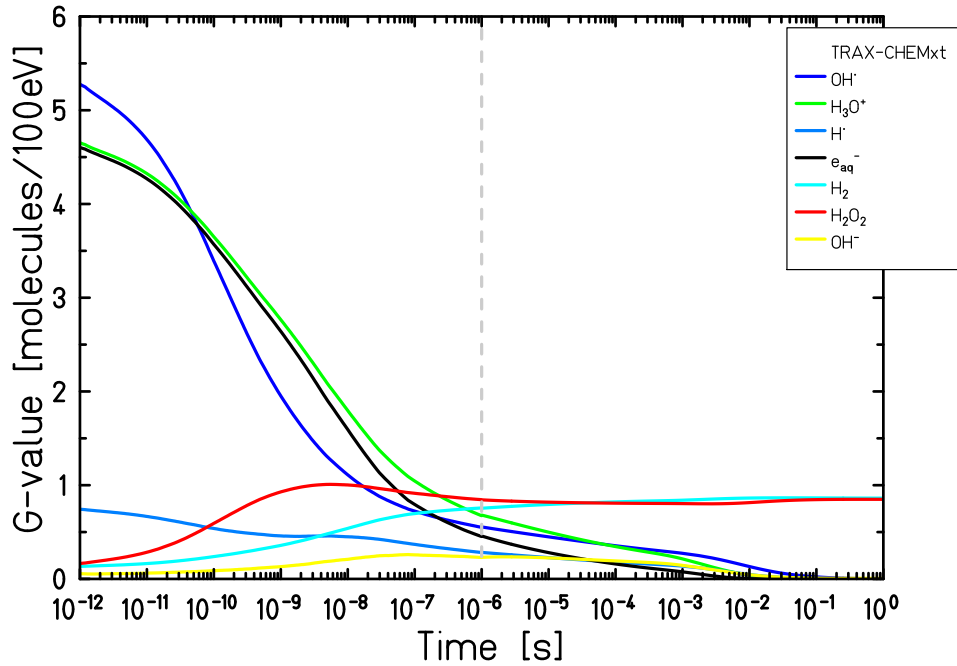


Figure 5.9: H_2O_2 yield trend predicted by TRAX-CHEMxt, for a 15 MeV u^{-1} carbon ion track (LET $\approx 115 \text{ keV}/\mu\text{m}$) in a water environment with $\text{pO}_2 = 0\%$. Initial data taken at $t_{\text{in}} = 1 \mu\text{s}$ (grey dashed line).

The theoretical track model calculation helps to understand the chemical dynamics featuring hydrogen peroxide [111, 112]. Referring to Table 3.1, after the initial increase in the H_2O_2 yield due to the recombination of two $\text{OH}^\bullet$ radicals, reaction (i), a decrease is registered, due to “second-order” reactions with hydrated electrons, hydrogen radicals, and hydroxyl radicals, respectively, (ix), (xi), and (v). The major effect of the reactions between hydrated electrons and H_2O_2 that reintroduce new hydroxyl radicals in the system was also pointed out by [42], for a 1.25 MeV u^{-1} ^4He irradiation (with a LET reported of $156 \text{ keV}/\mu\text{m}$). At the end, a slight rise due to reaction (i) is recorded. This result suggests that the track is still evolving around the μs time scale, corroborating what was found in Section 4.2.3 from the comparison with Kinetiscope. Therefore, the “intermediate” transitional phase, phase II, is crucial to carry on the information from the Monte Carlo algorithm, due to the still strong dependence of the chemical yields to the track evolution at the μs . As stated in [42], it is exactly due to the dependence of these second-order reactions on the reactants’ concentrations (and therefore on the LET) that high changes between various radiation qualities in the final species yields are registered. In fact, even though in principle the reactions happening within the heavy ion track occur in the photons spurs as well, the distinct spatial localisation and amount of species released induce different chemical dynamics. The application of homogeneous models under such conditions of high LET radiations resulted in incorrect yields predictions [43], implying that long-term water chemistry is not so trivial, and that accurate methods to simulate properly its dynamics are needed.

5.2 Biological environment

The following Section presents the findings of a study conducted to investigate the impact of a more complex biological environment in the final species yields produced by different radiation qualities. Some preliminary remarks are, however, necessary. As already mentioned in Section 3.4, the data on H_3O^+ , OH^- and H_2 are not shown, since their values might well be overestimated due to the stoichiometric decisions moved to define the new milieu kinematics. Moreover, also the yields on $\text{HO}_2^\bullet$, $\text{O}_2^{\bullet-}$, HO_2^- , R_2 and ROOR are not reported, as they always resulted in very low amounts compared to the other species generated. Conversely, despite the parameter pertaining to antioxidant depletion has been monitored especially for validation purposes (Section 4.3), given that secondary reactions are not included in the current reaction scheme, it is still considered to be of value, since it provides some useful information on the actual amount of XSH that intervened within the chemical network (cf. Section 5.2.2). Finally, the G-values for HS^- and $\text{X}^\bullet$ have been depicted with the same colour, because they are produced always in the same amount as evidenced by reaction (xxxvi) in Table 3.3.

5.2.1 Environment impact on chemical yields

The initial analysis entailed a comparison of the G-values generated by the same radiation quality in two distinct environments, respectively a water and a biological target. In Figure 5.10 are reported the primary chemical yields produced by a 90 MeV u^{-1} carbon ion track until, 1 s and under $p\text{O}_2 = 0.5 \%$.

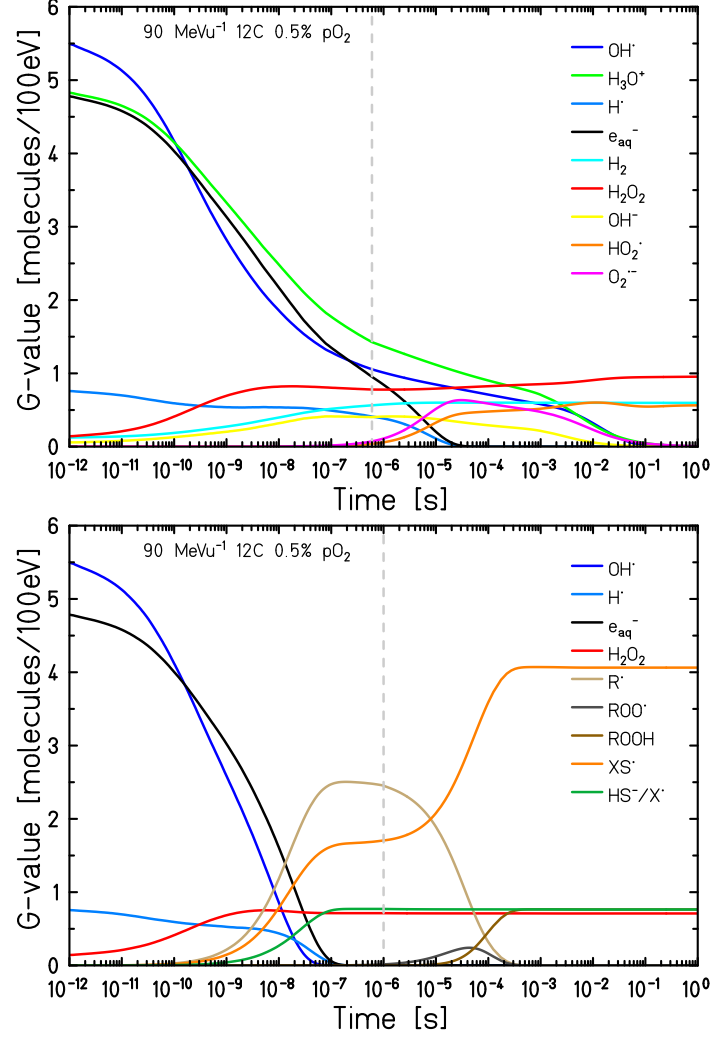


Figure 5.10: Example of G-value curves for the major species produced by a 90 MeV u^{-1} carbon track in a water (top) and biological (bottom) environment with $p\text{O}_2 = 0.5 \%$, recorded up to 1 s. Initial data taken respectively at 600 ns and $1 \mu\text{s}$ (grey dashed lines).

As far as the water environment is concerned, whilst the primary radicals are consumed at approximately a few tens of μs for $\text{H}^\bullet$ and e_{aq}^- , reaching almost 100 ms for $\text{OH}^\bullet$, the respective products, H_2 and H_2O_2 , exhibit a constant increase. Concomitantly, the oxygen present in the target starts to play a prominent role in the network after 1 μs , when both $\text{HO}_2^\bullet$ and $\text{O}_2^{\bullet-}$ are produced in a considerable increasing amount. This is due to the initial conditions simulated, i.e. low O_2 concentration and substantial energy deposited within the volume of interest. The combination of these parameters caused in fact a delay in the shifting from intratrack reactions to interactions with the oxygen in the environment. Some long-lasting reactions at around 1 ms can be spotted as well, mostly consuming completely the hydroxyl radicals and superoxide anions, producing further H_2O_2 and $\text{HO}_2^\bullet$. On the other hand, the situation changes quite drastically when dealing with the biological milieu. All primary water radicals are fully consumed already at around 100 ns, due to reactions with both RH and XSH at times as small as 1 ns. This implies not only that the introduction of the biological environment induces a severe change in the reaction network dynamics, indeed some species such as $\text{HO}_2^\bullet$ and $\text{O}_2^{\bullet-}$ are produced in way lower yields with respect to the pure water target, but also that, for a correct description of what is happening in the system, the biomolecules have to be included already within the Monte Carlo algorithm. Concurrently, it is possible to witness the increase of the biological radicals, both from the RH biomolecule, $\text{R}^\bullet$, and from the reactions with the antioxidant scavenger, mainly $\text{XS}^\bullet$, but also HS^- and $\text{X}^\bullet$. Similarly to the water environment, between tens and hundreds of μs , some long-lasting reactions are noticeable. The peak associated to $\text{ROO}^\bullet$ (stemming from the “fixation” reaction $\text{R}^\bullet + \text{O}_2$) is in fact registered at around 40 μs , after which it decreases as a result of the antioxidant intervention, yielding the “damaged” end-product ROOH . At the same time, reactions between the organic peroxy radicals and other RH occur, prompting both the further increase of the hydroperoxide, and the reintroduction of another $\text{R}^\bullet$. As for the XSH, it intervenes mostly in the direct neutralisation of the biological radicals. The O_2 impact is therefore shifted to later times as well, and the effects of these higher-order chemical reactions, highly environment-dependent, prove the necessity to cover longer time scales with the simulation.

5.2.2 Environment impact on radiolytic oxygen depletion

Following the observation of alterations in the G-values, the subsequent objective was to ascertain the impact of the novel biological environment on the radiolytic oxygen depletion. For this analysis, 500 keV electron tracks were simulated, which interacted with targets featured by oxygenation levels from hypoxic ($\text{pO}_2 = 0.3\%$ or $3.9\mu\text{M}$) to physoxic ($\text{pO}_2 = 7\%$ or $91\mu\text{M}$). A low LET radiation was selected, since its chemical yields are influenced to a greater extent by different O_2 concentrations than higher LET ones. Regarding the water environment, the data were recorded

at 1 μs , as in a previous study [18]. Recording these values at longer times would have resulted, in fact, in a simple saturation of the ROD to the upper limit value at low pO_2 levels. Conversely, the biological milieu dynamics was computed until 1 ms. This corresponds to the time at which the G-values for $\text{ROO}^\bullet$ and ROOH converge, respectively, to zero and to a constant, indicating that oxygen no longer plays a role in the reaction network. While it is evident from both Section 5.2.1 and [113] that oxygen depletion is occurring over an extended period of time, it is also clear from the findings of [22] that after approximately 5 to 10 ms, the presence of O_2 should no longer affect the susceptibility of the environment to radiation. The results of the analysis are summarised in Table 5.1 and depicted in Figure 5.11.

Table 5.1: ROD registered for a water (w) and biological (b) environment, under different oxygenation levels, after irradiation by a 500 keV electron track. The ROD data were saved for the two targets, respectively, at 1 μs and 1 ms.

pO_2 [μM]	ROD_w [$\mu\text{M}/\text{Gy}$]	ROD_b [$\mu\text{M}/\text{Gy}$]
3.9	0.02	0.07
6.5	0.04	0.12
13	0.07	0.24
19.5	0.10	0.34
26	0.13	0.45
39	0.17	0.64
52	0.21	0.81
65	0.23	0.96
78	0.25	1.10
91	0.27	1.22

As expected [65, 83, 114], larger depletion values are registered for the biological environment with respect to simple water. Whereas for the latter, the two reactions influencing ROD_w are due to $\text{H}^\bullet/\text{e}_{\text{aq}}^-$, for ROD_b the depletion is mostly due to reactions with $\text{R}^\bullet$. Nevertheless, a plateau for ROD_b is not observed, in contrast to the findings of other experiments [108, 114]. This is caused by the generalised milieu simulated [67] and by the rate constants implemented, and it is attributed to the combined effects of O_2 removal by organic radicals and reintroduction of other $\text{R}^\bullet$ by chain propagation events, respectively reactions (xxviii) and (xxxi) in Table 3.3.

