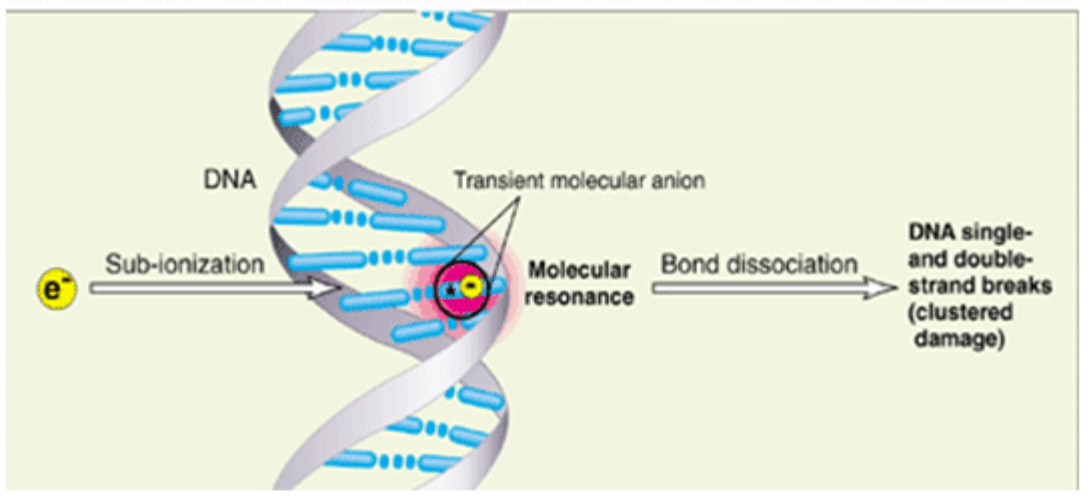
UV and electron induced damage of DNA



Nigel Mason

Centre of Molecular and Optical Sciences

The Open University, United Kingdom

In Europe this research has been developed through collaborative programmes Funded by EU

2002 Framework V Network EPIC 2002-2005
Electron and Positron Induced Chemistry

And more recently ESF Programme **Electron Induced Processing at the Molecular Level (EIPAM)** 2004-2009

EU COST Action P9 **RADAM Radiation damage** **2003-8**

Support for this Conference

Outline of Talk

- Summary of ionizing radiation processes
- Energetics of DNA damage
- Mechanisms of low energy electron induced molecular dissociation
- New electron/photon experiments on biomolecules
- Relationship to radiation chemistry

Radiation damage of biomolecules

- Ionising radiation damages biomolecules (including DNA) by inducing molecular dissociation/ionisation.

- Molecular damage occurs either by :

  - **Direct processes,** for example by direct ionisation of the biomolecule

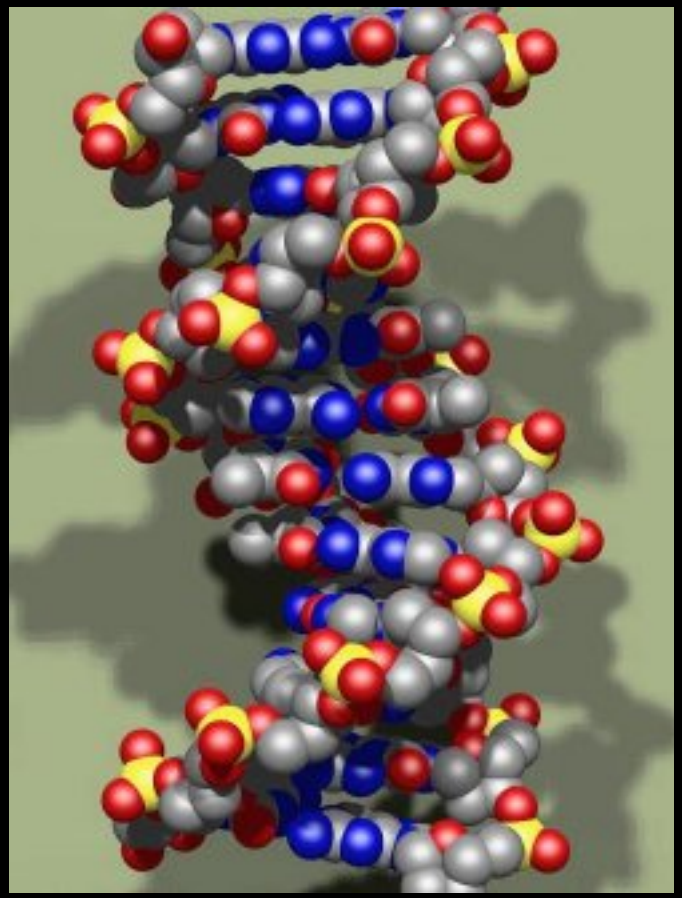
  - **Indirectly,** through the dissociation of water, and the formation of OH reactive radicals

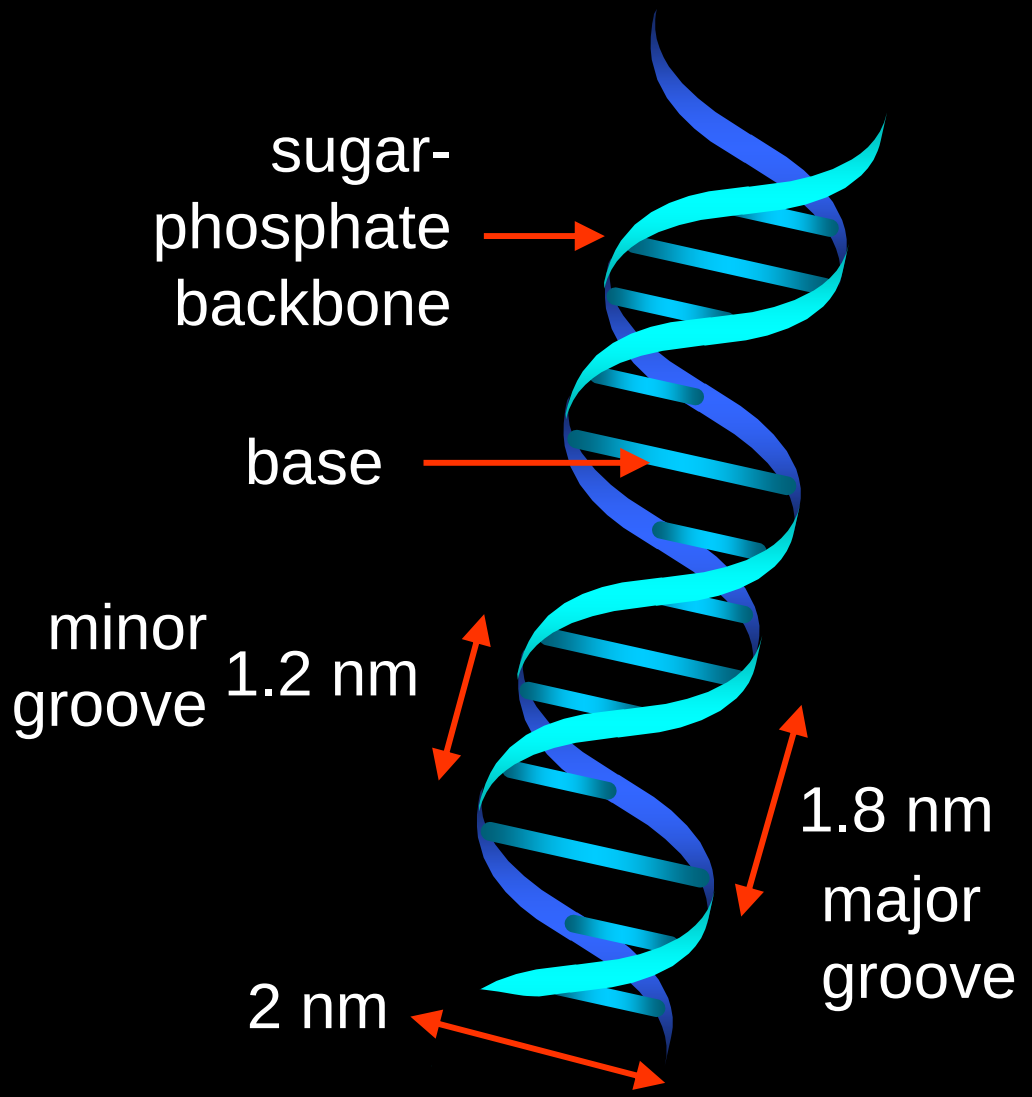
Radiation damage of biomolecules

- Hence in studying radiation damage we wish to study biofragmentation patterns.

- However to date there are still *'few'* studies on fragmentation and ionisation of biomolecules

The most **radiation-sensitive** biomolecule in living tissue is DNA





Studies in DNA damage

- What can we learn by studying the damage/fragmentation of DNA's constituent molecules ?

- Are the patterns of damage, fragmentation of constituent molecules reflected in DNA damage ?
  e.g in the energy dependence of strand breaks ?

This is one aim of WG1 and the COST programme

DNA damage energetics

- What is the minimum energy required to produce:

  A single-strand break ?

