Molecular simulations of radio-biological effects: DNA damage by ionizing radiation

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Pradip Biswas, NIH recently moved to Physics Dept. TU in Jackson MS, QMMM
one of developers of GROMACS-CPMD
Thomas Brabec, chair of photonics, Physics Dept., Ottawa U.

Fields Institute, U. Toronto,
Toronto, Dec. 16, 2011

Outline

Quick review on current computational methods for:

1)Radiation interaction with matter where the relevant length scale for dominant physical events ~ 0.1 nm.
Computational platform: Monte Carlo simulation based on empirical atomic scattering cross-sections. It is used as a method for accurate dosimetry and treatment plans in radiation therapy.

2) Cell damage-repair mechanisms, e.g., coarse-grained reaction-diffusion models with relevant length-scales ~ cell dimension ~ 10^3 nm ~ 10^4 atomic scale.

Our recent progress on:

- Computational/mathematical models for DNA damage with relevant length scale ~ 0.1-10^3 nm (filling a gap between atomic and coarse-grained scales).
- I focus on initial DNA damage, propagation of damage and chemical pathways in DNA-SSB, DNA-DSB and base damage …, and finally effect of environment.

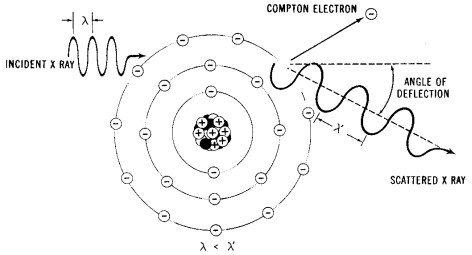
Proposals coming out of these studies:

- Mechanism(s) in controlling the initial damage through possible quantum manipulation by optical/chemical methods.
- Developing computational approaches for RBE at the molecular levels based on stochastic model of ion track structure in combination with molecular dynamics useful for low-dose limits where the experimental data is not easily accessible.

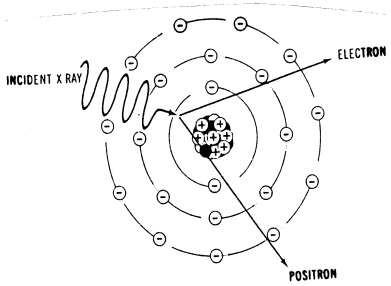
Radiation- propagation of energy in form of waves or particles (X-ray, n, p, e, C …)

Radiation interacts with matter within atomic scale ~ 0.1nm & deposits energy. ED unit: 1Gy=1J/Kg
Diagnostic radiation ~ 10-100keV, therapeutic radiation ~ 1-20 MeV X-ray. This energy decays in a cascade of reactions from high to low energies, such as:

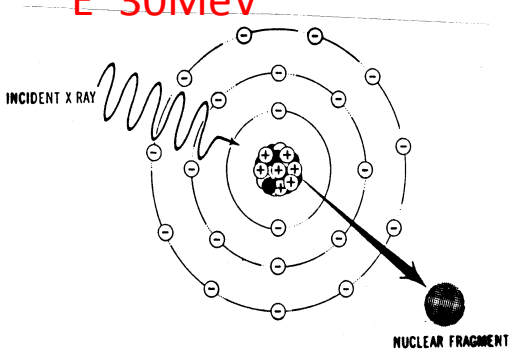
Compton scattering E~MeV



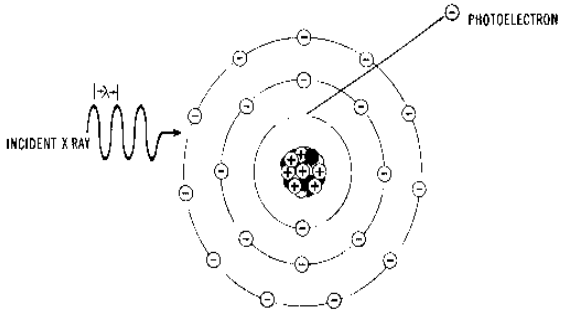
Pair Production E~1.2MeV



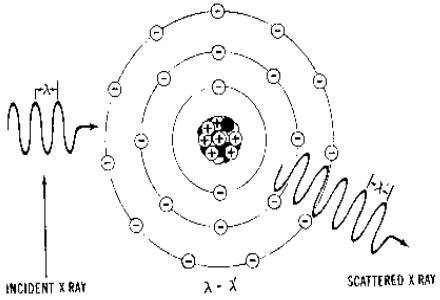
Nucleus-disintegration E~30MeV



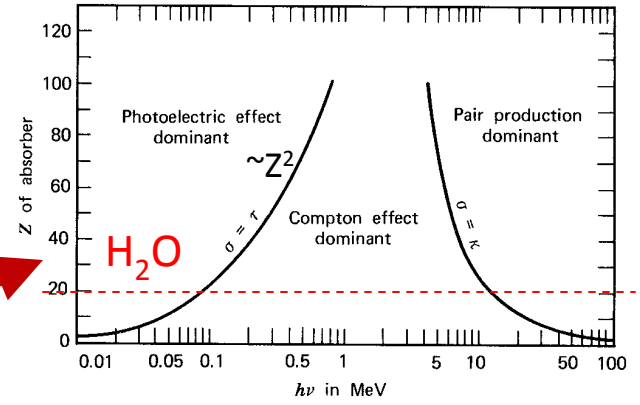
Photoelectric effect E~keV (imaging/diognostic/CT scan)



Coherent scatter E~eV



Relative importance



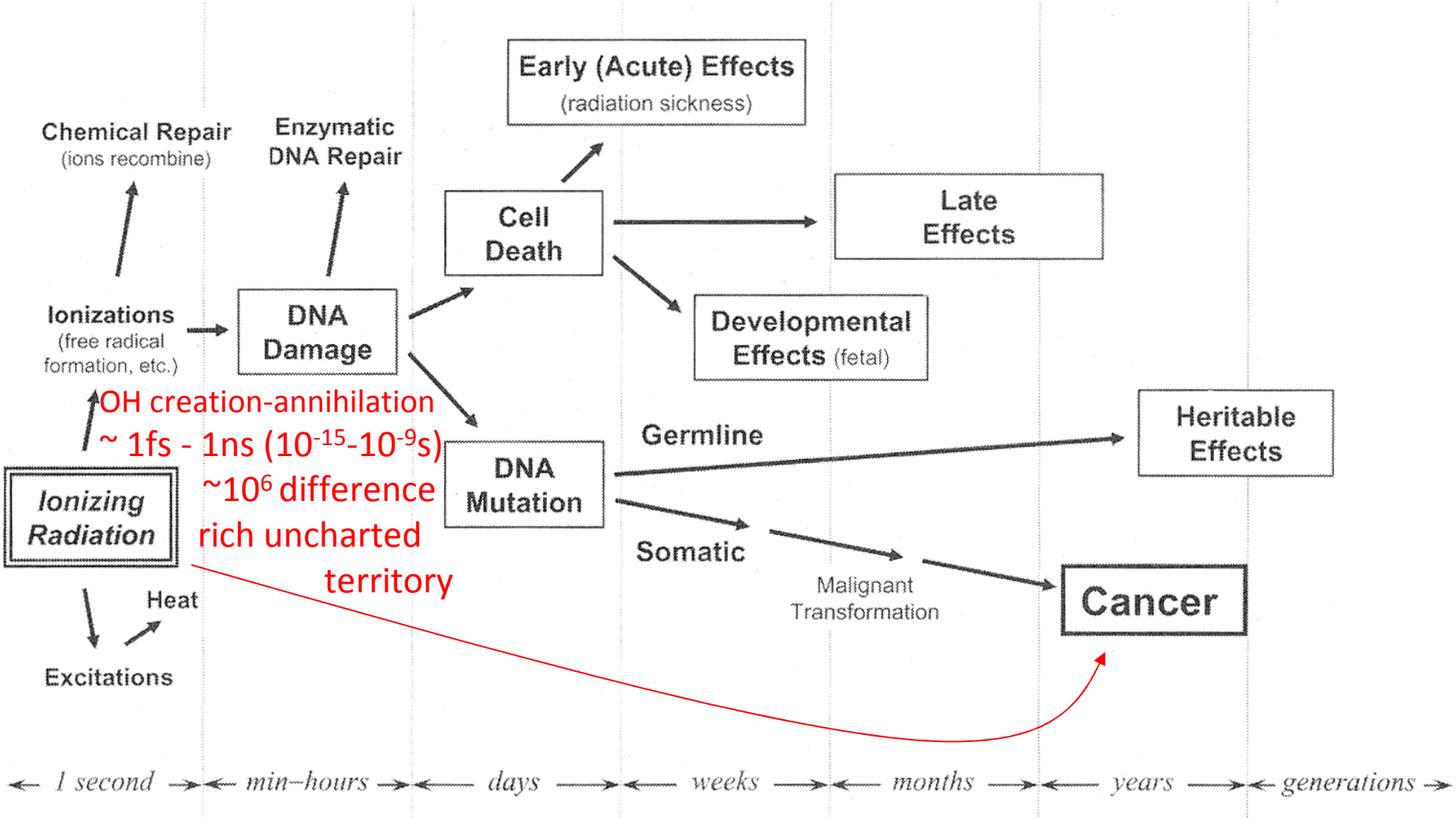
Water is a good approximation for tissues and human organs:
~ 80% of human body consist of water

Compton scattering predominant interaction in range 30 keV – 30 MeV

Classic Paradigm/hierarchy of Radiation Injury

Cancer development starts from initial DNA damage ~ 1fs – 1ns per hit

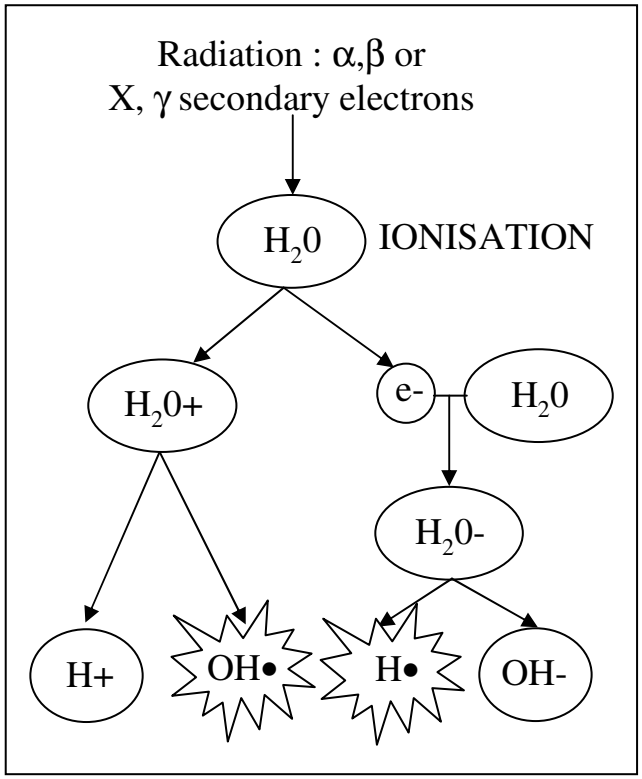
Ref: Eric Hall, Radiobiology for the Radiologist



OH free radical

- is charged neutral and has one unpaired electron with S=1/2
- Its motion is governed by thermal diffusion & Brownian motion (random walk)

Cascade of water radiolysis



Time scales of the events (in solution):

- Initial ionization: $1\,fs = 10^{-15}\,s$
- Primary radicals produced by ejection of the electrons: $100\,ps = 10^{-10}\,s$
- OH-radical life-time: $1ns = 10^{-9}\,s$

  Sies, Europ. J. Biochem. 215, 213 (1993).
- DNA-radical life-time produced by direct ionization or by indirect interaction with OH-radicals: $10\mu s = 10^{-5}\,s$
- Expression of biological effect due to DNA-damage: hours-days-years

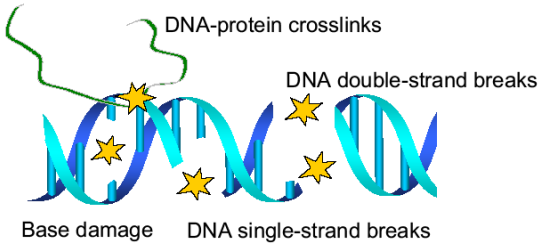
- 100 eV of absorbed photon/electron produce about 6 OH radicals, hence 1Gy of radiation produce n~10^{20} OH per m^{-3}

Initial DNA damage occurs by breaking/forming chemical bonds (not heating effect)

- DNA Single Strand Breaks (SSB)
- DNA Double Strand Breaks (DSB)
- Base damage (BD)

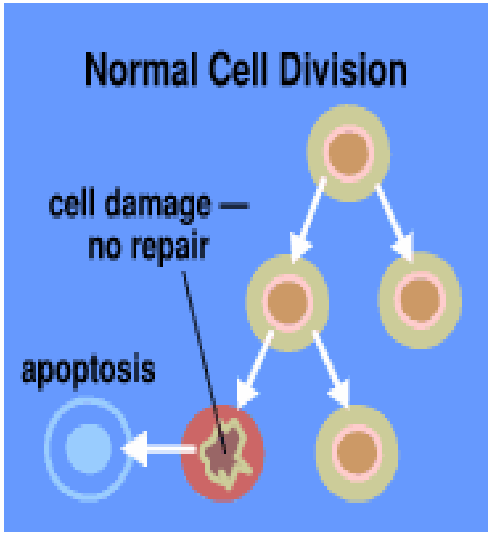
Refs on initial sites of damage (experiments):
- Pogozelski, Tullius, Chem. Rev., 98, 1089 (1998);
-Tullius, Greenbaum, Curr. Opin. Chem. Biol. 9, 127 (2005).

Radiation damage to biomolecules

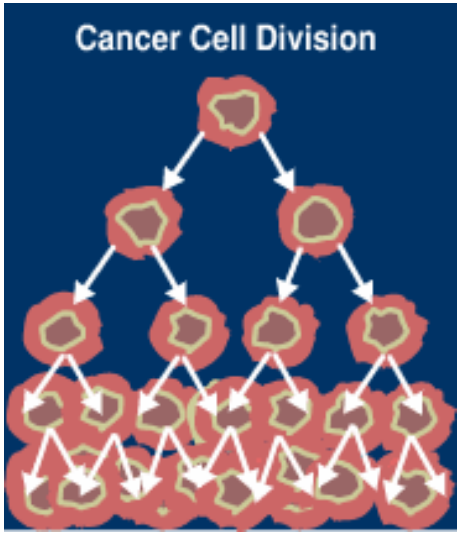


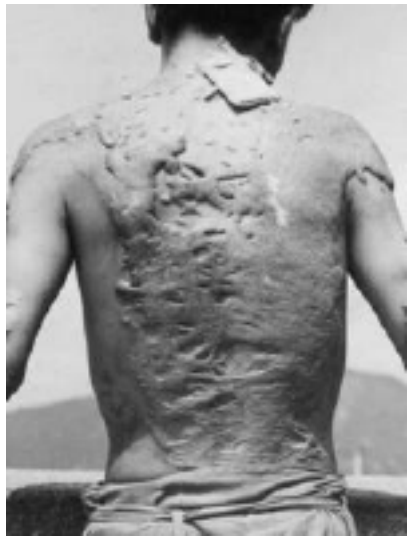
Types of DNA damage:	Approx. No. per gray:
1. Base damage	1000 – 2000
2. Cross-links	200 – 400
3. Single-strand breaks (ssb)	1000
4. Double-strand breaks (dsb)	40

- In SSB enzymes (ligase) use the other strand as a template to repair the damage
- In DSB the genetic information is deleted and there is a chance for repair enzymes to make mistakes.
- Any mistake in repair mechanism lead to genetic mutation which may be suppressed by cell death (apoptosis), otherwise possibly to carcinogenesis if tumor suppressor genes are deleted.



