Supplementary Information:

Modelling direct DNA damage for gold nanoparticle enhanced proton therapy

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1. An analytical model of secondary electron range probability generated from gold nanoparticles

The interaction probability of a proton with a GNP is composed by i) the geometrical interaction probability, which is the probability of a proton to meeting a GNP, and ii) the physical interaction probability, which is the probability of a proton to interact by a physical interaction process while crosses the GNP volume.

A. Geometrical interaction probability

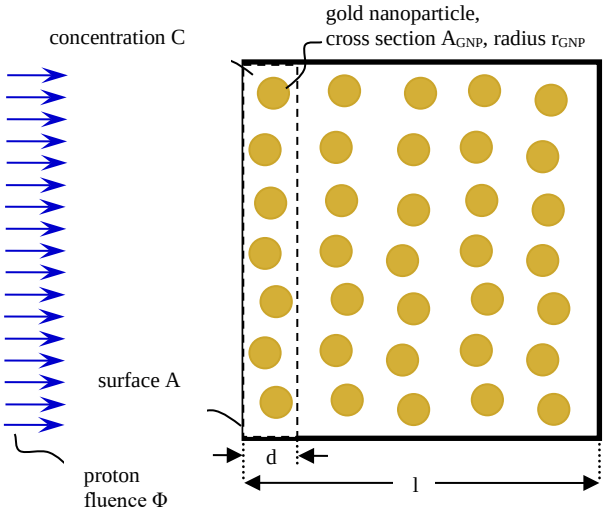

Figure I. Illustration of the geometry used for the development of the analytical model.

We assume a box filled with a concentration of gold nanoparticles and irradiated with protons. Let

Φ: the proton fluence (protons/surface), uniform irradiation

A: the surface of the box's side

C: the GNP concentration (GNP/volume)

A_{GNP}: the GNP's cross sectional surface. In this case is a circle with radius equal to the sphere's radius, $A_{GNP} = \pi * R_{GNP}^2$.

In a thin layer of dz thickness if the number of GNPs is ΔN_{GNP} the geometrical probability of a proton crossing a GNP will be

$$dP = \frac{\text{total GNP area}}{\text{total area}} = \frac{\Delta N_{GNP} * A_{GNP}}{A} = \frac{C * A * dz * A_{GNP}}{A} = C * dz * A_{GNP}$$

and the probability for the whole box will be

$$P = \int_0^l dP = \int_0^l C * A_{GNP} * dz = C * A_{GNP} * l.$$

A proton fluence, Φ, will have a probability to cross a GNP given by

$$P_{total} = \Phi * A * P = \Phi * A * C * A_{GNP} * l$$

where $\Phi * A$ is the number of incident protons.

Usually the concentration is given in %wt/wt. To convert to N_{GNP}/volume from a %wt/wt concentration, c,

$$\frac{N_{GNP}}{V_{H_2O}} = \frac{1}{V_{H_2O}} * \frac{M_{Au}}{m_{Au}} = \frac{1}{V_{H_2O}} * \frac{V_{H_2O} * \rho_{H_2O} * c/(1-c)}{{}^4/_3 \pi * \rho_{Au} * R_{GNP}^3} = \frac{c/(1-c)}{{}^4/_3 \pi * R_{GNP}^3} * \frac{\rho_{H_2O}}{\rho_{Au}}$$

Now the probability will be

$$P_{total} = \Phi * A * A_{GNP} * l * \frac{c/(1-c)}{{}^4/_3 \pi * R_{GNP}^3} * \frac{\rho_{H_2O}}{\rho_{Au}} =$$

$$= \Phi * A * l * \frac{3}{4} * \frac{c/(1-c)}{R_{GNP}} * \frac{\rho_{H_2O}}{\rho_{Au}}$$

For the case of the GNP filled box in front of the chromosomal territory described in the manuscript we have

R_{GNP} = 15 nm

c = 0.7% wt/wt gold

$\Phi * A$ = 25 protons @ 10 MeV, 92 protons @ 50 MeV; 2 Gy

$\frac{\rho_{H_2O}}{\rho_{Au}} = \frac{1}{19.2}$

and the total probability of a proton crossing a GNP per length will be

$${}^{P_{total}}/_{l} = 0.46 \text{ hits/μm or } {}^{P_{total}}/_{l} = 1.7 \text{ hits/μm}$$

for the 10 and 50 MeV irradiation respectively.