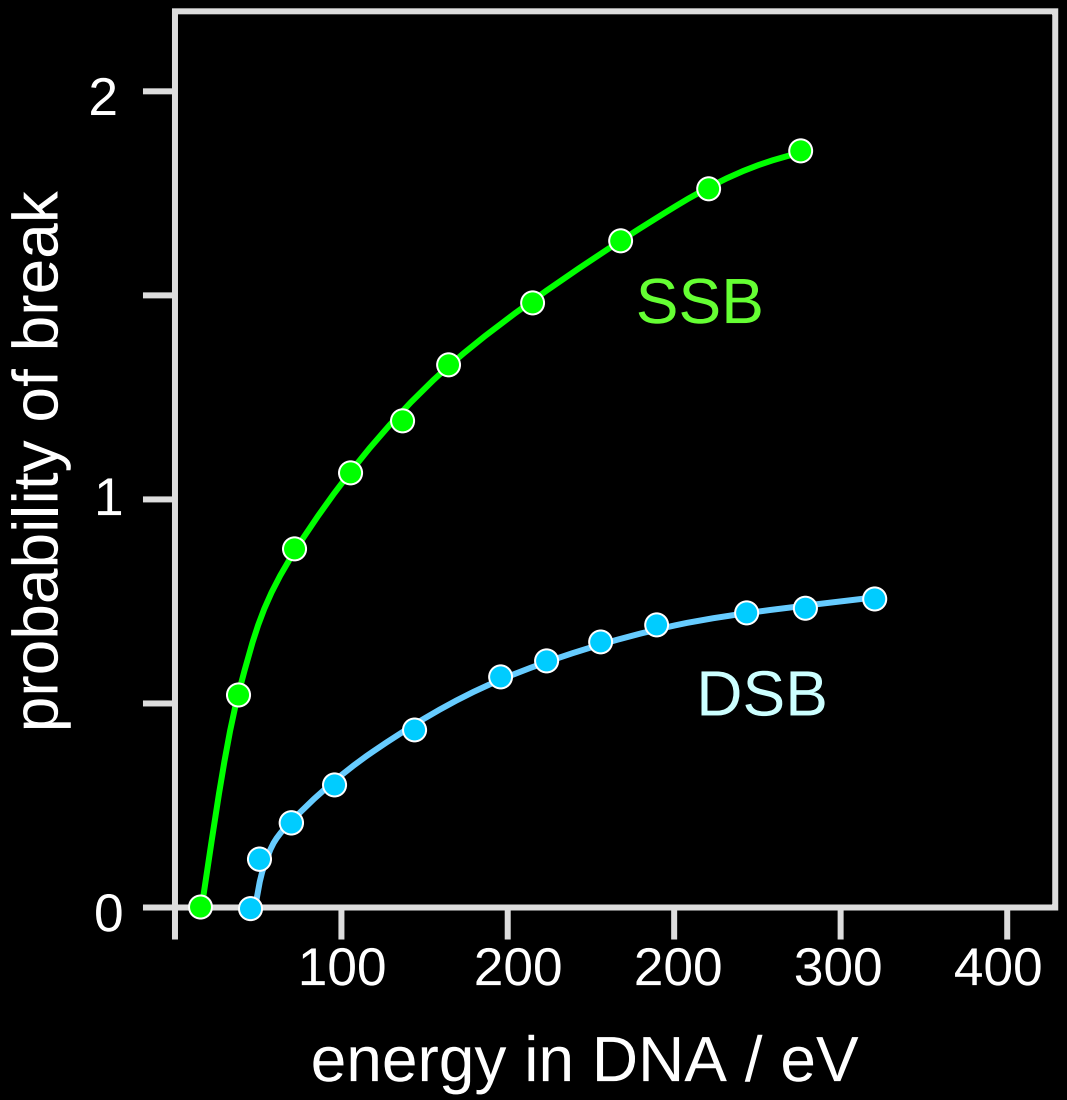
  Or a

  A double-strand break ?

DNA Damage

M Folkard slide !

- Minimum energy to produce SSB ~20 eV
  - ***Minimum energy to produce DSB ~50 eV***



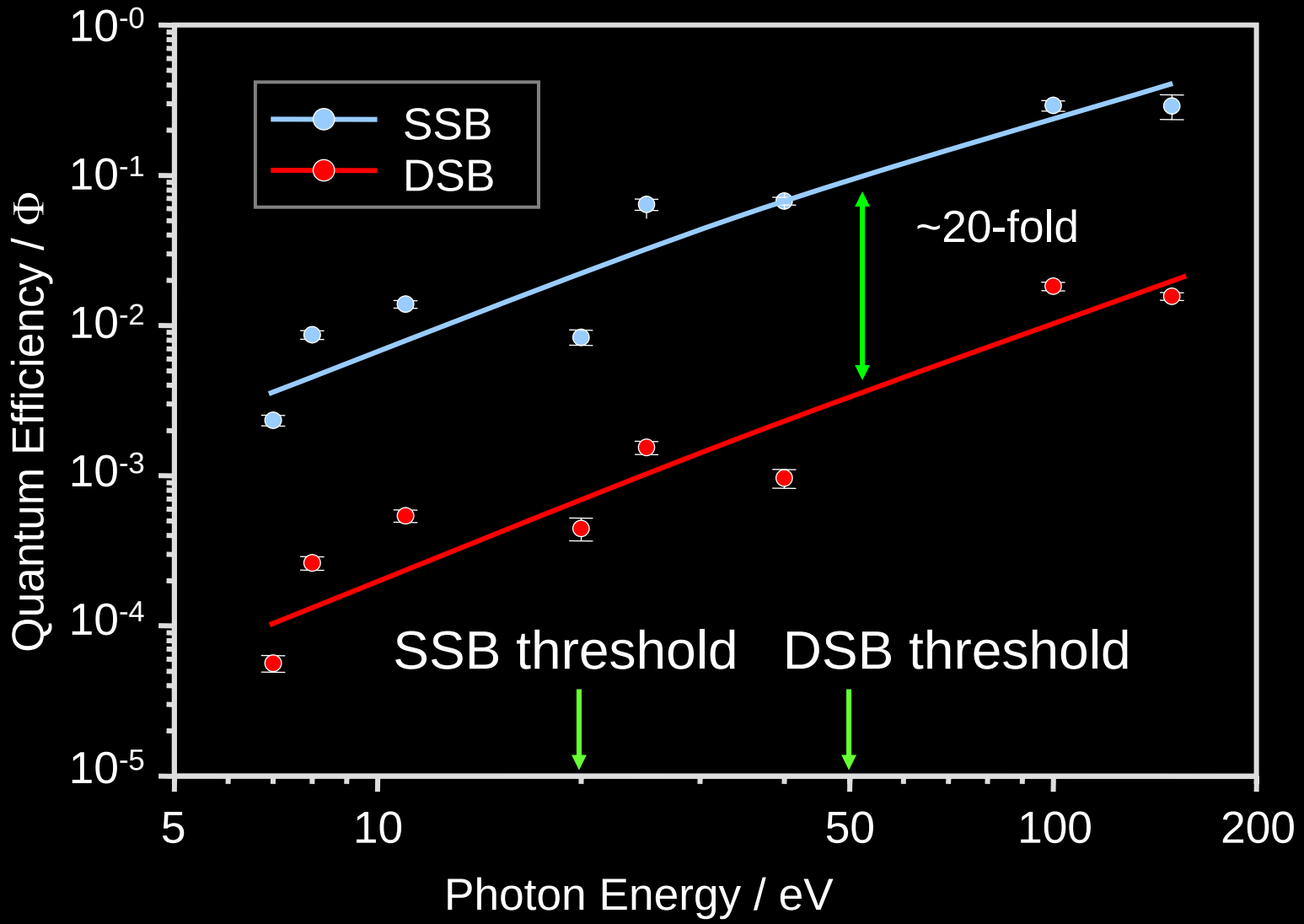
Nikjoo, Charlton, Goodhead, 1994

Are these energies correct ?

- Folkard, Prise Michael at GCI UK studied DNA damage using synchrotron radiation on ‘dry’ DNA
- Measured SSB and DSB

Prise, Folkard *et. al*, 1995, *Int. J. Radiat. Biol.* **76,** 881-90

Results of UV induced DNA damage (dry plasmid) M Folkard slide 2



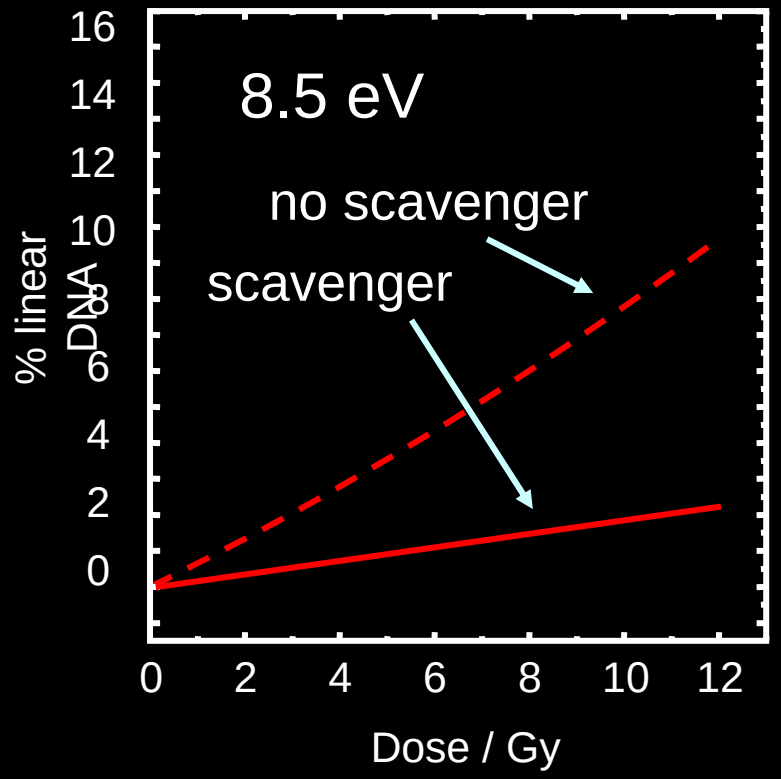
Prise, Folkard *et. al*, 1995, *Int. J. Radiat. Biol.* **76,** 881-90.

Conclusions

- SSB and DSB occur at much lower energies than model predicted
- WHY ??
- Indirect damage (Free Radical ?)

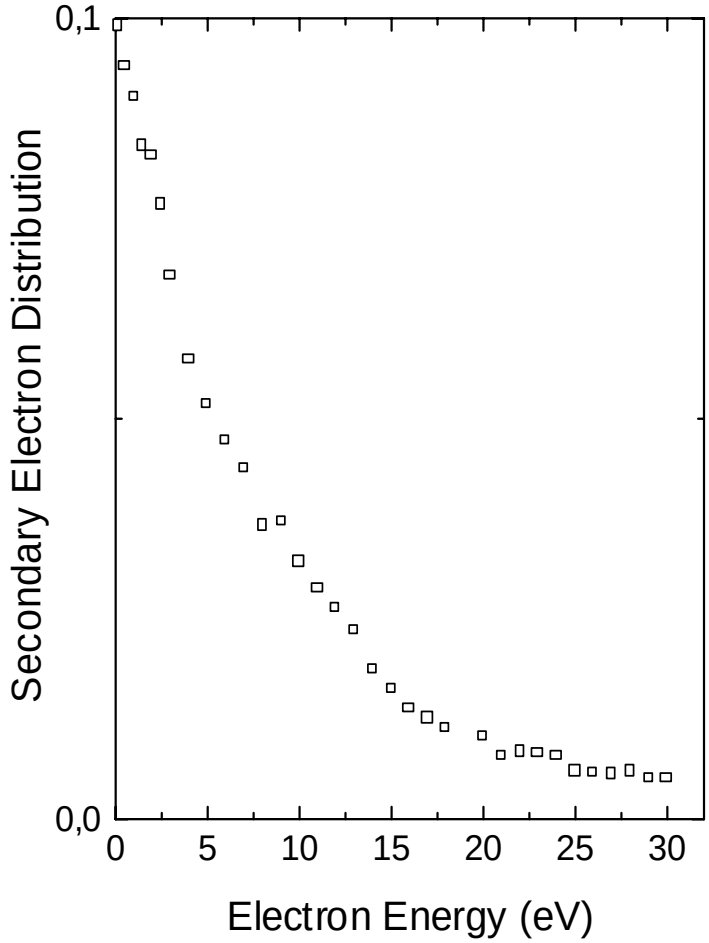
Radicals play a role but still some ‘direct’ damage

- Energies as low as **5 eV** can efficiently produce single- and double-strand breaks
- So what about low energy electrons ????