Cancer (carcinogenesis) is characterized by
- **uncontrolled cell division, abnormal growth of cells.**
- **ability of these cells to invade other tissues. If the spread is not controlled, it can result in death.**





Reaction-Diffusion models

non-homologous end-joining by enzymes

$$IR + DNA \rightarrow [DSB]$$

Bio-chemical repair kinetic model (reaction-diffusion models):

$$[DSB]+[E] \xrightarrow{k} [C] \xrightarrow{k'} [DNA]+[E]$$

E: enzyme
C: complex
k,k': reaction rate constants
D: IR dose
α: induction-rate per unit dose of DSB

(ignoring back reaction
C E+DSB)

Rate equations:

$$[E]+[C]=const.$$

$$\frac{d[DSB]}{dt} = \alpha \frac{dD}{dt} - k[E][DSB]$$

$$\frac{d[C]}{dt} = k[E][DSB] - k'[C]$$

Repair Mechanisms

non-homologous end-joining by enzymes

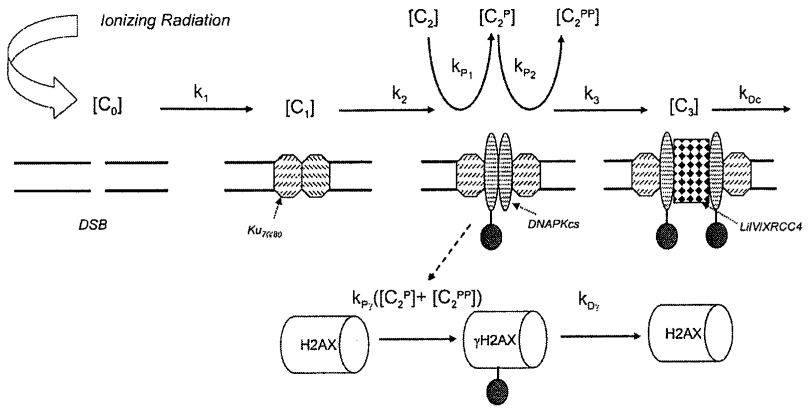
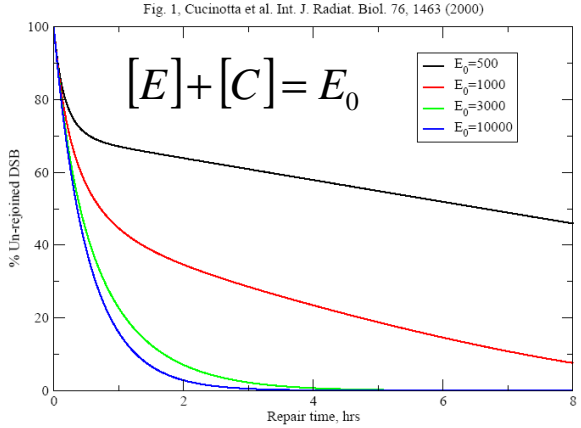


Fig. 1, Cucinotta et al. Int. J. Radiat. Biol. 76, 1463 (2000)



-Hammel et al. (UC Berkely +UTSW)
J. Bio. Chem. 285, 1414 (2010)
-Cucinotta, Nikjoo, O'Neill, Goodhead,
Int. Rad. Bio. 2000

Modalities in treatment of cancer

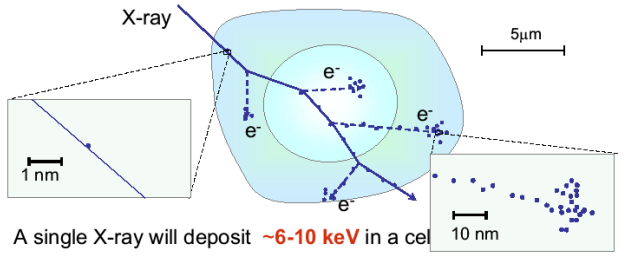
- diagnostic CT-scan/PET
- surgery
- chemo/gene/hormone therapy
- radiation therapy

Main Goal in RT:

Tumor ablation with minimal destruction to normal tissues to minimize chance of secondary cancer

Challenges in achieving the goals:

- Geometrically tumors and normal organs are entangled
- Human anatomy is not static
  - Respiratory cycle
  - Cardiac cycle
  - Thoracic and abdominal tumors can move as much as 3cm during Tx
  - Evolution of anatomy during treatments
- Protecting radio-sensitive organs at risk (OAR)
  - Dose volume constraint for OARs (SBRT, 3-5 fractions)
    - Spinal cord: 10 Gy
    - Lung: 20 Gy
    - Heart: 5-6 Gy

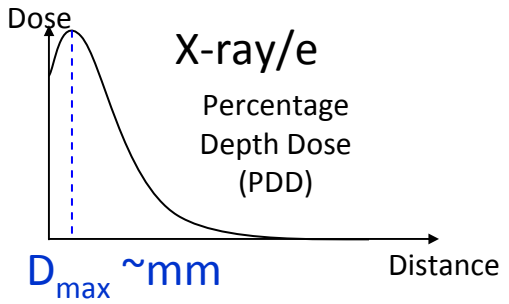


A single X-ray will deposit **~6-10 keV** in a cel

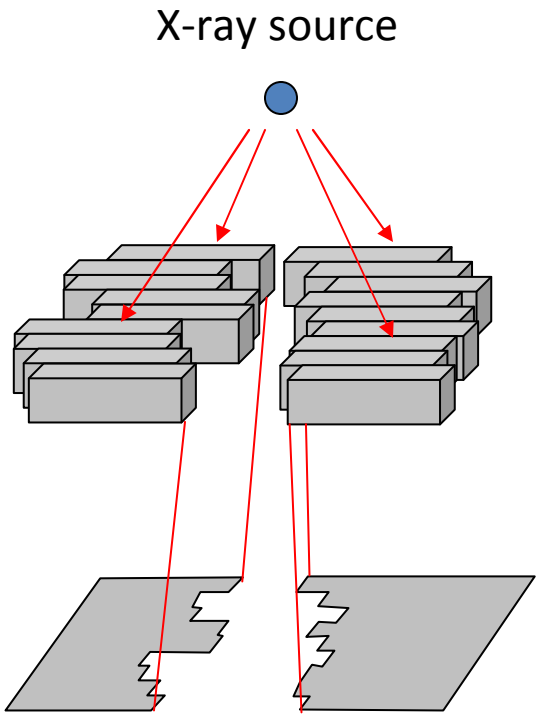
A single X-ray will produce **~300 ionizations** in a cell

~20 eV per ionization

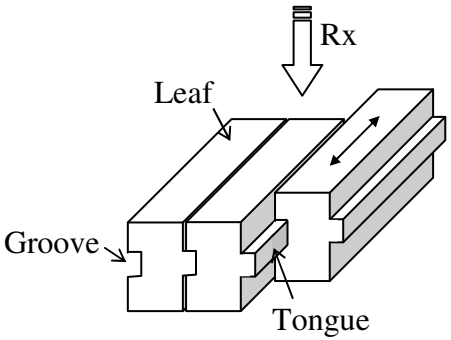
This is equivalent to **1 ionization every 40 nanometres**



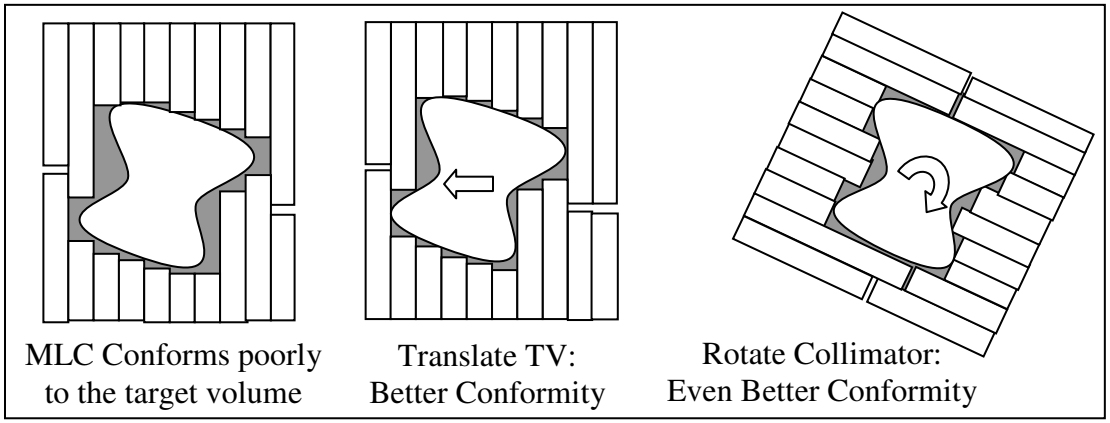
IMRT & Multi-leafs collimators in LINAC head

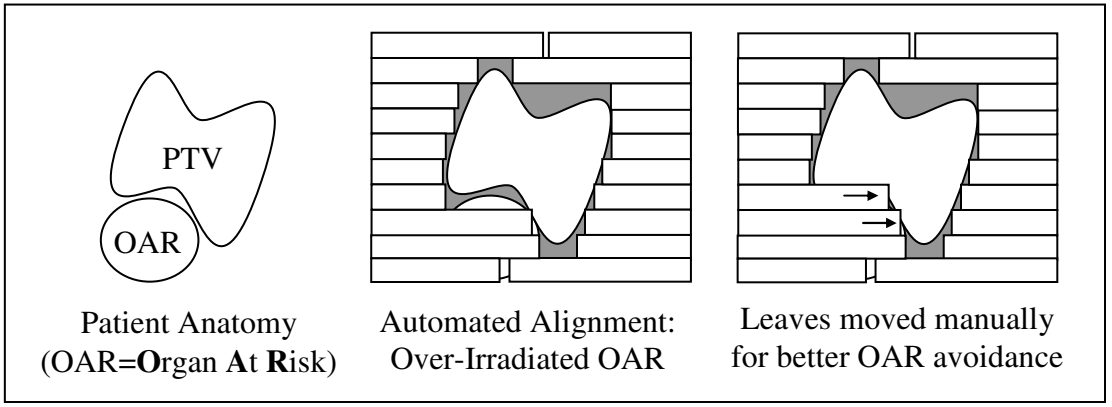


Shaped Field at Isocentre

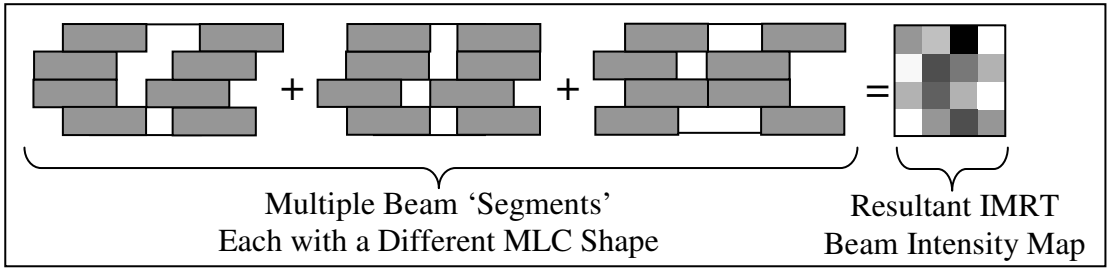


MLC Conformity





Step and Shoot mode:
common in clinical; stop then delivery

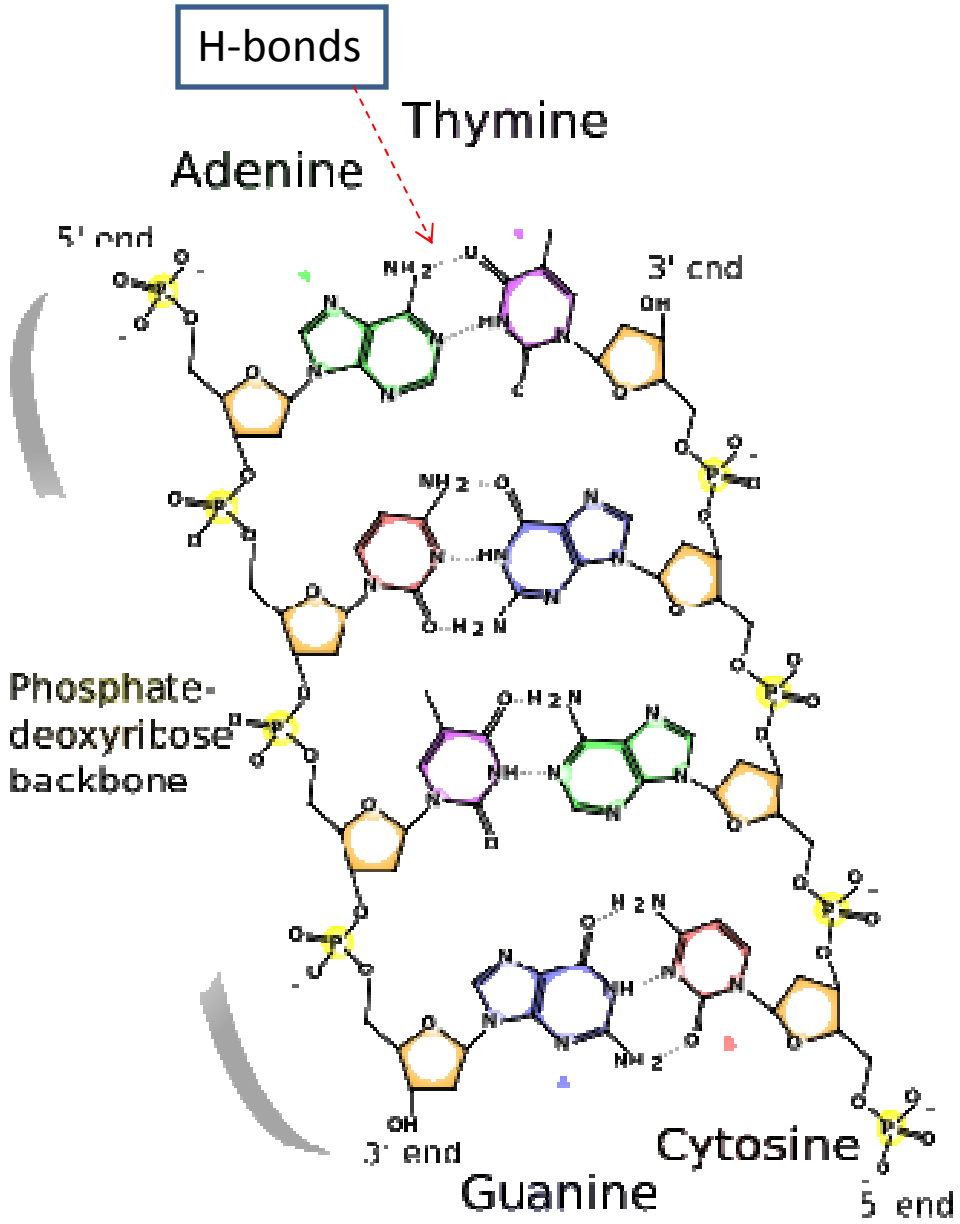


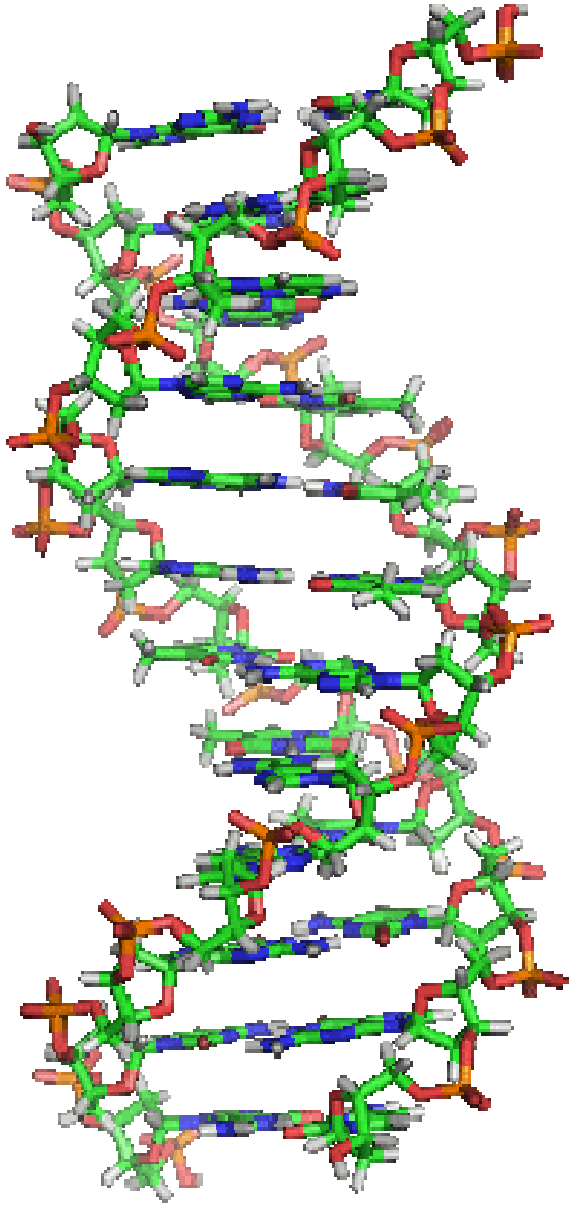
Dynamical collimation to compensate the tumor motion and protecting organs at risk optimized by “genetic algorithm”

Ref: Ramin Abolfath and Lech Papiez Med. Phys. 2009; 2010

Challenge: motion detection and lack of control system for dynamical collimation

Molecular Structure of DNA





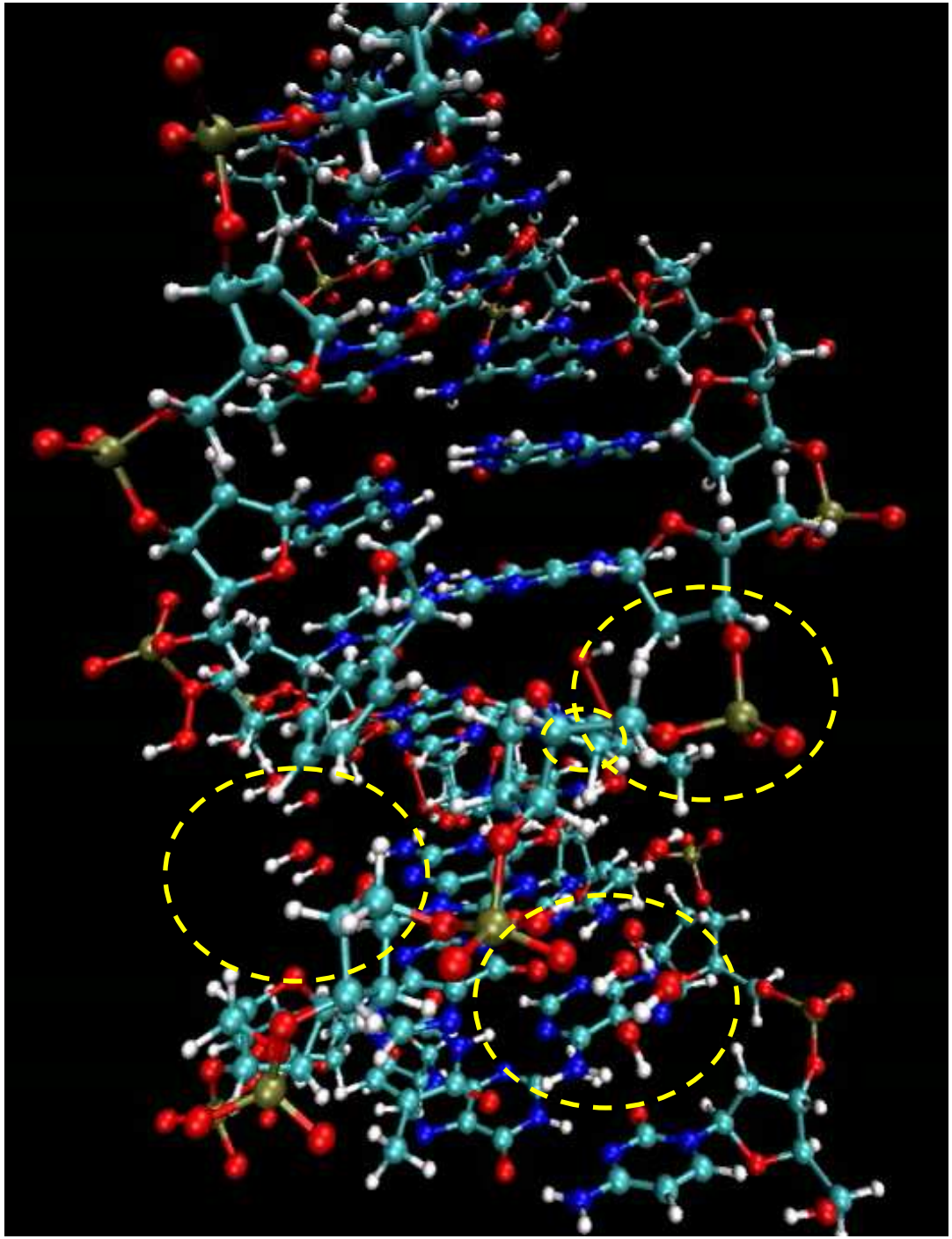
-This is a real time simulation performed up to 2.5 pico-second (2.5 10^{-12} s) using ReaxFF molecular dynamics. This simulation clearly demonstrates a single strand break on a realistic chunk of DNA for the first time (to the best of my knowledge). Ref: Abolfath, van Duin, Brabec, J. Phys. Chem. 2011 .