Moreover, it was resolved to corroborate what was reported in [65], specifically the assertion that at 4 % pO_2 , a negligible proportion of hydrated electrons will react with O_2 when released within a biological environment. By comparing the simulated yields of e_{aq}^- at 1 ps with those of $\text{O}_2^{\bullet-}$ at 1 μs , produced by a 500 keV electron track in 4 % oxygenated water, it was determined that approximately one-third of the sol-

vated electrons reacted with oxygen. Conversely, only less than 2% underwent this reaction to produce superoxide anion in an environment with both biomolecules and antioxidants. Also [22] reported that the amount of $O_2^{\bullet-}$ in a biological milieu is more than one order of magnitude smaller than the respective one in pure water.

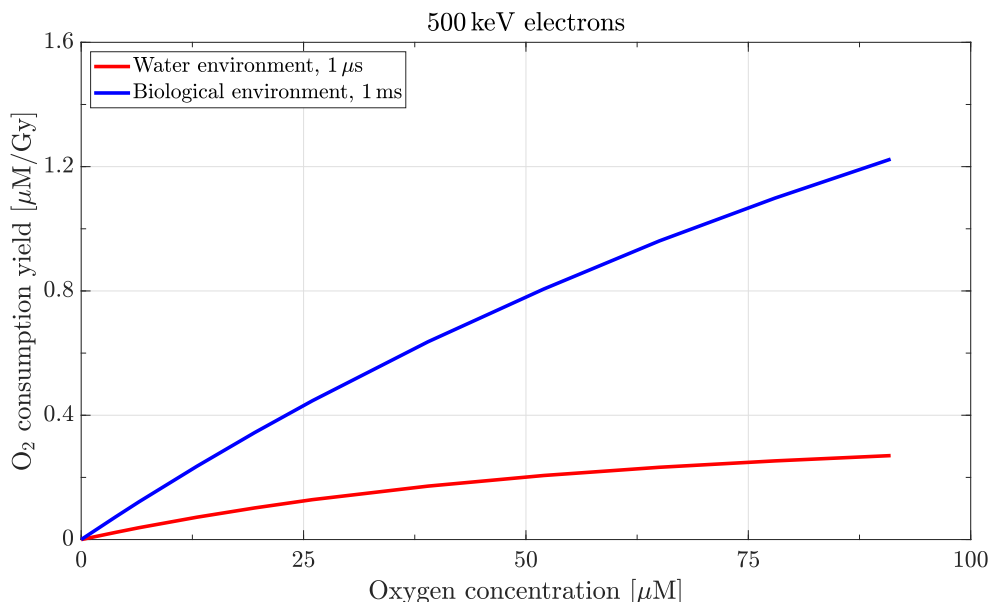


Figure 5.11: ROD registered for both a water and a biological environment, under different oxygenation levels, after irradiation by a 500 keV electron track. The ROD data were saved for the two targets, respectively, at 1 μ s and 1 ms.

For completeness, concerning the biological environment, the data regarding the depletion of the other scavengers – biomolecule RH and antioxidant XSH – were recorded as well. These are summarised in Table 5.2, from which it is possible to get an understanding of the “top scavenger” for each specific oxygenation level simulated. At small pO_2 values, there is a predominance of antioxidant with respect to O_2 or RH depletion, meaning that XSH intervenes mostly on the primary water radicals and on $R^{\bullet}$. Conversely, as the oxygen levels rise, a shift occurs within the reaction kinetics network, with the O_2 removal by biological radicals assuming a pivotal role. It is noteworthy that ROD and RBD exhibit comparable yields irrespective of the initial oxygenation status. This implies that the majority of oxygen consumed is directed to the formation of organic peroxy radicals, which in turn cause chain reactions with RH, producing both organic hydroperoxides and other $R^{\bullet}$. Therefore, a direct correlation can be established between ROD and ROOH [82]. On the other hand, RAD remains constant throughout the entire pO_2 domain simulated, therefore the antioxidant effect stays the same, with presumably a change in the reaction partner.

Indeed, at higher pO_2 levels, it intervenes mainly in chain terminating reactions, interacting with $ROO^\bullet$ [82].

Table 5.2: Radiolytic depletion of the three scavengers present in the simulated biological environment, i.e., O_2 (ROD), RH (RBD) and XSH (RAD), under different oxygenation levels. The data were saved at 1 ms after the irradiation of a 500 keV electron track.

pO_2 [μM]	ROD [$\mu M/Gy$]	RBD [$\mu M/Gy$]	RAD [$\mu M/Gy$]
3.9	0.07	0.08	0.72
6.5	0.12	0.12	0.72
13	0.24	0.24	0.72
19.5	0.34	0.34	0.72
26	0.45	0.44	0.71
39	0.64	0.63	0.71
52	0.81	0.80	0.71
65	0.96	0.95	0.71
78	1.10	1.08	0.70
91	1.22	1.21	0.70

5.2.3 LET impact on ROOH G-value

The organic peroxy radical $ROO^\bullet$ is a non-restorable form of the target RH, resulting from the damage fixation performed by oxygen [22]. Its amount can therefore be treated as an indicator of the indirect damage, particularly in presence of O_2 , as proposed by [115]. Consequently, it is important to monitor the yield of ROOH [114], which represents the final stable altered biomolecule [82]. This Section is dedicated to the investigation of the influence of LET on the ultimate yield of organic hydroperoxide. Electron and proton tracks, together with helium ions were simulated to cover the LET domain from 0.2 keV/ μm to 5 keV/ μm , whereas carbon ions with decreasing energies were exploited to mimic LET values up to 160 keV/ μm . A biological environment with a $pO_2 = 4\%$ (physoxic condition) was simulated for all the radiations considered. The data were saved at 1 ms, to ensure that the saturation of ROOH was reached in all scenarios. The resulting G-values are summarised in Figure 5.12.

It is of interest to observe the competition between the oxygen intervention in fuelling reactions with radicals and the intratrack recombination effects. Indeed, after the delivery of low LET radiations, the radicals are distributed in a way that favours reactions with the environment, first with RH and later with O_2 , leading then to the subsequent chain cascade. A quite high constant value around 7.7 molecules/100 eV is

registered under these conditions. As LET increases, so do the radical recombination rates, due to the enhanced proximity between the different chemical species produced. Consequently, the amount of radicals available for reacting with biomolecules is reduced, thereby limiting the formation of the final ROOH, which reaches a yield of approximately 2.9 molecules/100 eV for the largest LET simulated. This result clearly corroborates the assertion made in Chapter 2, namely that there are significant differences in the proportions of indirect damage with respect to the primary radiation quality under consideration.

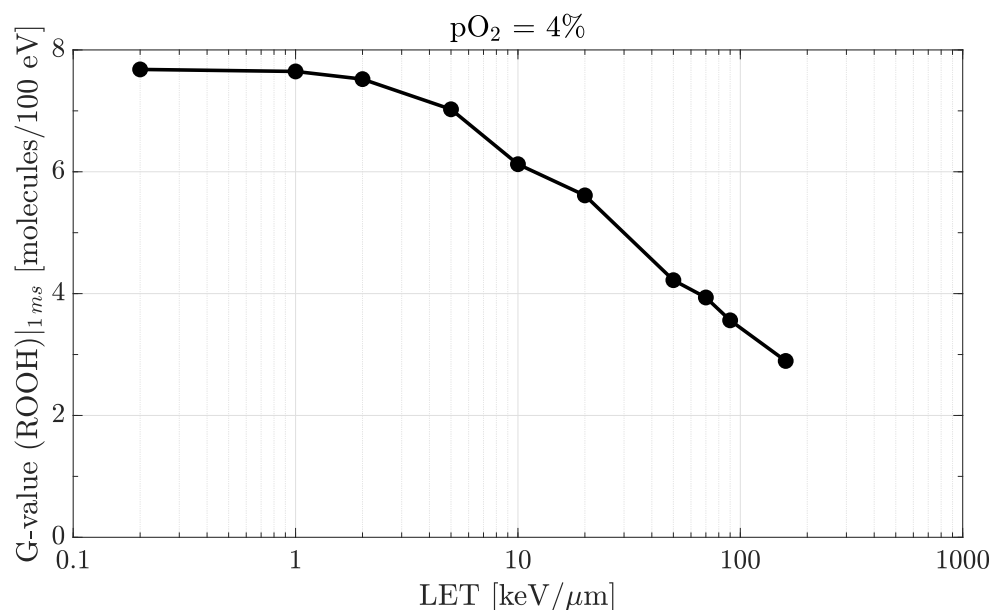


Figure 5.12: G-values for the organic hydroperoxide ROOH as a function of LET. The environment simulated was kept at a constant physiological oxygen level of 4 %, regardless of the primary radiation quality. The data were recorded at 1 ms.

5.2.4 Visualising contributions of in-loop reactions

The results showcased in Sections 5.2.2 and 5.2.3 indicate that both the predicted ROD values and ROOH yields are relatively high. This final Section aims at conducting a more comprehensive investigation into the underlying causes of these considerable amounts. A 500 keV electron track was selected as the probe for the analysis, interacting with a biological environment containing an oxygen level of 5 % pO₂ (65 μM). The ROD was already computed, 0.96 μM/Gy (or 9.3 molecules/100 eV, Table 5.2), whereas the G-value curves are shown in Figure 5.13. The entire system dynamics can be divided into approximately three principal and partially overlapping

temporal domains. The initial one, spanning from 1 ps to 100 ns, is characterised by reactions involving $\text{OH}^\bullet$, $\text{H}^\bullet$ and e_{aq}^- , which predominantly interact with the biological environment, although to a lesser extent also with one another. Some products that will remain stable for the rest of the evolution are already formed, namely H_2O_2 and $\text{HS}^-/\text{X}^\bullet$. During the second time domain, which encompasses six orders of magnitude from 1 ns to 1 ms, the chemical network is dominated by the presence of biological radicals, which exert a major influence on reaction events. The $\text{R}^\bullet$ radicals reach a maximum of approximately 3.5 molecules/100 eV at 100 ns, subsequently decreasing due to the presence of oxygen, whose effect starts to be discernible at around 1 μs [22, 67, 116]. A delay in the formation of $\text{ROO}^\bullet$ is observed, which is likely attributable to competing reactions with antioxidants [82]. Although a considerable amount of XSH intervenes already at the ns time scale, the majority react with biological radicals at later times, between tens and hundreds of μs (cf. increase in the $\text{XS}^\bullet$ curve). During this final time domain, covering from 10 μs to 1 s, a significant increase in reaction activity is observed for all three scavengers. It can also be noticed that the final amount of ROOH produced is greater than the initial yields of primary water radicals, suggesting a decisive contribution stemming from oxidation events occurring within chain reactions [82].

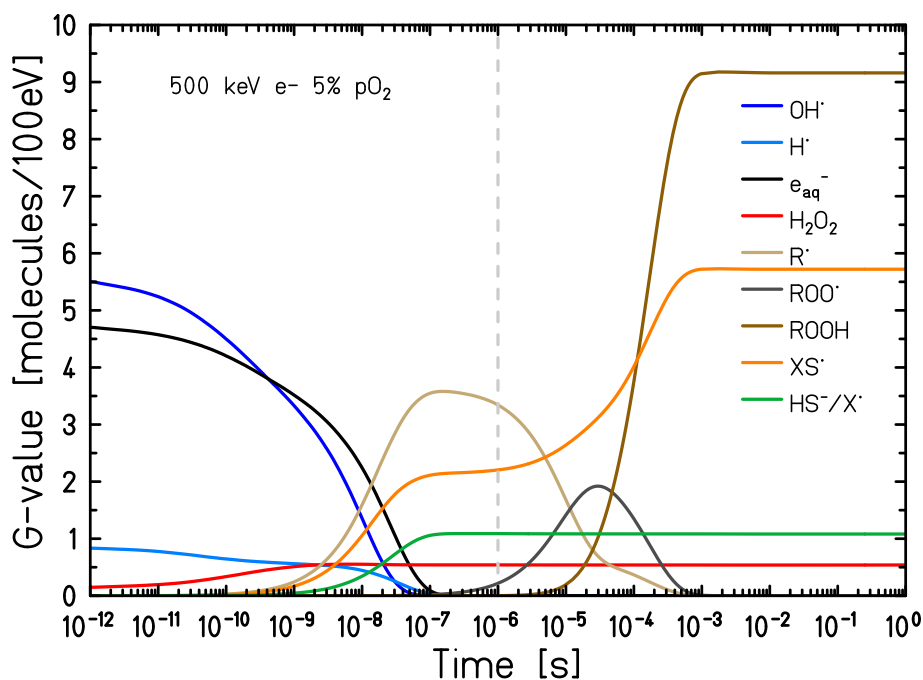
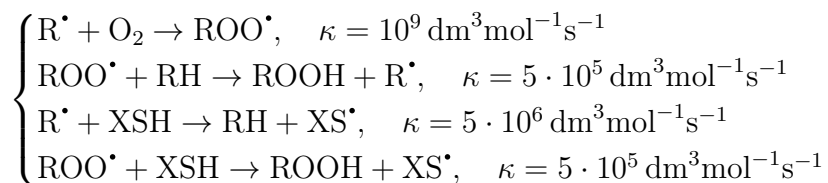


Figure 5.13: G-value curves for the major species produced by a 500 keV electron track in a biological environment with $p\text{O}_2 = 5\%$, recorded up to 1 s. Initial data taken at 1 μs (grey dashed line).

