Plasmids in Monte Carlo Simulations

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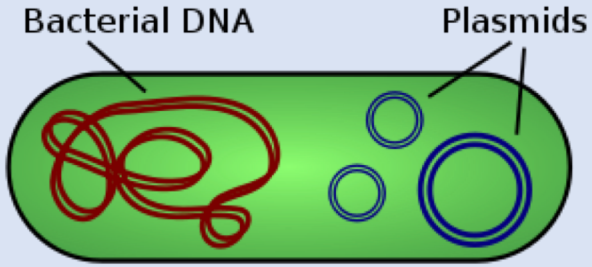
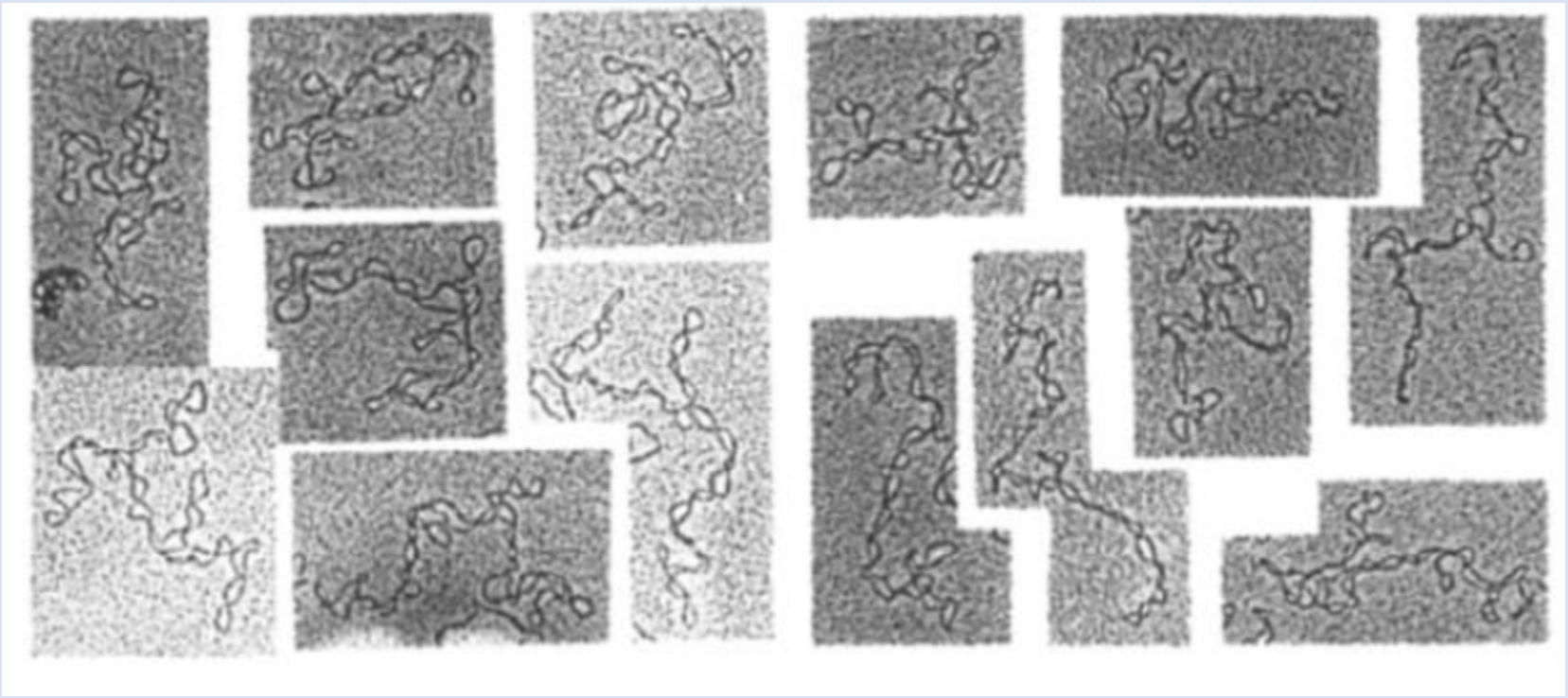
Outline

- General Introduction to Plasmids.
- Why to use Plasmids in Monte Carlo Simulations.
- The Algorithm.
- Important Cares to the Plasmid.
- The Use of Plasmids in MC Simulations.
- The Challenge behind their use.
- The Job at Hand.
- Conclusions.
- Future Work.

General Introduction to Plasmids





A set of electron micrographs taken from Boles TC. White JH, Cozzarelli NR. 1 990. Structure of plectonemically supercoiled DNA. 1. Mol. Bioi.213:931-51. This plasmids have a $\sigma = -0.03$ left, and $\sigma = -0.06$.

Plasmids are DNA molecules separated from the Chromosomal DNA and thus, they are able to replicate independently.

Plasmids carry additional DNA information that is used for the survival of the cells, that information is only used under certain circumstances (damage and repair of the cell).

Plasmids tend to adopt unknotted or supercoiled conformations.

Plasmids are classified by the origin of their cells, length (number of bps) and super helix density (σ).

Why to study plasmids.

Plasmids are commonly found in bacteria, but also exists in archaea and eukaryotes.

They provide one or more benefits to their host such as antibiotic resistance.

Plasmids used in labs are usually artificial, used to introduce foreign DNA to a cell.

The ease of modifying plasmids and their ability to self replicate within a cell makes them attractive tools for the life scientist of bioengineer.

For such a cell, having a plasmid inside its expensive, so they will only keep it if its worth it.