- Color codes: carbon, oxygen, nitrogen, phosphorus and hydrogen atoms are shown as green, red, blue, gold and white.

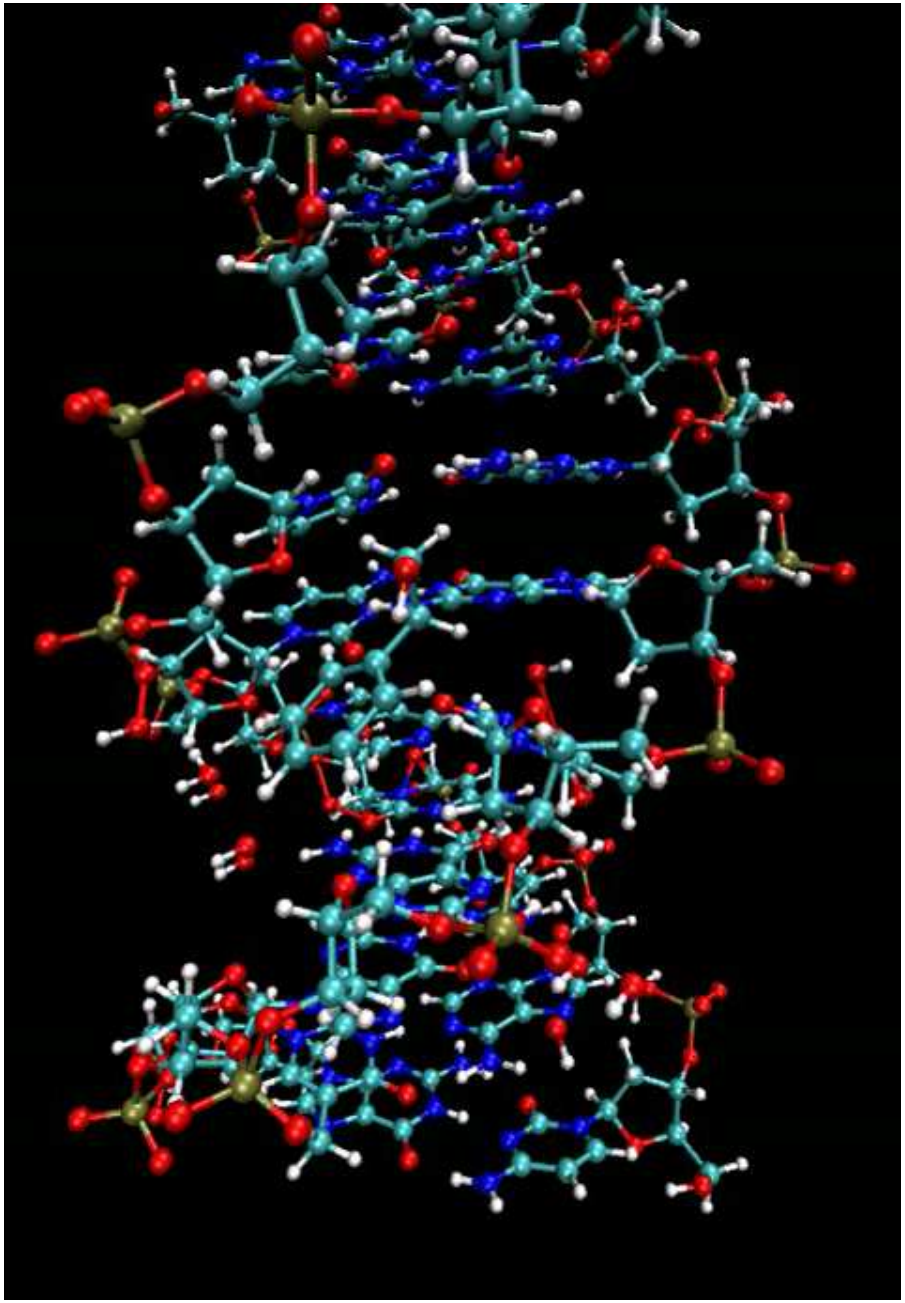
-Phosphorous (gold particle) in the right corner is being oxidized by OH and becomes mechanically separated from the backbone (single strand break).

-Two dash-lines show the broken bonds. The remaining H (the white particle) that was initially in OH, forms a bond with the Thymine (a base damage).

-The distorted thymine and sugar-ring destroys the base-pair hydrogen bonds and base stacking network.

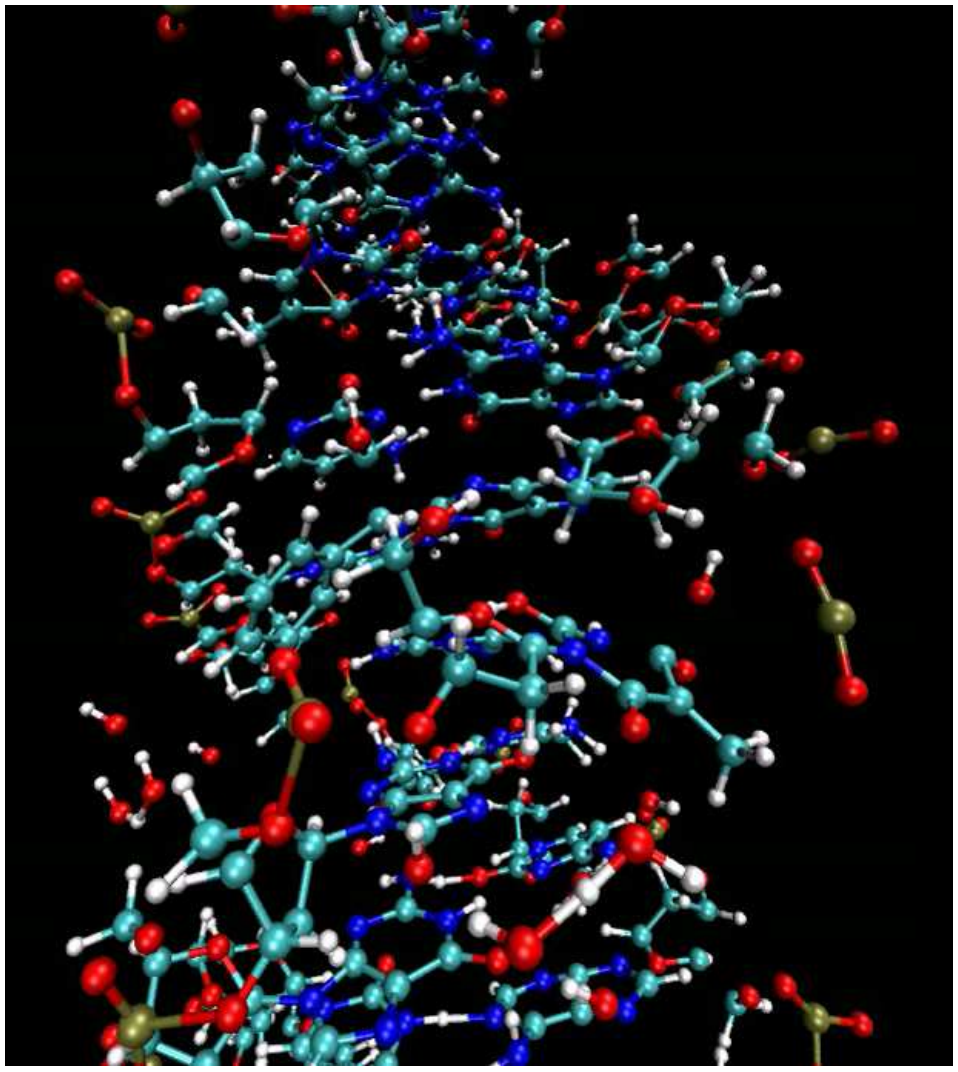


Time ~ 0-0.5 ps



Slow-motion movies

Time ~ 2-2.5 ps



Results and simulations based on recent development in ReaxFF

Abolfath, van Duin, Brabec
J. Phys. Chem. 2011

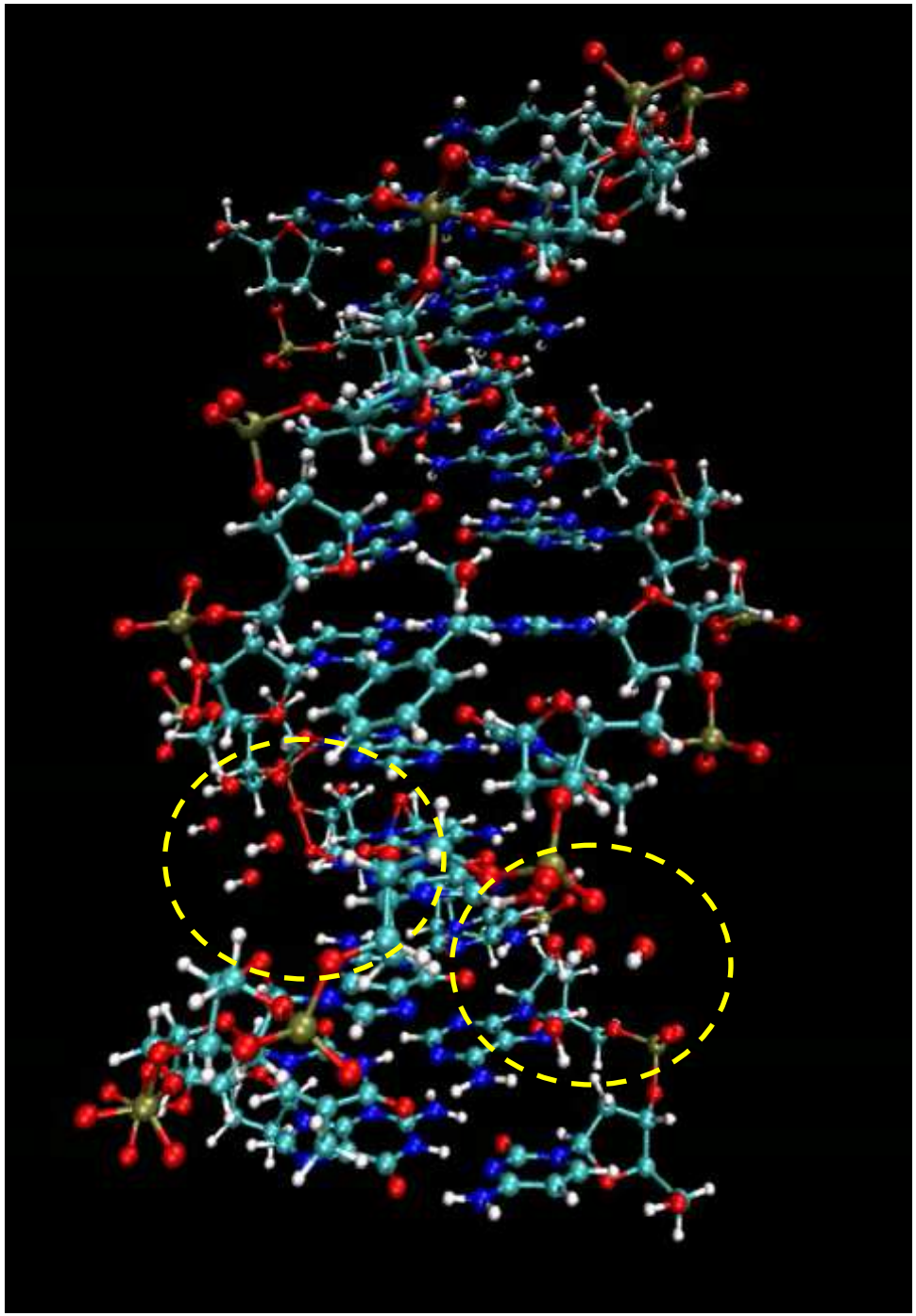
1) Back-bone damage: carbonyl/hydroxyl formation and broken sugar-rings