To clarify why both ROD and ROOH increase so much, the focus should be given to the specific reaction network implemented, and more in detail to the reactions featuring the three scavengers at times longer than the μs , deemed to be responsible for the aforementioned outcomes. In other terms, the competition between $\text{R}^\bullet + \text{O}_2$ (fixation) and $\text{R}^\bullet + \text{XSH}$ (neutralisation), and between $\text{ROO}^\bullet + \text{RH}$ (chain propagation) and $\text{ROO}^\bullet + \text{XSH}$ (chain termination) have to be studied. TRAX-CHEMxt permits to do so, by saving the contributions of the various reactions within one simulation loop. Figure 5.14 shows examples of these contributions, considering both major and minor reaction channels featuring the biomolecules at two different time points, comparing as well the cumulative final effects on the various species. Because the concentrations which define the reactions (cf. Section 3.1.3) depend on the selected bin and change over time due to the effects of diffusion, absolute values are not generally meaningful. For simplicity, the data reported relates to the first bins of each distribution. The minor channels, by definition, are featured by contributions to the respective species at least three orders of magnitude smaller with respect to the major ones. Consequently, they are only presented for the sake of completeness. Two time frames are chosen, one around the $\text{ROO}^\bullet$ peak, $30\ \mu\text{s}$, the other where the two biological radicals are converging to zero, $\approx 500\ \mu\text{s}$. Interestingly, the contributions stemming from the various reactions are almost constant, with only an overall decrease in magnitude (around two orders of magnitude) at longer time scales.

Figure 5.14 clarifies the substantial oxygen depletion observed at 1 ms. Taken alone, it is the second most significant reaction in the system, and its overall final effect depends on the result of a set of four competing reactions, which feature the chemistry of $\text{R}^\bullet$ and its organic peroxy $\text{ROO}^\bullet$



From one side, the first two events facilitate the continuation of the reaction activity, whereas from the other, the antioxidant intervenes to decelerate these channels, by interacting either with $\text{R}^\bullet$ and neutralising it, or by terminating the chain. The high ROD value is explained by the fact that the amounts of $\text{R}^\bullet/\text{ROO}^\bullet$ register a very slow decrease. Indeed, as a consequence of the chain reactions, the net impact on the radicals depletion is diminished, with around 80 % of the reacted species that are actually reintroduced in the milieu. The new $\text{R}^\bullet$, in turn, have high chances to consume other oxygen molecules, thus contributing to the overall ROD increase. Only at later times, after roughly 1 ms, when both $\text{R}^\bullet$ and $\text{ROO}^\bullet$ are almost entirely stabilised, the ROD reaches a stable value. Similarly, also the yield of ROOH increases rapidly, with the primary contribution arising from the loop reaction with a

biological substrate RH. This is as well the most significant reaction in the system. In fact, although having the same rate coefficient as the reaction with the antioxidant, the concentration of RH is one order of magnitude larger than that of XSH. This results in a contribution to the total ROOH yield of approximately 90 % from $\text{ROO}^\bullet + \text{RH}$, with the remaining 10 % derived from $\text{ROO}^\bullet + \text{XSH}$.

Lastly it is worth noting, from an alternative standpoint, the dependence between ROD and the G-value of ROOH. The only reaction consuming oxygen, namely $\text{R}^\bullet + \text{O}_2 \rightarrow \text{ROO}^\bullet$, is also the only one responsible to produce $\text{ROO}^\bullet$. At times larger than 30 μs , this radical is stabilised to a greater extent with respect to its production, thereby inducing the creation of ROOH, mostly via a chain reaction with another RH. Hence, as highlighted also in Section 5.2.2, the strict correlation between oxygen depleted and ROOH produced can be confirmed.

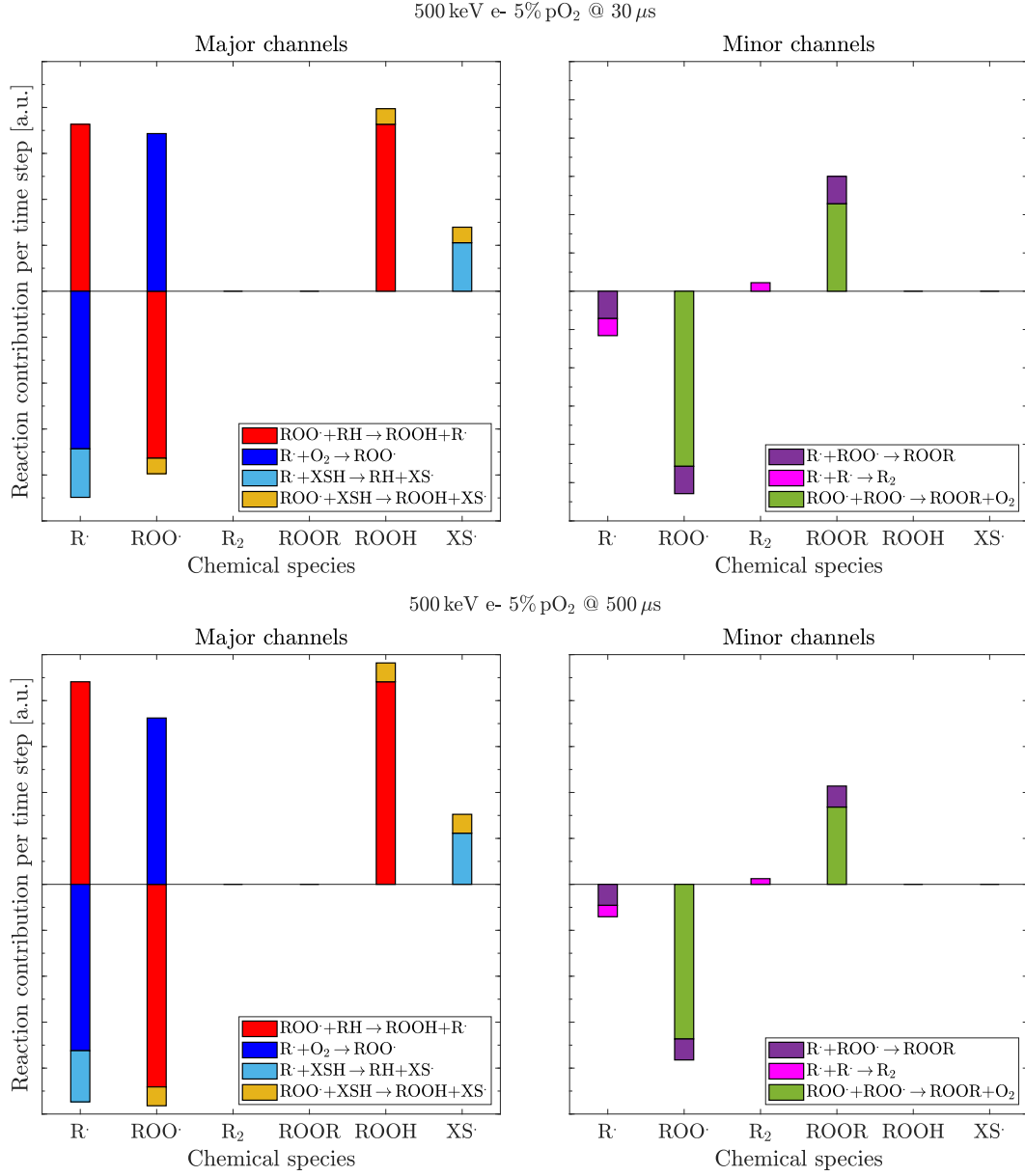


Figure 5.14: Reactions contributions (positive/production upwards and negative/reduction downwards) affecting the major biological species generated by a 500 keV electron track released in a biological environment with pO₂ = 5 %, saved at 30 μ s (top) and at 500 μ s (bottom). The results are obtained from the contributions associated to the first bin of the various concentration distributions. The minor channels contribute at least three orders of magnitude less to the respective species count than the major ones.

6. Discussion

The findings in Chapters 4 and 5 have given rise to interesting insights which are treated in further detail in this Chapter.

Initial tests were conducted to establish the parameters required to solve the reaction-diffusion problem. The set of time steps (Table 4.1) was determined by monitoring that the yields were consistent with those obtained through MC simulations, and that the species did not exhibit unphysical behaviours. Their implementation permitted to reliably simulate the system dynamics within the extension, up to time scales as long as 1000s. Following a similar approach, a bin width of 20 nm was selected to sample the spatial distributions of the radicals and molecules released around the primary track center. Values below 1 nm, reflecting an exaggerated sampling of the concentrations, would have resulted in considerable fluctuations between the bins. Lastly, time points ≥ 500 ns were found to be suitable to convert the information from TRAX-CHEM to TRAX-CHEMxt, Figure 4.1, with the largest differences registered for high LET radiations in a water target (Table 4.3). Too low transitional times suggest a still considerable track structure dependence of the yields. The derived values relate well with that obtained by applying the formula in [60] using an appropriate set of parameters (still in a simple water environment), together with other observations referring to the end of the spur expansion [27, 40, 49]. Nevertheless, t_{in} strongly depends on both the radiation examined and the chemical species investigated [60, 106]. The higher the species density close to the track core, the higher the recombination rate, and the later the local homogeneity between the histogram bins is reached. The importance of selecting an appropriate t_{in} was highlighted also in [49, 106]. These studies emphasised that the uncertainty in determining the transitional point between “heterogeneous” and “homogeneous” chemistry, for different LET values, leads to discrepancies between model predictions and experimental data. In principle, it may therefore be optimal to identify an appropriate switching time for each particle quality and initial energy. Consequently, the methodology employed in TRAX-CHEMxt to transform the data from a discrete approach into radial concentrations regardless of the primary radiation, is subject to an inherent degree of uncertainty, attributable to the initial clustering of the species. This represents the intrinsic uncertainty of the new resolution algorithm.

Starting with the oxygenated water environment, Tables 3.1 and 3.2, the novel algorithm demonstrates its accuracy through different comparison modalities. It showed an acceptable agreement with experimental data and another Monte Carlo IRT code at 10 μs , Table 4.6. Additionally, consistent outcomes with those from TRAX-CHEM were obtained, both in terms of radial concentrations, G-values, yield conversions ($e_{\text{aq}}^- / \text{H}^\bullet \rightarrow \text{O}_2^{\bullet-} / \text{HO}_2^\bullet$), and ROD, respectively Figures 4.2 and 4.3, and Tables 4.2 and 4.5. These results confirm also the aforementioned approximation employed for transferring the information at approximately 500 ns, with as well the importance of the second intermediate phase II. The set of validations was proposed for illustrative purposes, as they were in fact conducted for all the species released in the target, and for all the initial conditions presented in the various uncertainties Tables, 4.3 and 4.5. A possible additional cause of the registered deviations can be associated to the emission of relatively high energy (keV) secondary electrons from water molecules. By depositing energy away from the track center, these events will induce the formation of localised clusters of species, “breaking” the local radial symmetry, as can be seen in Figure 6.1.

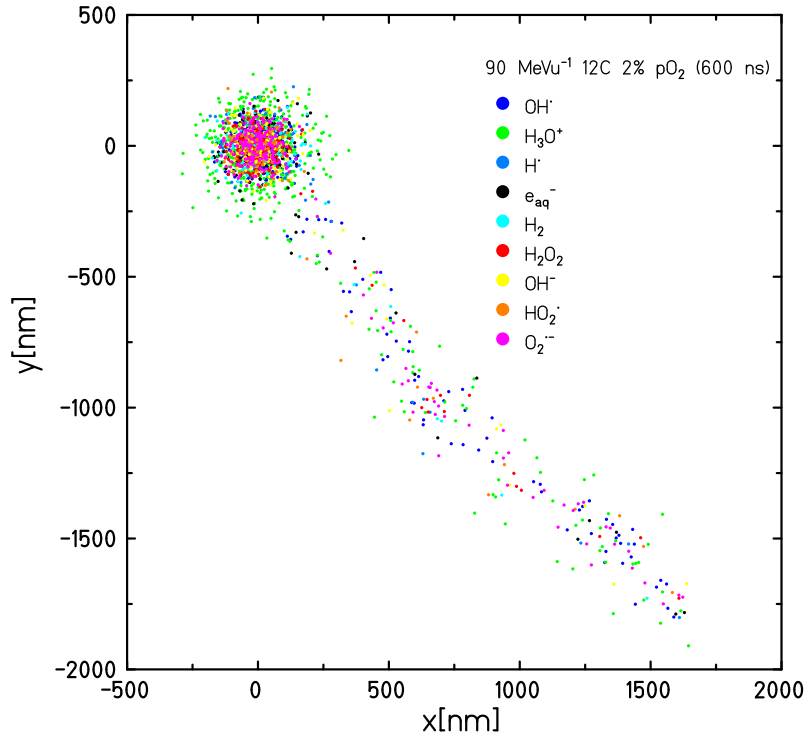


Figure 6.1: Species produced by a 90 MeV u^{-1} carbon ion track in a water target with $\text{pO}_2 = 2\%$ (generated by TRAX-CHEM). The snapshot was taken at 600 ns after the initial physical interactions. Clearly visible is the effect of an energetic secondary electron resulting in an energy deposition away from the track center.