Spectrum of low energy electrons

Experimental spectrum of secondary electron energy distribution



V. Cobut et al., *Rad.Phys.Chem.*, **51** 229 (1998)

Electron Induced reactions

At low energies electrons can do surprising things !

- They can ‘stick’ to the molecule
- To form a **negative ion** or ‘resonance’
- But only for a very short period of time (10^{-14} s)

- Then the electron detaches
- Leaving molecule excited or not (elastic scattering)
- But this process can also lead to the dissociation of the molecule

This is the process of
Dissociative Electron Attachment (DEA)

Dissociative Electron Attachment (DEA)

$$ABC + e^- \rightarrow \quad \mathbf{ABC}^{\#-} \quad \rightarrow \quad A^{(*)-} + BC$$

Resonance
$\tau \sim 10^{-14}$ s

$$A^{(*)-} + B + C$$

Applications of DEA (some !)

-Heterogeneous Chemistry (e.,g., Atmospheric Chemistry)
-Production of Negative Ions in industrial Plasmas

- and possibly DNA damage

Dissociative electron attachment therefore provides a method for breaking up molecules at low energies

Energies lower than the chemical bond energy !!!

DEA shows selective bond dissociation

- Different energy --- different pathways
- (E Illenberger talk)

In many molecules DEA leads to H atom loss

- This is most dominant process is in DEA to organic acids
- E.g. acectic, formic and …

DEA in propanoic acid

Dominant channel is H atom abstraction

$$e^- + CH_3CH_2COOH \rightarrow CH_3CH_2COO^- + H$$

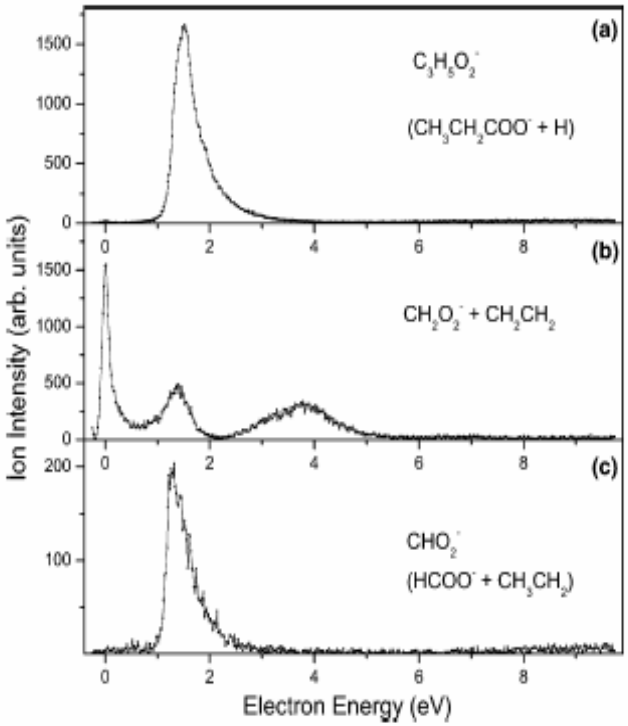


Fig. 1. Dominant fragment ions obtained from propanoic acid. The most intense channel ($C_3H_5O_2^-$) corresponds to hydrogen abstraction with a cross-section of 1.7×10^{-20} m^2 (see the text and Table 1).

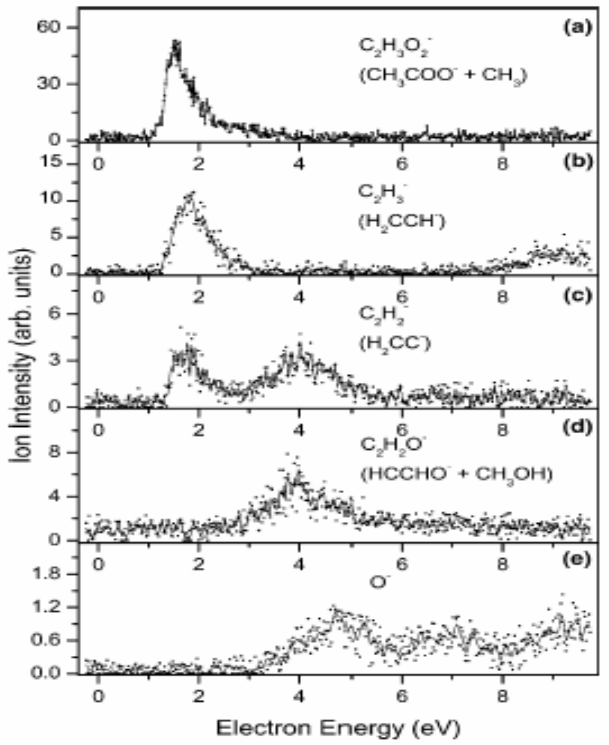


Fig. 2. Fragment ions observed from electron impact to propanoic acid.

$$e^- + CH_3CH_2COOH \rightarrow CH_3CH_2COO^- + H \quad (1)$$

$$e^- + CH_3CH_2COOH \rightarrow CH_2O_2^- + CH_2CH_2 \quad (2)$$

Table 1
Fragment ions observed from propanoic acid in the energy range 0–10 eV

Fragment ion	Structure	Peak position (eV)	Relative intensity
$C_3H_5O_2^-$	$CH_3CH_2COO^-$	1.5	1660
$CH_2O_2^-$	?	0/1.4/3.9	1550
CHO_2^-	$HCOO^-$	1.3	200
$C_2H_3O_2^-$	CH_3COO^-	1.5	53
$C_2H_3^-$	CH_2CH^-	1.9	11
$C_2H_2O^-$	$HCCHO^-$	4.0	8
OH^-	OH^-	0.3/≈9	5
$C_2H_2^-$	CH_2CH^-	1.9	5
$C_3H_5^-$	$CH_2CHCH_2^-$	≈4	4
O^-	O^-	≈4.8/≈7	1

The relative intensity refers to the peak value of the most intense peak. The cross-section of the most abundant fragment, $CH_3CH_2COOH^-$ can be transferred to an absolute DEA cross-section of 1.7×10^{-20} m^2 at an accuracy of one order of magnitude (see the text).

DEA and biomolecules

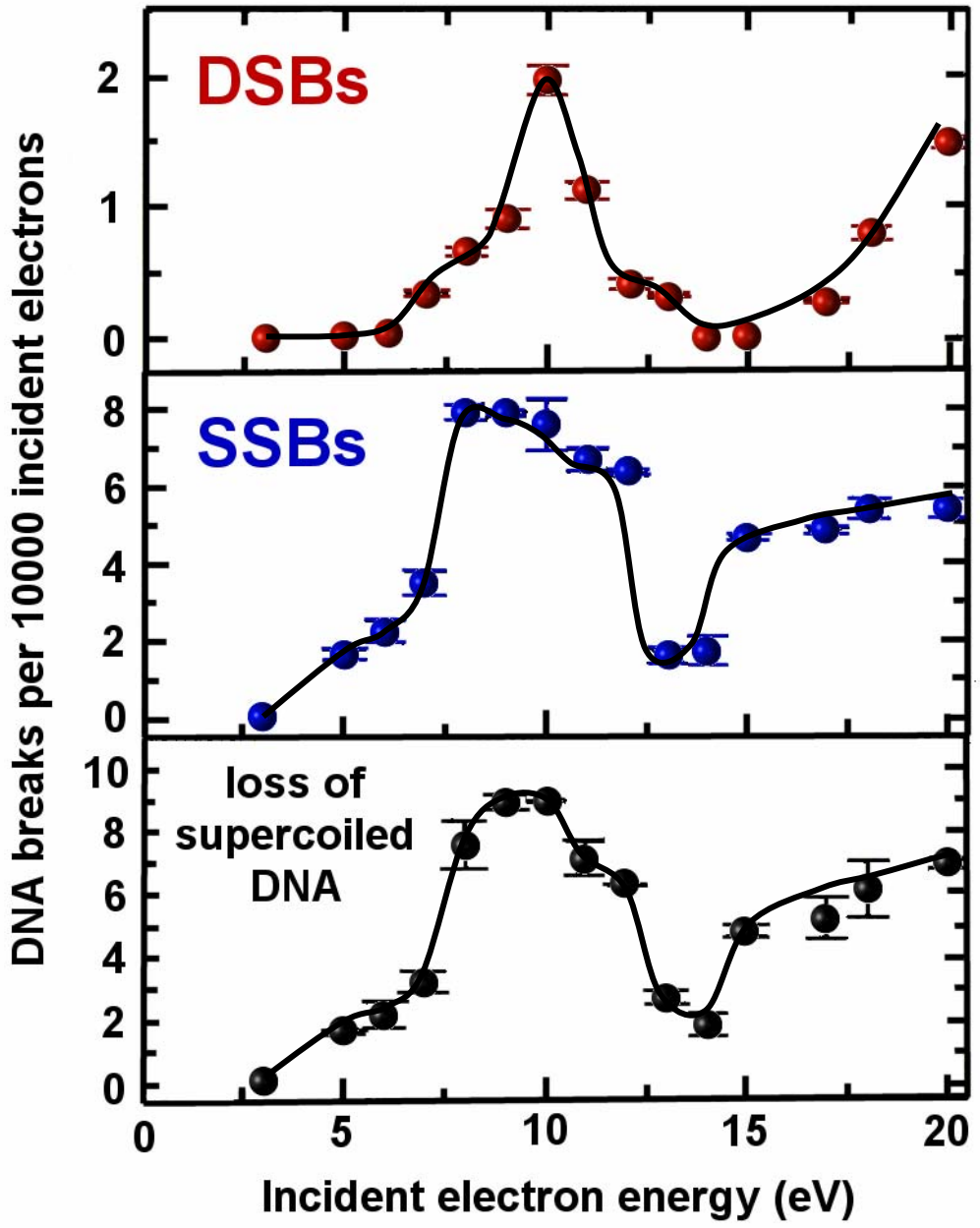
- DEA is a universal process
- So DEA will occur in biomolecules including those constituents of DNA
- So can DEA induced fragmentation lead to DNA damage ?