2) base-backbone separation

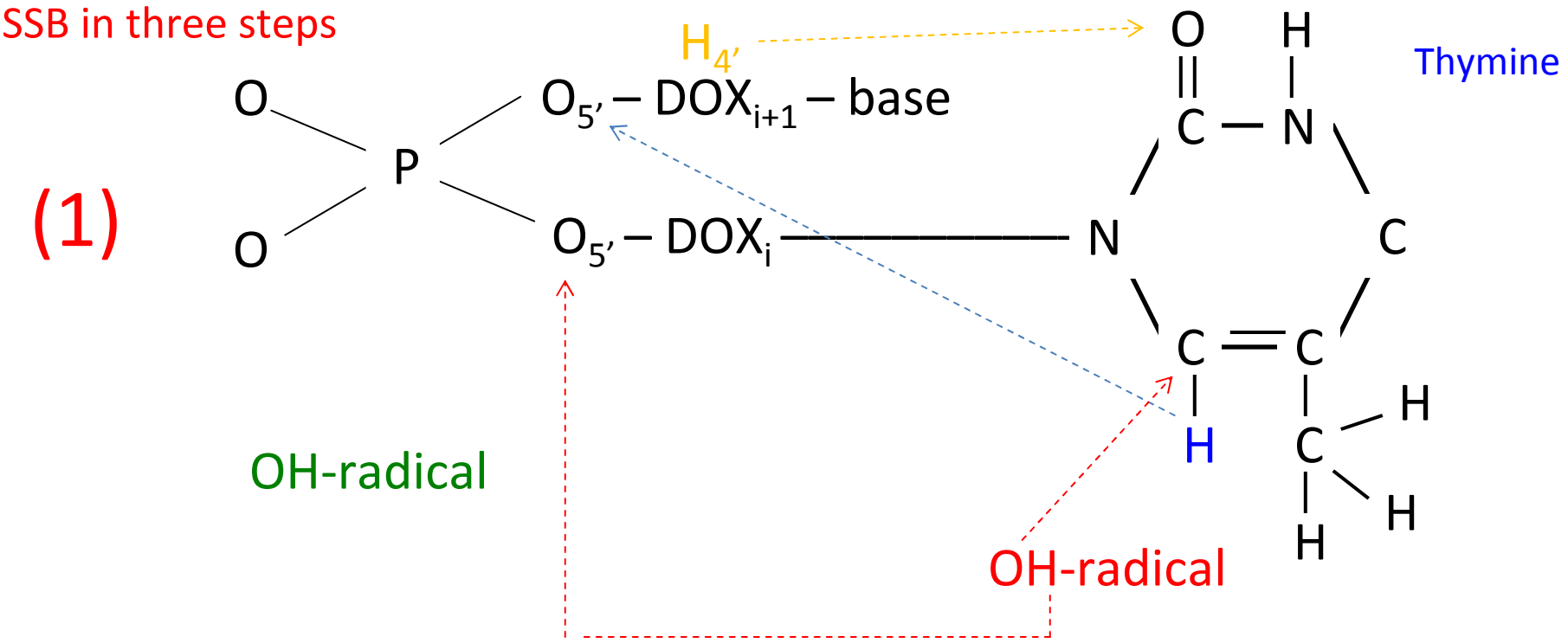
3) base-damage

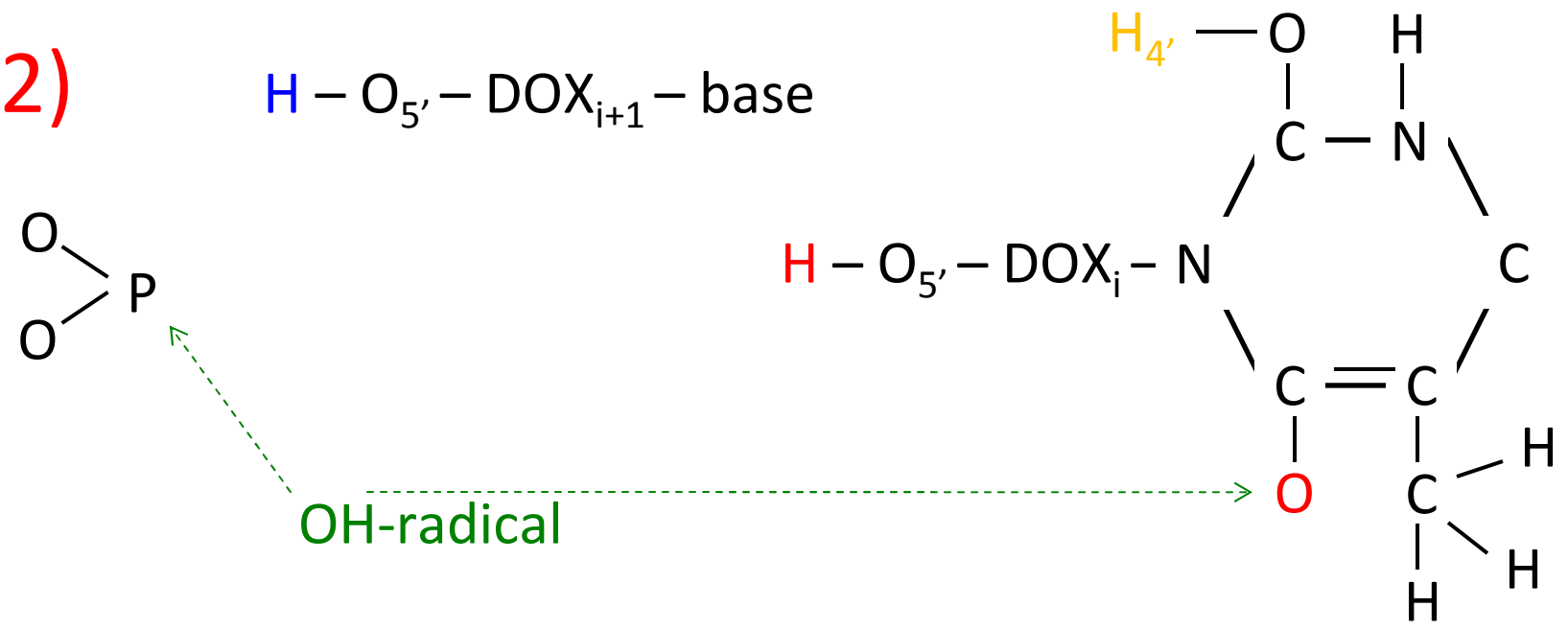
4) single-strand break

5)Double-strand damage

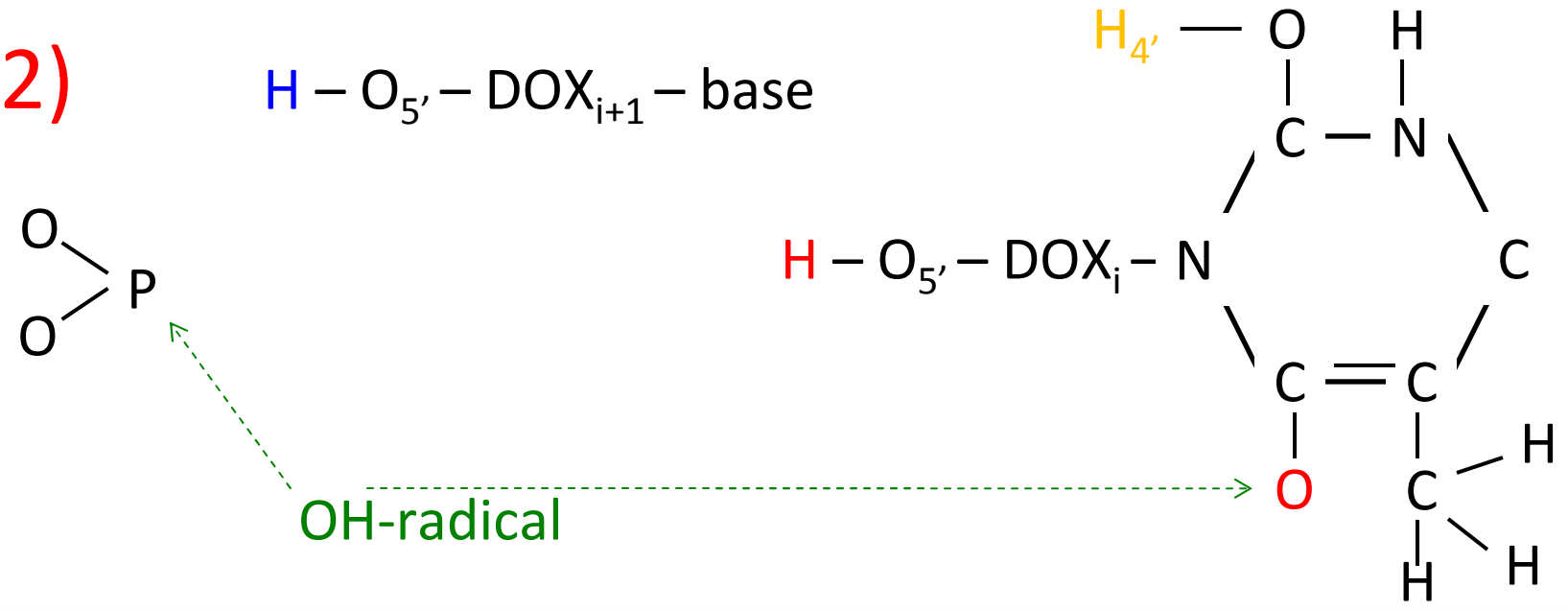


SSB in three steps

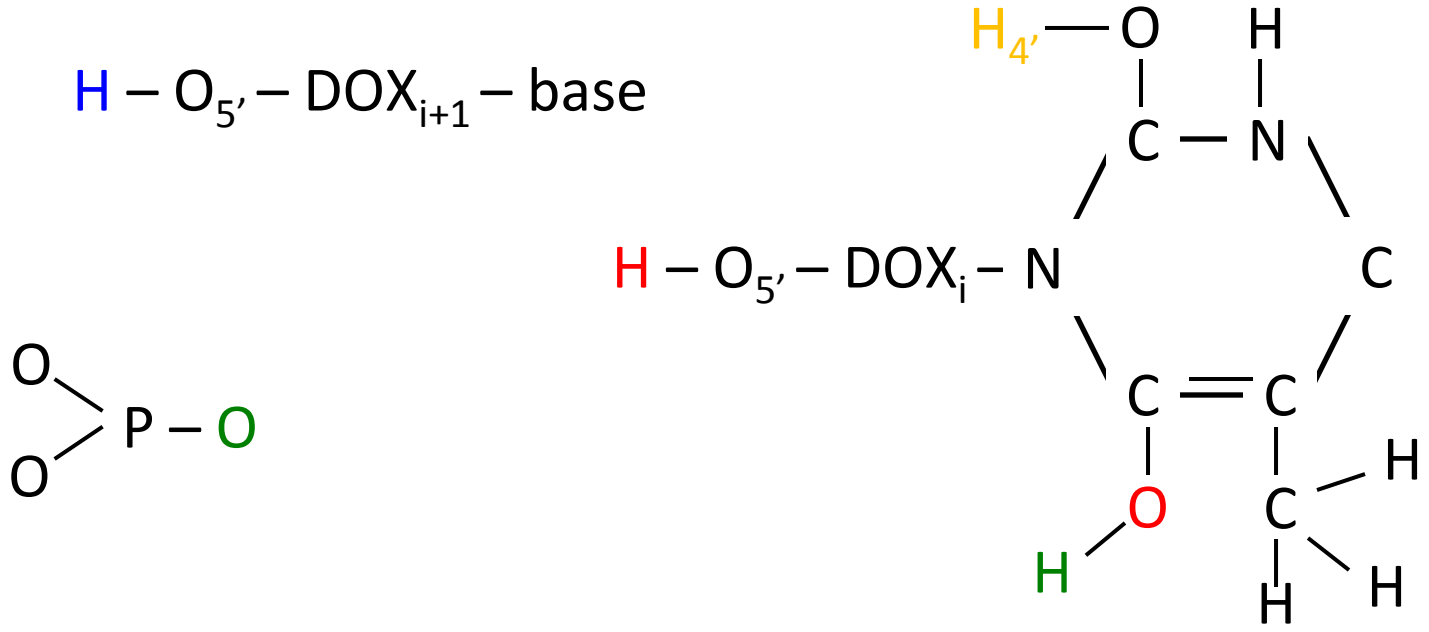




(2)


H – O5′ – DOXi+1 – base
H4′
O
H
C
N
H – O5′ – DOXi – N
C
C
C
H
O
C
H
H
H
O
P
O
OH-radical

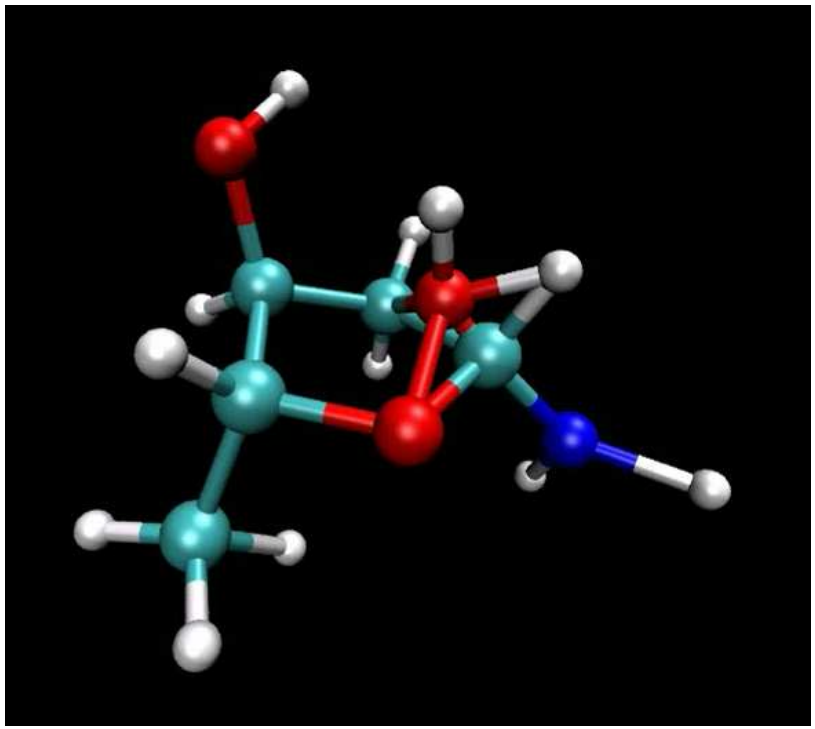
(3)


H – O5′ – DOXi+1 – base
H4′
O
H
C
N
H – O5′ – DOXi – N
C
C
C
H
O
C
H
H
H
O
P – O
O

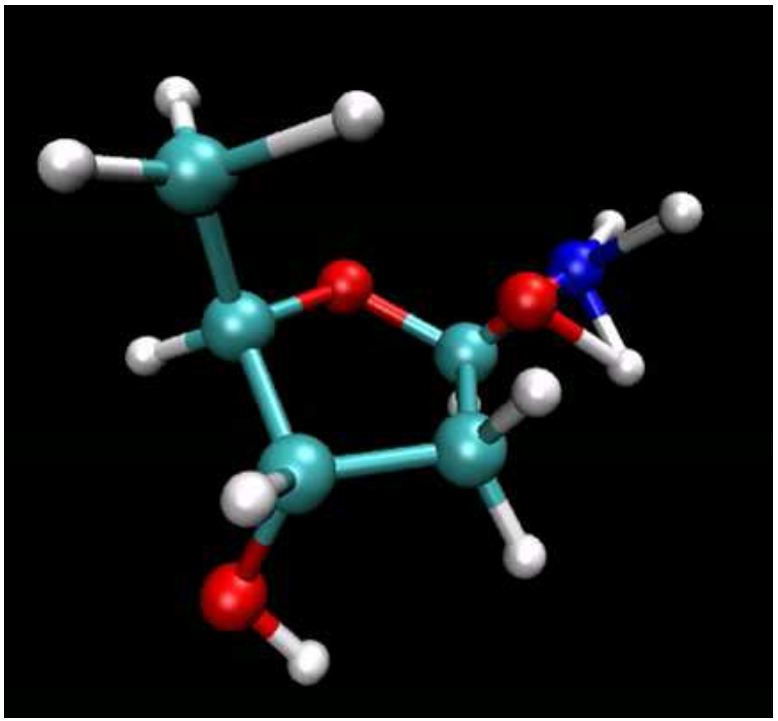
Checking with QM calculation:

- Energy of electrons is calculated by Schrodinger eq.
- It gives forces acting on nuclei that make them to move and a new configuration for electrons is generated
- This goes to a loop and generates time evolution of molecule under study
- Chemical reaction is simulated by calculation of the energy landscape on-the-fly

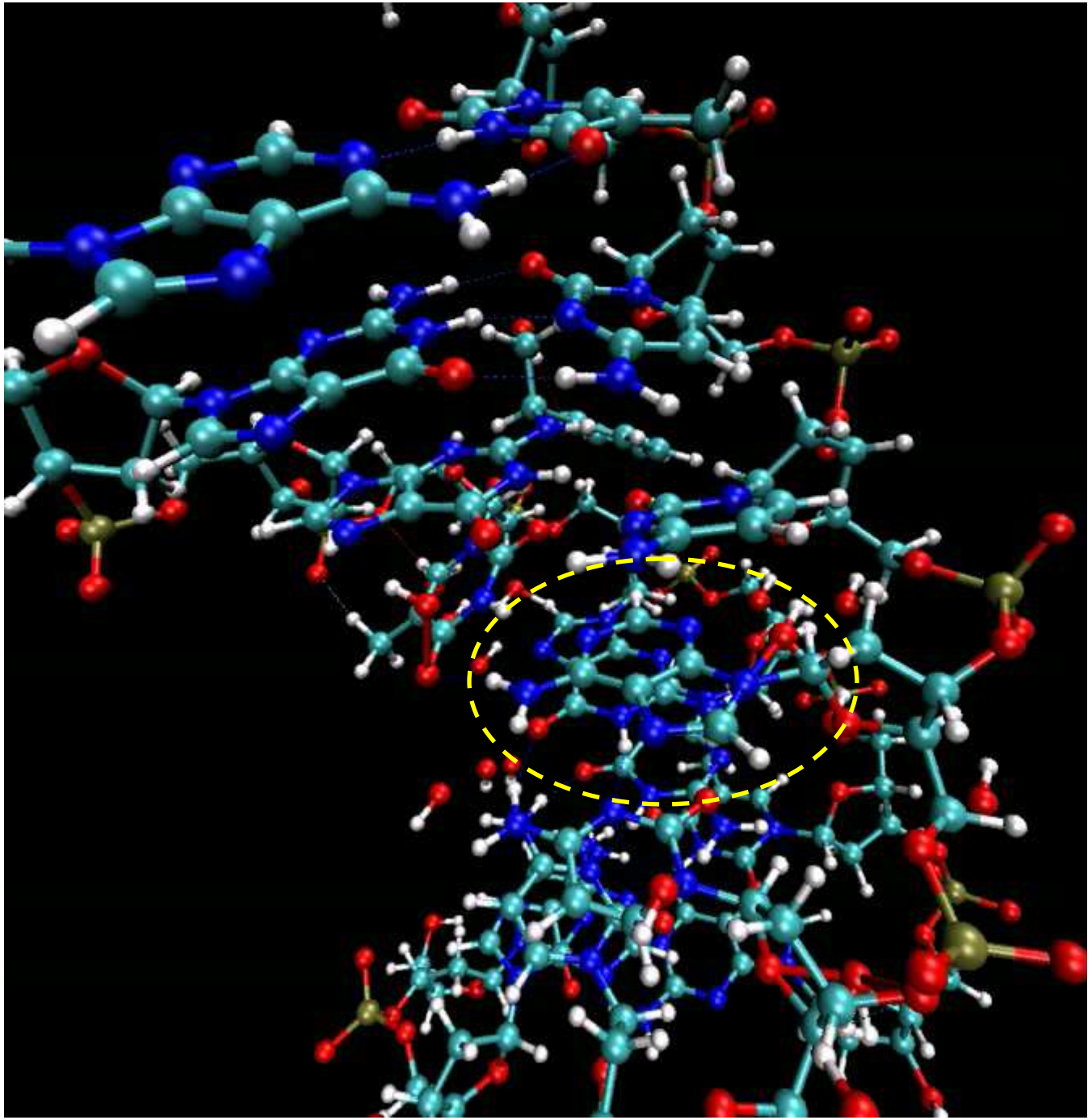
Breaking of sugar-ring and formation of hydroxyl



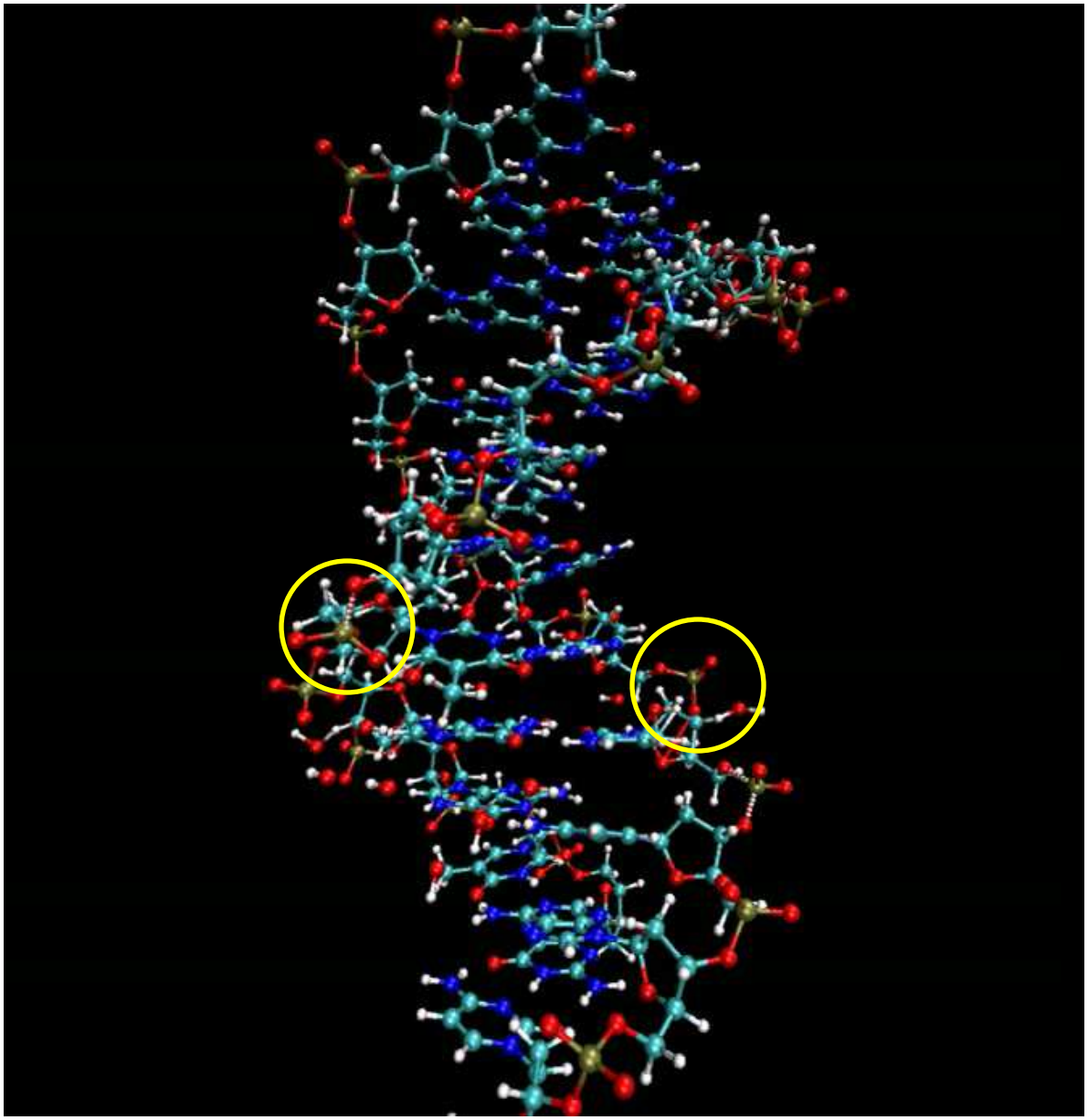
Breaking of amino-group base-backbone separation and formation of carbonyl



Base damage



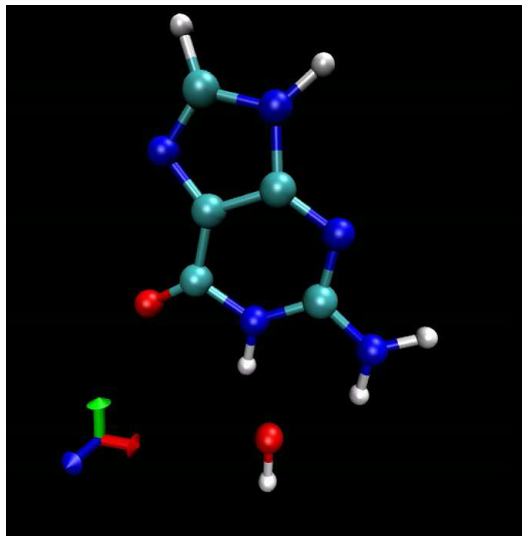
DSB: DNA-SSB shows criticality: any small deviation in position of additional OH radicals cause a dramatic change, is there any chaotic behavior?



