

SUMMARY OF THE Ph.D. THESIS: DNA damage by charged and neutral radiation at different spatial and temporal scales: integrating Monte Carlo simulations with *in vitro* experiments.

Radiation action in cells has been studied to understand the mechanisms related to the onset of damage inside targets of relevance for radiobiology, as it represents the fundamental knowledge for fields like oncological radiation therapy, radiation protection for human space programs and prevention of hazards from the use of nuclear power and nuclear weaponry.

The purpose of my Ph.D. thesis was to comprehensively investigate the mechanisms underlying the induction of DNA damage by different radiation qualities (*e.g.* heavy charged particles, X-rays and neutrons), using both experimental measurements and theoretical approaches to quantify the frequency and complexity of DNA lesions in different cell lines. Specific exposure scenarios were addressed, offering insight on possible applications that could benefit from results obtained in this study, such as clinical treatments, biodosimetry and design of radiation countermeasures.

In detail, I developed radiation transport and track-structure Monte Carlo (MC) simulations to describe *ab-initio* the physical and chemical events at the nanometre level and at very short time-scales (impossible to detect experimentally), that take place after the interaction of radiation with matter.

First, the characterization of DNA damage complexity as a function of the pattern of energy distributions (as measured by the Linear Energy Transfer – keV/um) was addressed, and I simulated DNA cluster lesions due to both light and heavy charged ions with the bio-physical PARTRAC code. A wide range of particle energies was probed, up to the low ones found in the distal end of Bragg peaks used in hadrontherapy.

Then, we aimed to address the debate about the available references for the neutron weighting factors currently used in radiation protection to take into account neutron biological effectiveness (neutron RBE), and to understand the physics implications underlying the risks of secondary tumors following proton-therapy in pediatric patients.

In this context, my contribution was on the simulation of neutron transport in matter and also of the energy depositions due to generated secondary particles. The output from the different simulations was coupled to model neutron RBE as a function of neutron energy, tracing it back to initial physical events and clustered DNA damage induction.

In another Chapter, simulation of DNA damage was extended to reproduce a detectable endpoint, that is DNA damage foci, meant as clusters of repair proteins recruited at the damage site. The aim here was to “unzoom” the observer point of view from the nanometre level (at which physical interactions take place) to the micro-scale. The phosphorylated form of the DNA histone H2AX (γ -H2AX focus) was considered, as it is one of the early events following the induction of double strand breaks in the DNA helix. This represents the main core of the experimental and modelling activities I performed during my Ph.D studies.

The goal of the modelling was to reproduce the read-out of the experimental endpoint: for this purpose, I developed a machine learning unsupervised algorithm that starts from initial damage (nm) and takes account of both: i) the physical extension of the genomic region interested by the phosphorylation and ii) the experimental detection technique. This approach was applied to different exposure conditions, *i.e.* radiations used in conventional- or hadron-therapy (X-rays and ^{12}C ions), or those generated by improvised nuclear devices (neutrons).

Benchmark of the newly developed modelling approach was carried out with biological data obtained *in vitro* from irradiation of cell cultures. At the RARAF facility, Center for Radiological Research, Columbia University Medical Center, New York, USA, I performed measurements with X-rays and an analogue of the neutron field generated by the Hiroshima bomb at 1.5 km from the hypocentre of the explosion. ^{12}C -ion irradiation was instead performed at the CNAO facility in Pavia, using a treatment plan for ^{12}C ions in a water phantom to mimic the irradiation of tumour cells in patients.

I measured foci induction experimentally through molecular biology assays as immunocytochemistry, and results from different microscopy techniques (conventional fluorescence vs confocal microscopy) were compared, to highlight the differences in the quantification of foci (and therefore of initial DNA damage) due to different radiation fields and technical limitations related to the read-out system.

Finally, I focused on space radiation, motivated by the more frequent human presence in space and by the great interest arisen for future long-term missions in deep space.

I reported the results I obtained from the modelling of DNA clustered damages due to the neutron field expected at the surface of Mars (as measured by the Radiation Assessment Detector), including estimates on the RBE of Martian neutrons. These could be eventually correlated to the onset of the late complications suffered by astronauts as a consequence of their permanence in space. This represents a unique predictive tool to estimate risks associated to the stay on the planet.

Moreover, I performed some calculations also at the tissue/organ level, to evaluate the dose reduction in organs that can display the onset of non-cancer short-term effects, achieved thanks to a personal radiation protection device. The shielding strategy consisted in a water-filled garment prototype that was designed and constructed in the framework of the PERSEO project (funded by the Italian Space Agency), to mitigate the harmful effects of cosmic radiation during solar particle events. The goodness of the garment was moreover tested on board of the International Space Station by the Italian

astronaut Paolo Nespoli. This set of results I obtained provided an example of radiation-transport modelling applied at the macroscopic scale.