Hence, by grouping the radicals and molecules in different histogram bins instead of treating each one separately, they are “smeared out” in the various rings around the track core, slightly reducing their consumption rate. Furthermore, the species dynamics are inextricably linked to the initial significant heterogeneities. Looking at the specific species ratios in Figure 4.1, especially for the carbon case, OH^- exhibited the most pronounced discrepancies with TRAX-CHEM, regardless of t_{in} , and H_3O^+ registered the least improvement as t_{in} increased. These findings suggest that, at the time of data conversion, the two species are more influenced than the others by the track dynamics. Thus, they have possibly not reached a local stabilisation at the transitional time point.

Nevertheless, the registered differences never exceeded 6 %, and if these uncertainties were obtained with an approach not completely homogeneous, by extrapolation it is expected that the predictions produced by a completely homogeneous method would give worse agreements (cf. Section 4.2.3, especially Figure 4.5 for high LET radiation). In fact, TRAX-CHEMxt results aligned well with both those from Monte Carlo approaches in the heterogeneous chemical stage, and the ones from pure homogeneous codes at low LET, suggesting that under these conditions such models can be applied to carry on the simulations data from 1 μs to longer time scales. Simultaneously, a good accuracy of the predictions was proven, when all the concentration distributions were uniform (phase III), as shown in a dummy analysis presented in Figure 4.6. Vice versa, for intermediate and high LET values, discrepancies higher than 20 % with the fully homogeneous approximation appeared. The consideration of a second “intermediate” phase, where the dependence of the species positions around the track is still accounted for (together with their diffusion events), even if in a less localised fashion, is therefore necessary for carrying on the information from TRAX-CHEM, and for trusting the simulation outcomes as well. This ensures that the extension can reliably simulate a broad spectrum of particle qualities. As a consequence, for intermediate and high LET radiations, homogeneous models can not be applied, at least around 1 μs [43]. As reported by [43], the transition from the heterogeneous to the homogeneous chemistry takes place over a much longer time scale in the track of a heavy ion with respect to lower LETs. In the analysis carried out by the cited group, a homogeneous model was used to predict the concentrations at long time scales, and issues for high LETs were registered. Therefore, such models must be used with caution under these conditions. From a different experimental study, [112], the data on radical yield variations suggested that the chemical tracks of radiations with LET larger than 10 keV/ μm are still evolving around the μs .

Once the reliability of TRAX-CHEMxt had been established, the feasibility of investigating time scales that were longer than had previously been possible was explored. This allowed the full potential and uniqueness of the code to be exploited, enabling the simulation of chemical kinetics for a wide range of projectile LET val-

ues, rather than limiting the investigation to only low values, as in [55, 60, 107, 117]. Species considered to have reached a stable yield around the μs showed instead variations in both their amounts and slopes at later times. A different development in H_2O_2 G-values under anoxic and oxic conditions was found, Figure 5.2. The competing reactions at hundreds of ns, together with the contribution from other higher-order ones, revealed their effects at the ms time scale, suggesting that hydrogen peroxide will reach a uniform yield only at later points, when O_2 is present. Hence, the opposite (decreasing) trend registered for H_2O_2 at ultra-high dose rates [118, 119] leads to considering the possible further interplay of other reaction channels included in the chemical network, probably induced by tracks released at subsequent times. This can result in scenarios where the primary projectile dynamics is not only affected by its “intrinsic” chemistry, but also by the “new” environment it encounters upon arrival, due to the previous tracks that have been delivered. A switch in the reaction channels can then be induced, whereby an initial situation in which an abundant amount of molecules is present, is transformed into one where these are diffused homogeneously enough from the track, such that the following radicals produced in the target are capable of promoting higher-order reactions, including the consumption of the aforementioned molecules. Altogether, a precise description of the dynamics at longer times may be very valuable, because it could elicit effects of secondary and slower reactions which are not perceived at early stages.

Probing scales beyond the μs gave insights also in terms of radicals’ and molecules’ dependence on LET and oxygenation. A direct relationship between the average intratrack products concentration (category ii) and LET was registered at 100 μs (Figure 5.3). The lower the LET, the more favoured were the reactions with O_2 with respect to recombination events, due to the broader spatial distribution of the species. This phenomenon was also observed in the analysis of the oxygen impact on chemical yields, where higher G-values for the sum ($\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$) were produced by the lower LET radiation, Figure 5.5. Furthermore, their increase during a fixed time interval (500 ns to 25 μs) and under hypoxic conditions was larger than that observed in the higher LET case. Figure 5.4, from a different standpoint, also suggests that the “reactivity” of the chemical network is LET-dependent, due to different intratrack effects. For the highest LET simulated, 160 keV/ μm , a roughly 20 % decrease in the overall G-value between 500 ns and 25 μs was registered, not showing major changes later until 100 μs . This however is deemed to be a “local minimum”, as some findings from comparisons with experimental outcomes at 1 s showed additional reactions occurring around the ms. The results from Figure 5.5 demonstrate additionally that the higher the pO_2 level in the target, the faster the saturation of the G-value($\text{HO}_2^\bullet + \text{O}_2^{\bullet-}$) is reached, regardless of the LET considered, even if the specific saturation levels do depend upon the primary projectile. Such a feature is also observable in Figure 5.6, where the mean values of the concentration distributions at 500 ns tend to converge

to the respective ones at 25 μs and 100 μs as the oxygenation increases, for both radiation qualities studied (e- and 12C).

To ascertain whether the extension yields consistent results over extended time periods (in the minute scale), three distinct experimental findings were evaluated, as documented in Figures 5.7, 5.8, and 5.9. The results are satisfactory, emphasising the significance of high-order reactions, particularly for high LET radiations, on the final products yields, due both to the species spatial distributions and their high concentration values. With regard to the carbon ion track (Figure 5.9), indeed, the simulation provides insights into the specific chemical dynamics associated with H_2O_2 , attributing to e_{aq}^- , $\text{H}^\bullet$ and $\text{OH}^\bullet$ the reason behind its decrease. Additionally, the outcomes give indirect evidence of the necessity to introduce phase II, which offers a good degree of accuracy by accounting for the dependence on the distance from the track center. In fact, the track is still evolving at the μs scale, validating the findings from [43] where it is reported that wrong predictions were obtained while using fully homogeneous models with high LET irradiations. At the same time, however, certain limitations of the simulations became evident. The hypothesis of track segment condition, despite its utility in studying the impact of a specific set of constant properties related to a particular radiation quality, proved to be a computational bottleneck in the experimental benchmarking process. An improvement in this regard would be the optimisation of the Monte Carlo code, either through the acceleration of the simulation itself, or the identification of an alternative methodology for the computation of the dynamics associated with the chemical yields released by different LET values, while maintaining the tracking of all species locations. Concurrently, additional reactions may be contemplated, to observe potential dynamics featuring higher-order kinetics occurring within the temporal domain spanning from μs to s. Noteworthy are the reaction schemes proposed by Pastina and LaVerne [43] and Elliot and McCracken [120]. Furthermore, the inclusion of environmental acidity and the associated acid-base pair equilibrium considerations, along with the assessment of intermediate and high dose rates (through the introduction of intertrack effects in TRAX-CHEM and/or TRAX-CHEMxt), would enable a more precise representation of the actual chemical processes occurring in real experimental settings.

A well-constructed computational model can facilitate the distinction between disparate primary projectile qualities, from a physical and chemical standpoint. This is an important characteristic that has been highlighted by [22, 115]. The implementation of new species in the reaction network of both TRAX-CHEM and TRAX-CHEMxt, namely RH and XSH, has enabled an investigation into the chemical effects of radiation in a biological target as a function of initial particle and environmental conditions, over an extended time period (Section 3.4). The concentration values, diffusion coefficients and reaction rate constants were derived from a large pool of values and are therefore subject to significant errors. Despite the simple biological

milieu modelled is not yet able to mimic all characteristics of the real intracellular environment, it represents a significant advance in this direction. The results are still semi-quantitative, and provide interesting insights into the chemical reaction network featuring the biological species simulated. The concentrations assigned to the generic biomolecule and antioxidant were respectively 43 mM and 4 mM, with kinematics detailed in Tables 3.3 and 3.4. As far as XSH is concerned, it is crucial to acknowledge that within living cells it is capable of undergoing a “switching state” process. This phenomenon occurs when a molecule reacted, prompting the activation of another one via signalling events, effectively “filling the gap” and thereby maintaining its equilibrium. Taking the glutathione case as an example, its amount is “restored” by a new synthesis or by the reduction of glutathione disulfide (GSSG) [82]. Therefore, the parameter associated to its depletion, RAD, was monitored especially for computational reasons, to demonstrate the reliability of TRAX-CHEMxt in simulating the actions of multiple scavengers. Nevertheless, this also provided valuable insight into its intervention within the network. Good agreements were found with TRAX-CHEM, for both concentration distributions, Figure 4.7, G-values, Table 4.7, and radiolytic depletion of the three scavengers present in the environment, Table 4.8, regardless of the initial condition considered or the specific substance under investigation. Only in cases of particularly low yields, associated with the species R_2 and ROOR, considerable uncertainties arose. These discrepancies are attributed to the inherent differences between the “quantised” Monte Carlo approach, and the “continuous” concentration distribution shortcut, with additionally the absence of species ricochets at the target longitudinal borders in TRAX-CHEM.

Figure 5.10 illustrated the impact of diverse environmental conditions on the reaction channels featuring primary water radicals and on the ultimate species yields. These effects are attributable to distinct chemical network dynamics. Regarding the water environment, chemical species behaved in accordance with expectations, meaning that the radicals were consumed and the respective molecular products were generated, with also a production of $HO_2^\bullet$ and $O_2^{\bullet-}$ around the μs , since at this time scale the species distributions were more diffused and could be affected by the surrounding (low amount of) O_2 . Despite the simple environment, some long-lasting reactions at the ms frame were present as well, with a final temporary equilibrium of the species H_2O_2 and $HO_2^\bullet$ at 1 s, as hydroperoxyl radicals recombination are expected at later times. It is evident that incorporating biological components at the Monte Carlo stage is crucial, since all primary water radicals were consumed within a time scale of less than 1 μs (i.e., even before being imported into the extension). This finding is in accordance with the observations reported in [82]. It is thus expected that initially a great amount of primary radicals react with biomolecules [114], with the generation of an intermediate radical ($R^\bullet$) [82], and only subsequently additional reactions may occur with other species. It can thus be posited that in certain compu-

tational implementations, such as that described in [55], it would be more accurate to incorporate the biological milieu directly into the Monte Carlo code at the outset, rather than introducing it simply at 1 μ s. Contemporary, it can be observed that oxygen plays a role at a slightly later point in time than the approximately 1 μ s registered in the case of oxygenated water, with the fixation of $R^\bullet$ and the production of organic peroxy radicals between 10 μ s and 200 μ s. As reactions with a larger plethora of target molecules were permitted, the chemical species were able to react with O_2 /RH/XSH also after the track structure was loose [17]. Consequently, the equilibrium is not attained at the μ s scale, necessitating a meticulous simulation of the dynamics (maintaining phase II). For instance, regarding the $XS^\bullet$ production from the antioxidants reacting with radicals (both from water, at early stages, and from the biological environment, $ROO^\bullet$ and $R^\bullet$, at longer times), its yield continued to rise until around 200 μ s, when it stabilised at approximately 4 molecules/100 eV. At the same time, a change in the slope of $R^\bullet$ was observed, mostly due to the involvement of antioxidants, despite oxygen exerted a contributory influence as well. Also the production of $ROOH$, from XSH and chain reactions, occurred within a range of tens to hundreds of μ s. All these outcomes prove the importance of simulating longer time scales. The discrepancies in the yields between the two environments emphasise the decisiveness of the actual environment simulated in determining the final yields. Consequently, it is imperative that any conclusions drawn are subjected to rigorous scrutiny, as they may vary considerably depending on whether only water or a bio-mimicking system is considered, with the latter being also contingent upon the specific biological molecules involved. Therefore, simple water systems may not be sufficient to elucidate certain mechanisms, such as the FLASH effect [114].