Mechanisms for ssb and dsb induction at low-energies

- Boudaiffa et al. (Leon Sanche, Sherbrooke Canada) demonstrated that there apperas tpo be a corrrelation between patterns of ssb and dsb induced in DNA and **DEA of constituent molecules**

Resonant Formation of DNA Strand Breaks by Low-Energy (3 to 20eV) Electrons. Science **287**, *1658-1660* (2000). B. Boudaiffa, P. Cloutier, D. Hunting, M.A. Huels et L. Sanche.

DNA-strand breaks



Sanche a.c. Science, 287 (2000) 1659

DEA to Uracil (Innsbruck)

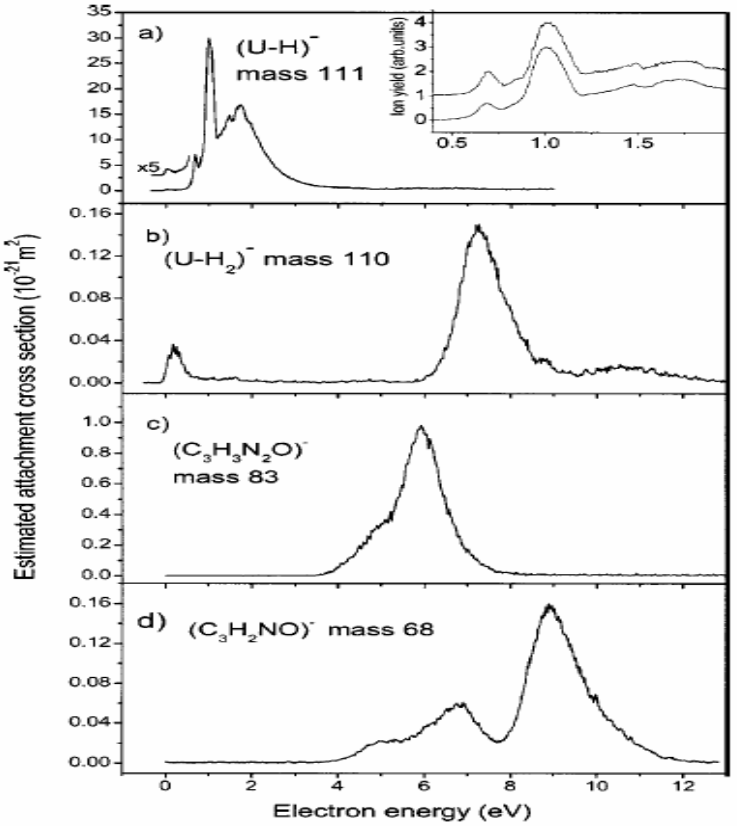


FIG. 3. Ion yield of $(U\text{-}H)^-$, $(U\text{-}H_2)^-$, $(C_3H_3N_2O)^-$, and $(C_3H_2NO)^-$ for dissociative electron attachment to uracil as a function of electron energy. These ion yields were measured without any presence of a calibration gas. The partial cross section scale was determined relative to the Cl^-/CCl_4 ion yield and has an accuracy within one order of magnitude. The inset in (a) shows the ion yield of $(U\text{-}H)^-$ measured at an electron energy resolution of 60 meV (upper curve) and 90 meV (lower curve), respectively.

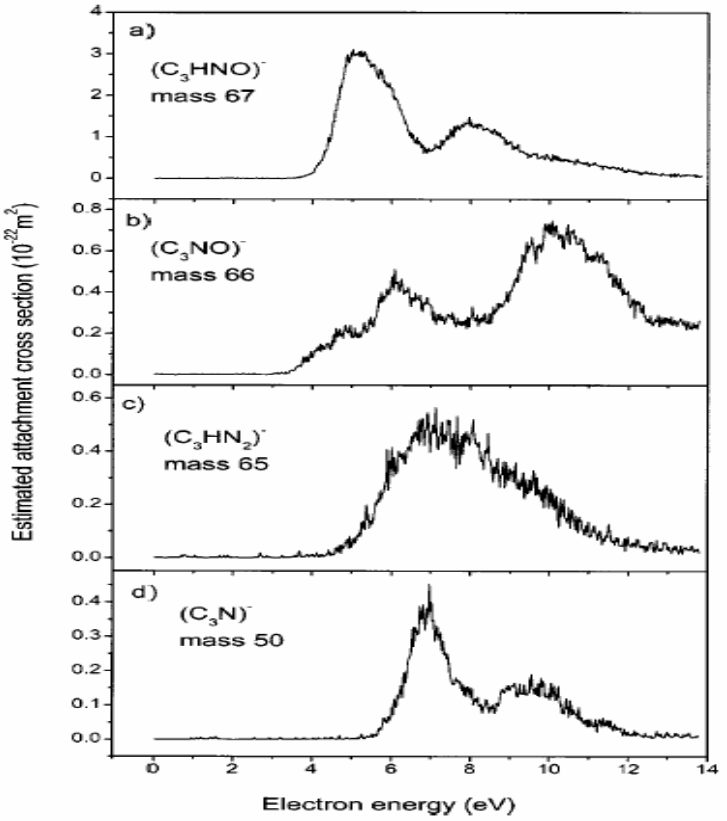
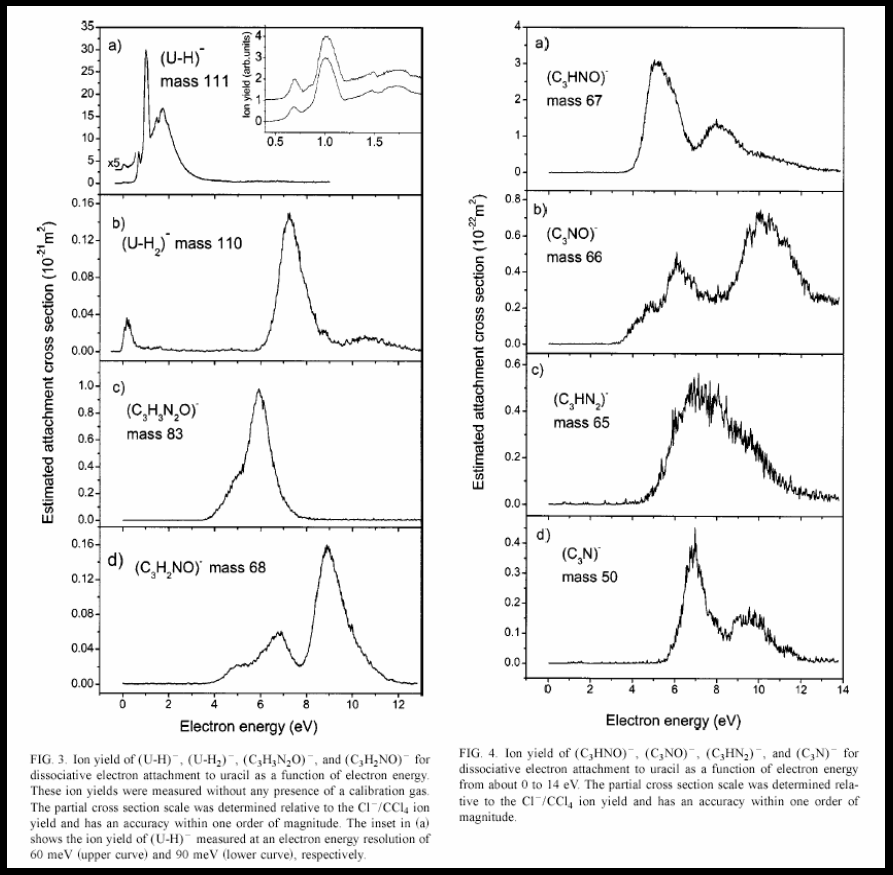


FIG. 4. Ion yield of $(C_3HNO)^-$, $(C_3NO)^-$, $(C_3HN_2)^-$, and $(C_3N)^-$ for dissociative electron attachment to uracil as a function of electron energy from about 0 to 14 eV. The partial cross section scale was determined relative to the Cl^-/CCl_4 ion yield and has an accuracy within one order of magnitude.

DEA to Uracil (Innsbruck)



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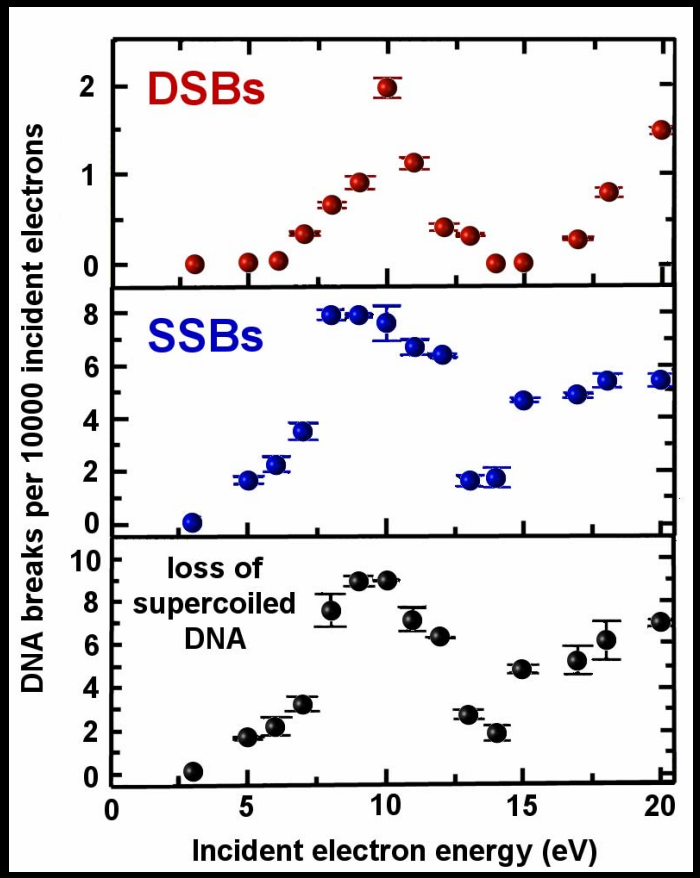


What about lower energies ?