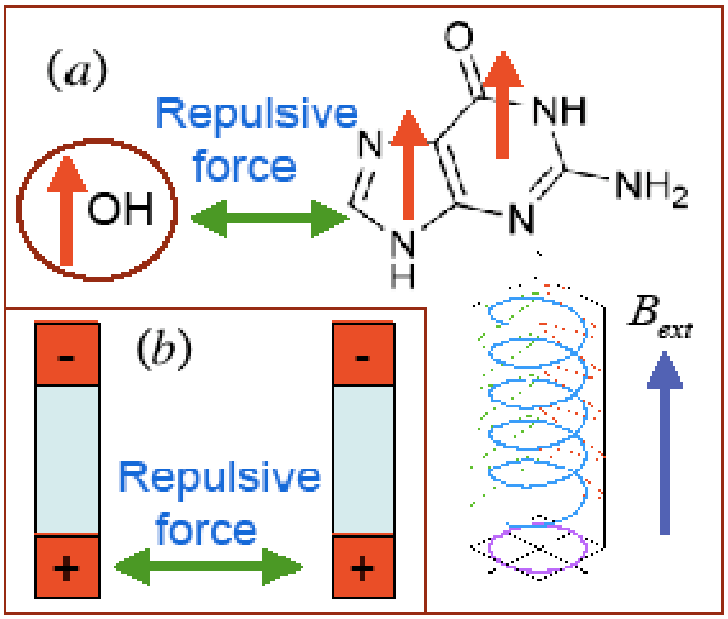
Spin blockade control of chemical reactions by free radicals

RMA, JPC (09) & JCC (10), DFT-MD

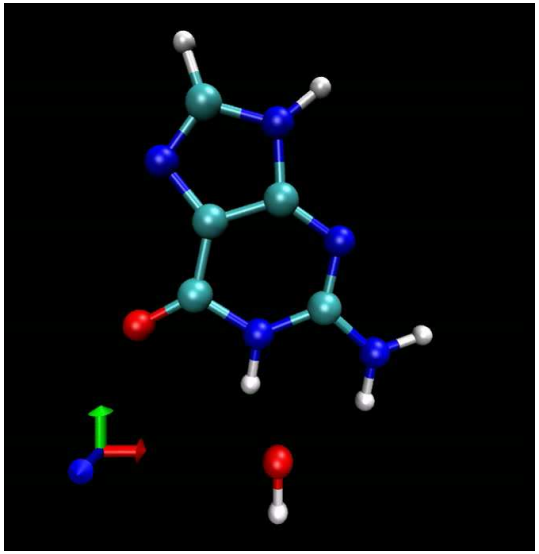
Total spin doublet (singlet molecule)

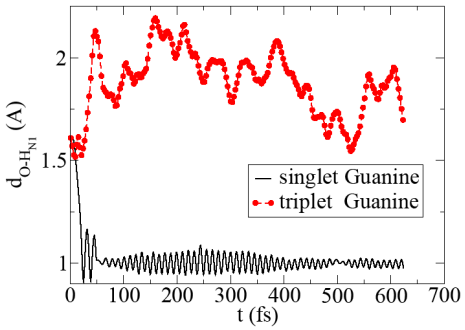


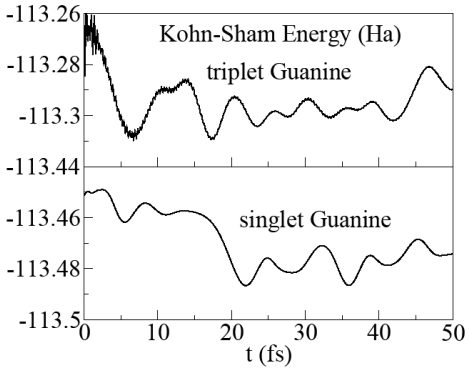
$\lambda = 463nm$ (blue)



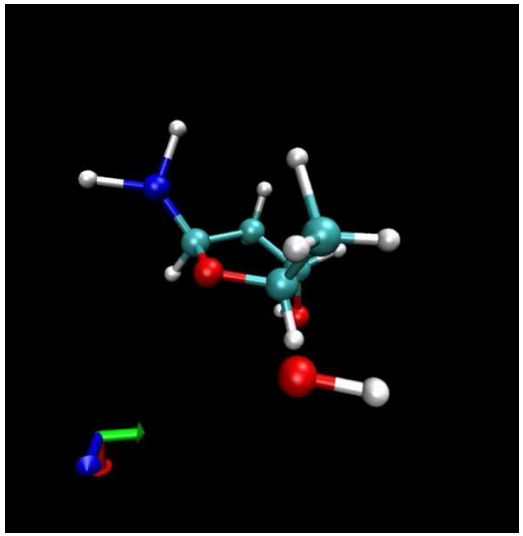
Total spin quartet (triplet molecule)

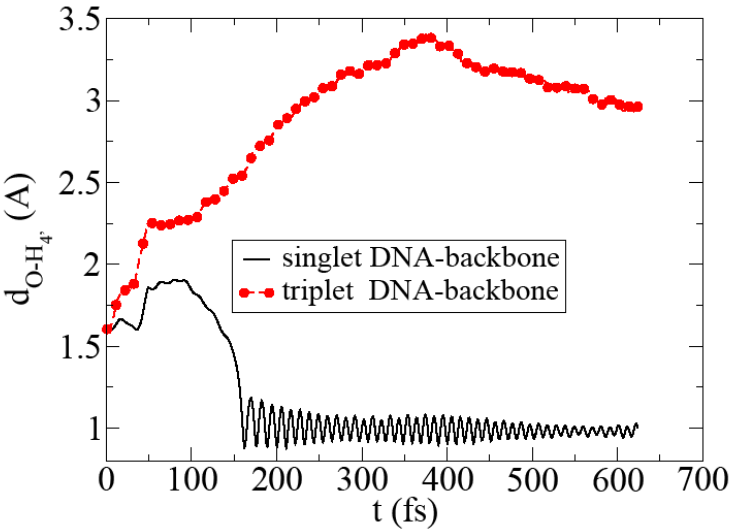




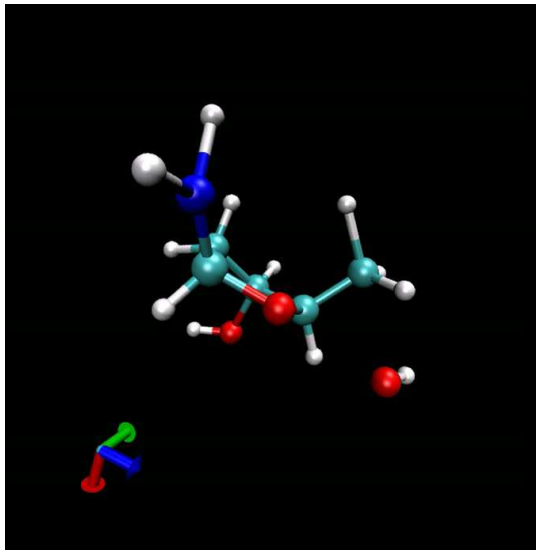


Total spin doublet (singlet molecule)

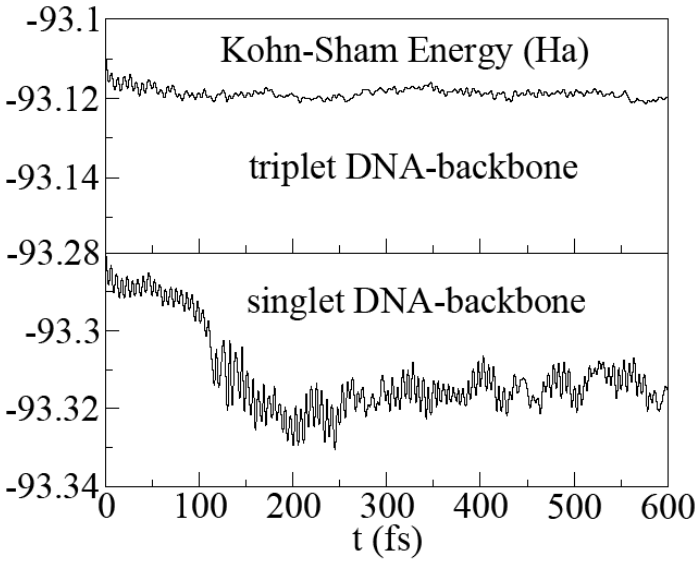




Total spin quartet (triplet molecule)



$\lambda = 521nm$ green



Effects of solution (QMMM simulation)

Guanine: QM (CPMD)
Water + Sugar + P : MM (GROMACS)

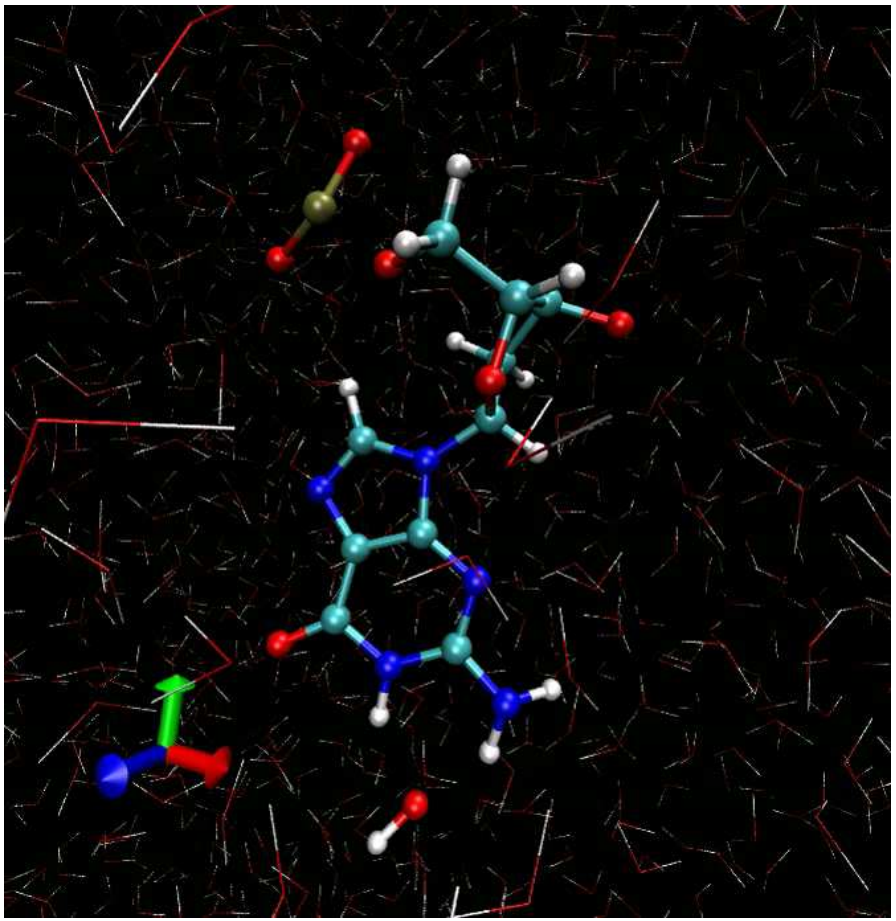
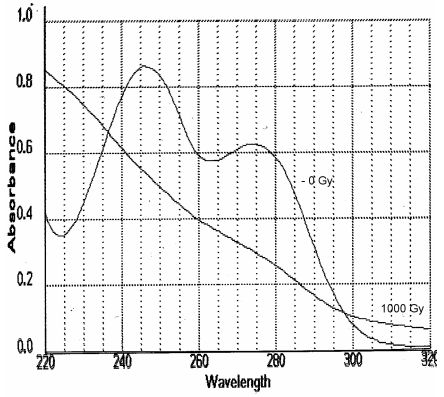
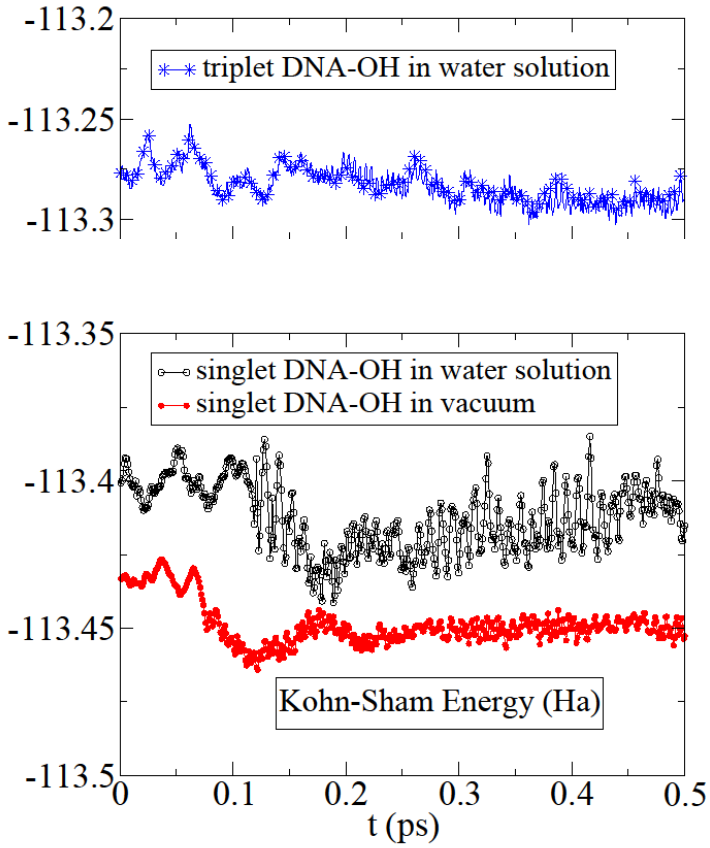