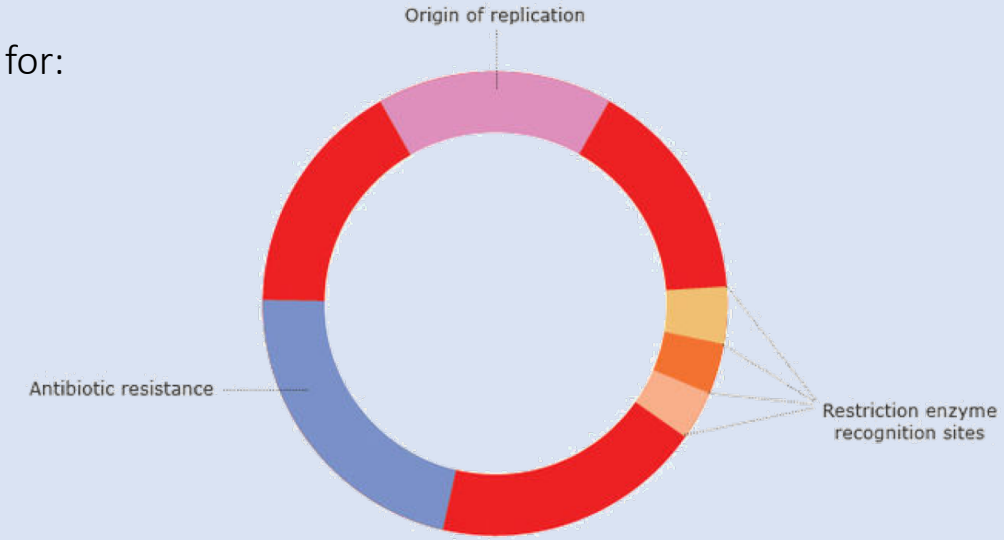
Some plasmids even take their cell hostage by providing a poison and antidote at the same time.

They tend to aport so much to the cell, that they become indispensable for such a cell to continue living under it's current conditions.

Altough, many use the circular plasmid representation, it is to be noted that the only kind of plasmid found in vivo are the supercoiled

Plasmids are if of interes for:

Biologists.
Radio chemistries.
Medical Physics.
Bioengineers.
Pharmaceuticals.
Etc.



[Ref] Nicola, Casali, Preston Andrew. E. Coli Plasmid Vectors. Humana Press (2003). New Jersey.

The algorithm

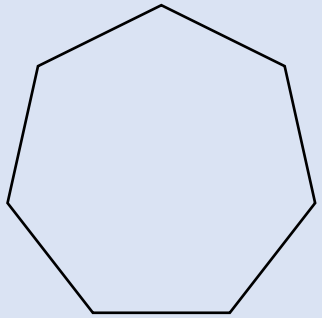
- The best method to recreate plasmids is by using Monte Carlo simulations, in particular, the so-called adaptative method [Ref1].
- In this method, a plasmid are generated from regular polygon of *N* sides, each one of a fixed length (usually of 9.962 nm which corresponds to 1 Kuhn statistical length).
- Subsequently, an iterative process of applying trial deformations to the chain is performed. The new conformations are accepted only if the total energy of the chain satisfies:

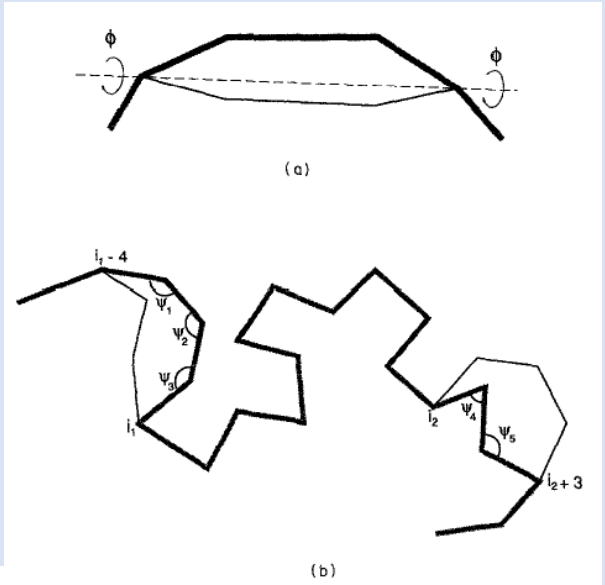
$$\exp\left(\frac{E_i - E_{i+1}}{K_B T}\right) > uniformRand[0,1]$$

Where:

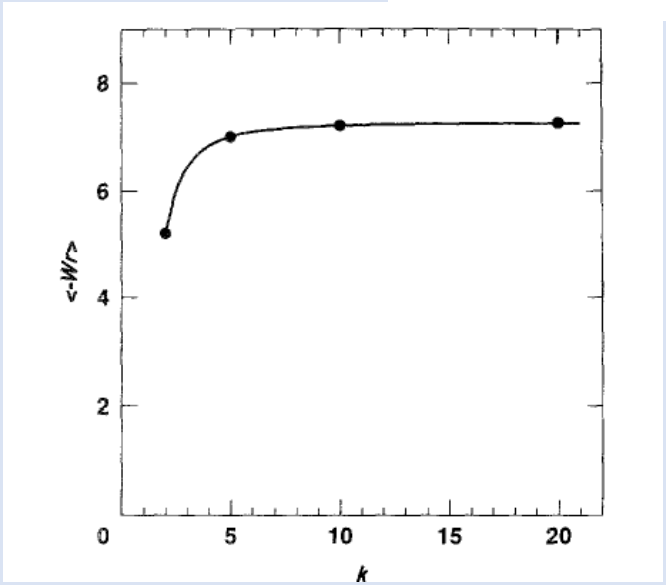
$$E = K_B T \alpha \underbrace{\sum_{i=1}^{N} \Theta_i^2}_{\text{Bending}} + \underbrace{\left(\frac{2\pi C}{L}\right)(\Delta L_K - W_r)}_{\text{Torsional}}$$