B. Physical interaction probability

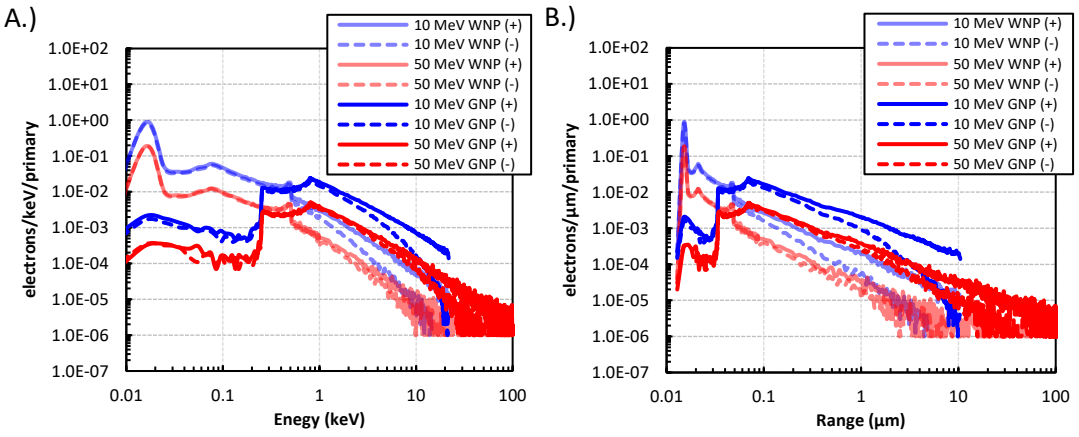

Figure II. The spectrum A.) and range B.) of electrons escaping the 15 nm WNP or GNP towards the direction of the beam (+), and opposite (-); per primary proton of 10 or 50 MeV.

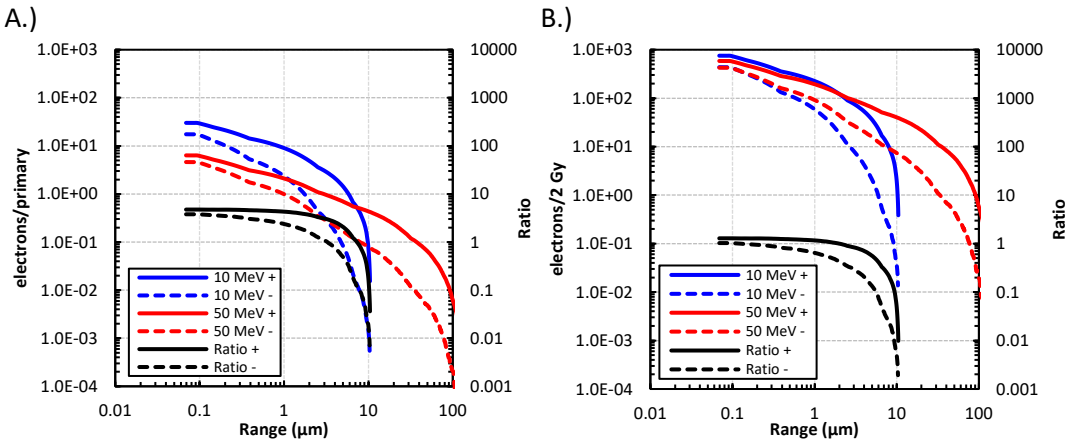

Figure III. Number of electrons produced in the forward (+) or opposite (-) direction and ratio that have a range above the specified value, per primary A.), and B.) for 2 Gy delivered with 10 or 50 MeV protons in the GNP filled box of the previous section. The given ratio is the cumulative number of electrons escaping the GNP at 10 to 50 MeV, in the forward (+) or opposite (-) direction. Values are calculated above the mean excitation potential, which is 790 eV for gold; see text for details.

