Ion irradiation of large biomolecules and clusters

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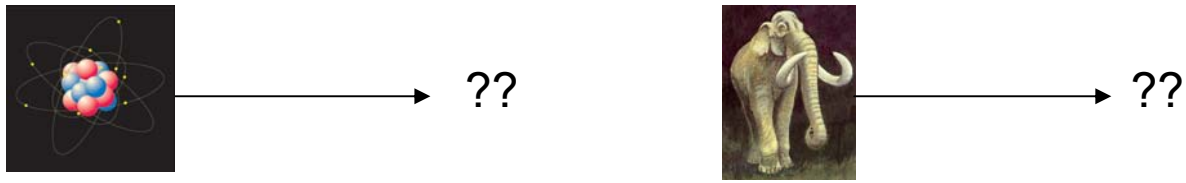
Outline

- Collision physics ⟶ radiobiology

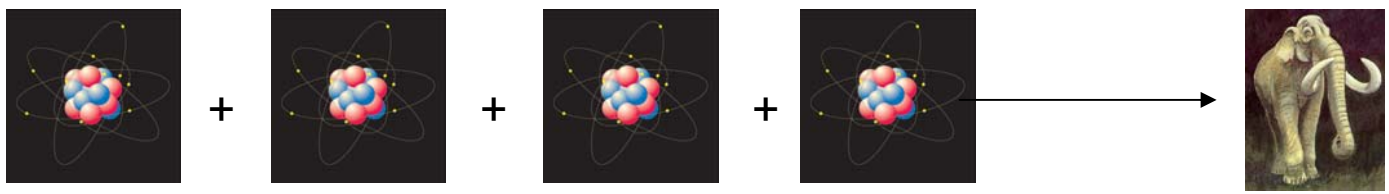
- Complex molecules with low energy ions
  - Which molecules and why?
  - Techniques
    - Condensed phase
    - Gas phase
    - clusters
  - A few results

Interdisciplinary work…

- Physics starts with the small but biology starts with the large.



- To do physics in a biological setting, we have to go beyond our normal environment!
- Why?! Can't we just use simple systems and build them up?



  - Yes… but… biologists are yet to be convinced by this and physicists have yet to provide definite evidence that this works in larger system

Biological studies

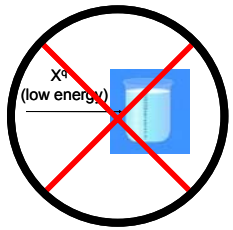
- Keep most if not all of the critical components present within the experiment
  - Living creatures e.g. humans/Hiroshima

  - At the very least, solvated complex molecules

  - Why?
    - 'Other' molecules add reactive components e.g. water giving hydroxyl radicals
      - Indirect effects, late effects

Low energy ions in radiobiology

- Problem
  - Limited range so we cannot fire a low energy ion at either a large organism or into a solution and expect it to do anything!

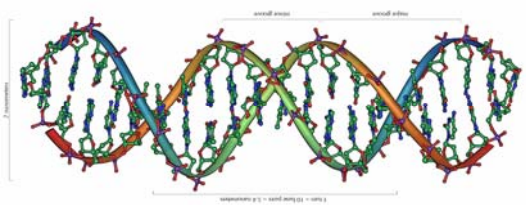


- Therefore we cannot do these experiments as a biologist would ideally like

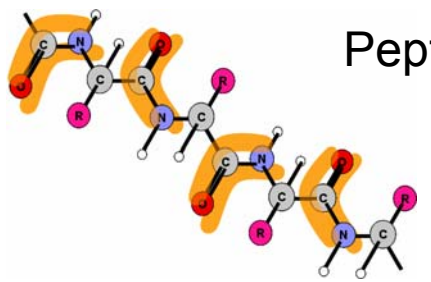
- What can we do?
  - Use complex molecules (somewhere between the small and the very large!)
  - Add other environmental factors later (e.g. water)
  - Even some techniques which can produce targets including complex molecules together with some water

Some complex molecules

- DNA, RNA, amino acid molecules (+ water)
  - 'fundamental' molecules within the biological system e.g. DNA often considered to be the critical target when considering radiation damage
    - Nb. Bystander effect!



DNA



Peptide

How might we study these?