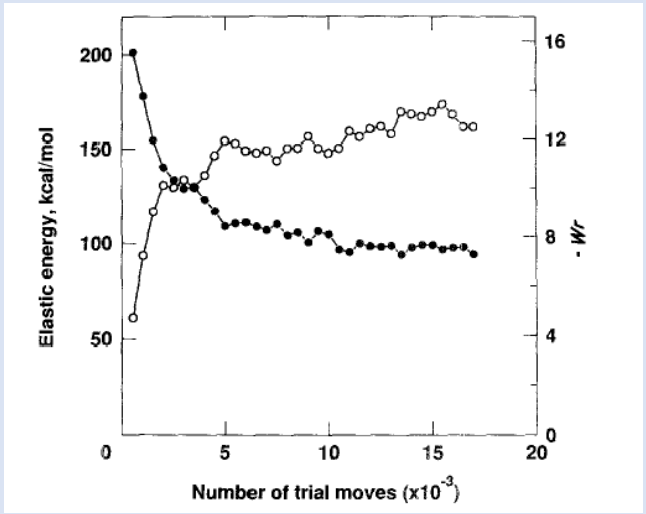
- To stop the iterative process, we take a sample of 1 to 4 million steps for the desired equilibrium quantity (i. e. energy or Wr) and calculate its standard deviation, if the difference between the initial value and the last value its less than the standard deviation, we said we have reached the equilibrium.





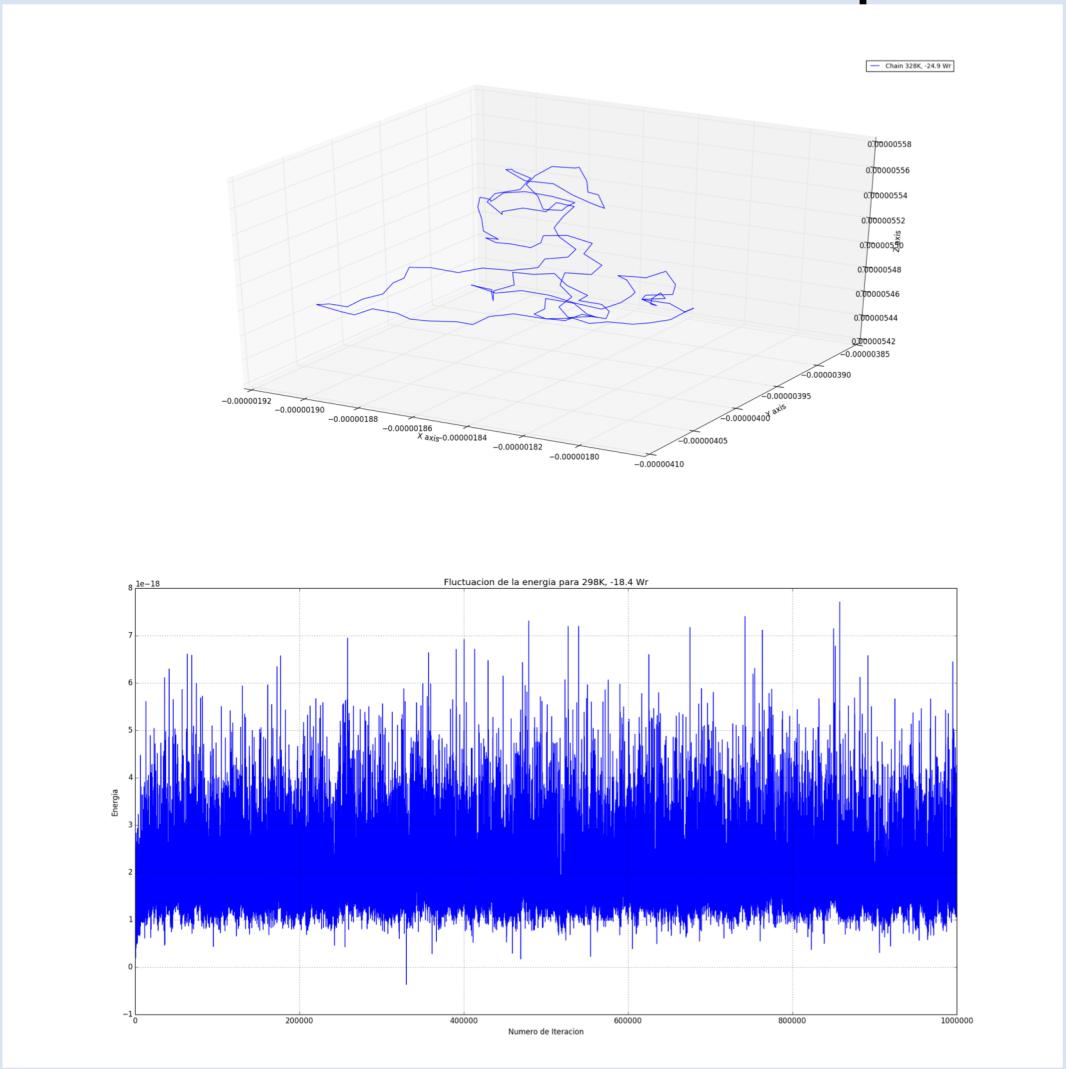
Figures. Normal behavior for the plasmid characteristics, taken form the article of Vologodskii [Ref2].





[Ref1] Konstantin V. Klenin, et. al. Computer Simulation of DNA Supercoilling, J. Mol. Bid. (1991) 217, 413-419.
[Ref2] Alexander V. Vologodskii, et. al. Conformations and Thermodynamics properties of supercoiled DNAAnnu. Rev. Biaphys. Biamal. Seruel. 1994.23:609--43

Issues to consider for plasmid conformation.