- DEA is open channel at zero energy !!
- Sanche reported ssb at energies below 1 eV (though not dsb)

Electrons and DNA damage

- SSB and DSB can be induced by low energy electrons. *FACT*

- Lowest energy for DSB about 4 eV *FACT*
- Lowest energy for SSB .. 0 eV !!!!! *FACT*

- *Mechanisms of DNA damage from DEA processes ?*

Thymine H atom loss

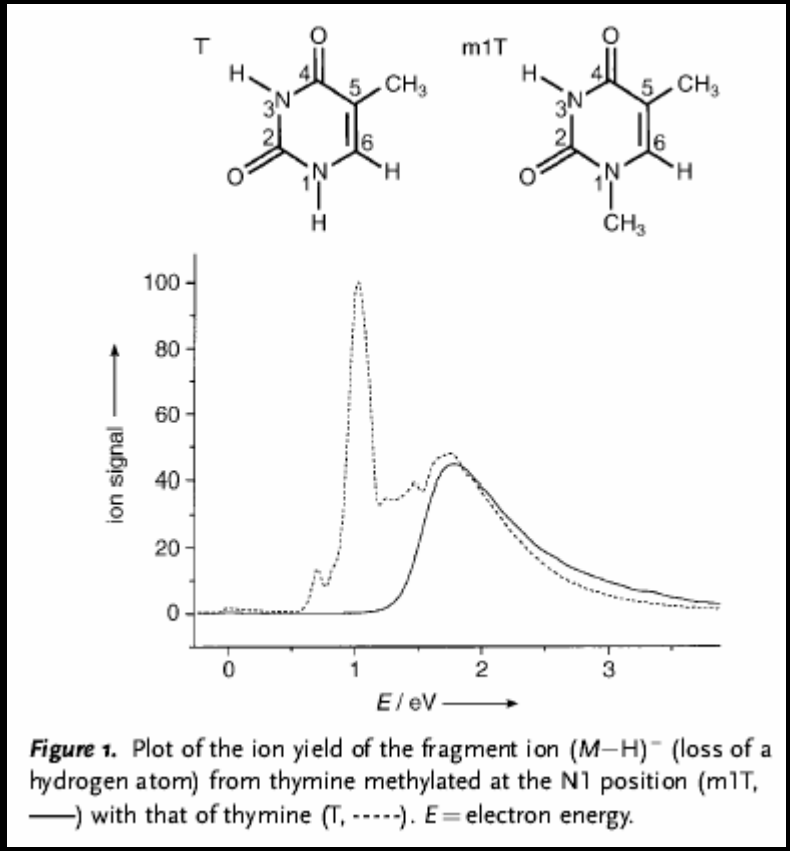


Figure 1. Plot of the ion yield of the fragment ion $(M-H)^-$ (loss of a hydrogen atom) from thymine methylated at the N1 position (m1T, ——) with that of thymine (T, -----). E = electron energy.

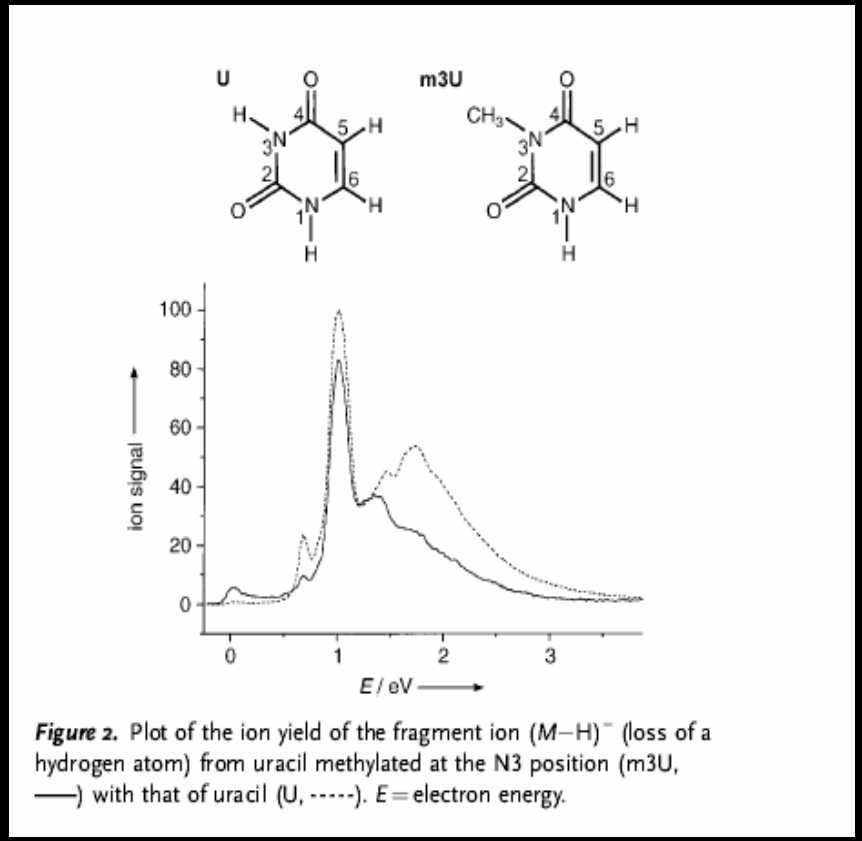


Figure 2. Plot of the ion yield of the fragment ion $(M-H)^-$ (loss of a hydrogen atom) from uracil methylated at the N3 position (m3U, ——) with that of uracil (U, -----). E = electron energy.

DEA to deoxyribose - H loss

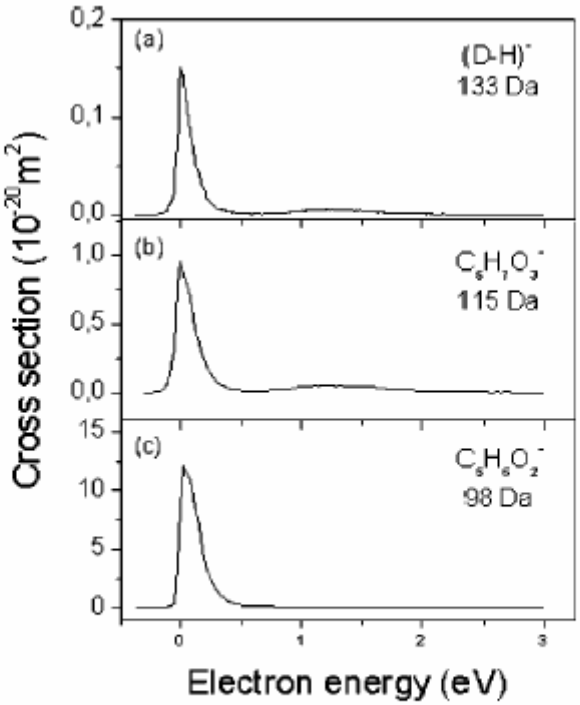
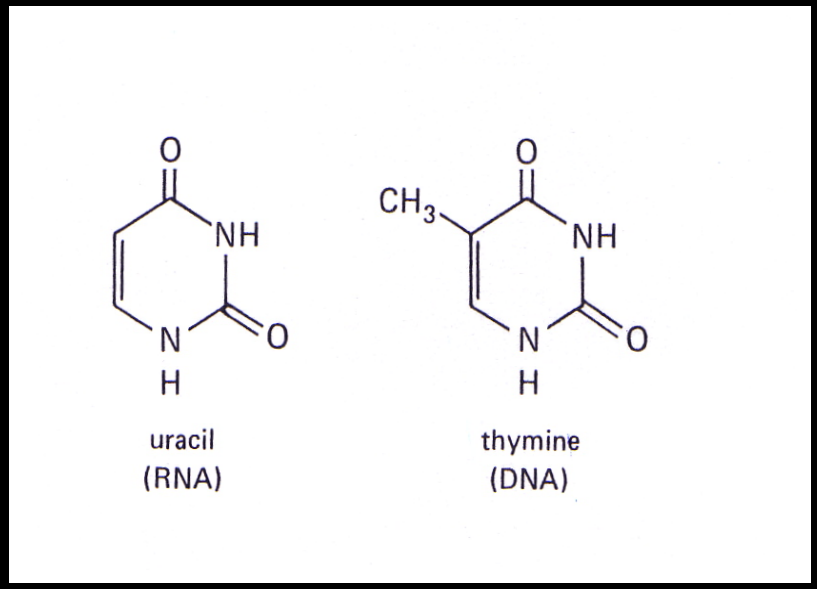


FIG. 3. Absolute cross sections of $(D-H)^-$, $C_5H_7O_3^-$, and $C_5H_6O_2^-$ formed via dissociative electron attachment to deoxyribose as a function of the electron energy from about 0 to 3 eV.