Overall, the results I presented in my thesis could concur to the creation of a large database to be queried for practical applications such as biodosimetry following e.g. accidental exposure, optimization of treatment plans in oncological radiation therapy and space radiation protection.

List of publications in which the work carried out in the thesis has been finalized, including my personal contributions and additional data.

As first author:

1. **S.Barbieri**, G.Baiocco, G.Babini, J.Morini, W.Friedland, M.Buonanno, V.Grilj, D.J.Brenner, A.Ottolenghi. Predicting DNA damage foci and their experimental readout with 2D microscopy: a unified approach applied to photon and neutron exposures. *Scientific Reports* 9, 2019. DOI:10.1038/s41598-019-50408-5
2. **S.Barbieri**, G.Baiocco, G.Babini, J.Morini, W.Friedland, M.Buonanno, V.Grilj, D.J.Brenner, A.Ottolenghi. Modelling γ -H2AX foci induction to mimic limitations in the scoring technique. *Radiation Protection Dosimetry*, online ahead of print, 2018. DOI: 10.1093/rpd/ncy217-- CITATIONS: 2

As a co-author:

3. A.Bertolet, A.Baratto-Roldán, G.Baiocco, **S.Barbieri**, A.Carabe, M.A. Cortés-Giraldo. Dose average LET calculation for proton track segments using microdosimetric Monte Carlo simulations. *Medical Physics*, 2019. DOI: 10.1002/mp.13643 -- CITATIONS: 1
4. J.Schuemann, A.McNamara, J.W.Warmenhoven, N.T.Henthorn, K.Kirkby, M.J.Merchant, S.Ingram, H.Paganetti, K.D.Held, J.Ramos-Mendez, B.Faddegon, J.Perl, D.Goodhead, I.Plante, H.Rabus, H.Nettelbeck, W.Friedland, P.Kundrat, A.Ottolenghi, G.Baiocco, **S.Barbieri** et al. A new Standard DNA Damage (SDD) data format. *Radiation Research*, online ahead of print, 2019. DOI:10.1667/RR15209.1 -- CITATIONS: 6
5. H.Rabus, **S.Barbieri**, G.Baiocco, A.Ottolenghi, U.Giesen. Investigation into uncertainty contributions in foci-based assays. *Radiation Protection Dosimetry*, online ahead of print, 2018. DOI: 10.1093/rpd/ncy251
6. G.Baiocco, L.Bocchini, M.Giraud, **S.Barbieri**, L.Narici, C.Lobascio, A.Ottolenghi. Innovative solutions for personal radiation shielding in space. *Radiation Protection Dosimetry*, online ahead of print, 2018. DOI: 10.1093/rpd/ncy216
7. G.Baiocco, G.Babini, **S.Barbieri**, J.Morini, W.Friedland, C. Villagrasa, H.Rabus, A. Ottolenghi. What roles for track structure and microdosimetry in the era of -omics and systems biology?. *Radiation Protection Dosimetry*, online ahead of print, 2018. DOI: 10.1093/rpd/ncy221-- CITATIONS: 1
8. G.Baiocco, M.Giraud, L.Bocchini, **S.Barbieri**, et al. A water-filled garment to protect astronauts during interplanetary missions tested on board the ISS. *Life Science in Space Research* (18), 1-11, 2018. DOI:10.1016/j.lssr.2018.04.002 -- CITATIONS: 3
9. G.Baiocco, **S.Barbieri**, G.Babini, J.Morini, W.Friedland, P.Kundrat, E.Schmitt, M.Puchalska, U.Giesen, R.Nolte, A.Ottolenghi. At the physics-biology interface: the neutron affair. *Radiation Protection Dosimetry*, 1-4, 2017. DOI:10.1093/rpd/ncx222.
10. G.Baiocco, M.Vuolo, **S.Barbieri**, L.Bocchini, M.Giraud, T.Gheysens, C.Lobascio, A.Ottolenghi. Exploring innovative radiation shielding approaches in space: a material and design study for a wearable radiation protection spacesuit. *Life Sciences in Space Research* (15), 69-78, 2017. DOI:10.1016/j.lssr.2017.08.003, WOS:000416873500009 -- CITATIONS: 8
11. W.Friedland, E.Schmitt, P.Kundrat, G.Baiocco, **S.Barbieri**, A.Ottolenghi. Comprehensive track-structure based evaluation of DNA damage by light ions from radiotherapy relevant energies down to stopping. *Scientific Reports* (7), 45161, 2017. DOI: 10.1038/srep45161, WOS:000397824900001 -- CITATIONS: 57
12. G.Baiocco, **S.Barbieri**, G.Babini, J.Morini, D.Alloni, W.Friedland, P.Kundrat, E.Schmitt, M.Puchalska, L.Sihver, A.Ottolenghi. An ab initio approach to trace back the origin of neutron biological effectiveness as a function of energy. *Scientific Reports*, 2016. DOI:10.1038/srep34033, WOS:000383697500001 -- CITATIONS: 21

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