Knots

- All that said, with the previous algorithm alone, plasmid cannot be well formed.
- As the W_r increases with each iteration (due to decreasing the energy), the more the plasmid coils with itself. Therefore, the conformations with lower energy tend to form knots.
- Knots are not found in plasmids in reality. Hence, the need of a knot detection algorithm arises.
- Knot detection algorithm is the most computationally demandant part in the simulation of plasmids.

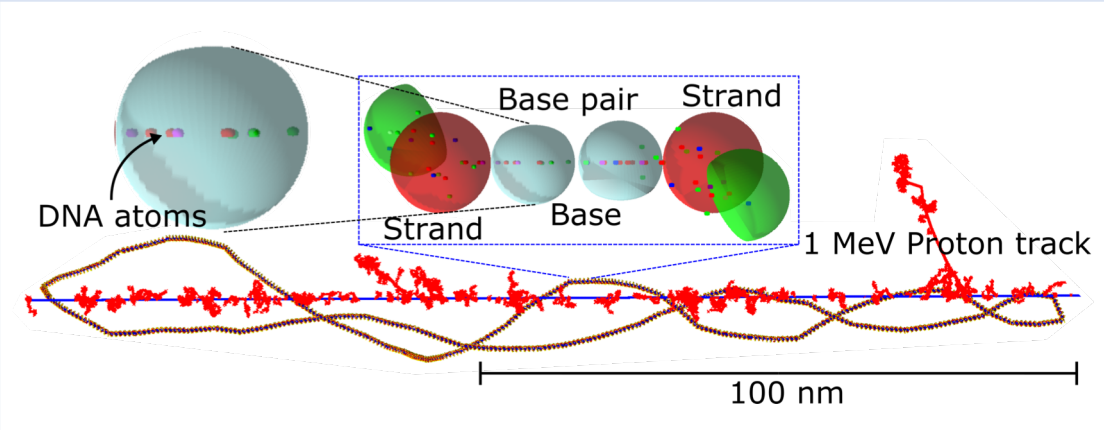
DNA molecular geometry model

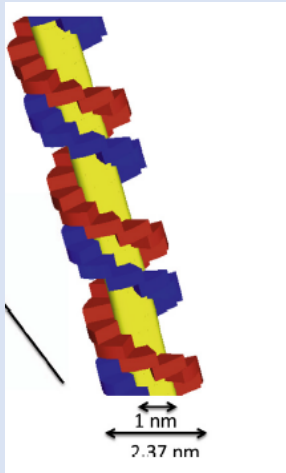
For this work, plasmids are aimed to study the accuracy of Geant4-DNA in simulating DNA strand breaks caused by ionizing irradiation, then further conditions apply:

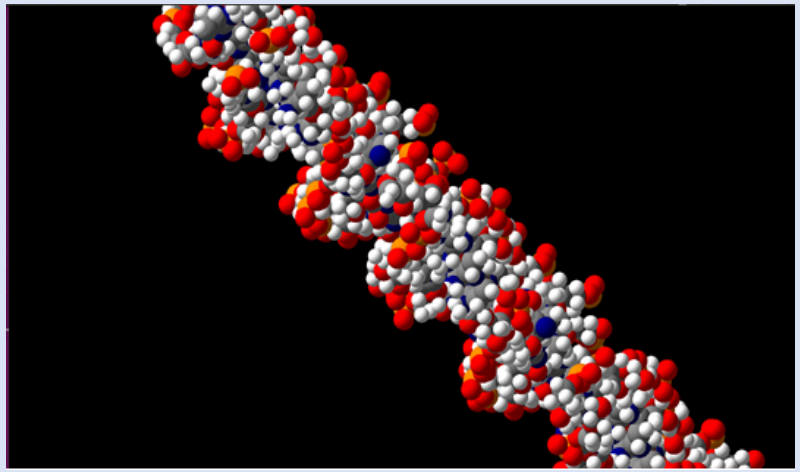
1. A DNA molecular model need to be wrapped around the plasmid structure to score energy deposition events.
2. Then, each segment length must be proportional to 1 bp (0.34 nm).
3. Overlaps between volumes must be avoided.
4. Set a criteria to stop the iterative process of plasmid formation within a reasonable computation time, e.g. saturation of W_r or the Energy or both. In our case we used the rotation radius (Gyration Radius)[Ref3].

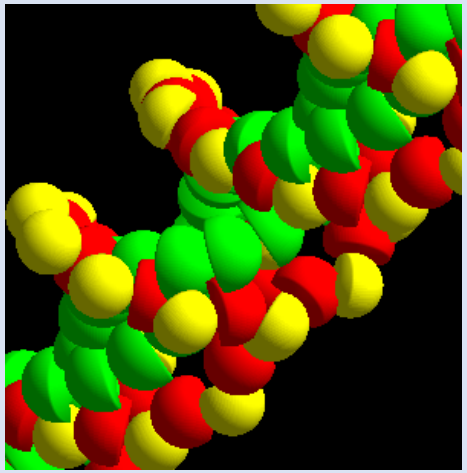
[Ref3] Bryant S. Fujimoto and J. Michael Schurr. Monte Carlo Simulations of Supercoiled DNAs Confined to a Plane. Biophysical Journal Volume 82 February 2002 944–962.

The use of plasmids In MC track-structure simulations









DNA Geometry model

- The model of double-helix of James Watson and Francis Crick in a B-DNA conformation is commonly used.
- Aimed to score energy deposition, the model may consider a effective volume that represent each base and sugar or phosphate moiety. E.g. a simple cylindrical model where bases are represented by cylinders and strands by cylindrical segments of thickness 0.34 nm [Ref5]
- If one wants to study the chemical damage due to the creation of chemical species produced by radiolysis, then an atomic model of the DNA may be needed.
- In this work, both models are combined into a single DNA model aimed to simulate the physico-chemical processes of interaction of radiation and hence, to estimate the DNA damage due to direct and indirect events.