The introduction of the biological milieu, especially due to $R^\bullet$, has resulted in higher oxygen depletion values being registered with respect to the water environment under the same low LET radiation, as evidenced in Table 5.1. There is a 3 to 4.5-fold increase with respect to ROD_w , within a pO_2 domain spanning from 3.9 μ M to 91 μ M. However, the ROD_b are notably high, and do not reach saturation as instead reported by some experimental findings [108, 114]. Despite these data refer to extracellular depletion, meaning that in principle greater values could be expected within the cell, the substantial different behaviour of the simulated data is caused by both the general biological milieu considered, treating only two species, RH and XSH, and by the reactions and reaction rate values implemented to describe the chemical network. As reported by [114], the rate of reaction (xxviii) in Table 3.3 can be up to two orders of magnitude lower in a viscous system. On top, ROD values change quite considerably depending on the possible buffer involved (acidity of the environment), as confirmed by [83]. Notwithstanding the discrepancies registered with experiments, the kinetics simulated represents a small advancement towards a more complex scenario, as supported by [108]. Concomitantly, from the data obtained, it is evident

that under the hypotheses of conventional doses and dose rates, it is impossible to induce a total oxygen consumption, as the change in the environmental pO_2 is minimal. This observation has been demonstrated in previous experimental findings [67, 108, 114, 121].

Furthermore, the impact of the remaining scavengers was also documented, cf. Table 5.2. The homogeneous distributions hypotheses exposed at the beginning are confirmed. This is evidenced by the fact that, despite the Monte Carlo algorithm is not accounting for concentration variations of any of the three uniformly distributed species (as in other codes such as Geant4-DNA [107]), the overall depletion values at the switching time point with TRAX-CHEMxt, $t_{in} = 1 \mu s$, are not high enough to induce noticeable changes and decrease their initial concentrations (at least under conventional dose and dose rate assumptions). The antioxidant turned out to be the major scavenger under low pO_2 , whereas oxygen (and the generic biomolecule) dominated at higher O_2 levels. Additionally, ROD and RBD had always similar values, suggesting a direct and strong correlation between oxygen removal and ROOH creation regardless of initial pO_2 , due to the simultaneous effect of the two reactions $R^\bullet + O_2 \rightarrow ROO^\bullet$ and $ROO^\bullet + RH \rightarrow ROOH + R^\bullet$. Conversely, RAD was always constant. This means that the antioxidants always intervene in the same amount, changing however the reaction partner, from $R^\bullet$ to $ROO^\bullet$, as the initial oxygenation increases. Also the experimental outcomes reported in [82] – HL-60 cells irradiated by γ -Co⁶⁰ source – demonstrated that intracellular glutathione and ascorbate can suppress protein chain peroxidation due to their reactions with both protein radicals and peroxy radicals. The “scavenging capacity” concept, i.e., the predisposition of a specific species to react with another one, given by the product of its concentration value with the reaction rate coefficient, can be used to clarify the observation of the change in the XSH reaction partner. For $R^\bullet + XSH \rightarrow RH + XS^\bullet$ and $ROO^\bullet + XSH \rightarrow ROOH + XS^\bullet$, the antioxidant scavenging capacities are respectively $2 \cdot 10^4 s^{-1}$ and $2 \cdot 10^3 s^{-1}$. Also the scavenging capacity for the reaction $ROO^\bullet + RH \rightarrow ROOH + R^\bullet$ is constant, and equal to $2.2 \cdot 10^4 s^{-1}$. Different is the case for oxygen, since various oxygenation values have diverse probabilities in interacting with the biological radicals. For the reaction $R^\bullet + O_2 \rightarrow ROO^\bullet$, at $pO_2 = 0.5 \%$, the scavenging capacity is $6.5 \cdot 10^3 s^{-1}$. Therefore, in this case, as reflected by the amount of RAD and ROD, XSH is favoured for reacting and recovering the damage induced to the biomolecule. On the other hand, for a $pO_2 = 7 \%$, the scavenging capacity is $9.1 \cdot 10^4 s^{-1}$, shifting the preference towards the fixation of the damage by an oxygen molecule. Note that these results are related to the approximations moved regarding the various scavengers concentrations and reaction rate coefficients. A differentiated environment where all the biological species are treated separately will provide different outcomes. In the current implemented scenario, due to the high concentration of RH, a great extent of all the primary water radicals will react with it, regardless of

the initial oxygenation. Only later on, the produced $R^\bullet$ will be featured by distinct preferred reaction channels depending on the other scavengers in play. Assigning different values of concentrations, reaction rate constants and diffusion coefficients may also favour the occurrence of other termination reactions, thereby increasing their overall contribution, such as the recombination of two biological radicals to produce a R_2 molecule.

An investigation was also conducted to examine the impact of LET on the final ROOH yield, representing the stable end product stemming from the non-restorable form of the original target RH, i.e., $ROO^\bullet$. An inverse relation was found, illustrated in Figure 5.12, which depicted the competition between oxygen fixation and radicals (intratrack) recombination, as observed previously during the analysis of LET/ pO_2 impact in a water environment, but this time on the final biological indirect damage. Under low LETs, reactions with the milieu occur with a higher frequency, due to the broader distribution of the radicals, whereas for larger energy depositions recombination events between the chemical species dominate, resulting in lower interactions with RH. The slope of the curve underwent indeed a change at approximately $2 \text{ keV}/\mu\text{m}$, transitioning from a constant amount to a linear decrease. This results in a G-value of the organic hydroperoxide for the largest LET simulated that is more than 50 % lower with respect to the one observed at low LETs, showcasing how considerably the indirect damage changes with respect to the radiation considered.

Given the substantial amounts of both ROOH produced and ROD observed, approximately 9 molecules/100 eV for the former (Figure 5.13), and $1 \mu\text{M}/\text{Gy}$ for the latter (Table 5.2), a final analysis was conducted to elucidate the underlying mechanism responsible for these phenomena. TRAX-CHEMxt is distinguished by its capacity to monitor and quantify individual (user-defined) reactions within a single simulation loop, due to their implementation via Δ functions (Section 3.1.3), and to evaluate these relative contributions with respect to the overall amount of each species, which is useful for investigating the causes behind specific trends. This method was employed to study the impact of competing reactions within the scavengers network (fixation against neutralisation, chain propagation against termination), Figure 5.14. Reactions $ROO^\bullet + RH \rightarrow ROOH + R^\bullet$ and $R^\bullet + O_2 \rightarrow ROO^\bullet$ result to be the two most significant ones in the network. Besides, the effects of fixation (for $ROO^\bullet$, consuming an oxygen molecule) and chain propagation (for $R^\bullet$, reintroducing it in the reactive milieu) are identified as the primary factors responsible for the observed increase in oxygen depletion and hydroperoxide formation, establishing a direct correlation between the two parameters originating from the strict concatenated influences. As a consequence of this combination, the net depletion rate of both biological radicals is considerably slowed down, since within each loop, approximately 80 % of the reacted species are reintroduced into the network by the chain reaction. The effect in question remains almost constant between $30 \mu\text{s}$ and $500 \mu\text{s}$, despite a general decrease in

the overall intensity at longer times. This emphasises both a strong impact of the aforementioned reactions over a wide temporal domain, and a possible role played by them in the final biological damage aggravation. Additionally, albeit the overall ROD is larger due to the contribution of the events occurring before 1 μ s, it is expected that the final G-value for ROOH will be greater than the ROD registered between 1 μ s and 1 ms. Indeed, the total ROD for $pO_2 = 5\%$ is 9.3 molecules/100 eV. Approximately 0.4 molecules/100 eV comes from oxygen reactions at times preceding 1 μ s. The remaining 8.9 molecules/100 eV are attributed to the kinetics occurring between 1 μ s and 1 ms, where also a very small amount of O_2 is generated via the recombination of two organic peroxy radicals. Vice versa, ROOH is created only within this latter time domain, with a 90% contribution coming from the reaction with RH, and the remaining 10% from the intervention of XSH, and reaches a final G-value of approximately 9.2 molecules/100 eV, as can be seen in Figure 5.13. Here, the effects of the chain reaction are visible as well, by simply noticing the higher yield of ROOH with respect to the primary water radicals.

It is crucial to reiterate that all these findings are exclusively associated with the simulated generalised environment, encompassing both concentration values, reaction rate constants and diffusion coefficients. Consequently, comparisons with experimental results are inherently challenging [108]. TRAX-CHEMxt limits to a few reactions and classes of biochemical species, and does not discriminate between them (proteins, amino acids and nucleotides, or GSH and AscH⁻), meaning that the radicals can have different degrees of affinity with each specific substrate, resulting in substantial variations in the final chemical yields, as pointed out also by [22, 83]. To achieve an optimal analysis of the indirect damage via the exploration of the biochemical stage, some possible future improvements can be made. For instance, the actual separation of the various components can be performed, regarding both the biomolecules and antioxidants, in order to gain a more detailed understanding of the primary pathways involved in the induction and neutralisation of the damage. For example, ascorbate has been demonstrated to be more effective than glutathione in restoring protein radicals [82]. The definition of different concentration values and reaction channels for antioxidants could also assist in distinguishing the effects between healthy and tumour environments, due to their differing abilities in restoring R[•] [22]. Indeed, as reported in [83], different intervention rates are to be expected as a function of the substrates under consideration.

Other species can be included. Candidates for consideration are superoxide dismutase (SOD), catalase (CAT), α -tocopherol (vitamin E), as antioxidants, as well as Fe²⁺ (iron), Cu⁺ (copper), and other metallic ions participating in the “Fenton reaction”. In the presence of transition metals, H₂O₂ can generate OH[•] via $H_2O_2 + Fe^{2+}/Cu^{+} \rightarrow OH^{\bullet} + OH^{-} + Fe^{3+}/Cu^{2+}$. Moreover, other cellular structures such as membranes and DNA could be taken into account. Some data in this

regard have already been gathered. Nevertheless, in order to simulate specific cell compartments for a more variegated milieu, the hypothesis proposed in this approach might be challenged. This suggests that a different description of the system may be required, as for instance an alternative method to solve the diffusion equations when dealing with radicals crossing lipid bilayers. Additionally, dedicated tuning of the simulation settings, geometry and advanced assumptions should be performed. Similarly as with the water environment, the introduction of additional reactions may be necessary to account for potential higher-order effects at longer times [43]. The consideration of an appropriate buffer, such as phosphate-buffered saline and bicarbonate buffer, with the function of maintaining constant the pH of the system, and as well different acidity conditions through the “ionic strength” (a measure of ion concentrations within the solution) that affect both reaction rate constants, acid-base equilibria and molecular yields [111], could be beneficial improvements. In fact, antioxidants are only effective within a specific pH range. It would therefore be worthwhile to implement the ability to handle different pH values and to maintain them at a constant level via an appropriate buffer, allowing for a more realistic overall simulation. The explicit consideration of acid-base pairs, which according to [43] are particularly relevant at longer time scales, may facilitate the enhancement of the predictions precision with respect to the experimental results, cf. Figures 5.7 and 5.8. Conversely, if different temperatures are to be treated, this will necessitate a significant amendment to numerous parameters delineating the dynamics of the system. This is because not only will the reaction rate coefficients be influenced, but also the Henry’s constant for converting gaseous oxygen into a concentration value, the diffusion coefficients of the various species, and even the secondary electrons thermalisation distances during the physico-chemical stage. It is also important to consider that all parameters used are derived from water measurements. However, the cell milieu is more viscous, which consequently induces a slowdown of both diffusion coefficients and reaction rate constants [22, 65, 114]. Irrespective of the environment under consideration, these parameters are subject to significant uncertainties, and in order to simulate this “steric hindrance”, adjustments must be made at the physico-chemical stage (TRAX-CHEM), as for the temperature regulation, which necessitates a subsequent retuning of the dissociation channels for the initial radicals/molecules created at 1 ps. Finally, the introduction of oxygen resupply by blood capillaries at times larger than 1 ms [122, 123, 124] could represent a significant advancement towards a more precise simulation of a real system.

7. Conclusion and Outlook

The objective of this study was to develop and validate TRAX-CHEMxt, extension of the track structure code TRAX-CHEM, in order to probe longer times (until s) and biomolecular environments, under a wide range of particle qualities, energies, LET values and oxygenation conditions. TRAX-CHEMxt is consistent with the existing validated simulations. The results demonstrate the capacity to continue simulation with the data provided by the Monte Carlo counterpart over extended time periods, keeping its accuracy due to the smooth transition between the codes. Simultaneously, the possibility to investigate the biochemical phase of radiation-induced damage (Figure 2.1) was also implemented, by introducing additional biomolecules within the simulated environment. The reliability of TRAX-CHEMxt depends on a computationally efficient algorithm, based on concentration distributions, which has been demonstrated to be of pivotal importance for the accurate treatment of the diverse reactants observed around the track center of different particle qualities (Figure 2.2). Indeed, maintaining control of the spatial information beyond the μs range ensured the accurate simulation of the yields for intermediate and high LET radiations. The necessity of monitoring all species positions, as required by the SBS approach, was circumvented by employing the histogram outputs provided by TRAX-CHEM. This 1D representation collected the various radicals and molecules located around the primary radiation track, and facilitated their transformation into concentrations (cf. Figure 3.8). As a result, the simulation could be conducted in a less localised manner, under the assumption of cylindrical symmetry, with a computational gain of at least three orders of magnitude for the water environment (Table 4.4). Furthermore, an additional approximation implemented internally in TRAX-CHEMxt enabled the reformulation of the distributions into uniform values in phase III (Figure 3.7), thereby allowing the novel code to cover temporal domains of few minutes in reasonable times (approximately two hours).