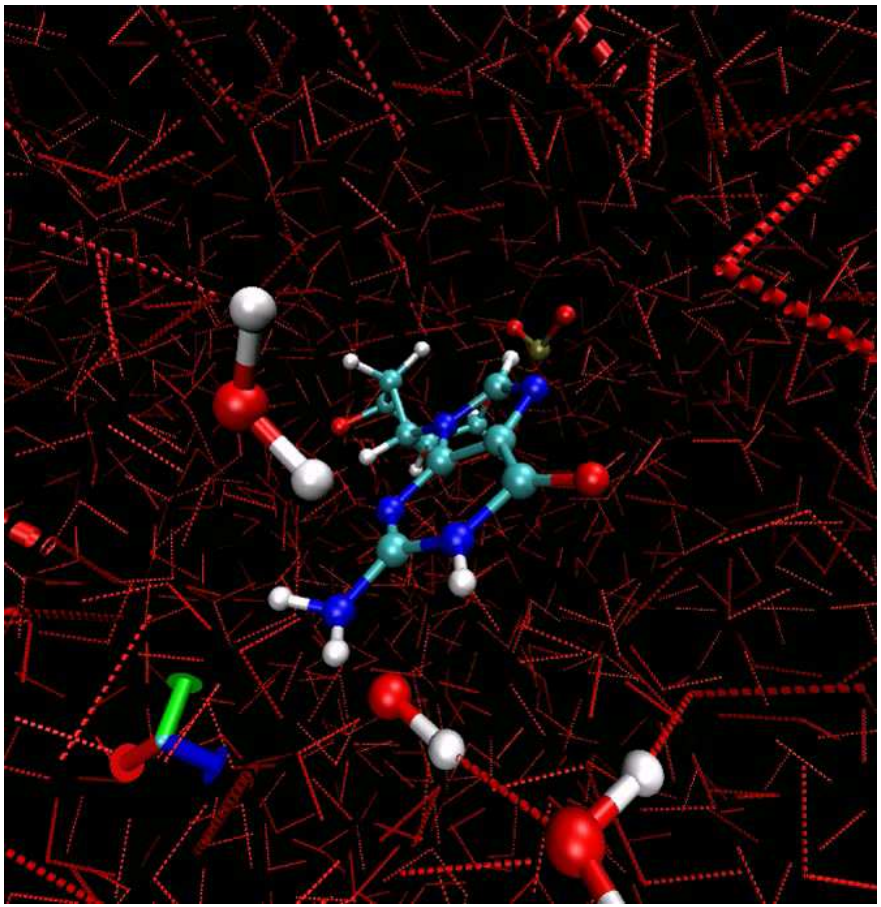
Photo-absorption experiments

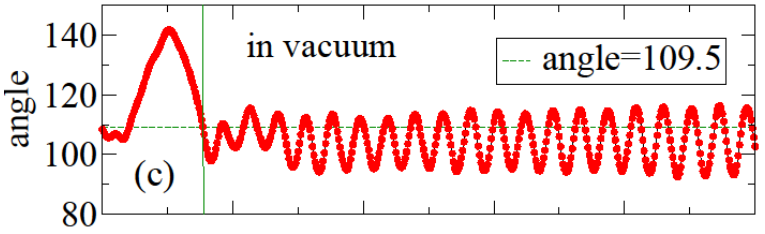


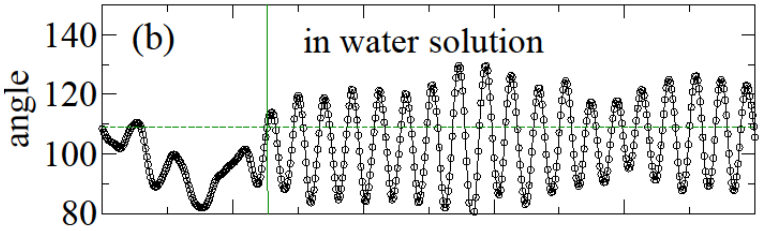


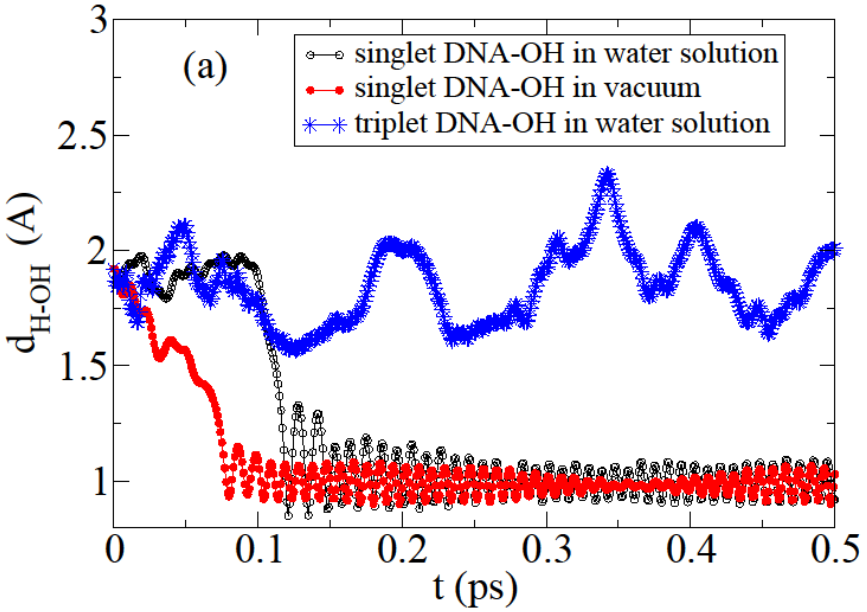
RMA, PKB et al. submitted to J. Phys. Chem.

Reaction rate in solution
is slower than in vacuum
Due to a network of hydrogen
bonds in solution



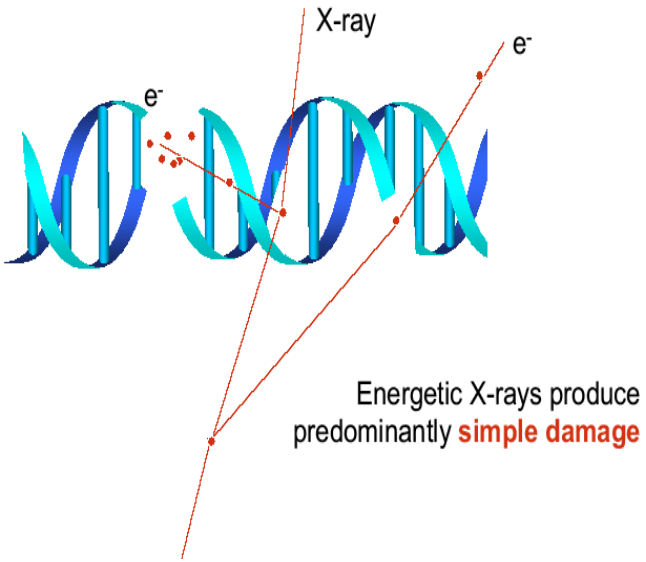


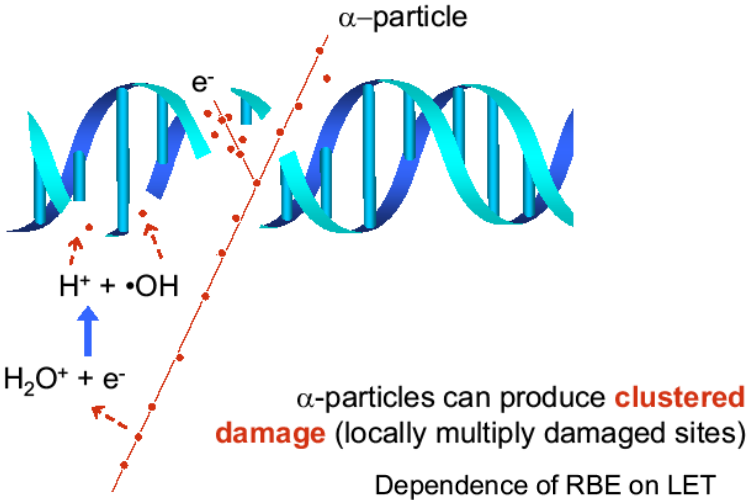




Open questions and possibilities for future works:

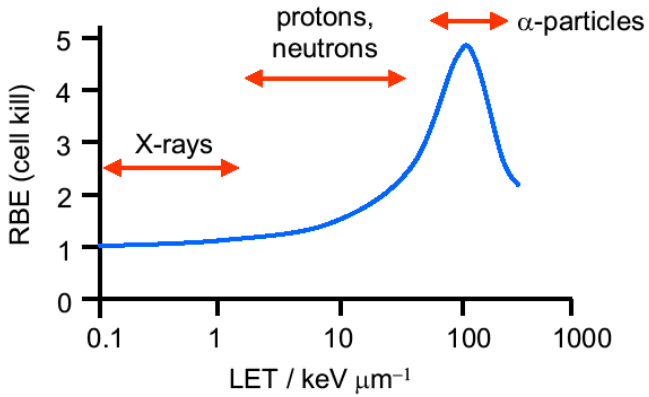
1) Modeling realistic DNA-environment in-vivo:
   - How DNA in water resembles DNA in cell?
   - What is the form of water in cell and how OH radicals diffuse in such environment?
3) Making molecular simulation for repair mechanisms.
4) How can we match MC with MD to model realistic RBE? Developing new dosimetry?





Relative biological effectiveness (RBE) is traditionally defined as the ratio of a dose of a standard low linear energy transfer X-ray beam (X-ray of 250KeV energy)(D_X) to the dose of the test radiation type or configuration (D_T), required to cause the same biological level of effect. Thus: $RBE=D_X/D_T$

Dependence of RBE on LET



Summary:

-There are plenty of empirical information on DNA damage-repair mechanisms and well developed MC simulation and dosimetry techniques.

We attempt to understand it from molecular simulations:

- DNA damage occurs mainly by chemical change, requires developing reactive computational platforms, not accessible by non-reactive FF, e.g., AMBER, CHARMM, GROMACS.

- ab-initio/DFT methods based on quantum mechanics that calculates the energy landscapes and transition states on-the-fly are suitable for simulating breaking and forming chemical bonds, but they are heavily slow calculation. A QM-MM approach that allows QM treatment of active site and non-reactive treatment of environment is one solution.

- We recently built and tested ReaxFF-MD. Consistent with empirical data, we demonstrated OH-DNA reaction, DNA-SSB, DSB, BD (Abolfath, van Duin, Brabec JPC A, 2011).

- Computer modeling allow identifying mechanisms in controlling DNA radio sensitivity, using chemical/biological /optical/magnetic methods, e.g., a possibility in forming magnetic energy barrier (within 1ns life time, comparable with life time of OH-radicals) that results to spin blocking of diffusion controlled OH-radicals (Abolfath JPC B 2009).

- QM simulations allow access to locally resolved information, possibly not available from experiment. Such studies may lead to computationally design complex pathways for drug-design, chemo-radio treatments, and provide valuable information on low dose RBE of IR.

Summary

- DNA damage is mainly by chemical change, requires developing simulation platforms suitable for chemical reactions.
- There are various simulators in market, e.g., AMBER, CHARMM, GROMACS, … suitable for non-reactive processes, not suitable for chemical reactions
- ab-initio/DFT methods based on quantum mechanics that calculates the energy landscapes and transition states on-the-fly are suitable, but they are heavily slow calculation
- A QM-MM approach that allows QM treatment of active site and non-reactive treatment of environment is one solution
- ReaxFF showed success in simulating chemical reactions in non-organic materials such as graphene-oxides and carbon nano-tubes
- We recently showed first application of ReaxFF for DNA-damage
  Abolfath, van Duin, Brabec, JPC 2011

Summary:

- 80% of ionizing radiation turns to production of OH-radicals in cells
- OH-radicals are charged neutral, with spin-1/2 magnetic moment & half-filled electronic shell structure with life-time of 1 ns
- Chemical pathway of OH-DNA is diffusion controlled
- OH-DNA reaction leads to DNA-SSB, DSB, BD, simulated by computer modeling, consistent with empirical data
- Computer simulation shows a possibility in forming magnetic energy barrier (with 1ns life time) that results to spin blocking of diffusion controlled OH-radicals

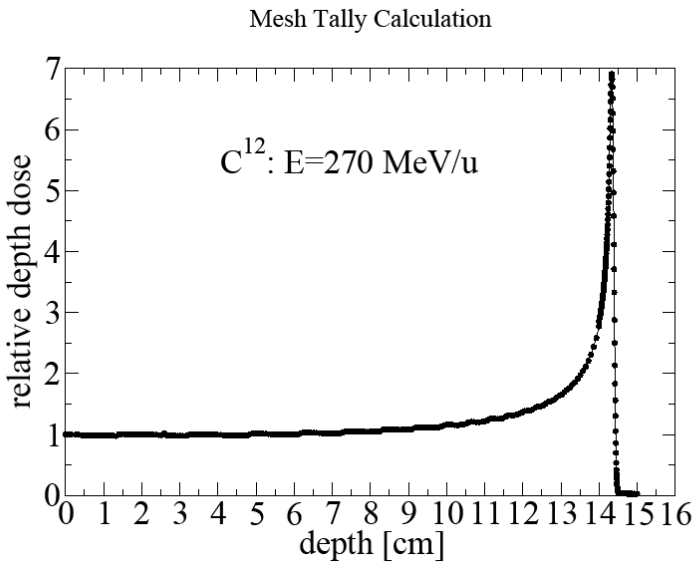
Other issues, problems and challenges:

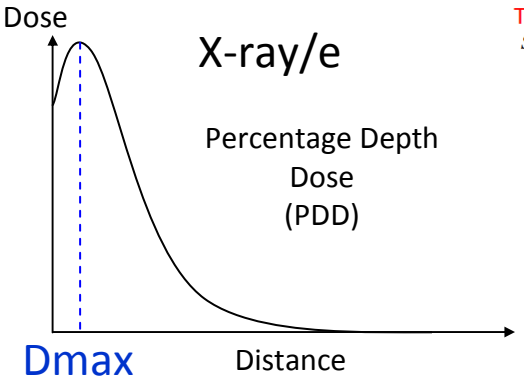
1) There is a scaling mismatch between radio-biological events
- energy scale in ionizing radiation events ~ MeV
- energy scale in biological effects induced by IR ~ eV

modeling radio-biochemical events within eV-MeV requires multi-scale modeling

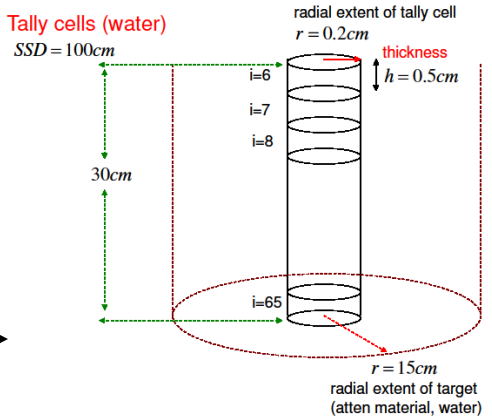
2) Modeling realistic DNA-environment in-vivo can be done
   How DNA in water resembles DNA in cell?
   What is the form of water in cell and how OH radicals diffuse in such environment?
3) How can we match MC with MD to model realistic RBE? ReaxFF-MC (in progress)
4) QM simulations allow access to locally resolved information, possibly not available from experiment. Such studies may lead to computationally design complex pathways for drug-design, chemo-radio treatments, and provide valuable information on low dose RBE of IR, not easily accessible experimentally
5) Making molecular simulation of repair mechanism

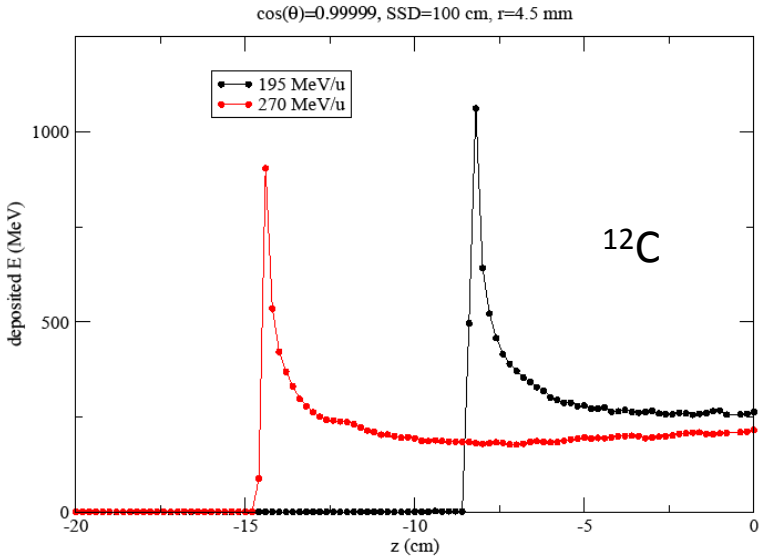
Dosimetry and treatment planning by Monte Carlo sampling, e.g., MCNPX