The Use of Plasmids In MC Simulations: Importance of Use

Plasmids are the natural state of the DNA required for the survival of the cell. It gives the information of how to repair itself and how to defend the cell. [Ref 4]

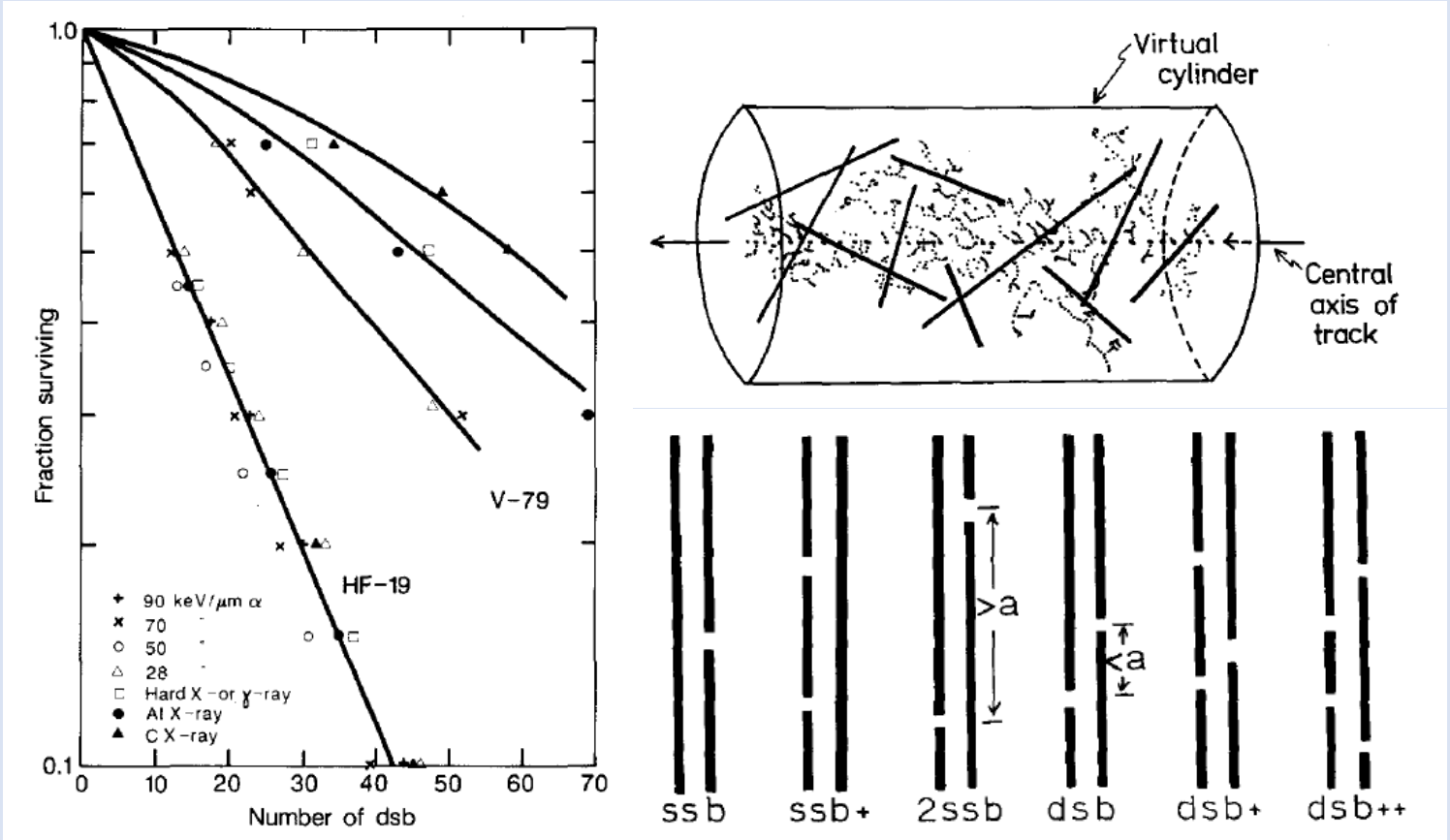
If the Plasmid DNA “breaks” after a certain point, it will no longer be able to repair. Subsequently, the cell which contains such a plasmid will die [Ref 5].

Many simulations in past years have tried to use DNA models and to count the number of breaks in the DNA e.g. [Ref1] [Ref2]

Although many of them show a good agreement to experimental data (<8%), the gap between experimental and simulation data may lay in the lack of detail in the Plasmids model in addition to the physical cross sections (mainly for vapor water).

Although, the use of plasmids is difficult and has many challenges in order to incorporate into a simulation, in this work we aim to quantify the difference in results from combining two complex geometries (plasmids with DNA models) with physical cross-sections for DNA materials and the IRT for the estimation of indirect damage.

All this facilitated by the TOPAS-nBio Monte Carlo simulation tool.



Images taken from [Ref 4] D.E Charlton Calculation of initial yields of single- and double-strand breaks in cell nuclei from electrons, protons and alpha particles, INT. J . RADIAT . BIOL ., 1989, VOL. 56, NO. 1 , 1-19
[Ref 5] Aimee McNamara, et. al. Validation of the radiobiology toolkit TOPAS-nBio in simple DNAGeometries. Physica Medica 33 (2017) 207–215.

Challenges behind their use

As with each complex geometry, the implementation is always a challenge.