In view of the current state of the code, which has been validated and prepared in accordance with the requisite specifications, it is now ready to be deployed in the next series of applications, and to undergo further refinement depending on the process under analysis. The following steps will entail the simulation of a more variegated cell-like environment, accompanied by the benchmarking of the code predictions with

additional experimental results. As derived from this analysis, the chemical species yields depend in fact markedly on the biomolecules under consideration. Data on O_2 depletion [83, 108, 115] may prove valuable in this context. An alternative approach would be to implement a bespoke set-up, in order to represent a specific experimental scenario, with the Fricke dosimeter representing a potential candidate for this purpose [40]. Furthermore, validations with other simulations, such as those regarding the scavenging effect of GSH [56], or the production of organic peroxy radicals under several irradiation conditions [55], could be conducted.

A further study that can be performed by exploiting the novel algorithm is an investigation into the characteristic reaction network featuring additional critical biomolecules. This may provide insights into the preferred channels and competing pathways by which some compounds behave as protectors [27], and at the same time how other species act as radiation sensitizers [26]. Introducing specific solutes may also be beneficial for the comprehension of how radiolysis-induced yields contribute to more complex experimental techniques of current interest, such as UHDR irradiations [5, 116, 118], minibeam radiation therapy [125], or use of nanoparticles as chemical radiosensitizers [126, 127]. For instance, the combination of the precise Monte Carlo step-by-step simulations with TRAX-CHEMxt fast and accurate extension to longer temporal domains could be a useful tool, as suggested by [128], to test and disentangle hypotheses regarding the FLASH effect [115], such as oxygen depletion, radical-radical interactions, and intertrack phenomena. The ability to probe time scales between ms and s, at which these processes are taking place [22], will prove invaluable.

In light of the aforementioned evidence, it would be worthwhile to undertake an analysis of the dose rate dependencies of the species yields. The simulation of multiple tracks can be performed in both TRAX-CHEM and TRAX-CHEMxt, with some investigations already carried out within the Monte Carlo framework. Here, this possibility has been implemented by computing the physical stages of the various projectiles independently, and introducing the related chemical species at user-specified times, corresponding to the arrival of the subsequent tracks. To establish the unit cell dimension from the desired instantaneous dose (cf. Figure 3.7), equation (3.6) can be employed and used to set the corresponding track distance in TRAX/TRAX-CHEM as well as the calculation volume (placement of boundary condition) in TRAX-CHEMxt. Moreover, particular complex dose rate patterns, deriving from specific pulse doses and timings, could be handled by inserting into the extension different generations of the MC output after each other. The simulations could thereby assist in comprehending special and, to some extent, counterintuitive findings associated with FLASH experiments, as the observed decrease in H_2O_2 [118, 129]. The various reaction channels involving the primary radicals could be investigated as a function of dose rate. In relation to this topic, TRAX-CHEMxt can also

elucidate whether LET effects impact FLASH [22]. A further avenue for investigation would be to conduct a parallel study, to compare the outcomes and monitor for similarities between high LET and UHDR radiations. Indeed, as reported by [28], under UHDRs the overlap region of tracks is enlarged, and the G-values exhibit behaviours similar to those elicited by the same ion but with a higher LET. At present, a reduction in ROOH formation (Figure 5.12) has been observed, attributed to elevated local radical densities. This could indicate a sparing effect, reflecting the findings of Labarbe [55], even if in this case the UHDR study was on lipids. As the author proposed, at higher dose rates, the amount of $R^\bullet$ accumulating in close proximity to one another would be greater, facilitating their recombination (dimerisation) before producing $ROO^\bullet$. Consequently, the chain reaction would have a diminished impact in the chemical network. Further potential indicators of resemblances could include a reduction in the G-value(H_2O_2), an increase in the likelihood of radical-radical reactions (intertrack for ultra-high dose rates, intratrack for high LETs), and a decrease in oxygen pressure.

Finally, a particularly noteworthy potential application of TRAX-CHEMxt is in the context of precise chemotherapy. This promising treatment modality exploits the radiation-induced chemical activation of cancer prodrugs by primary water radicals, achieving a combined chemo-radio effect at the tumour site [130]. As a result, indirect and prodrug damages enhance the therapeutic effect, with an improved (selective) targeting and reduced toxicity. The current approach, which is predominantly performed with photon irradiations [131, 132], can be simulated to both facilitate the understanding of the underlying mechanisms, and to test different LETs. The latter point will enable the verification of the persistence of this phenomenon, and the assessment of whether it can be further enhanced with the use of charged particles (protons, helium and carbon ions).

List of Scientific Contributions

Contributions at conferences

- C.i E. Scifoni, D. Boscolo, M. Krämer, U. Weber, **G. Camazzola**, M. Durante, and M. Fuss. “Radiation chemistry based analysis of carbon FLASH feasibility”. **Talk:** PTCOG59, 59th annual meeting of the Particle Therapy Co-Operative Group (*virtual* – 2021).
- C.ii D. Boscolo, E. Scifoni, M. Fuss, **G. Camazzola**, M. Durante, M. Krämer, and U. Weber. “Radiation-chemistry for FLASH treatments with ion beams”. **Talk:** Joint conference of the ÖGMP, DGMP and SGSMP (*virtual* – 2021).
- C.iii M. Fuss, D. Boscolo, **G. Camazzola**, M. Durante, M. Krämer, and E. Scifoni. “Radical-radical recombinations at ultra-high dose rate for different LET and particle type”. **Poster:** RRS 2021, Radiation Research Society’s 67th annual international meeting (*virtual* – 2021).
- C.iv **G. Camazzola**, D. Boscolo, E. Scifoni, M. Durante, M. Krämer, and M. Fuss. “Current status of the TRAX-CHEM extension to the homogeneous chemical stage”. **Poster:** FRPT 2021, 1st Flash Radiotherapy and Particle Therapy conference (*virtual* – 2021).
- C.v **G. Camazzola**, D. Boscolo, E. Scifoni, A. Dorn, M. Durante, M. Krämer, and M. Fuss. “Towards the homogeneous chemical stage of radiation damage: new extension of the TRAX-CHEM code”. **Talk:** FRIDA 2022, FLASH Radiotherapy with hIgh Dose-rate particle beAms general meeting (*virtual* – 2022).
- C.vi **G. Camazzola**, D. Boscolo, E. Scifoni, A. Dorn, M. Durante, M. Krämer, and M. Fuss. “Towards the homogeneous chemical stage of radiation damage – update on the TRAX-CHEM code”. **Talk:** FRPT 2022, 2nd Flash Radiotherapy and Particle Therapy conference (*Barcelona, Spain* – 2022).

- C.vii **G. Camazzola**, D. Boscolo, E. Scifoni, A. Dorn, M. Durante, M. Krämer, V. Abram, and M. Fuss. “Accurate and faster: probing the biochemical stage of radiation damage with the new TRAX-CHEMxt code”. **Talk:** INSIGHTS workshop, 1st INnovative Systems In radiation therapy: breakthrouGHs novel detectors, Treatments and AI techniqueS meeting (*Pisa, Italy* – 2023).

Peer-reviewed articles

Publications with direct relation to this thesis are marked with “*”:

- P.i D. A. Kartini, O. Sokol, J. Wiedemann, W. Tinganelli, M. Witt, **G. Camazzola**, M. Krämer, C. Talabnin, C. Kobdaj, and M. C. Fuss. “Validation of a pseudo-3D phantom for radiobiological treatment plan verifications”. In: *Phys. Med. Biol.* 65 (2020).
- P.ii J. Seco, C. C. King, **G. Camazzola**, J. Jansen, L. Tirinato, M. G. Marafioti, R. Hanley, F. Pagliari, and S. P. Beckman. “Modulating nucleus oxygen concentration by altering intramembrane cholesterol levels: creating hypoxic nucleus in oxic conditions”. In: *Int. J. Mol. Sci.* 23 (2022).
- P.iii ***G. Camazzola**, D. Boscolo, E. Scifoni, A. Dorn, M. Durante, M. Krämer, V. Abram, and M. C. Fuss. “TRAX-CHEMxt: towards the homogeneous chemical stage of radiation damage”. In: *Int. J. Mol. Sci.* 24 (2023).
- P.iv L. Castelli, **G. Camazzola**, M. C. Fuss, D. Boscolo, M. Krämer, and E. Scifoni. “Probing intertrack effects at ultra-high dose rates with a new implementation of simultaneous multiple tracks in TRAX-CHEM”. *In preparation* (2024).

Bibliography

- [1] A. Alanazi, J. Meesungnoen, and J. P. Jay-Gerin. “A computer modeling study of water radiolysis at high dose rates. Relevance to FLASH radiotherapy”. In: *Radiat. Res.* 195 (2020). DOI: 10.1667/RADE-20-00168.1.
- [2] E. Scifoni. “Radiation biophysical aspects of charged particles: from the nanoscale to therapy”. In: *Mod. Phys. Lett. A* 30 (2015). DOI: 10.1142/S0217732315400192.
- [3] A. Mairani et al. “Roadmap: helium ion therapy”. In: *Phys. Med. Biol.* 67 (2022). DOI: 10.1088/1361-6560/ac65d3.
- [4] P. O’Neill and P. Wardman. “Radiation chemistry comes before radiation biology”. In: *Int. J. Radiat. Biol.* 85 (2009). DOI: 10.1080/09553000802640401.
- [5] M. C. Vozenin, J. H. Hendry, and C. L. Limoli. “Biological benefits of ultra-high dose rate FLASH radiotherapy: sleeping beauty awoken”. In: *Clin. Oncol.* 31 (2019). DOI: 10.1016/j.clon.2019.04.001.
- [6] P. Montay-Gruel et al. “FLASH radiotherapy with photon beams”. In: *Med. Phys.* 49 (2022). DOI: 10.1002/mp.15222.
- [7] W. Tinganelli et al. “Ultra-high dose rate (FLASH) carbon ion irradiation: dosimetry and first cell experiments”. In: *Int. J. Radiat. Oncol. Biol. Phys.* 112 (2022). DOI: 10.1016/j.ijrobp.2021.11.020.
- [8] W. Tinganelli et al. “FLASH with carbon ions: tumor control, normal tissue sparing, and distal metastasis in a mouse osteosarcoma model”. In: *Radiother. Oncol.* 175 (2022). DOI: 10.1016/j.radonc.2022.05.003.
- [9] A. A. Friedl et al. “Radiobiology of the FLASH effect”. In: *Med. Phys.* 49 (2022). DOI: 10.1002/mp.15184.
- [10] H. Kacem et al. “Understanding the FLASH effect to unravel the potential of ultra-high dose rate irradiation”. In: *Int. J. Radiat. Biol.* 98 (2022). DOI: 10.1080/09553002.2021.2004328.
- [11] U. A. Weber, E. Scifoni, and M. Durante. “FLASH radiotherapy with carbon ion beams”. In: *Med. Phys.* 49 (2022). DOI: 10.1002/mp.15135.

- [12] V. Wasselin-Trupin et al. “Hydrogen peroxide yields in water radiolysis by high-energy ion beams at constant LET”. In: *Radiat. Phys. Chem.* 65 (2002). DOI: 10.1016/S0969-806X(01)00682-X.
- [13] G. Camazzola et al. “TRAX-CHEMxt: towards the homogeneous chemical stage of radiation damage”. In: *Int. J. Mol. Sci.* 24 (2023). DOI: 10.3390/ijms24119398.
- [14] D. Boscolo et al. “TRAX-CHEM: a pre-chemical and chemical stage extension of the particle track structure code TRAX in water targets”. In: *Chem. Phys. Lett.* 698 (2018). DOI: 10.1016/j.cplett.2018.02.051.
- [15] M. Krämer and G. Kraft. “Calculations of heavy-ion track structure”. In: *Radiat. Environ. Biophys.* 33 (1994). DOI: 10.1007/BF01219334.
- [16] C. Wälzlein et al. “Advancing the modeling in particle therapy: from track structure to treatment planning”. In: *Appl. Radiat. Isot.* 83 (2014). DOI: 10.1016/j.apradiso.2013.01.019.
- [17] D. Boscolo et al. “Impact of target oxygenation on the chemical track evolution of ion and electron radiation”. In: *Int. J. Mol. Sci.* 21 (2020). DOI: 10.3390/ijms21020424.
- [18] D. Boscolo et al. “May oxygen depletion explain the FLASH effect? A chemical track structure analysis”. In: *Radiother. Oncol.* 162 (2021). DOI: 10.1016/j.radonc.2021.06.031.
- [19] D. Schardt, T. Elsässer, and D. Schulz-Ertner. “Heavy-ion tumor therapy: physical and radiobiological benefits”. In: *Rev. Mod. Phys.* 82 (2010). DOI: 10.1103/RevModPhys.82.383.
- [20] M. Durante and H. Paganetti. “Nuclear physics in particle therapy: a review”. In: *Rep. Prog. Phys.* 79 (2016). DOI: 10.1088/0034-4885/79/9/096702.
- [21] M. Durante, J. Debus, and J. S. Loeffler. “Physics and biomedical challenges of cancer therapy with accelerated heavy ions”. In: *Nat. Rev. Phys.* 3 (2021). DOI: 10.1038/s42254-021-00368-5.
- [22] V. Favaudon, R. Labarbe, and C. L. Limoli. “Model studies of the role of oxygen in the FLASH effect”. In: *Med. Phys.* 49 (2022). DOI: 10.1002/mp.15129.
- [23] H. Ledford. “Why are so many young people getting cancer? What the data say”. In: *Nature* 627 (2024). DOI: 10.1038/d41586-024-00720-6.
- [24] R. R. Wilson. “Radiological use of fast protons”. In: *Radiology* 47 (1946). DOI: 10.1148/47.5.487.