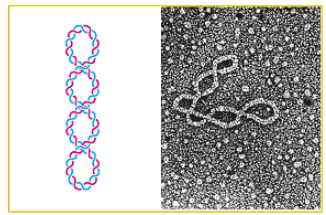
- Condensed phase e.g. plasmid DNA/Oligonucleotides
- Gas phase e.g. oligonucleotides/protein chains
- Clustered with water
- Each of these has limitations with regards to the information which can be obtained

Solid phase

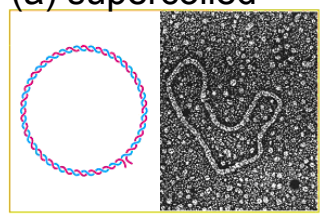
- Provides a wide range of potential targets since the only necessity is that the material can be dried on a surface
- Can even try and achieve within an icy matrix (i.e. some water included in the target)
- Relatively straight forward with many techniques to analyse
- BUT…
- Often difficult to obtain a 'clean' target
- Small quantities complicate analysis
- Targets are very difficult to characterise.

Example – plasmid DNA and electrophoresis

- Large ‘twisted’ molecule which changes conformation when damaged
- Sensitive to strand breakage
  - SSB — nicked DNA
  - DSB — linear DNA
  - Multiple DSBs — fragments



(a) supercoiled



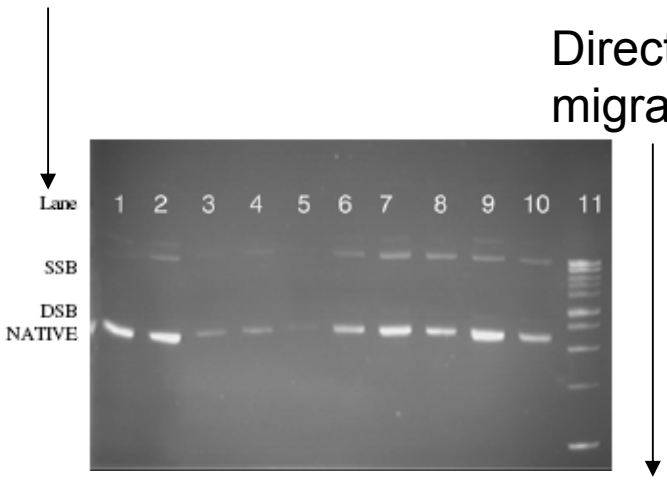
(b) nicked

- Analysis using gel electrophoresis
  - Different forms migrate at different rates through the gel (negative ions in solution migrate in electric field)
- Deposit and freeze dry solution onto gold (tantalum?) surface
- Irradiate in vacuum
- Recover into solution and analyse

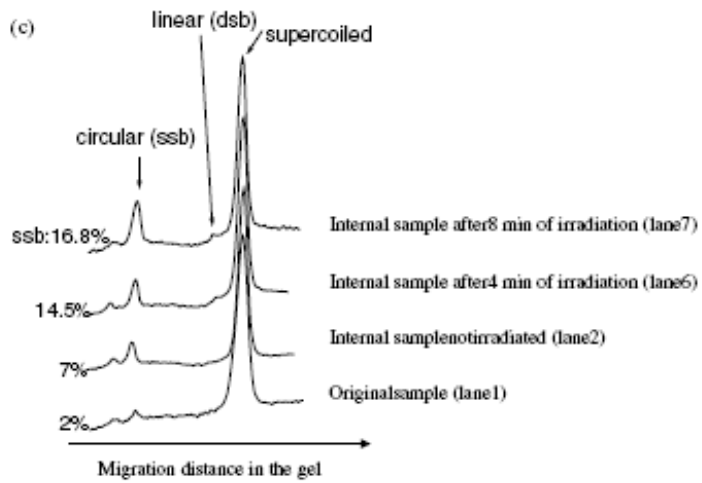
Results

- Gel electrophoresis (Lacombe et al., Phys. Med. Biol. **49** (2004) N65–N73)
- Ar^+ at low keV energies
  - Single and double strand breakages occur