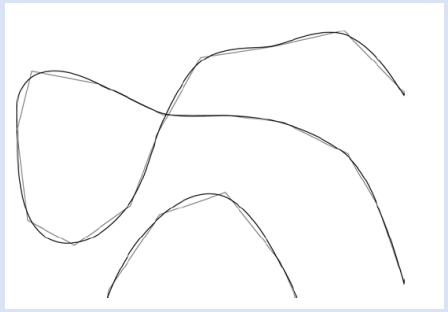
From the irradiation method to the general setup of the simulation.

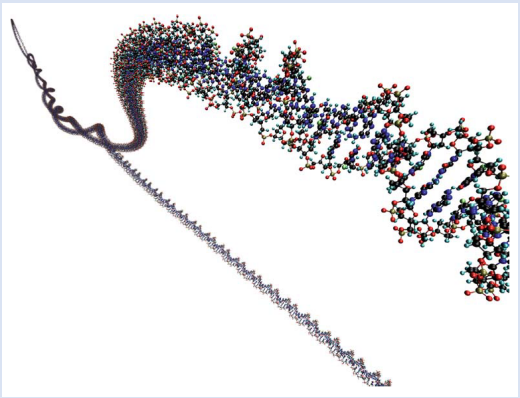
In this case, the most common challenges are: irradiation, overlapping, smoothing, break counts, etc.

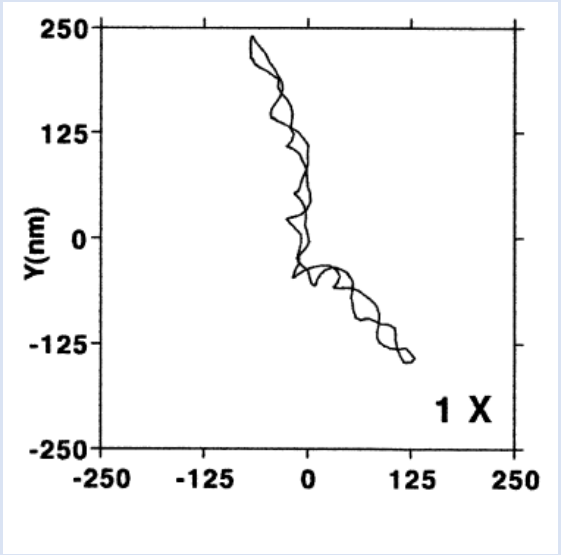
As to the general algorithm of the plasmid which tends to take days, or weeks.

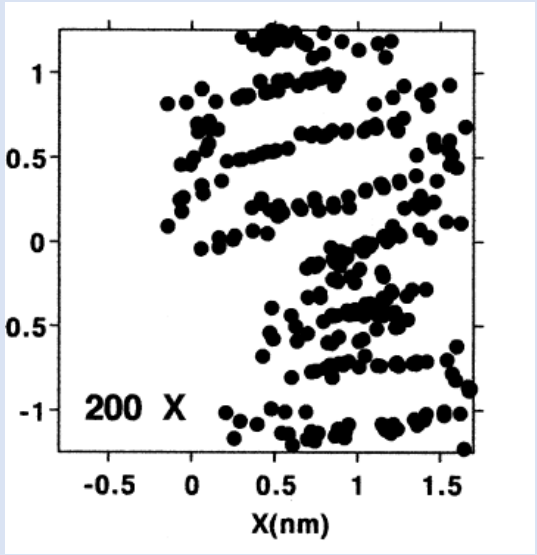
Because of that, many researchers tend to exclude their use as much as possible. Focusing only on the irradiation method in geometries based on primitive shapes (cylinders) and break classification algorithms.

A general tool to generate plasmids will be much appreciated, but many articles tends to don't give the important details about plasmid simulations.