The additional number of electrons produced in the presence of the GNP when a proton crosses a GNP can be calculated from the spectra generated by the irradiation of a GNP and a WNP (water nanoparticle – a hypothetical nanoparticle composed of water). In particular, we used the method described by Sotiropoulos et al[1] and generated the spectra of a 15 nm WNP and GNP irradiated with 10 and 50 MeV protons (Figure II. A.). The energy spectra of the escaping electrons was converted to range distribution (Figure II B.) using the data in *Figure 2* from Francis et al[2]. Then the additional electrons generated in the presence of the GNPs with range of more than a specific value were calculated (Figure III) from the previously generated spectra by subtracting the WNP from the GNP values; per primary proton, and 2 Gy delivered in the GNP filled box of the previous section.

From Figure III we can calculate the number of excess electrons per proton crossing the GNP generated in the forward direction. Due to the limitation of the Geant4 models to correctly produce the energy spectra for energies below the mean excitation potential[1], we only account for electrons generated above the mean excitation potential of the two material, i.e. 790 eV. About 30% and 33% of the excess electrons will have range greater than 1 μm, while about 5% and 11% will have range greater than 5 μm, for the 10 and 50 MeV respectively. In absolute numbers, about 9 and 1.4 more electrons per proton crossing the GNP will be generated with range more than 1 and 5 μm, respectively, for the 10 MeV protons. For the 50 MeV protons the same numbers are 2.1 and 0.7 more electrons in the presence of the GNPs.

We can also consider the GNP filled box of the previous section. We get that 4.14 and 0.6 more electrons will reach 1 and 5 μm respectively, for 2 Gy irradiation with 10 MeV protons (3.6 and 1.2 electrons for the 50 MeV protons). If those numbers combined with the occupancy of the DNA sensitive volume in the nucleus (about 15-20%) and the probability to convert an energy deposition in the sensitive volume to strand break, we can conclude that the DNA damage predicted by this simplified model should be negligible.

Similar trends are observed in the case of the 30 nm GNP (data not shown).

C. Model predictions

The simple model developed in the previous sections could be used to make some interesting observations. We postulate that while the absolute numbers of electrons produced represent

the GNP filled box of the previous section, the relative values should hold for any case. Therefore the ratio presented in Figure IIIB.) can be used in any scenario.
As can be deducted from Figure IIIB.), the model predicts a similar enhancement for both energies when ranges of up to about 1 μm are considered. On the other hand, when distances of more than 1 μm are considered we expect the higher energy to create more electrons. Interestingly, the difference gets accentuated when we consider the case of the sensitive volume surrounded by the GNPs, where we have contribution from both the forward and opposite direction.
Obviously, we have to consider (potentially unrealistic) high concentrations to observe any effect. This model do not account for the clustering of the ionization events created by the electrons that lead to the formation of the DSBs, so the observations should be limited to the SSB yields only.