-
- [25] T. Friedrich, M. Durante, and M. Scholz. “Simulation of DSB yield for high LET radiation”. In: *Radiat. Prot. Dosim.* 166 (2015). DOI: 10.1093/rpd/ncv147.
- [26] H. B. Michaels and J. W. Hunt. “A model for radiation damage in cells by direct effect and by indirect effect: a radiation chemistry approach”. In: *Radiat. Res.* 74 (1978). DOI: 10.2307/3574754.
- [27] R. Meesat et al. “Utilization of the ferrous sulfate (Fricke) dosimeter for evaluating the radioprotective potential of cystamine: experiment and Monte Carlo simulation”. In: *Radiat. Res.* 177 (2012). DOI: 10.1667/RR2829.1.
- [28] M. S. Kreipl, W. Friedland, and H. G. Paretzke. “Interaction of ion tracks in spatial and temporal proximity”. In: *Radiat. Environ. Biophys.* 48 (2009). DOI: 10.1007/s00411-009-0234-z.
- [29] S. R. McKeown. “Defining normoxia, physoxia and hypoxia in tumours - implications for treatment response”. In: *Br. J. Radiol.* 87 (2014). DOI: 10.1259/bjr.20130676.
- [30] A. Ito et al. “Contribution of indirect action to radiation-induced mammalian cell inactivation: dependence on photon energy and heavy-ion LET”. In: *Radiat. Res.* 165 (2006). DOI: 10.1667/RR3557.1.
- [31] T. Schlathölter et al. “Improving proton therapy by metal-containing nanoparticles: nanoscale insights”. In: *Int. J. Nanomed.* 11 (2016). DOI: 10.2147/IJN.S99410.
- [32] R. Hirayama et al. “Radioprotection by DMSO in nitrogen-saturated mammalian cells exposed to helium ion beams”. In: *Radiat. Phys. Chem.* 78 (2009). DOI: 10.1016/j.radphyschem.2009.07.006.
- [33] J. D. Chapman et al. “Radioprotection by DMSO of mammalian cells exposed to X-rays and to heavy charged-particle beams”. In: *Radiat. Environ. Biophys.* 16 (1979). DOI: 10.1007/BF01326894.
- [34] R. Grün et al. “Is the dose-averaged LET a reliable predictor for the relative biological effectiveness?” In: *Med. Phys.* 46 (2019). DOI: 10.1002/mp.13347.
- [35] A. Colliaux et al. “Simulation of ion-induced water radiolysis in different conditions of oxygenation”. In: *Nucl. Instrum. Methods Phys. Res., Sect. B* 365 (2015). DOI: 10.1016/j.nimb.2015.08.057.
- [36] J. E. Turner, H. A. Wright, and R. N. Hamm. “A Monte Carlo primer for health physicists”. In: *Health Phys.* 48 (1985). DOI: 10.1097/00004032-198506000-00001.
- [37] J. E. Turner et al. “Physical and chemical development of electron tracks in liquid water”. In: *Radiat. Res.* 96 (1983). DOI: 10.2307/3576111.

- [38] F. Ballarini et al. “Stochastic aspects and uncertainties in the prechemical and chemical stages of electron tracks in liquid water: a quantitative analysis based on Monte Carlo simulations”. In: *Radiat. Environ. Biophys.* 39 (2000). DOI: 10.1007/s004110000060.
- [39] S. M. Pimblott, M. J. Pilling, and N. J. B. Green. “Stochastic models of spur kinetics in water”. In: *Int. J. Radiat. Appl. Instrum. Part C* 37 (1991). DOI: 10.1016/1359-0197(91)90006-N.
- [40] J. Ramos-Méndez et al. “LET-dependent intertrack yields in proton irradiation at ultra-high dose rates relevant for FLASH therapy”. In: *Radiat. Res.* 194 (2020). DOI: 10.1667/RADE-20-00084.1.
- [41] J. Ramos-Méndez et al. “Independent reaction times method in Geant4-DNA: implementation and performance”. In: *Med. Phys.* 47 (2020). DOI: 10.1002/mp.14490.
- [42] J. A. LaVerne. “Track effects of heavy ions in liquid water”. In: *Radiat. Res.* 153 (2000). DOI: 10.1667/0033-7587(2000)153[0487:TEOHII]2.0.CO;2.
- [43] B. Pastina and J. A. LaVerne. “Effect of molecular hydrogen on hydrogen peroxide in water radiolysis”. In: *J. Phys. Chem. A* 105 (2001). DOI: 10.1021/jp012245j.
- [44] W. G. Burns and H. E. Sims. “Effect of radiation type in water radiolysis”. In: *J. Chem. Soc., Faraday Trans. 1* 77 (1981). DOI: 10.1039/f19817702803.
- [45] M. Karamitros et al. “Diffusion-controlled reactions modeling in Geant4-DNA”. In: *J. Comput. Phys.* 274 (2014). DOI: 10.1016/j.jcp.2014.06.011.
- [46] J. Schuemann et al. “TOPAS-nBio: an extension to the TOPAS simulation toolkit for cellular and sub-cellular radiobiology”. In: *Radiat. Res.* 191 (2019). DOI: 10.1667/RR15226.1.
- [47] R. Watanabe and K. Saito. “Monte Carlo simulation of water radiolysis in oxygenated condition for monoenergetic electrons from 100 eV to 1 MeV”. In: *Radiat. Phys. Chem.* 62 (2001). DOI: 10.1016/S0969-806X(01)00195-5.
- [48] I. Plante. “A Monte-Carlo step-by-step simulation code of the non-homogeneous chemistry of the radiolysis of water and aqueous solutions. Part I: theoretical framework and implementation”. In: *Radiat. Environ. Biophys.* 50 (2011). DOI: 10.1007/s00411-011-0367-8.
- [49] I. Plante. “A Monte-Carlo step-by-step simulation code of the non-homogeneous chemistry of the radiolysis of water and aqueous solutions – Part II: calculation of radiolytic yields under different conditions of LET, pH, and temperature”. In: *Radiat. Environ. Biophys.* 50 (2011). DOI: 10.1007/s00411-011-0368-7.

-
- [50] H. Zhu et al. “Modeling of cellular response after FLASH irradiation: a quantitative analysis based on the radiolytic oxygen depletion hypothesis”. In: *Phys. Med. Biol.* 66 (2021). DOI: 10.1088/1361-6560/ac226d.
- [51] A. Hu et al. “CPU-GPU coupling independent reaction times method in NA-SIC and application in water radiolysis by FLASH irradiation”. In: *Biomed. Phys. Eng. Express* 8 (2022). DOI: 10.1088/2057-1976/ac52d9.
- [52] Z. Tian, S. B. Jiang, and X. Jia. “Accelerated Monte Carlo simulation on the chemical stage in water radiolysis using GPU”. In: *Phys. Med. Biol.* 62 (2017). DOI: 10.1088/1361-6560/aa6246.
- [53] Y. Lai, X. Jia, and Y. Chi. “Modeling the effect of oxygen on the chemical stage of water radiolysis using GPU-based microscopic Monte Carlo simulations, with an application in FLASH radiotherapy”. In: *Phys. Med. Biol.* 66 (2021). DOI: 10.1088/1361-6560/abc93b.
- [54] I. Plante. “A review of simulation codes and approaches for radiation chemistry”. In: *Phys. Med. Biol.* 66 (2021). DOI: 10.1088/1361-6560/abbd19.
- [55] R. Labarbe et al. “A physicochemical model of reaction kinetics supports peroxyl radical recombination as the main determinant of the FLASH effect”. In: *Radiother. Oncol.* 153 (2020). DOI: 10.1016/j.radonc.2020.06.001.
- [56] A. Colliaux et al. “O₂ and glutathione effects on water radiolysis: a simulation study”. In: *J. Phys.: Conf. Ser.* 261 (2011). DOI: 10.1088/1742-6596/261/1/012007.
- [57] C. Wälzlein et al. “Simulations of dose enhancement for heavy atom nanoparticles irradiated by protons”. In: *Phys. Med. Biol.* 59 (2014). DOI: 10.1088/0031-9155/59/6/1441.
- [58] M. J. Berger et al. *ESTAR, PSTAR, and ASTAR: computer programs for calculating stopping-power and range tables for electrons, protons, and helium ions*. National Institute of Standards and Technology, 2005. URL: <http://physics.nist.gov/Star>.
- [59] T. Elsässer et al. “Impact of track structure calculations on biological treatment planning in ion radiotherapy”. In: *New J. Phys.* 10 (2008). DOI: 10.1088/1367-2630/10/7/075005.
- [60] H. N. Tran et al. “Geant4-DNA modeling of water radiolysis beyond the microsecond: an on-lattice stochastic approach”. In: *Int. J. Mol. Sci.* 22 (2021). DOI: 10.3390/ijms22116023.
- [61] P. Clifford et al. “Stochastic models of multi-species kinetics in radiation-induced spurs”. In: *J. Chem. Soc., Faraday Trans. 1* 82 (1986). DOI: 10.1039/F19868202673.

- [62] A. Quarteroni. *Numerical models for differential problems*. Springer, 2014.
- [63] H. Brezis. *Functional analysis, Sobolev spaces and partial differential equations*. Springer, 2010.
- [64] R. A. Usmani. “Inversion of Jacobi’s tridiagonal matrix”. In: *Comput. Math. Appl.* 27 (1994). DOI: 10.1016/0898-1221(94)90066-3.
- [65] P. Wardman. “Approaches to modeling chemical reaction pathways in radiobiology”. In: *Int. J. Radiat. Biol.* 98 (2022). DOI: 10.1080/09553002.2022.2033342.
- [66] V. Cobut, C. Corbel, and J. P. Patau. “Influence of the pH on molecular hydrogen primary yields in He²⁺ ion tracks in liquid water. A Monte Carlo study”. In: *Radiat. Phys. Chem.* 72 (2005). DOI: 10.1016/j.radphyschem.2004.05.052.
- [67] X. Cao et al. “Quantification of oxygen depletion during FLASH irradiation in vitro and in vivo”. In: *Int. J. Radiat. Oncol. Biol. Phys.* 111 (2021). DOI: 10.1016/j.ijrobp.2021.03.056.
- [68] M. von Smoluchowski. “Versuch einer mathematischen Theorie der Koagulationskinetik kolloider Lösungen”. In: *Z. Phys. Chem.* 92 (1918). DOI: 10.1515/zpch-1918-9209.
- [69] R. M. Noyes. “Effects of diffusion rates on chemical kinetics”. In: *Prog. React. Kinet.* 1 (1961).
- [70] R. A. Alberty and G. G. Hammes. “Application of the theory of diffusion-controlled reactions to enzyme kinetics”. In: *J. Phys. Chem.* 62 (1958). DOI: 10.1021/j150560a005.
- [71] S. A. Rice et al. “A solution of the Debye-Smoluchowski equation for the rate of reaction of ions in dilute solution”. In: *J. Chem. Phys.* 70 (1979). DOI: 10.1063/1.438020.
- [72] N. J. B. Green. “Reaction probability and diffusion-controlled rate constants for ionic reactions in solvents of high permittivity”. In: *Chem. Phys. Lett.* 107 (1984). DOI: 10.1016/S0009-2614(84)80260-2.
- [73] H. Van Beijeren, W. Dong, and L. Bocquet. “Diffusion-controlled reactions: a revisit of Noyes’ theory”. In: *J. Chem. Phys.* 114 (2001). DOI: 10.1063/1.1350642.
- [74] M. J. Davies. “Protein oxidation and peroxidation”. In: *Biochem. J.* 473 (2016). DOI: 10.1042/BJ20151227.
- [75] C. L. Hawkins and M. J. Davies. “Generation and propagation of radical reactions on proteins”. In: *Biochim. Biophys. Acta, Bioenerg.* 1504 (2001). DOI: 10.1016/S0005-2728(00)00252-8.