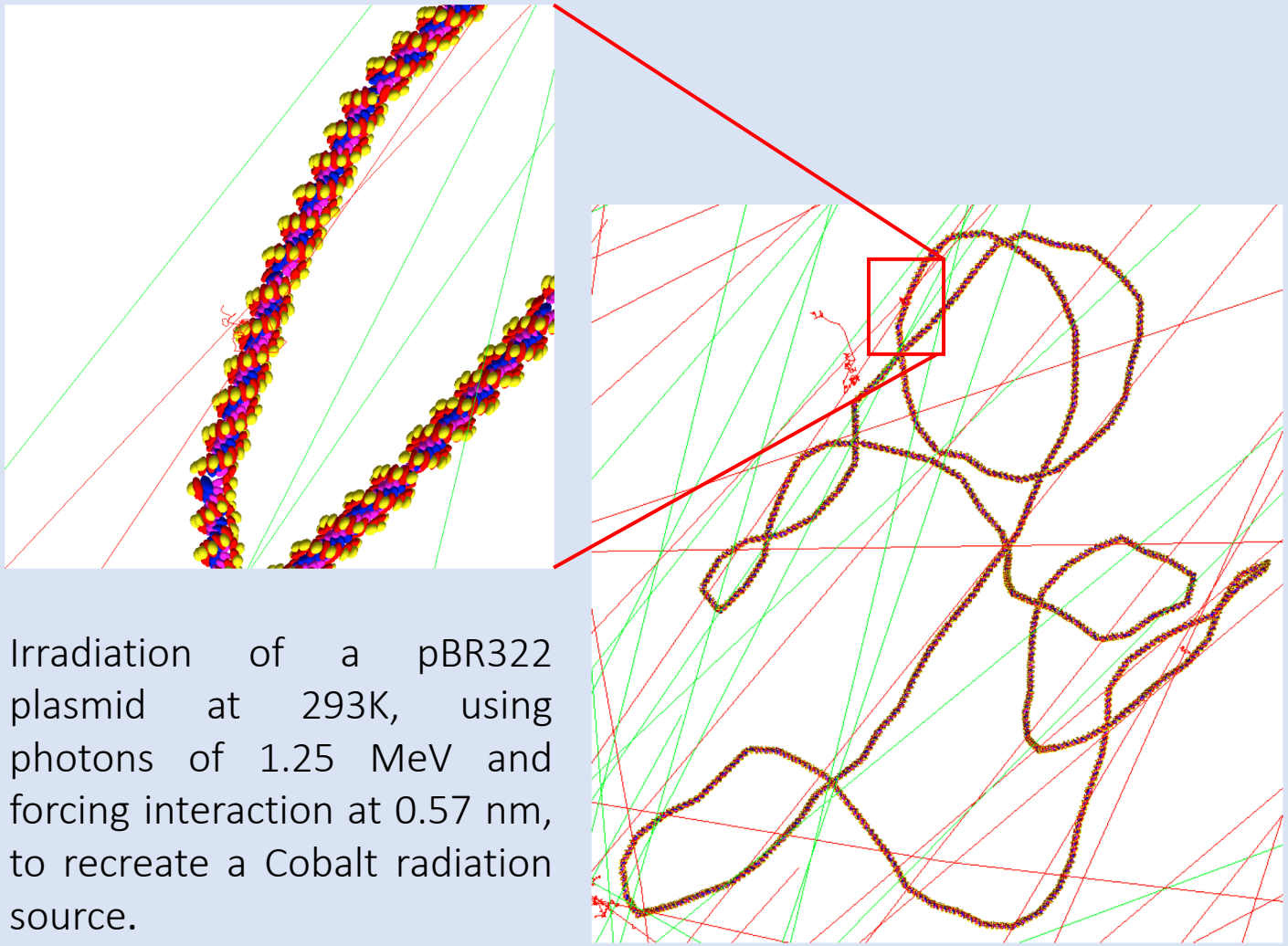






The upper figures are from Kummerle, E. Pomplum 2005 article in which is seen a smoothing algorithm for their plasmids.
The Lower images are from Tomita 1998 pBR322 article in which determines the damage of a plasmid due to chemical interactions.

The Job at Hand



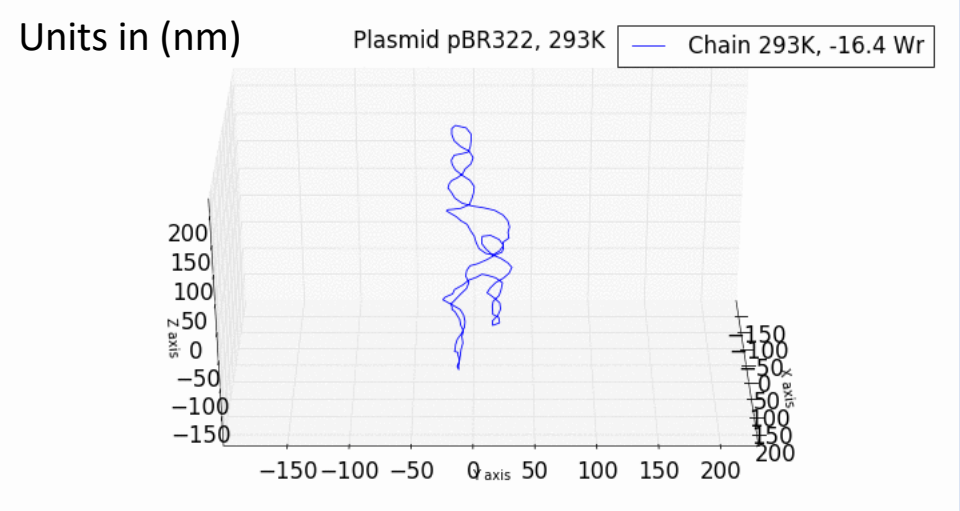
Irradiation of a pBR322 plasmid at 293K, using photons of 1.25 MeV and forcing interaction at 0.57 nm, to recreate a Cobalt radiation source.

With all that said, the work we are developing in its final stages its:

To create a TOPAS-nBio extensions which will create a plasmid from parameters given by the user, and wrap it using a predefined DNA model or a user given one.

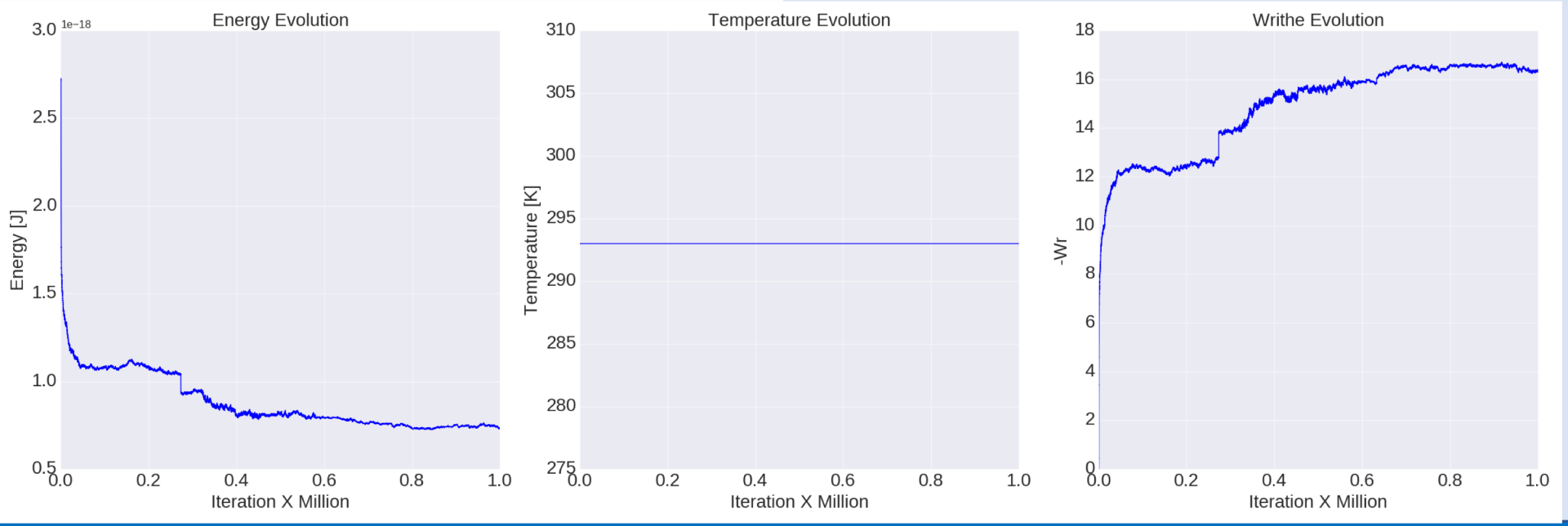
The DNA model must be compatible IRT approximation and be able to classify damage due to physical and chemical interactions.