2. Dependence of the clustering algorithm to the energy threshold values

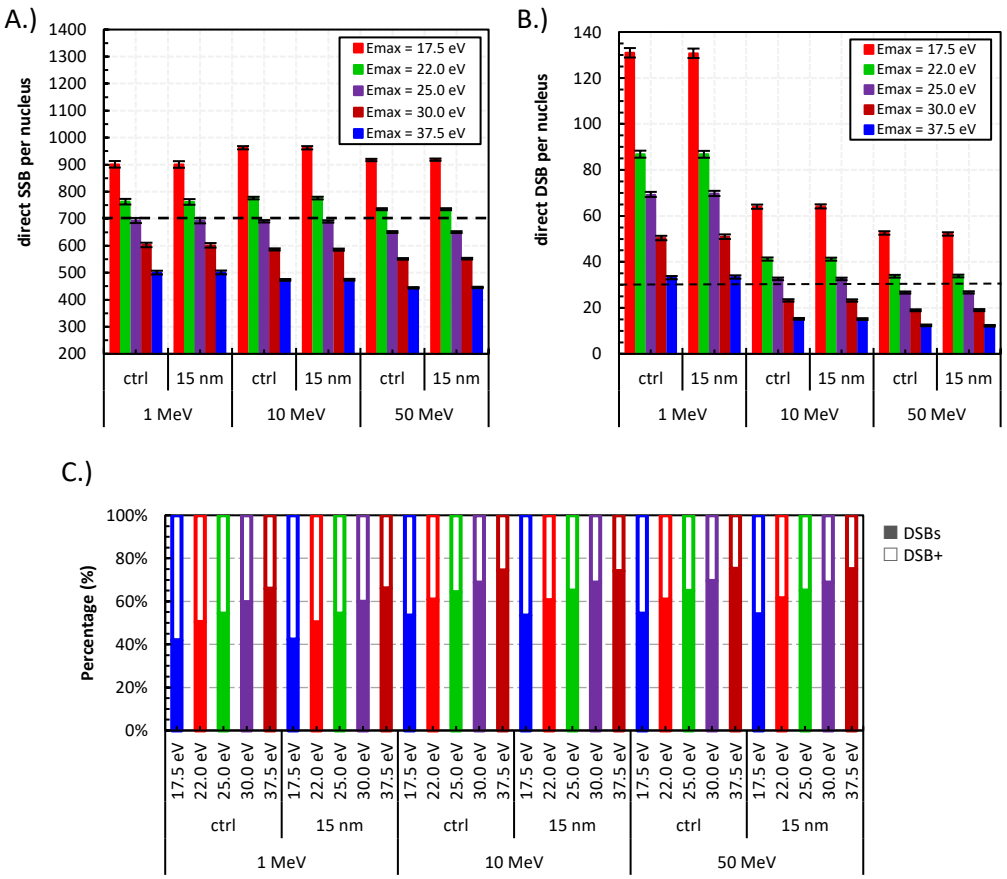

Figure IV. Influence of the E_{max} parameter to the direct A.) single (SSB), B.) double strand breaks (DSB), and C.) percentage contribution of simple (DSB, closed column) and complex (DSB+, open column) double strand breaks per nucleus irradiated with 2 Gy of 1, 10, or 50 MeV protons, with and without 0.7% wt/wt 15 nm gold nanoparticles. Error bars represent the standard error of the mean between 1000 repeats. The dashed line represents the reference value of 700 SSBs and 30 DSBs for 2 Gy.

The complexity of the mechanisms involved in the induction of the strand breakage and the simplifications on the geometrical representation of the DNA do not allow the ab-initio calculation of the strand breaks. Instead assumptions have to be made of the conversion of an ionization, excitation, or energy deposition event in the DNA backbone to strand break. As

discussed in further detail by Pater et al[3], the single and double strand break yields are very sensitive to the parameterization in the conversion process.

Although the main purpose of this work is not to produce accurate strand break yields, but rather to compare the DNA damage in the presence of gold nanoparticles, effort has been made for producing realistic yields. In this work we adapt the conversion scheme introduced in the PARTRAC[4] simulation code as implemented by Francis et al[5]. In this parameterization, a linear probability is assigned between a minimum (E_{min}) and maximum (E_{max}) energy. Energy deposition below the E_{min} does not create a strand break, while above E_{max} always creates a strand break. We select the values of E_{min} = 5 eV and E_{max} = 25.0 eV, in contrast to the E_{max} = 37.5 eV that has been used before, as this produces more consistent yields when compared with the literature. The effect of the E_{max} value on the single and double strand break numbers and break complexity can be seen in Figure IV. While the break complexity and strand break yields are sensitive to this value, similar trends are observed regardless the E_{max} value.