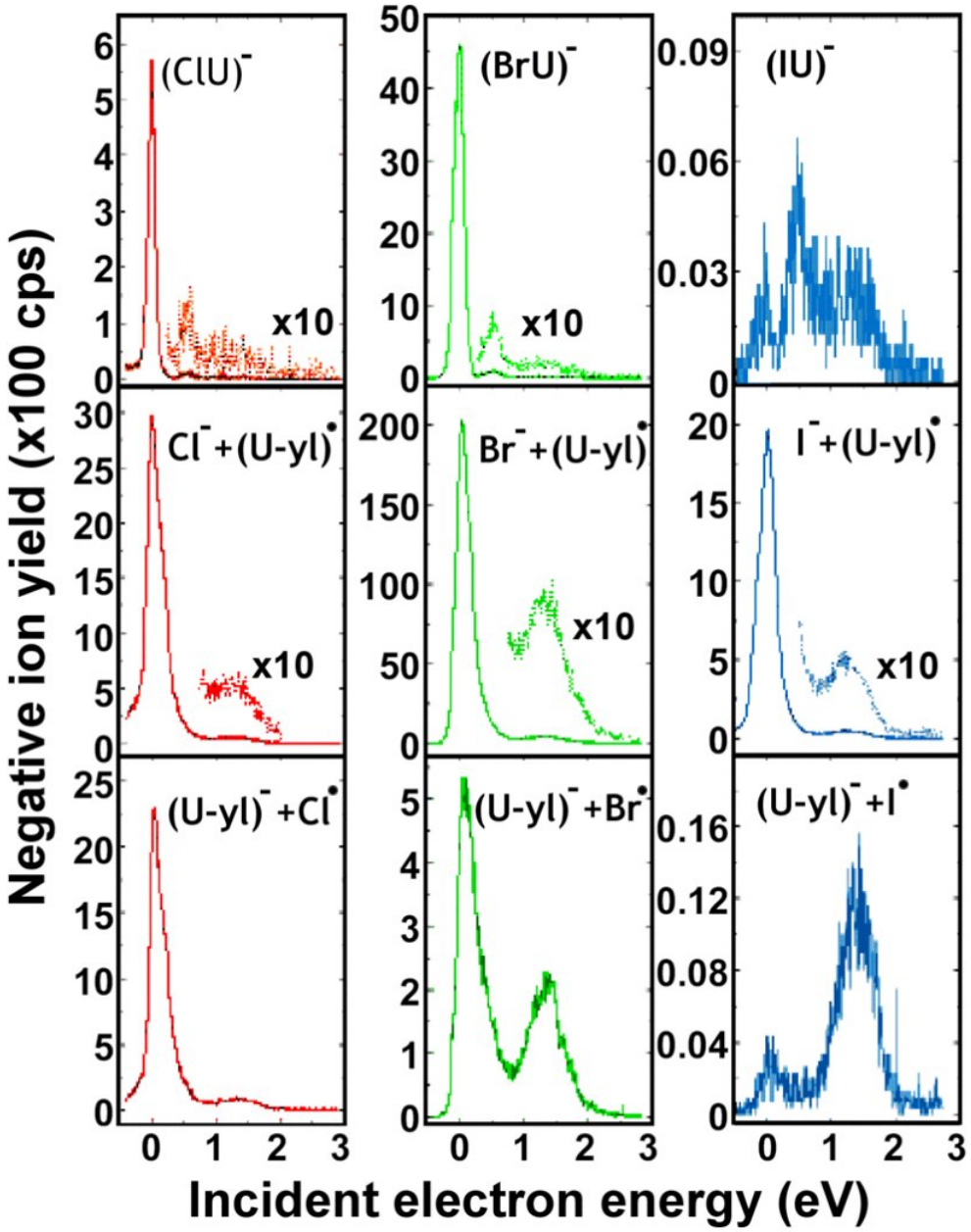
Does DEA explain effectiveness of some radiosensitizers ?

- Observation of correlation between carcinogens and DEA rates ?
- Effectiveness of halogenated compounds as radiosensitizers



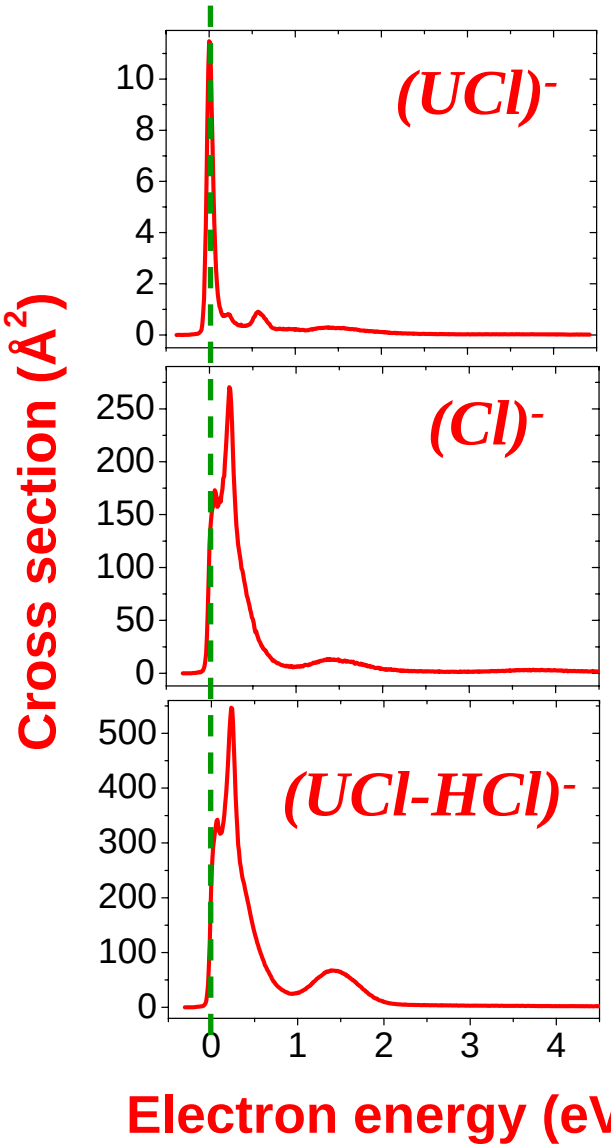
Uracil Thymine Bromouracil
(Radiosensitizer)

Halo-uracils measured by Illenberger et al Berlin

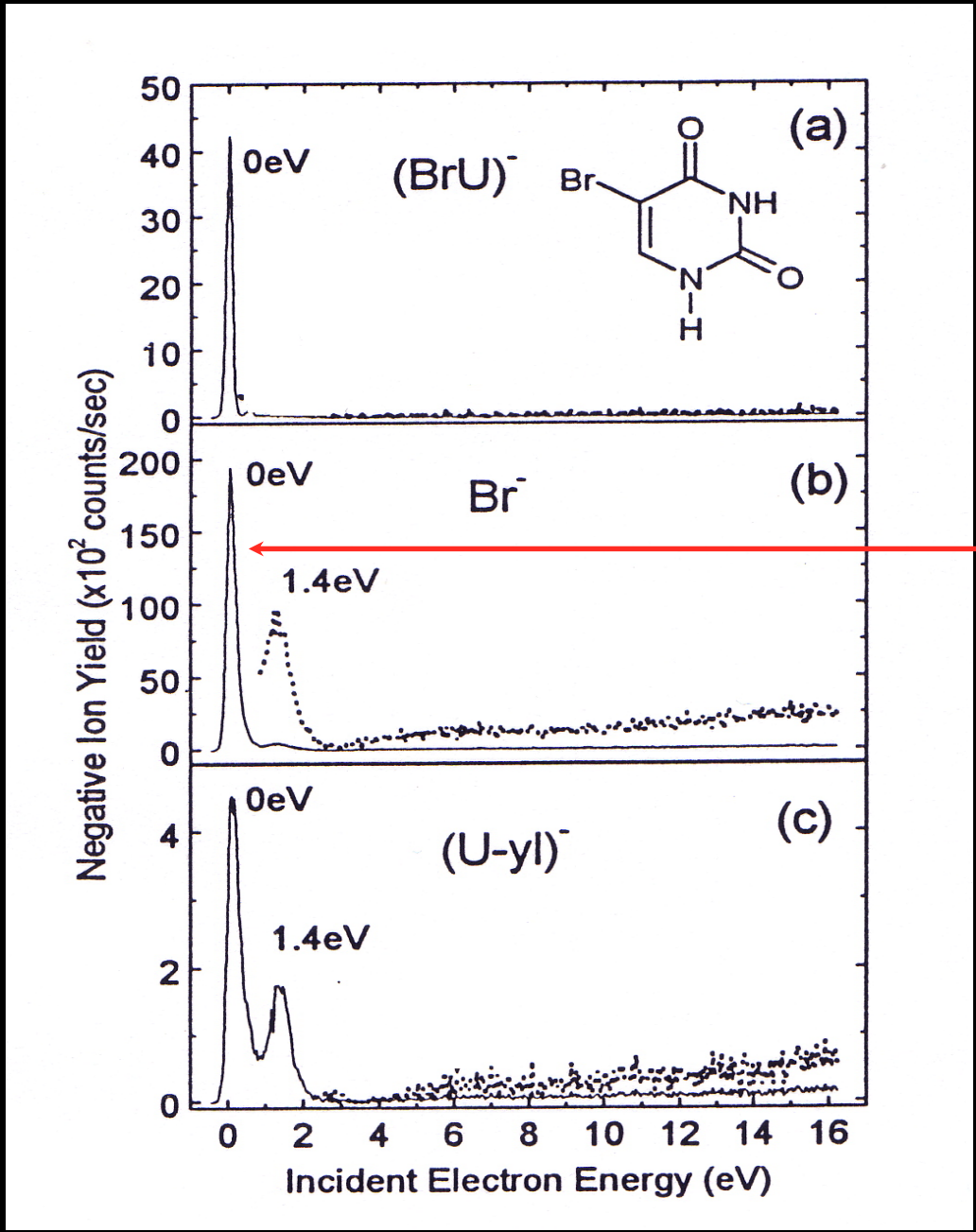


Ratios: $X^-/(U\text{-}yl)^-$ = 1.3, 40, 490 (X=Cl,Br,I)

$UCl + e^- \rightarrow$ anions



Present: $Cl^-/(U\text{-}yl)^- = 0.47$



Freie University Berlin

$\sigma \approx 600$ Å^2

Hypothesis for Mechanism of SSB and DSB?