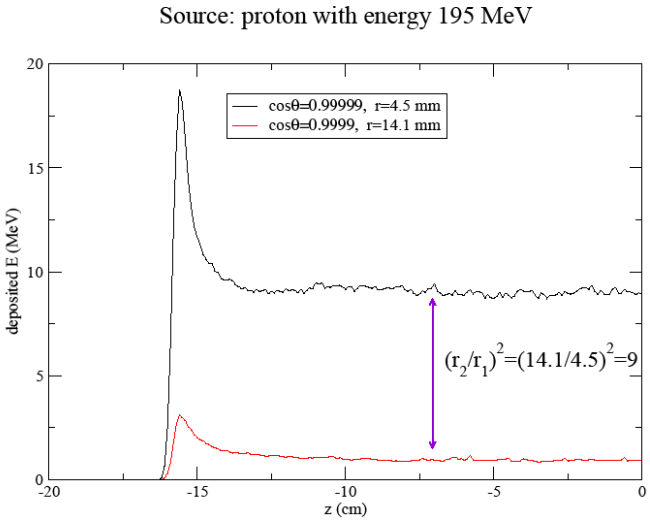




Geometrically defined target



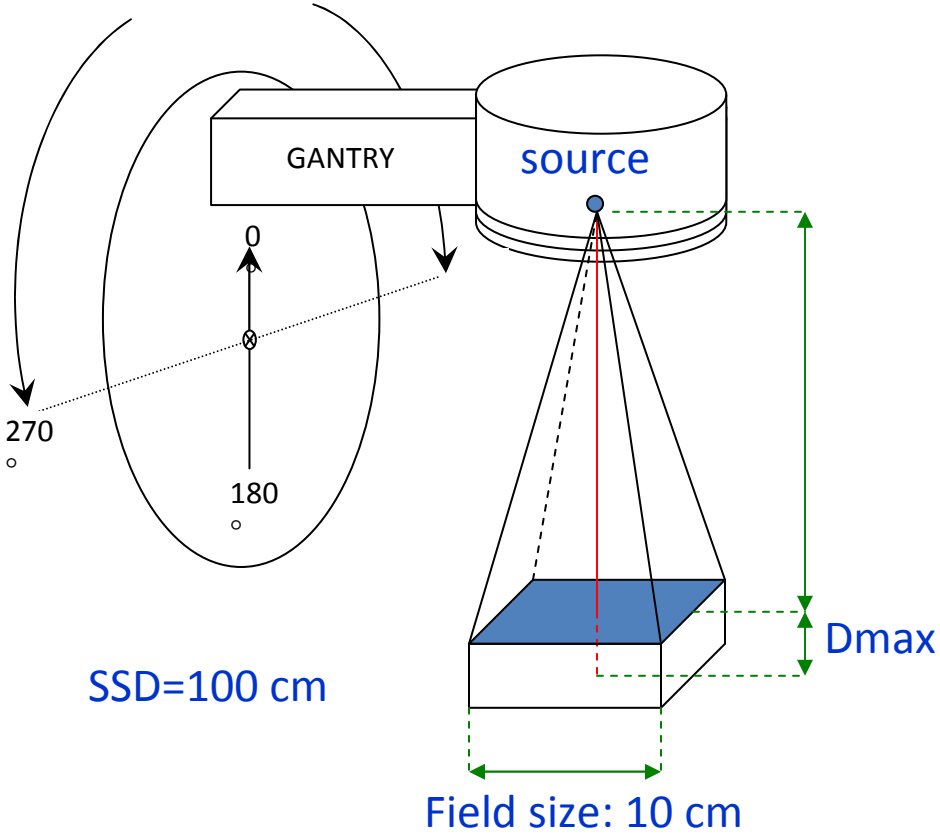


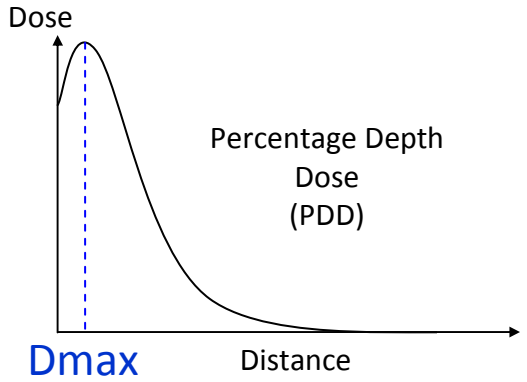


Delivering Radiation

Intensity Modulated Radiation Therapy: IMRT

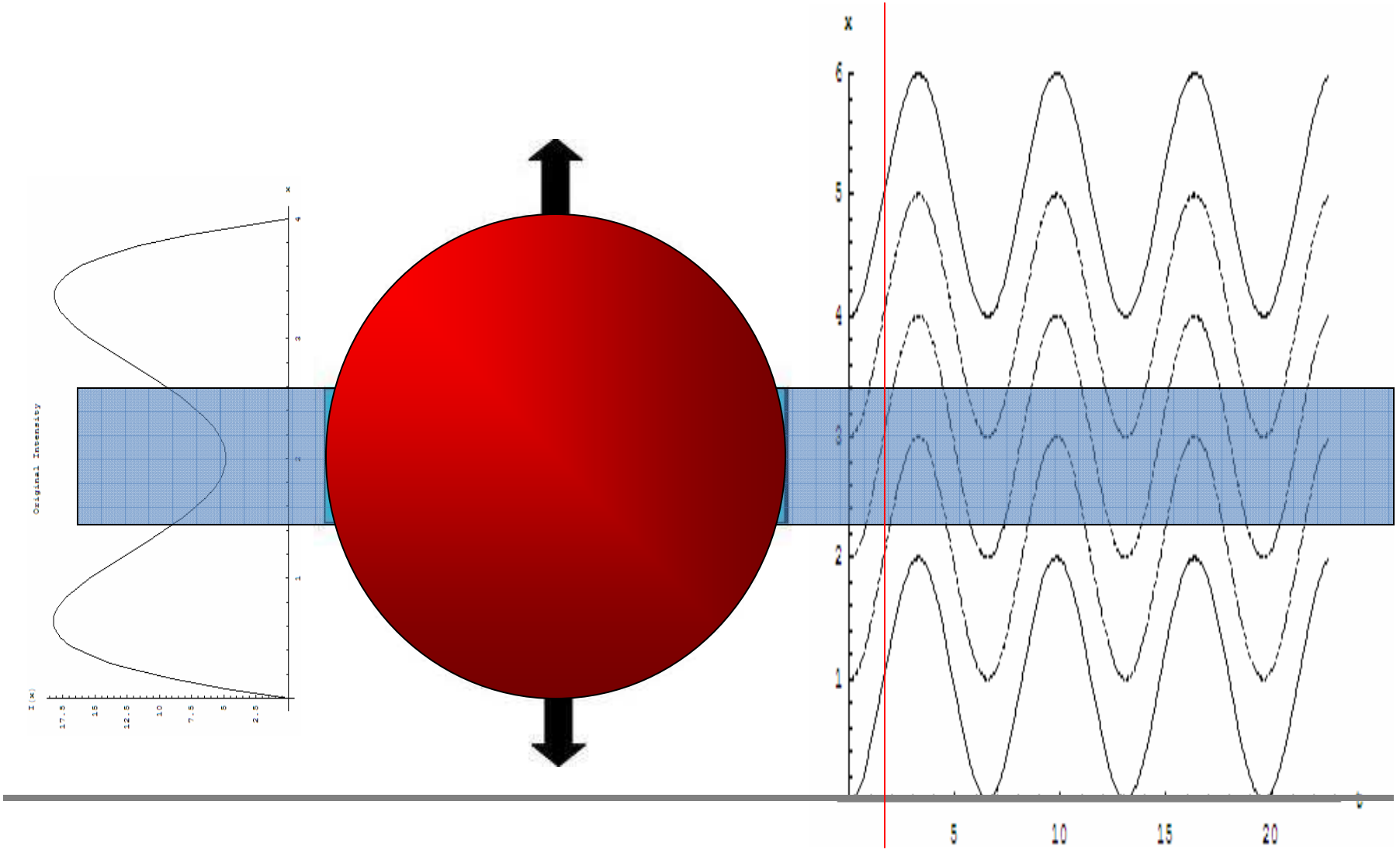
1MU = 1cGy @ Dmax if:





MU can be used as a measure of the time if dose rate (MU/min) is given

IMRT to moving target and static organ at risk (OAR)



Singlet-triplet energy gap of deoxyribose (No OH radical):

$$\Delta_0 = E_{triplet} - E_{\sin glet} \approx 2.38eV$$

Quartet-doublet energy gap of deoxyribose + OH radical:

$$\Delta_1 = E_{quartet} - E_{doublet} \approx 3.05eV$$

Excessive magnetic energy:

$$\Delta_1 - \Delta_0 = (E_{quartet} - E_{triplet}) - (E_{doublet} - E_{\sin glet}) \approx 0.67eV \approx 6000K$$

$$\Delta_1 - \Delta_0 >> 300K$$

Few words on optical pumping:

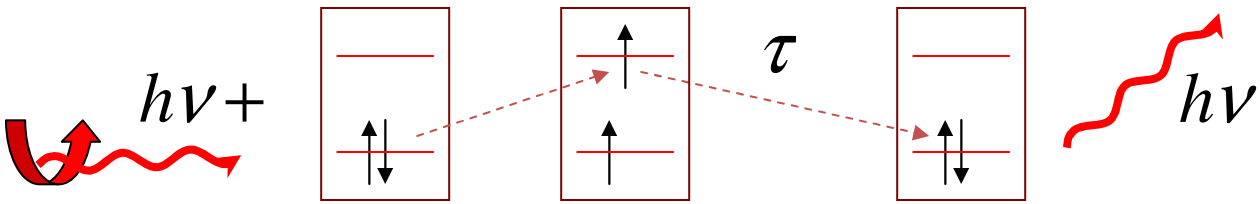
Electron-photon interaction follow:

1) Energy conservation law

2) Angular momentum conservation law

$$h\nu = E_2 - E_1$$

$$\frac{\varepsilon_x \pm \varepsilon_y}{\sqrt{2}} \rightarrow \Delta S$$



Circularly Polarized Light

Spin polarized exciton

Spin singlet-triplet transition (forbidden by dipole selection rule if SO-coupling =0)

Decay mechanisms:

1) Spontaneous emission

PNAS 104, 4794 (07)

$\tau \approx 0.1 - 1ns$ (from measurement)

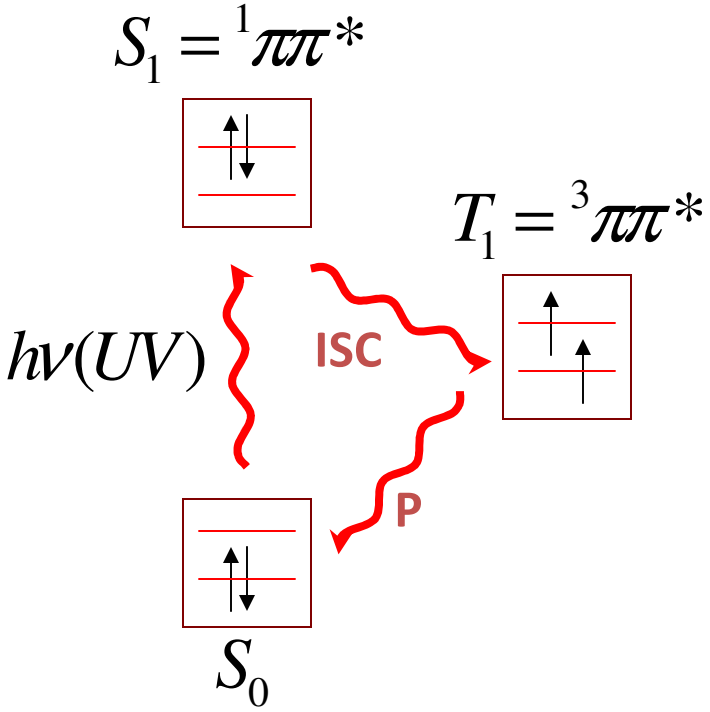
2) Non-radiative channels:

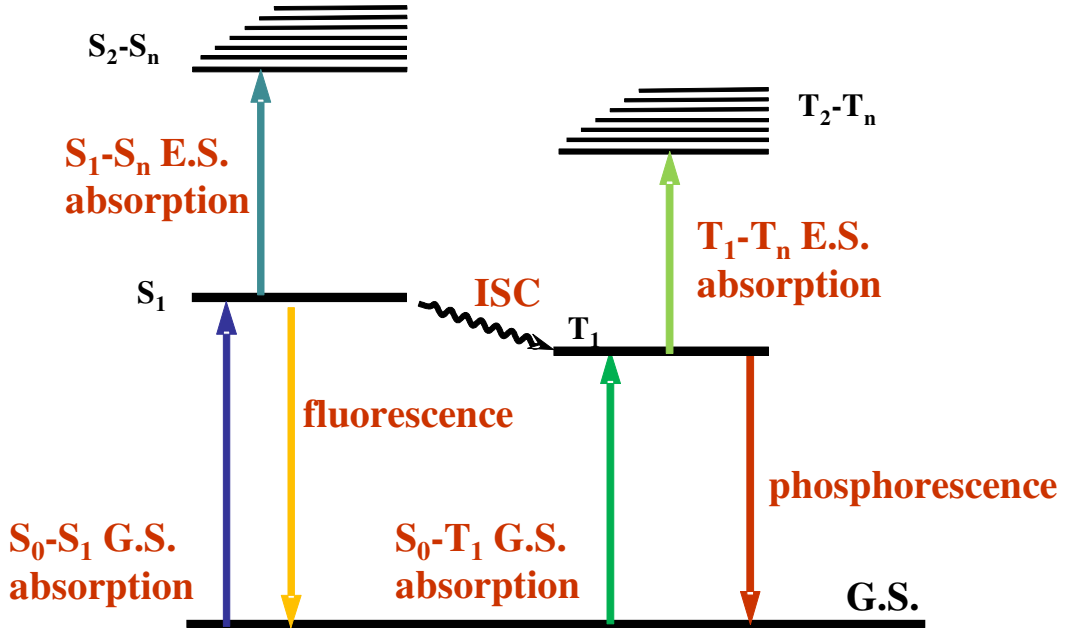
- Spin-orbit coupling, collisions
- Hyperfine interaction

$\tau \approx 0.1 - 1ns$

(from Fermi Golden rule)

Optical spectrum of nucleobasis from pump-probe femto second laser spectroscopy:





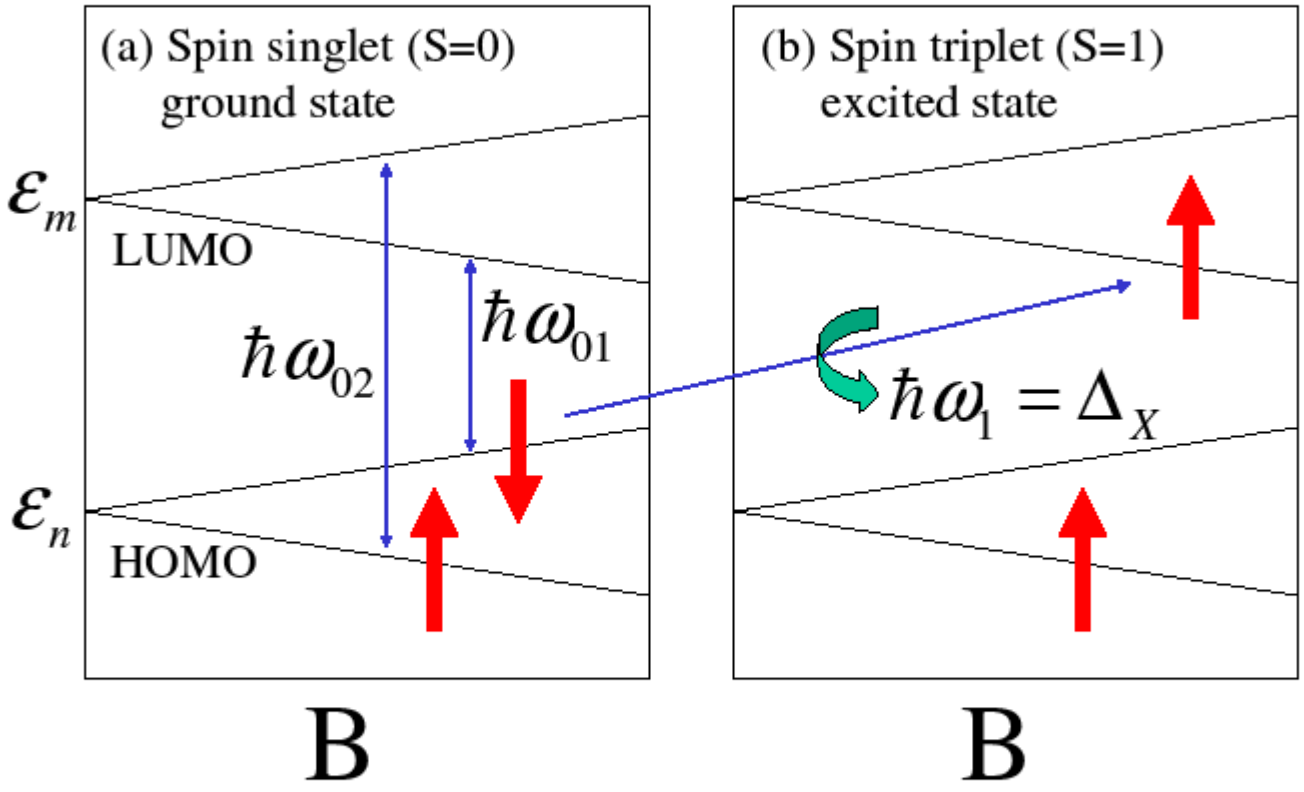
ISC: inter-system crossing (non-radiative decay, spin-orbit coupling)

P: phosphorescence

Ref: Middleton et al. Annu. Rev. Phys. Chem. 60, 217 (2009)

- **A direct transition from S0 T1 has not been reported (dipole selection rule, weak SO-coupling???)**
- **There are triplets below UV**
- **How we can access DIRECTLY to triplets using optical pumping?**

Electron spin resonance: a way to pump selective spin

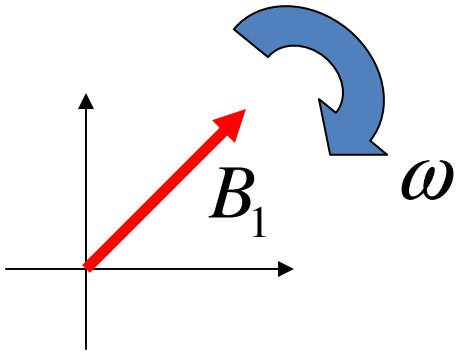


Spin singlet (S0) ⟶ Spin triplet (T)

$$\hbar\omega_1 = \Delta_X = \varepsilon_m - \varepsilon_n - \hbar\gamma B - < n,m | \frac{e^2}{|\vec{r}_e - \vec{r}_h|} | m,n >$$

4-level Hamiltonian model

$$\vec{B} = B_0\hat{z} + B_1(\vec{r})\left[\hat{x}\cos\omega t - \hat{y}\sin\omega t\right]$$



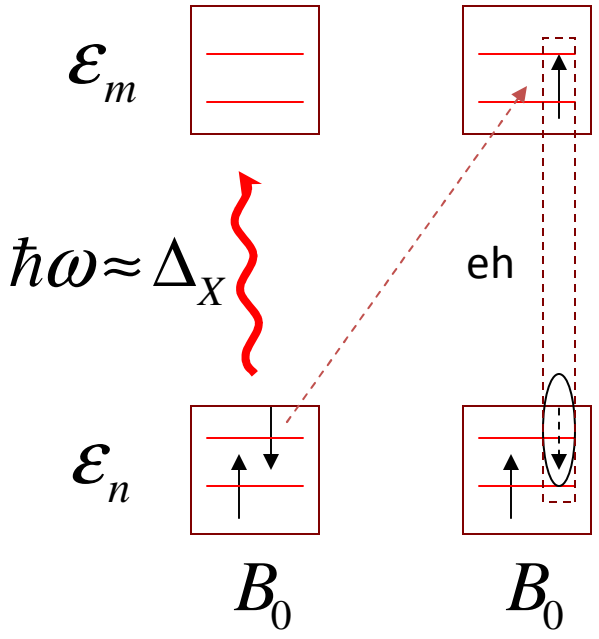
$$H = -\vec{\mu}\cdot\vec{B} = H_1 \oplus H_2$$

Spin Resonance

$$\Delta = -\frac{\hbar\gamma}{2}\langle n \mid B_1(\vec{r}) \mid m\rangle$$

$$H_1 = \begin{pmatrix} \varepsilon_{n\downarrow} & \Delta e^{i\omega t} \\ \Delta^* e^{-i\omega t} & \varepsilon_{m\uparrow} \end{pmatrix}$$

Rabi-oscillations



$$P_{n\downarrow\to m\uparrow} = \frac{|\Delta|^2}{\Omega_R^2}\sin^2\left(\frac{\Omega_R t}{2}\right)$$