-
- [76] K. A. Piez and H. Eagle. “The free amino acid pool of cultured human cells”. In: *J. Biol. Chem.* 231 (1958). DOI: 10.1016/s0021-9258(19)77326-8.
- [77] T. W. Traut. “Physiological concentrations of purines and pyrimidines”. In: *Mol. Cell. Biochem.* 140 (1994). DOI: 10.1007/BF00928361.
- [78] T. Nauser, W. H. Koppenol, and C. Schöneich. “Protein thiyl radical reactions and product formation: a kinetic simulation”. In: *Free Radical Biol. Med.* 80 (2015). DOI: 10.1016/j.freeradbiomed.2014.12.006.
- [79] N. S. Kosower and E. M. Kosower. “The glutathione status of cells”. In: *Int. Rev. Cytol.* 54 (1978). DOI: 10.1016/S0074-7696(08)60166-7.
- [80] P. Wardman and C. von Sonntag. “[3] Kinetic factors that control the fate of thiyl radicals in cells”. In: *Methods Enzymol.* 251 (1995). DOI: 10.1016/0076-6879(95)51108-3.
- [81] R. B. Mikkelsen and P. Wardman. “Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms”. In: *Oncogene* 22 (2003). DOI: 10.1038/sj.onc.1206663.
- [82] C. C. Liu and J. M. Gebicki. “Intracellular GSH and ascorbate inhibit radical-induced protein chain peroxidation in HL-60 cells”. In: *Free Radical Biol. Med.* 52 (2012). DOI: 10.1016/j.freeradbiomed.2011.10.450.
- [83] C. J. Koch, M. M. Kim, and R. D. Wiersma. “Radiation-chemical oxygen depletion depends on chemical environment and dose rate: implications for the FLASH effect”. In: *Int. J. Radiat. Oncol. Biol. Phys.* 117 (2023). DOI: 10.1016/j.ijrobp.2023.04.001.
- [84] S. Gebicki and J. M. Gebicki. “Formation of peroxides in amino acids and proteins exposed to oxygen free radicals”. In: *Biochem. J.* 289 (1993). DOI: 10.1042/bj2890743.
- [85] C. L. Hawkins and M. J. Davies. “Detection, identification, and quantification of oxidative protein modifications”. In: *J. Biol. Chem.* 294 (2019). DOI: 10.1074/jbc.REV119.006217.
- [86] H. S. Tan et al. “Modeling ultra-high dose rate electron and proton FLASH effect with the physicochemical approach”. In: *Phys. Med. Biol.* 68 (2023). DOI: 10.1088/1361-6560/ace14d.
- [87] R. Huie. *NDRL/NIST solution kinetics database on the web*. National Institute of Standards and Technology, 2003. URL: <https://kinetics.nist.gov/solution/>.
- [88] P. Neta, R. E. Huie, and A. B. Ross. “Rate constants for reactions of peroxy radicals in fluid solutions”. In: *J. Phys. Chem. Ref. Data* 19 (1990). DOI: 10.1063/1.555854.

- [89] C. von Sonntag. *Free-radical-induced DNA damage and its repair*. Springer, 2006.
- [90] J. A. LaVerne and S. M. Pimblott. “Yields of hydroxyl radical and hydrated electron scavenging reactions in aqueous solutions of biological interest”. In: *Radiat. Res.* 135 (1993). DOI: 10.2307/3578391.
- [91] F. Leinisch et al. “Peroxyl radical- and photo-oxidation of glucose 6-phosphate dehydrogenase generates cross-links and functional changes via oxidation of tyrosine and tryptophan residues”. In: *Free Radical Biol. Med.* 112 (2017). DOI: 10.1016/j.freeradbiomed.2017.07.025.
- [92] C. López-Alarcón et al. “The role of protein-derived free radicals as intermediaries of oxidative processes”. In: *Biomol. Concepts* 5 (2014). DOI: 10.1515/bmc-2014-0004.
- [93] M. Yu et al. “The investigation of protein diffusion via H-cell microfluidics”. In: *Biophys. J.* 116 (2019). DOI: 10.1016/j.bpj.2019.01.014.
- [94] J. F. Torres et al. “Measurement of the molecular mass dependence of the mass diffusion coefficient in protein aqueous solutions”. In: *DDF* 326 (2012). DOI: 10.4028/www.scientific.net/DDF.326-328.452.
- [95] B. Tang et al. “Quantitative interpretation of protein diffusion coefficients in mixed protiated-deuteriated aqueous solvents”. In: *J. Phys. Chem. B* 126 (2022). DOI: 10.1021/acs.jpccb.2c03554.
- [96] P. M. Boyer and J. T. Hsu. “Experimental studies of restricted protein diffusion in an agarose matrix”. In: *AIChE J.* 38 (1992). DOI: 10.1002/aic.690380212.
- [97] S. Miyamoto and K. Shimono. “Estimation of the diffusion coefficients of small molecules by diffusion measurements with agar-gel and theoretical molecular modeling”. In: *Chem-Bio Inform. J.* 22 (2022). DOI: 10.1273/cbij.22.13.
- [98] Y. Ma et al. “Studies on the diffusion coefficients of amino acids in aqueous solutions”. In: *J. Chem. Eng. Data* 50 (2005). DOI: 10.1021/je049582g.
- [99] C. M. Romero et al. “Limiting diffusion coefficients of α,ω -amino acids in water and in sodium chloride aqueous solutions at 298.15 K”. In: *Eur. Phys. J. E* 42 (2019). DOI: 10.1140/epje/i2019-11856-1.
- [100] Z. Wang, H. Krieger, and S. Wiegand. “Thermal diffusion of nucleotides”. In: *J. Phys. Chem. B* 116 (2012). DOI: 10.1021/jp3032644.
- [101] W. Stricks and I. M. Kolthoff. “Polarography of glutathione”. In: *J. Am. Chem. Soc.* 74 (1952). DOI: 10.1021/ja01138a060.
- [102] W. Jin and H. Chen. “A new method of determination of diffusion coefficients using capillary zone electrophoresis (peak-height method)”. In: *Chromatographia* 52 (2000). DOI: 10.1007/BF02490786.

-
- [103] M. Shamim and S. M. A. Baki. "Diffusion measurements in aqueous L-ascorbic acid solutions". In: *Aust. J. Chem.* 33 (1980). DOI: 10.1071/CH9801857.
- [104] H. V. S. Ganesh, M. Noroozifar, and K. Kerman. "Epigallocatechin gallate-modified graphite paste electrode for simultaneous detection of redox-active biomolecules". In: *Sensors* 18 (2018). DOI: 10.3390/s18010023.
- [105] A. N. Soriano et al. "Estimation of diffusion coefficients of ascorbate-based ions at infinite dilution through electrolytic conductivity measurements". In: *IOP Conf. Ser.: Earth Environ. Sci.* 191 (2018). DOI: 10.1088/1755-1315/191/1/012028.
- [106] N. Autsavaporn et al. "Monte Carlo simulation study of the effects of acidity and LET on the primary free-radical and molecular yields of water radiolysis – Application to the Fricke dosimeter". In: *Can. J. Chem.* 85 (2007). DOI: 10.1139/V07-021.
- [107] F. Chappuis et al. "Modeling of scavenging systems in water radiolysis with Geant4-DNA". In: *Physica Med.* 108 (2023). DOI: 10.1016/j.ejmp.2023.102549.
- [108] A. L. Van Slyke et al. "Oxygen monitoring in model solutions and in vivo in mice during proton irradiation at conventional and FLASH dose rates". In: *Radiat. Res.* 198 (2022). DOI: 10.1667/RADE-21-00232.1.
- [109] J. Delattre, J. L. Beaudoux, and D. Bonnefont-Rousselot. *Radicaux libres et stress oxydant: aspects biologiques et pathologiques*. Editions Tec & Doc, 2005.
- [110] D. T. Gillespie. "A general method for numerically simulating the stochastic time evolution of coupled chemical reactions". In: *J. Comput. Phys.* 22 (1976). DOI: 10.1016/0021-9991(76)90041-3.
- [111] O. Roth and J. A. LaVerne. "Effect of pH on H₂O₂ production in the radiolysis of water". In: *J. Phys. Chem. A* 115 (2011). DOI: 10.1021/jp1099927.
- [112] B. Pastina and J. A. LaVerne. "Hydrogen peroxide production in the radiolysis of water with heavy ions". In: *J. Phys. Chem. A* 103 (1999). DOI: 10.1021/jp984433o.
- [113] H. B. Michaels. "Oxygen depletion in irradiated aqueous solutions containing electron affinic hypoxic cell radiosensitizers". In: *Int. J. Radiat. Oncol. Biol. Phys.* 12 (1986). DOI: 10.1016/0360-3016(86)90224-5.
- [114] M. El Khatib et al. "Ultrafast tracking of oxygen dynamics during proton FLASH". In: *Int. J. Radiat. Oncol. Biol. Phys.* 113 (2022). DOI: 10.1016/j.ijrobp.2022.03.016.

- [115] W. Thomas et al. “Proton and electron ultrahigh-dose-rate isodose irradiations produce differences in reactive oxygen species yields”. In: *Int. J. Radiat. Oncol. Biol. Phys.* 118 (2024). DOI: 10.1016/j.ijrobp.2023.07.042.
- [116] D. R. Spitz et al. “An integrated physico-chemical approach for explaining the differential impact of FLASH versus conventional dose rate irradiation on cancer and normal tissue responses”. In: *Radiother. Oncol.* 139 (2019). DOI: 10.1016/j.radonc.2019.03.028.
- [117] J. N. D-Kondo et al. “An integrated Monte Carlo track-structure simulation framework for modeling inter and intra-track effects on homogenous chemistry”. In: *Phys. Med. Biol.* 68 (2023). DOI: 10.1088/1361-6560/acd6d0.
- [118] G. Blain et al. “Proton irradiations at ultra-high dose rate vs. conventional dose rate: strong impact on hydrogen peroxide yield”. In: *Radiat. Res.* 198 (2022). DOI: 10.1667/RADE-22-00021.1.
- [119] H. Kacem et al. “Comparing radiolytic production of H₂O₂ and development of Zebrafish embryos after ultra high dose rate exposure with electron and transmission proton beams”. In: *Radiother. Oncol.* 175 (2022). DOI: 10.1016/j.radonc.2022.07.011.
- [120] A. J. Elliot and D. R. McCracken. “Computer modelling of the radiolysis in an aqueous lithium salt blanket: suppression of radiolysis by addition of hydrogen”. In: *Fusion Eng. Des.* 13 (1990). DOI: 10.1016/0920-3796(90)90028-5.
- [121] J. Jansen et al. “Does FLASH deplete oxygen? Experimental evaluation for photons, protons, and carbon ions”. In: *Med. Phys.* 48 (2021). DOI: 10.1002/mp.14917.
- [122] G. Pratx and D. S. Kapp. “A computational model of radiolytic oxygen depletion during FLASH irradiation and its effect on the oxygen enhancement ratio”. In: *Phys. Med. Biol.* 64 (2019). DOI: 10.1088/1361-6560/ab3769.
- [123] P. Wilson et al. “Revisiting the ultra-high dose rate effect: implications for charged particle radiotherapy using protons and light ions”. In: *Brit. J. Radiol.* 85 (2012). DOI: 10.1259/bjr/17827549.
- [124] H. Weiss et al. “Oxygen depletion in cells irradiated at ultra-high dose-rates and at conventional dose-rates”. In: *Int. J. Radiat. Biol.* 26 (1974). DOI: 10.1080/09553007414550901.
- [125] R. Dal Bello et al. “Proposal of a chemical mechanism for mini-beam and micro-beam efficacy”. In: *Front. Phys.* 8 (2020). DOI: 10.3389/fphy.2020.564836.

-
- [126] S. Penninckx et al. “The role of thioredoxin reductase in gold nanoparticle radiosensitization effects”. In: *Nanomed.* 13 (2018). DOI: 10.2217/nnm-2018-0171.
- [127] B. Rudek et al. “Radio-enhancement by gold nanoparticles and their impact on water radiolysis for x-ray, proton and carbon-ion beams”. In: *Phys. Med. Biol.* 64 (2019). DOI: 10.1088/1361-6560/ab314c.
- [128] Y. Shiraishi, Y. Matsuya, and H. Fukunaga. “Possible mechanisms and simulation modeling of FLASH radiotherapy”. In: *Radiol. Phys. Technol.* 17 (2024). DOI: 10.1007/s12194-023-00770-x.
- [129] P. Montay-Gruel et al. “Long-term neurocognitive benefits of FLASH radiotherapy driven by reduced reactive oxygen species”. In: *Proc. Natl. Acad. Sci. U. S. A.* 166 (2019). DOI: 10.1073/pnas.1901777116.
- [130] J. Geng et al. “Switching on prodrugs using radiotherapy”. In: *Nat. Chem.* 13 (2021). DOI: 10.1038/s41557-021-00711-4.
- [131] X. Li et al. “Radiotherapy-triggered prodrug activation: a new era in precise chemotherapy”. In: *Med* 3 (2022). DOI: 10.1016/j.medj.2022.08.004.
- [132] N. J. Farrer, G. S. Higgins, and I. H. Kunkler. “Radiation-induced prodrug activation: extending combined modality therapy for some solid tumours”. In: *Br. J. Cancer* 126 (2022). DOI: 10.1038/s41416-022-01746-1.

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