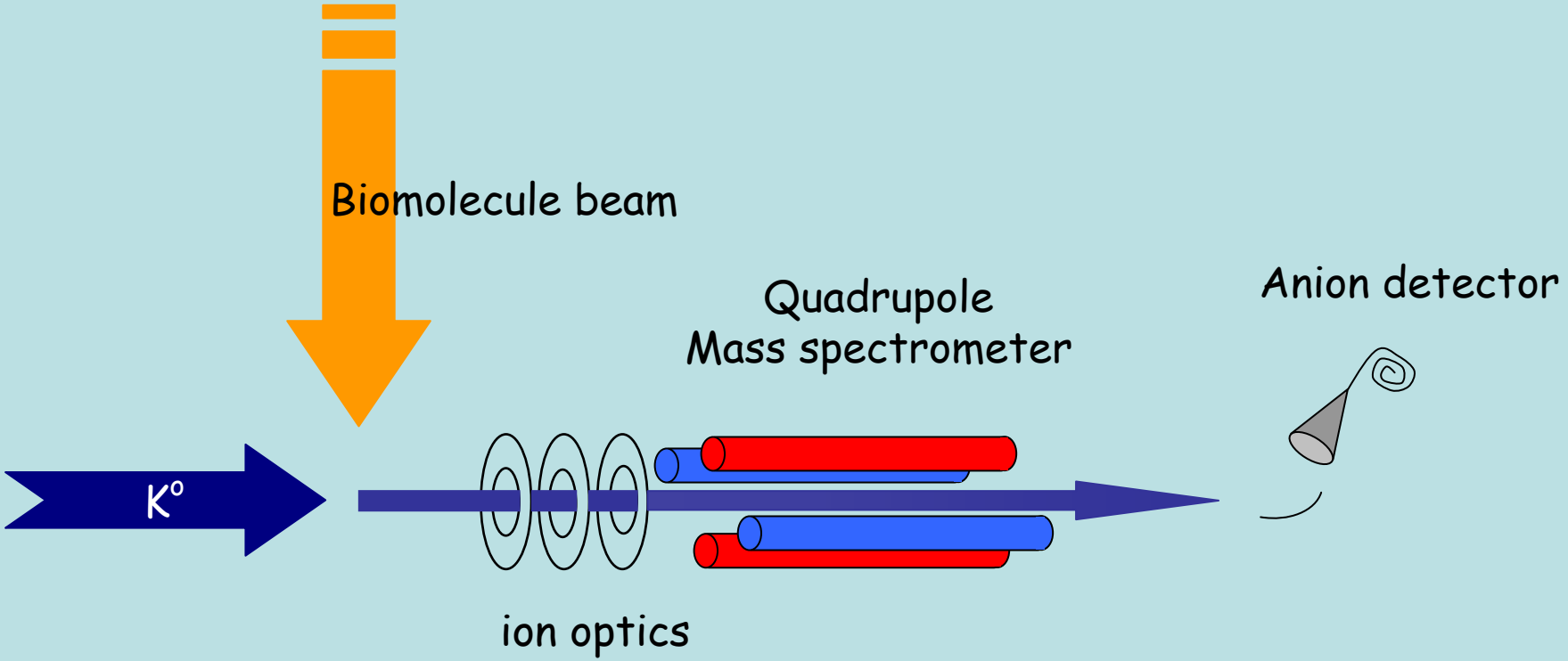
- Electron attachment liberates H atoms
  - This can induce an SSB
  - DSB induction occurs when fragmentation components react with the opposite strand

But is the ‘free electron’ model a good one for electrons in biology ?

- Electrons are not ‘free’ or ‘ballistic’ in nature

Electron Charge transfer experiment

Universidade Lisboa P Limao-Vieira et al



This experiment will study the ionic fragments produced and compare them with those observed by free electron dissociative attachment.

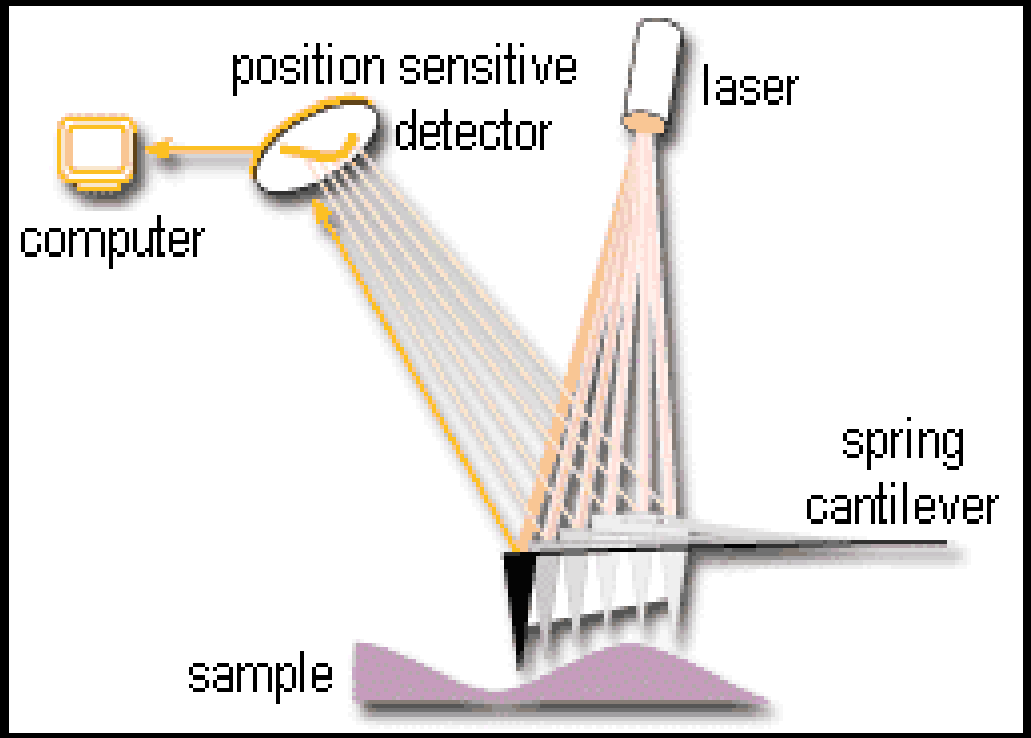
Are Fragmentation patterns the same ? What are kinetic energies of fragments ?

New Methods for Viewing DNA Damage

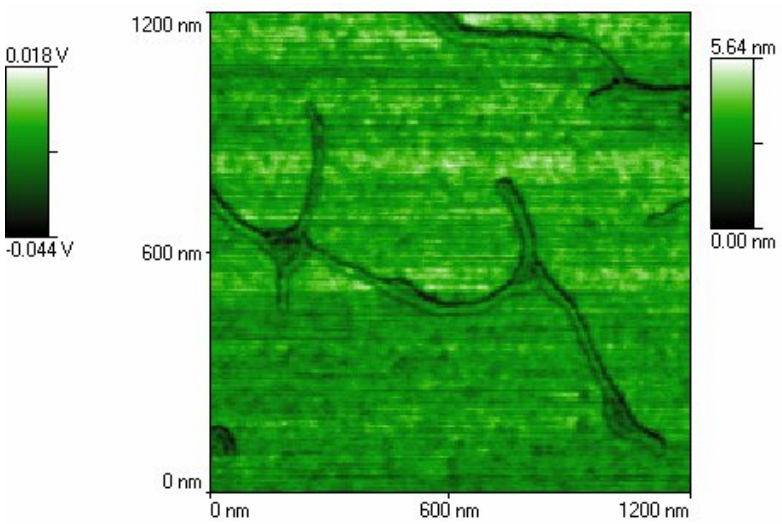
- Electrogelphoresis measures SSB and DSB

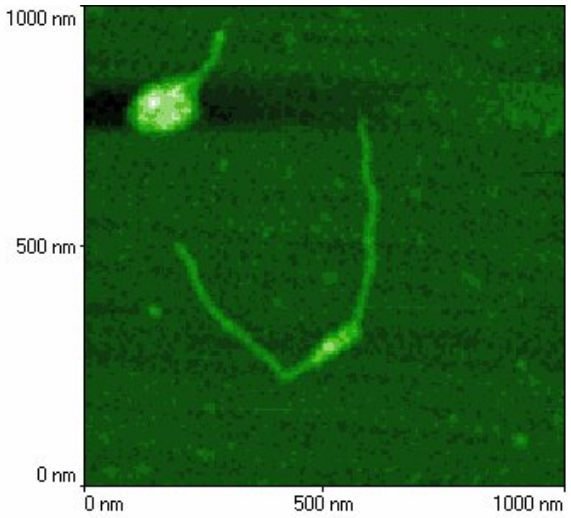
But can we ‘see’ DNA Damage directly ?