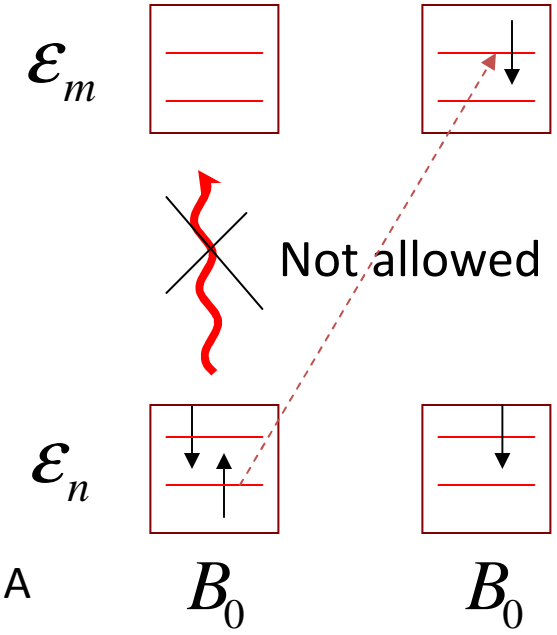
$$\Omega_R = \sqrt{(\omega-\omega_1)^2 + \Delta^2}$$

$$\hbar\omega_1 = \Delta_X = \varepsilon_m - \varepsilon_n - \hbar\gamma B_0 - \langle n,m \mid \frac{e^2}{|\vec{r}_e - \vec{r}_h|} \mid m,n\rangle$$

~500nm

Off-resonance mode

$$H_2 = \begin{pmatrix} \varepsilon_{n\uparrow} & \Delta e^{-i\omega t} \\ \Delta^* e^{i\omega t} & \varepsilon_{m\downarrow} \end{pmatrix}$$



- Forbidden transition/absorption by clock-wise polarization of photon
- By changing polarization to counter clock wise the first transition is forbidden and second transition is allowed
-spin/angular-momentum conservation
- polarization of the light controls the spin orientation of the DNA
- spin resonance happen at the peak of P: $\omega \rightarrow -\omega$

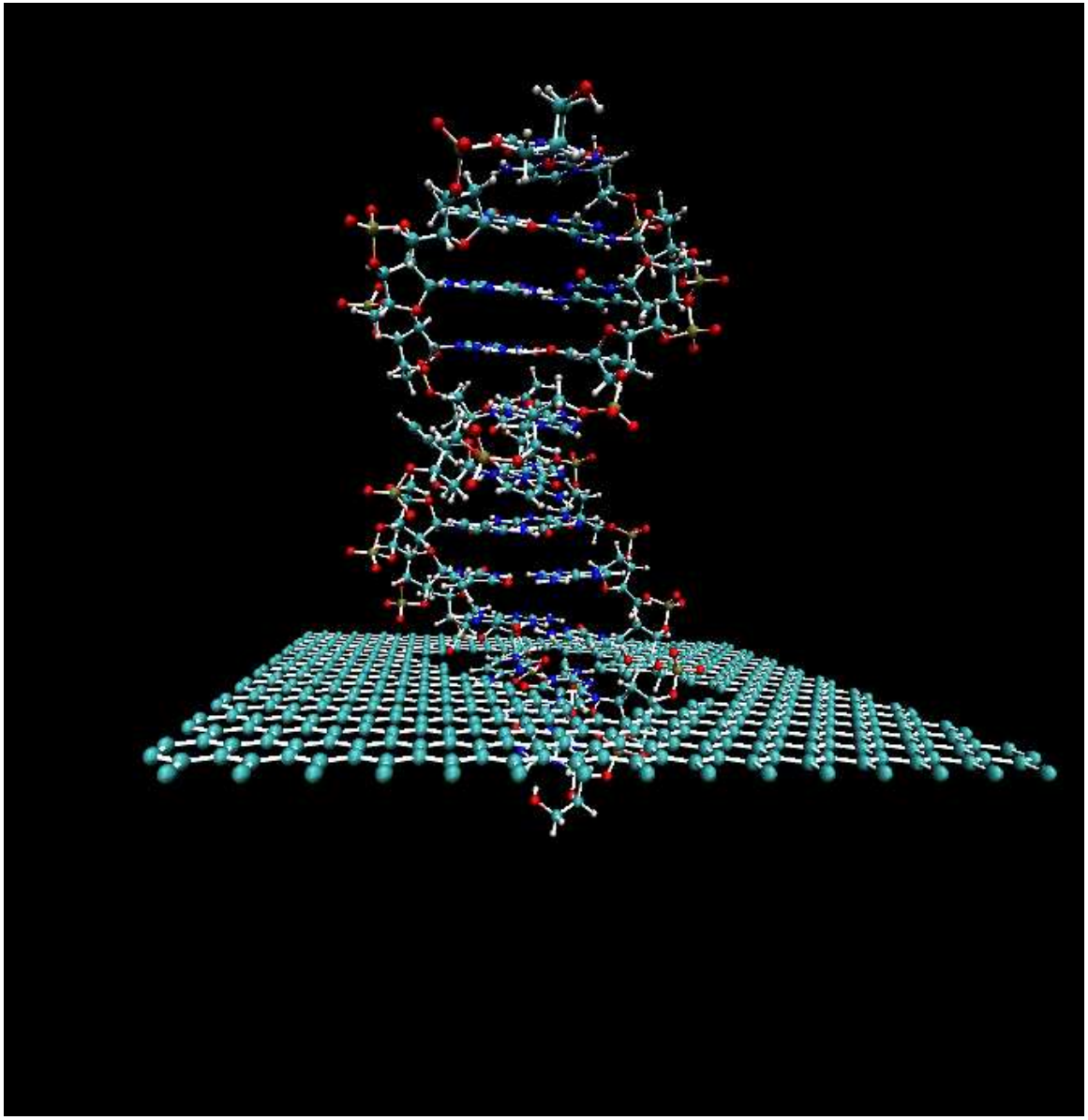
$\omega = \omega_1$ If polarization is clock-wise

$$\hbar\omega_1 = \Delta_X = \varepsilon_m - \varepsilon_n - \hbar\gamma B_0 - < n,m | \frac{e^2}{|\vec{r}_e - \vec{r}_h|} | m,n >$$

$\omega = \omega_2$ If counter clock-wise

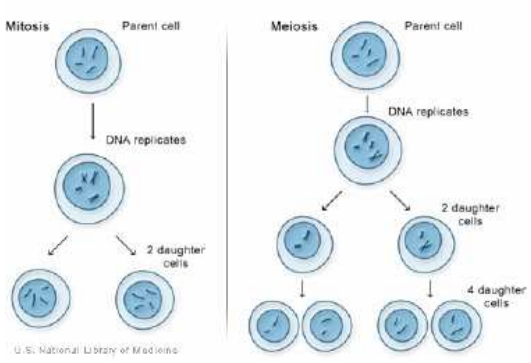
$$\hbar\omega_2 = \Delta_X = \varepsilon_m - \varepsilon_n + \hbar\gamma B_0 - < n,m | \frac{e^2}{|\vec{r}_e - \vec{r}_h|} | m,n >$$

Interface of biology/bio-chemistry with nano-technology DNA gene-sequencing



Cell division in colony (binary rules)

[10^{14} cells in the human body]



Generation (n)	# cells in one generation	time (min)
1	$2^0 = 1$	1
2	$2^1 = 2$	2
3	$2^2 = 4$	3
4	$2^3 = 8$	4
.....		
10	$2^{10} \sim 10^3 = 1K$	10
.....		
20	$2^{20} \sim 10^6 = 1M$	20
.....		
30	$2^{30} \sim 10^9 = 1G$	30
.....		
40	$2^{40} \sim 10^{12} = 1T$	40
.....		
50	$2^{50} \sim 10^{15} = 1H$	50

# of errors/mutations in a DNA duplication: 10^{-9} /bp/cell-division
1gene ~ 1000 bp (number of bp need for protein transcription)
In human 24 chromosomes in a cell ≒ 3,079,843,747 bp/cell ~ 3 X 10^9 bp/cell
Total # of cell division in human body per day ~ 10^{12}
Total # of mutations in human body per day: (10^{12} cells) (3 . 10^9 bp) 10^{-9} = 3 . 10^{12} ~ 3T

How about computational models between atomic scales and cells ????

- Studies based on coarse-grained MC sampling on a static structure of DNA has been performed. The fitted empirical data Can be used as phenomenological models?

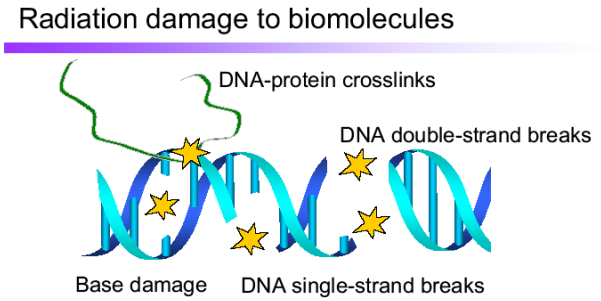
Monte Carlo Modeling on DNA:

- Semenenko, and Stewart, Radiat. Res. 164, 180, (2005); *ibid.* 164, 194 (2005): Purdue U./Washington U.
- Aydogan, Bolch, Swarts, Turner, Marshall, Radiat. Res. 169, 223 (2008):U. Chicago, Oak Ridge National Lab, U. Rochester

Stochastic model of ion track structure:

- Wilson, Paretzke, Radiat. Prot. Dosimetry 52, 249 (1994).
- Nikjoo, O'Neill, Terrissol, Goodhead, Int. J. Radiat. Biol. 66, 453 (1994).

- How about events, missed/non-accessible to experiments? Can we do first-principle modeling?
It requires dynamical simulation of DNA and its environment using quantum mechanics.



Types of DNA damage:	Approx. No. per gray:
1. Base damage	1000 – 2000
2. Cross-links	200 – 400
3. Single-strand breaks (ssb)	1000
4. Double-strand breaks (dsb)	40

Sites of damage (experiments):

- Pogozelski, Tullius, Chem. Rev., 98, 1089 (1998);
-Tullius, Greenbaum, Curr. Opin. Chem. Biol. 9, 127 (2005)

General remarks and criteria on computational platforms:

-DNA damage induced by chemical change, requires developing simulation platforms suitable for chemical reactions, breaking-forming bonds.

- There are various simulators in market, e.g., AMBER, CHARMM, GROMACS, … suitable for non-reactive processes, not suitable for chemical reactions

- ab-initio/DFT methods based on quantum mechanics that calculates the energy landscapes and transition states on-the-fly are suitable, but they require very large memories with large number of CPU's (limited to small molecules)

- A QM-MM approach that allows QM treatment of active site and non-reactive treatment of environment is one solution

- ReaxFF recently developed platform developed in Caltech and Sandia National Lab in Goddard's group is another possibilities. ReaxFF, a topology free computational platform showed success in simulating chemical reactions in non-organic materials such as graphene-oxides and carbon nano-tubes. For DNA damage: Abolfath, van Duin, Brabec, JPC 2011

Animations (go to Normal presentation) !!!

Reaction-Diffusion models

non-homologous end-joining by enzymes

$$IR + DNA \rightarrow [DSB]$$

Bio-chemical repair kinetic model (reaction-diffusion models):

$$[DSB]+[E] \xrightarrow{k} [C] \xrightarrow{k'} [DNA]+[E]$$

E: enzyme
C: complex
k,k': reaction rate constants
D: IR dose
α: induction-rate per unit dose of DSB

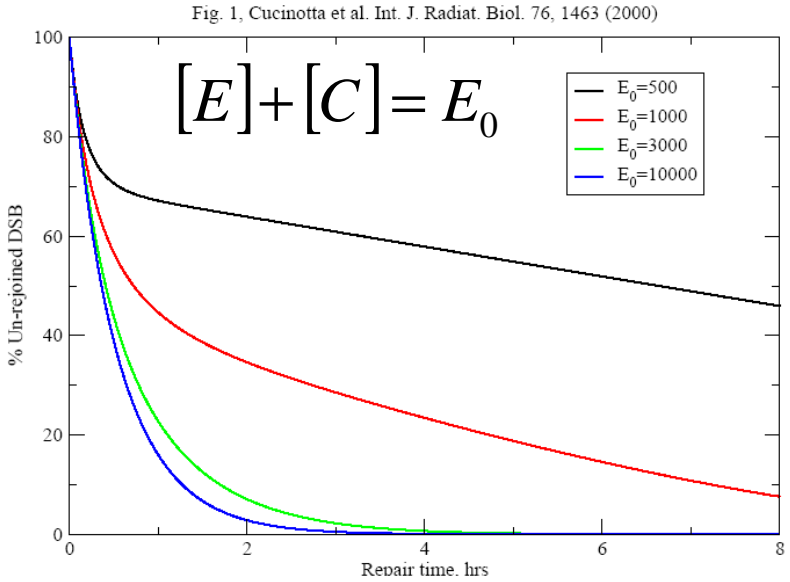
(ignoring back reaction C E+DSB)

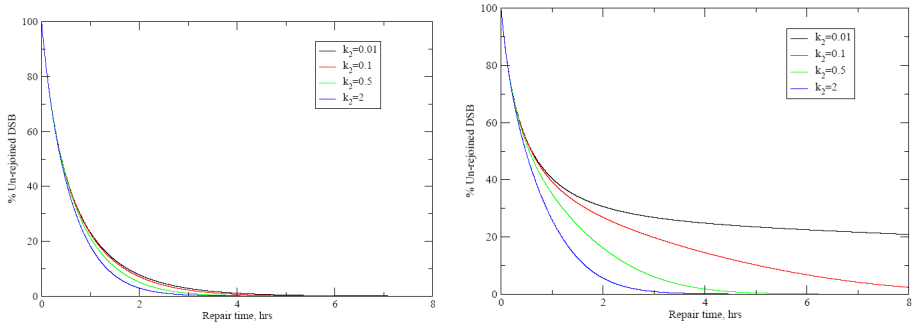
Rate equations:

$$[E]+[C]=const.$$

$$\frac{d[DSB]}{dt} = \alpha \frac{dD}{dt} - k[E][DSB]$$

$$\frac{d[C]}{dt} = k[E][DSB] - k'[C]$$

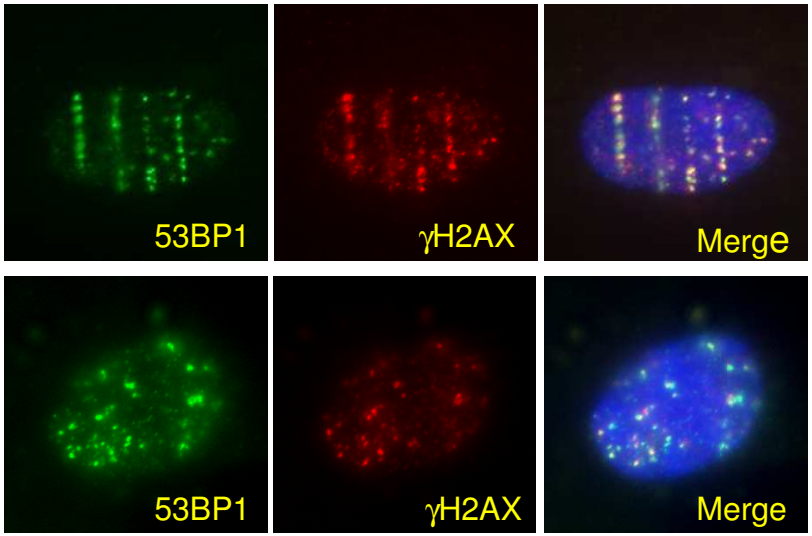




-Hammel et al. (UC Berkely +UTSW)
J. Bio. Chem. 285, 1414 (2010)
-Cucinotta, Nikjoo, O'Neill, Goodhead,
Int. Rad. Bio. 00

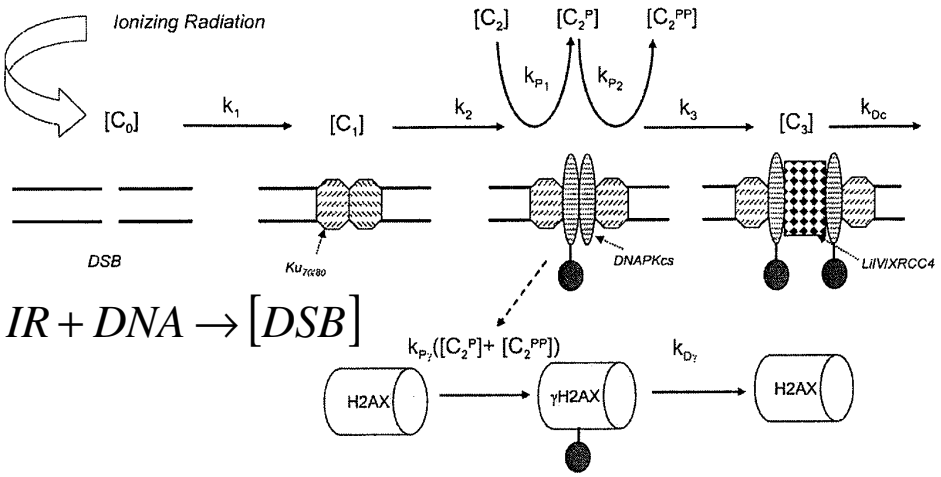
Computational models suitable for cells damage-repair

Fluorescence Recovery After Photobleaching (FRAP)



Repair Mechanisms

non-homologous end-joining by enzymes



Bio-chemical pathway of repair:

1) An initial complex bound by Ku70/80 hetero-dimer
2) Ku-mediated DNA-PKcs binding
3) The regulation of the DSB-DNA-PKcs complex through auto-phosphorylation by DNA-PK
4) A final repair complex involving the ligase heterodimer denoted LigIV

macroscopic reaction-diffusion (a coarse-grained model) for damage-repair mechanism

Radio Biological Effects:

Relative biological effectiveness (RBE) is traditionally defined as the ratio of a dose of a standard low linear energy transfer X ray beam (x ray of 250KeV energy)(D_X) to the dose of the test radiation type or configuration (D_T), required to cause the same biological level of effect. Thus: $RBE=D_X/D_T$

- Scattering cross-section of the IR with water & biological materials depend on the type of IR source (X-ray, gamma-ray, p, n, C,..), their energy, beam geometry, … that determine the distribution of OH free radicals within blubs/clusters with random size and spatial distribution