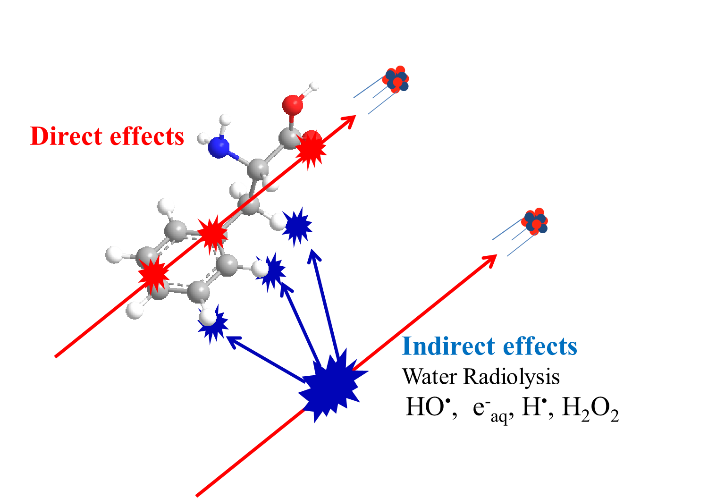
***Contribution to the 2020-2030 prospective reflection***

*Nuclear Science and Health*

Radiolysis of biomolecules by accelerated ions



Among treatments against cancer, radiation therapy is recognized as a successful method, and is widely represented in hospitals worldwide. In most cases, it consists in high-energy photon irradiation. However, some tumors exhibit radiation-resistance, which is one of the most important limitations of the method. Luckily, these tumors are often sensible to high-LET radiations, especially carbon ions. Moreover, use of ions allows a very localized energy deposition, essentially concentrated in the Bragg peak, thus preserving the healthy tissues. This is why radiotherapy with accelerated ions, or hadron therapy, is strongly developing worldwide, as a complementary radiotherapy.

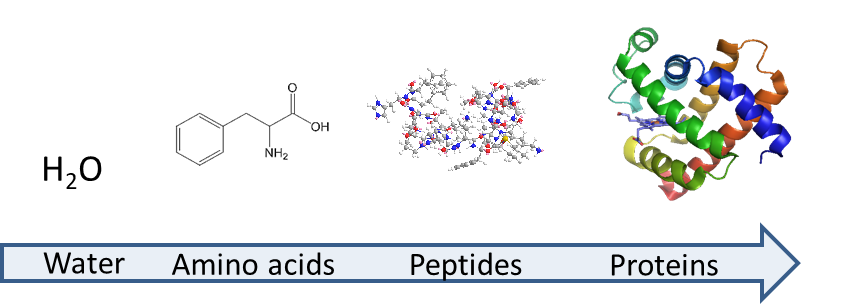
Figure 1. Scheme representing the direct effects of ion irradiation, when energy is deposited directly to the molecule of interest, and indirect effects, through reactive species formed by water radiolysis.

This development of hadrontherapy is accompanied by many research studies in various domains: clinical, biology, physics, chemistry. Many studies have been performed with various irradiation conditions by radiobiologists on the biological effects, from the cell level to the whole organism level. The results obtained have provided precious information, not only on the radiations risks but also on the benefits for therapeutic applications such as radiation diagnosis and therapy. Meanwhile, the radiation physics and measurements have been well established, which can give access to physical quantities of radiation deposited in matter. **However, the detailed processes occurring in the biomolecules between the initial interaction (physical process) and the final results (biological process) through complex chemical reactions is still far from being understood**.

In this framework, we propose an innovative research on the effects of accelerated ions at the molecular scale on biomolecules. The knowledge gained on the chemical processes occurring under ion irradiation and the mechanisms of the modifications of biomolecules will be of great interest to better understand phenomena observed under irradiation in a living cell, and therefore the effect of the radiation at the biological stage.