3. Double strand break enhancement ratio values for all studied cases

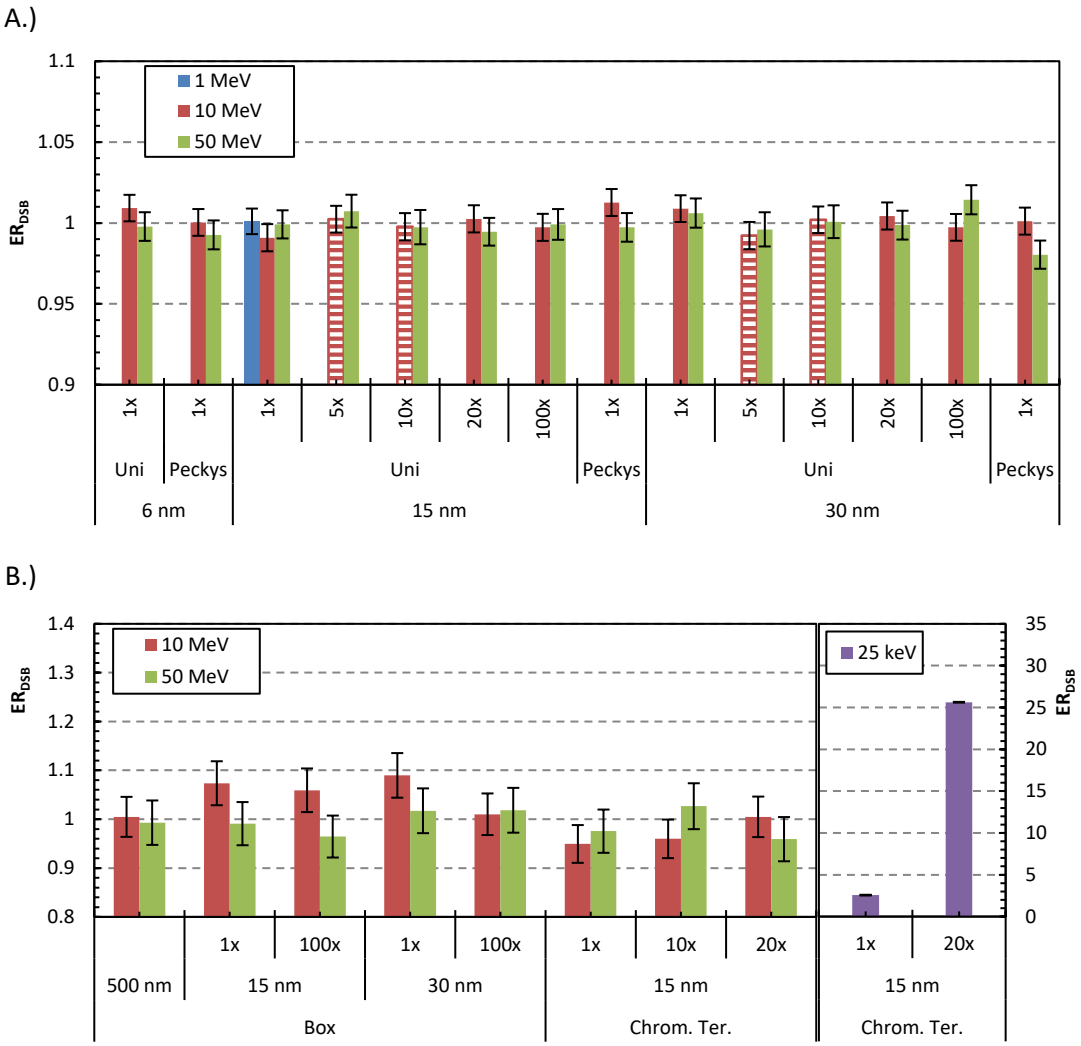

Figure V. DSB enhancement ratio for (A.) the cell model and (B.) the chromosomal territory at all the cases studied. The GNP concentration is given in multiples of the GNP numbers of the reference concentration of 0.7% wt/wt. “Uni” and “Peckys” are the uniform and the vesicle based distribution of the GNPs respectively. When not specified, uniform distribution of GNPs is assumed. “Box” denotes

the case of the solid gold or GNPs filled box impinging the chromosomal territory. “Chrom. Ter.” denotes the chromosomal territory filled with GNPs. All irradiations were at 2 Gy. Error bars represent the standard error of the mean between 1000 repeats; or 600 repeats for the striped lines filled bars.

4. References

1 M. Sotiropoulos, M. J. Taylor, N. T. Henthorn, J. W. Warmenhoven, R. I. Mackay, K. J. Kirkby and M. J. Merchant, *Biomed. Phys. Eng. Express*, 2017, **3**, 25025.

2 Z. Francis, S. Incerti, M. Karamitros, H. N. Tran and C. Villagrasa, *Nucl. Instruments Methods Phys. Res. Sect. B Beam Interact. with Mater. Atoms*, 2011, **269**, 2307–2311.

3 P. Pater, J. Seuntjens, I. El Naqa and M. A. Bernal, *Med. Phys.*, 2014, **41**, 121708.

4 W. Friedland, M. Dingfelder, P. Kundrát and P. Jacob, *Mutat. Res. - Fundam. Mol. Mech. Mutagen.*, 2011, **711**, 28–40.

5 Z. Francis, C. Villagrasa and I. Clairand, *Comput. Methods Programs Biomed.*, 2011, **101**, 265–270.