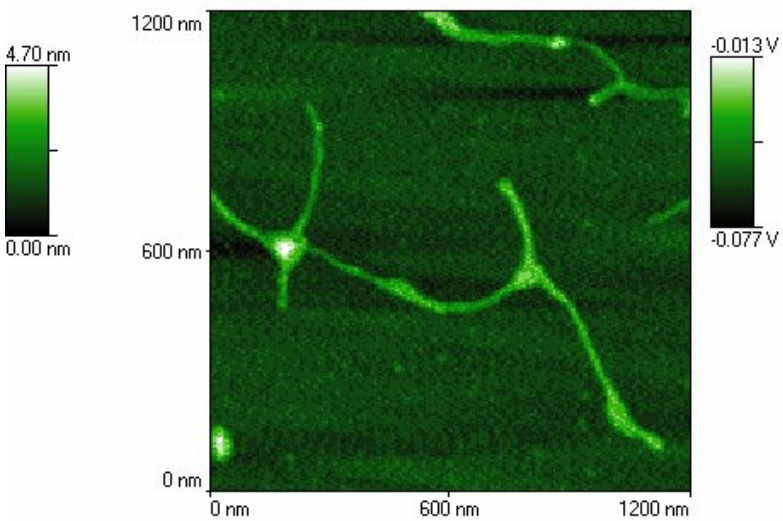
Atomic Force Microscope

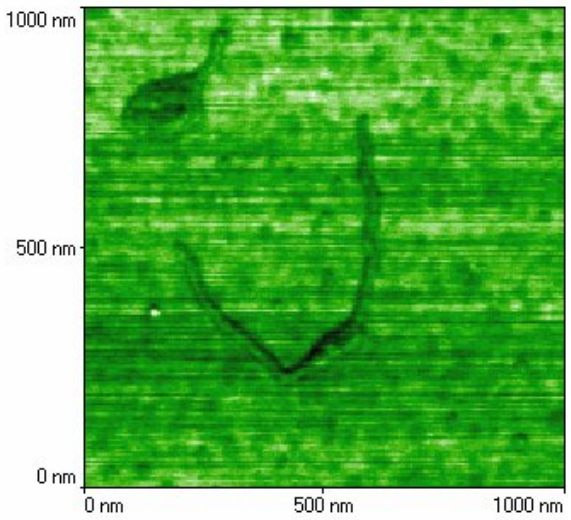


Recent DNA Images on OU AFM

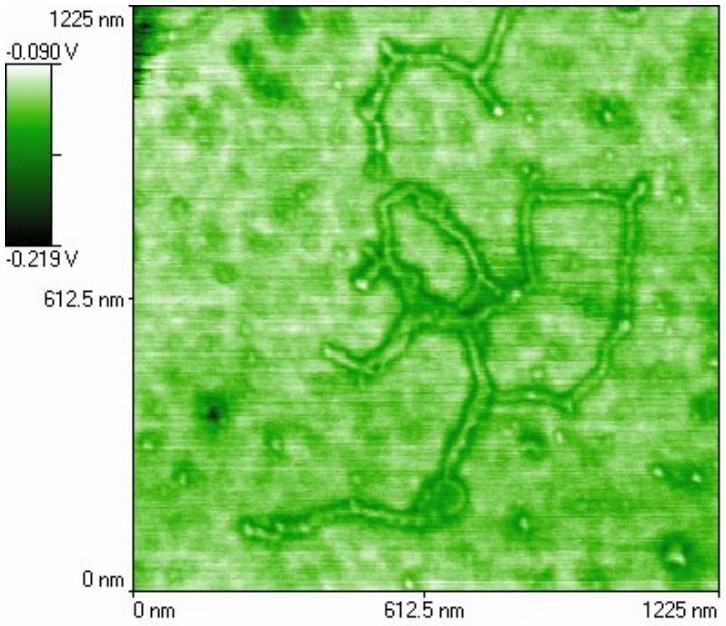


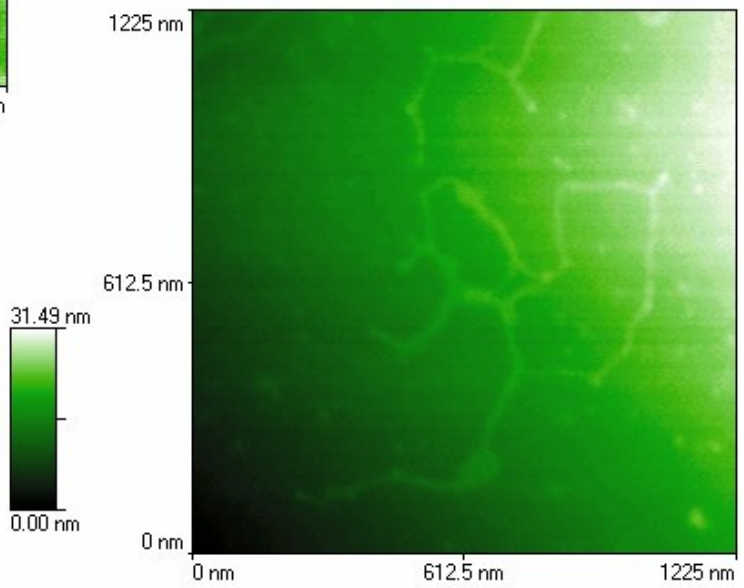




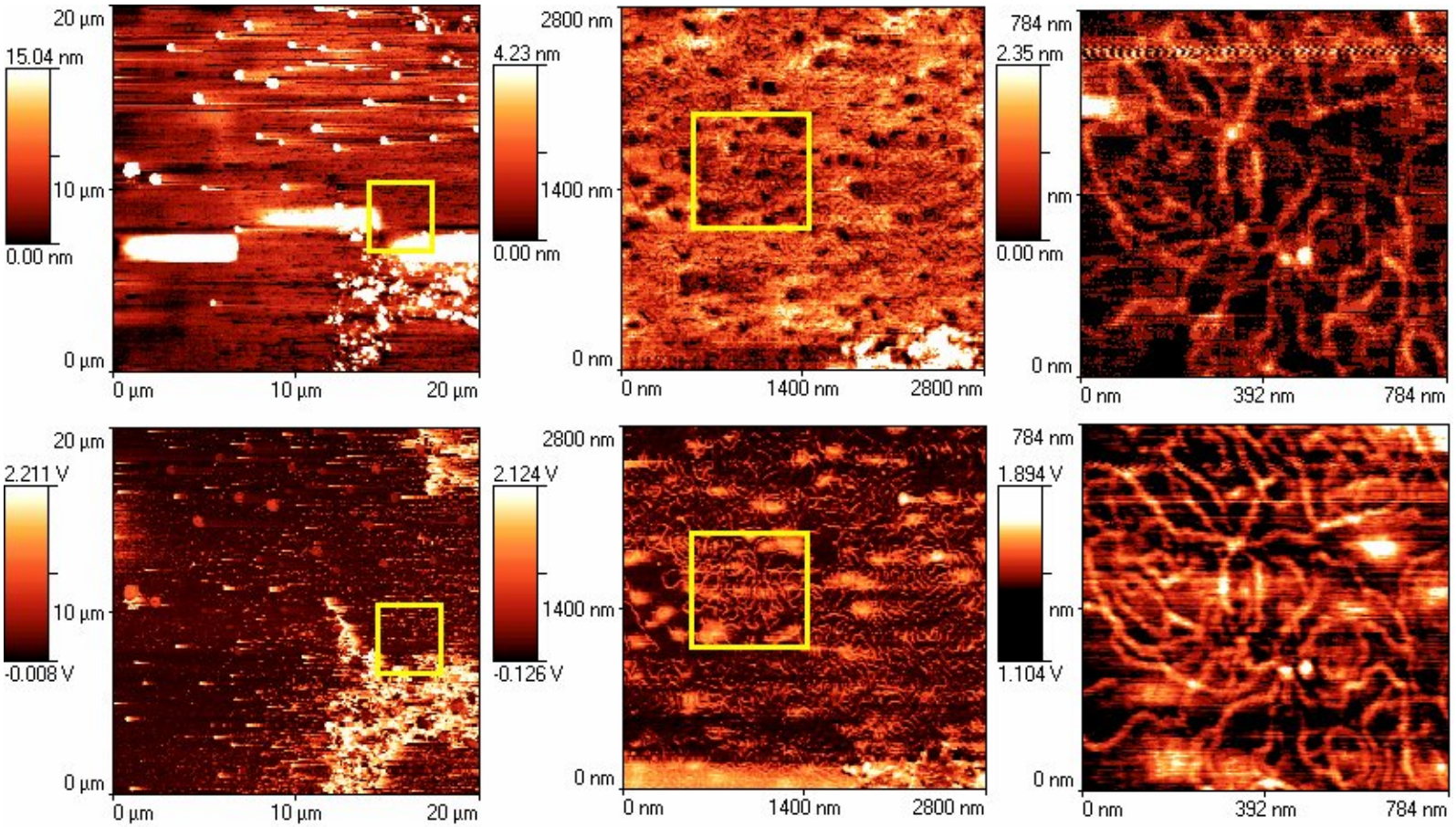


RECENT !! DNA images on OU AFM





Rat DNA plus 0.1% Tween-20



DNA extracted from rats blood
50 mM HEPES/10mM NiCl2 (pH 8) + 0.1% Tween-20
sample rinsed + dried in air prior to imaging

top: topography
bottom: phase image
yellow box shows approximate area of zoom in for subsequent image

Using AFM to probe DNA Damage

- Place DNA on mica and irradiate
- Examine irradiated sample for damage
- Quantify strand breaks and length of fragments

Using AFM to probe DNA Damage

- Can irradiate same sample several times
- Accumulative damage (enhanced sensitivity ?)
- Can change wavelength, energy
- Look for thresholds

Final Conclusions

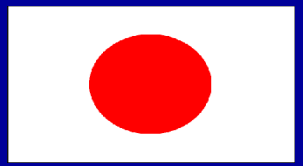
- DNA damage be induced at low energies
- Possibly related to molecular dissociation by low energy secondary electrons ?
- If so, what is the process ? DEA ??
- And what are the consequences for radiation chemistry and track damage models ?

The team – with thanks

- **Paulo Vieira, Samuel Eden,**
- **Paul Kendall, Anita Dawes**
- **Philip Holtom, Robin Mukerji,**
- **Dagmar Jaksch, Mike Davis,**
- **Sarah Webb, Liz Drage,**
- **Eva Vasekova, Gorsa Smialek,**
- **Bhala Sivaraman,**
- **Patrick Cahillane, William Stevens.**
- **and all colleagues**
- **Staff and Technical**

A collaborative exercise

- Tilmann Märk, Paul Scheier et al , Universität Innsbruck, Austria
- David Field, Nykola Jones,Soren Hoffmann University Aarhus, Denmark
- Eugen Illenberger and group Frei University Berli
- Stefan Matejcik, Jan D. Skalny, Comenius University, Bratislava
- Gustavo Garcia, Madrid, Spain
- Paulo Vieira Lisbon. Portugal
- Michael Alan Freiburg Switzerland
- Marie Jeanne Hubin Franskin, Jacques Delwishe, Liege, Belgium
- E. Krishnakumar, S.V.K. Kumar, Tata Institute, Bombay
- H Tanaka Sophia Univeristy Tokyo and Y Itikawa , Japan
- **M FOLKARD AND K PRISE GCI, UK**



EIPAM

EPIC

COST RADAM

A EU funded Project
for scientific exchange

2003-2008

COST RADAM

Physics action on Radiation damage

Launched November 2003 but

First Steering meeting

Lyons June 2004

Five year progamme

So end date around end 2008

COST RADAM

Members

Austria, Belgium, Bulgaria, Czech, Denmark, France, Germany, Hungary, Iceland, Ireland, Italy, Latvia,Netherlands,Poland,

Portugal, Serbia, Slovakia, Spain, Sweden, United Kingdom

COST RADAM

Arranged in 5 working groups

1 UV and electron damage

2. Ion induced damage

3. Cellular and radiation chemistry

4. Theoretical (Molecular structure)

5. Track models

COST RADAM

Activities

Short visits 1 to 4 weeks

Conferences/workshops

COST RADAM

Short visits

Up to 1 month

Simple application procedure and report

www.isa.au.dk/cost

Must continue to use and develop links !

COST

Can be applied anytime but in 2006 will be arranged in two parts

January to June

July to December

Expect about 50 ? visits

COST RADAM

Conferences

RADAM 1 Lyons June 2004

RADAM 2 Berlin March 2005

RADAM 3 Groningen June 3-6 2006

RADAM 4 Dublin 2007

RADAM 5 ???

COST RADAM

Conferences

ESF conference in Obergurgl AUSTRIA

June 26 -29 (?) 2006

Theme; Biomolecules and physical and Chemical processes

50,000 Euros about 100 attendees

COST RADAM

Reminders

www.isa.au.dk/cost

Chair N J Mason UK
n.j.mason@open.ac.uk

UK Secretary Beverley Harker
b.j.harker@open.ac.uk