While the main component of the cell is water (~65 %), proteins are its second most abundant component (~20 %), when DNA represent less than 0.1% of its mass. DNA damage is critically linked to cell-death, making it naturally a key target for studies. Nevertheless, because of their concentration in the cell, **proteins are the biomolecules that will statistically undergo most of the damages** by direct deposition of the ions energy (*direct effect*), or by *indirect effect* through reactions with water radiolysis species (Figure 1). The study of their fate under ion irradiation will give valuable insight to better understand the phenomena observed in the living cell. However, **at this moment, there are no systematic studies on the radiolysis of biomolecules by accelerated ions.**

Our proposal for a contribution is therefore the realization of a **systematic molecular radiolysis study, with scales ranging from water to proteins, through amino acids and peptides, to better understand the influence of these biomolecules in cell damage and death under irradiation.**



**Details of the proposal**

When ionizing radiations penetrate the cell, an important part of their energy is deposited in water. This leads to water radiolysis and production of active species: hydroxyl radical, aqueous electron, hydrogen atom and hydrogen peroxide, which can then react with biomolecules. Therefore, studies of the radiolysis of biomolecules are closely linked to that of water radiolysis. Production of water radiolysis species, especially hydroxyl radical HO•, needs therefore to be quantified precisely under ion irradiation.

The smallest building blocks of proteins are amino acids. Their reactivity towards water radiolysis products, and in particular HO• is not the same for all of them. A comprehensive study of the most reactive ones irradiated by accelerated ions, in which all the radiolysis products will be identified and quantified, would be crucial to determine the mechanisms of their radiolysis.

Then, small peptides of a few tens of amino-acids will bridge the gap between amino acids and proteins, as they are small enough to allow precise chemical analysis and quantification at the molecular level, and have the chemical structure of proteins. Peptide studies will allow determining the changes in the radiolysis mechanisms when the amino acids are linked together, which could especially involve radical-transfers not occurring with single molecules.

Whole proteins will be studied at various scales, given the complexity of these molecules, by examining the effects of ions on their secondary structure, that is their 3D organization, and on their chemical structure.

Interactions between proteins or peptides with DNA under irradiation need also to be scrutinized, to understand how proteins could be the cause of damages to DNA under irradiation.

Influence of water on the damage to biomolecules needs also to be understood. Therefore, studies will be conducted with biomolecules in diluted solutions and in very concentrated ones, close to the concentrations in the cell, as well as in dry samples. This would lead to evaluation of the *indirect* *effects* and the *direct effects* of the ions on the radiolysis of these biomolecules.

The quantities of reactive species generated and the chemical mechanisms of formation of radiolysis products can vary greatly depending on density of energy deposition, or Linear Energy Transfer, and the nature of the ionizing radiation. All the studies will therefore need to be conducted with various ions (H, C, He etc.) of energies from the therapeutic ones to the Bragg-peak, to draw general conclusions on the results obtained. Radiolysis with some low-LET radiations (gamma, X-Rays or electrons) will also have to be performed for comparison of the mechanisms.

**Prospective feasibility**

Our team has developed an expertise in the field of water and biomolecules radiolysis. We have recently completed a study on phenylalanine, an aromatic amino acid, showing the formation of radiolysis products specifically under ion irradiation, and their mechanisms of formation have been determined. The radiolysis of several model proteins, chosen for their secondary structures, has also been studied, in diluted solutions (indirect effects) and concentrated ones (towards direct effects). Recently, our team has also started working on small peptides, and the results obtained were very promising. We have developed setups for the irradiation of solutions with ions of high and low energies, equipped with on-line analysis techniques (infrared, UV-Visible, Fluorescence).

As for the accelerated ions sources, experiments were conducted on the 4 MV Van de Graaff accelerator of Icube (UMR 7357), on which our team has developed an air extracted beam line, and on the Himac, Japan, thanks to a strong collaboration with Japanese research team. Future experiments will also be done on the Precy platform, built around IPHC cyclotron Cyrcé.

**Other projects**

This project is strongly connected to simulations, especially Geant4-DNA. The experimental results obtained, with water radiolysis products to begin with, will be used to improve simulation codes, taking into account the mechanisms determined and the experimental radiolytic yields. This prospective could also have ties with “Inside” project, coordinated by Franck Gobet in MoVi master project on the radiolysis of DNA by protons.