The Results to Date

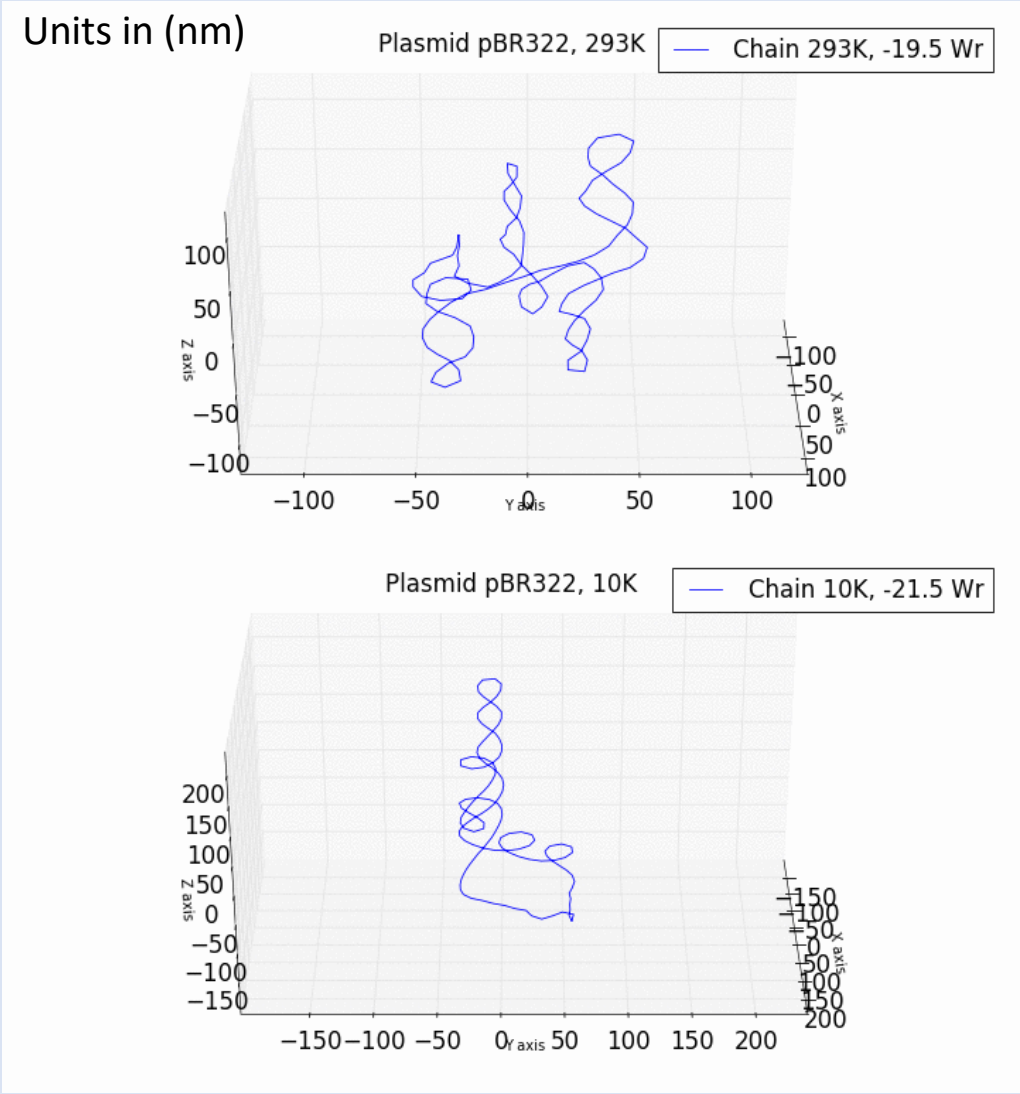


The Plasmid Generatig C++ Tool to be incorporated in to a TOPAS Extension.

- We have an Adaptative (Monte Carlo) Plasmid Generating tool that uses the Geant4 CHLEP libraries to decrease computing time.
- This plasmids can be made in 4 hrs, on average .
- Very good agreement of W_r with those reported in references with differences of $\pm 1, was\ found$.



Conclusions



The Plasmid Generator C++ Tool, to be incorporated in to a TOPAS Extension.

- A Plasmid Generator tool that uses the Geant4 CHLEP libraries.
- Plasmids are created in a short computation time with good agreement with the literature.
- This plasmid generating tool will be implemented in TOPAS-nBio and will be available to the users.

Future Work

- This algorithm can be have further improvements:
- Electromagnetic potentials and potentials from biding planes.
- Compatibility with the TOPAS-nBio chemistry transport and IRT.
- We are currently at the simulations phase: estimation of DNA strand break for low LET particles.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