DNA starts here

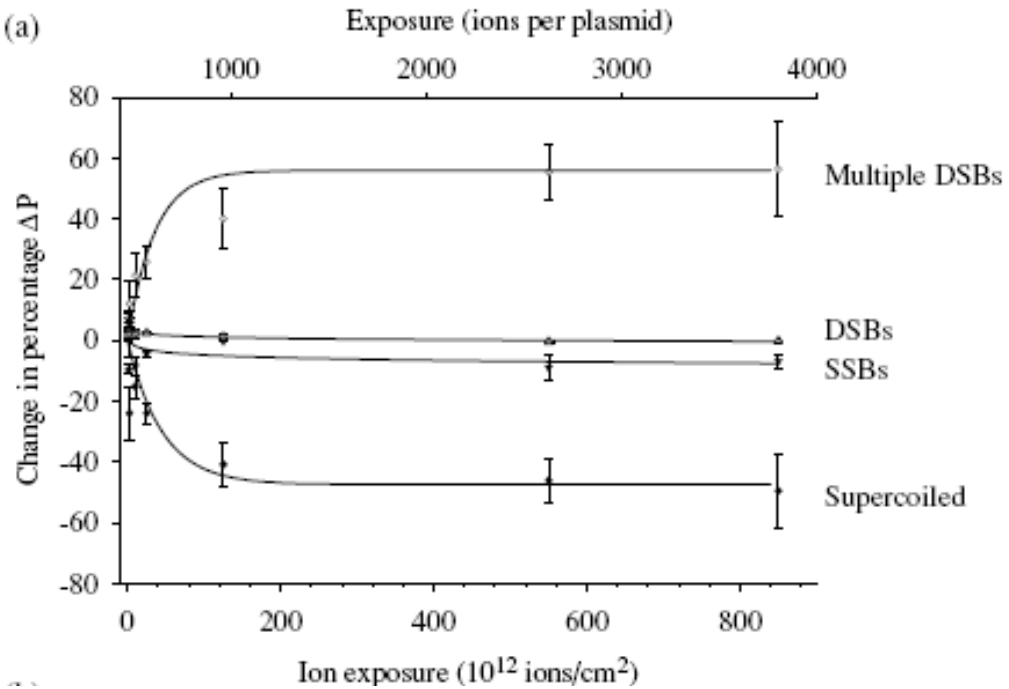


Direction of migration



Results

- Similar technique but extending to include a fluence relationship



- C^+ at 2 keV
  - Saturation effects, formation of fragments, cross sections may be calculated
    - $P^S = A(1 - e^{-\sigma_S^d})$ – *for supercoiled, cross section for destruction of supercoiling* = $(2.2 \pm 0.5) \times 10^{-14}$ cm^2
- Hunniford et al., Phys. Med. Biol. **52** (2007) 3729–3740

Analysis

- Low energy ions can produce both single and double strand breakages and may do so rather effectively

- Notes
  - relatively large error range
    - Variation in Lacombe gel
  - Limited mechanistic information
    - Only strand breakage info, no site specificity
  - Large molecules like these inherently will have contamination
    - Produced in buffer, stored in buffer, cleaned in buffer, buffers are everywhere!

Gas Phase

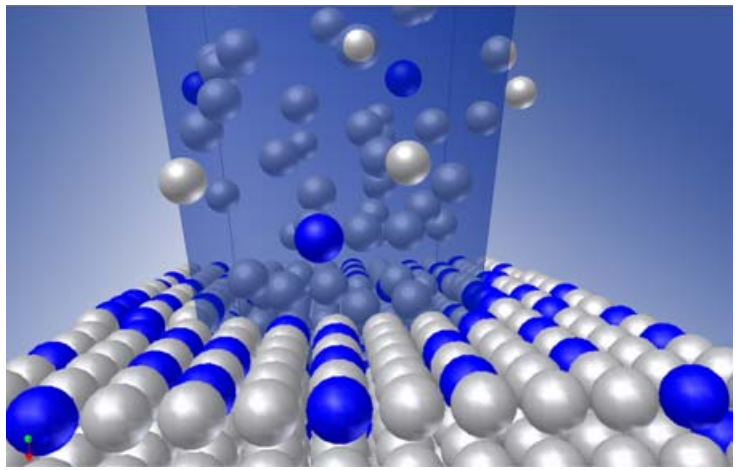
- An area where significant expertise exists (us!)
- Targets may be well characterised
- Targets may be clean (!)
- Very sensitive analytical techniques available
  - E.g. TOF-MS, FTICR-MS etc.
- BUT….
- Targets are hard to produce (molecules fall apart!)
- Characterisation is not trivial
  - Target thickness, number density (fragmentation?)

How to make a gas phase target?

- Several techniques being investigated
- Heating destroys molecules
- Electrospray Ionisation source combined with electrostatic trapping
  - Ionic target, KVI
- Laser desorption techniques
  - Neutral target, QUB
- Others (e.g. sonic spray, sonic vaporisation)

Example – Laser desorption

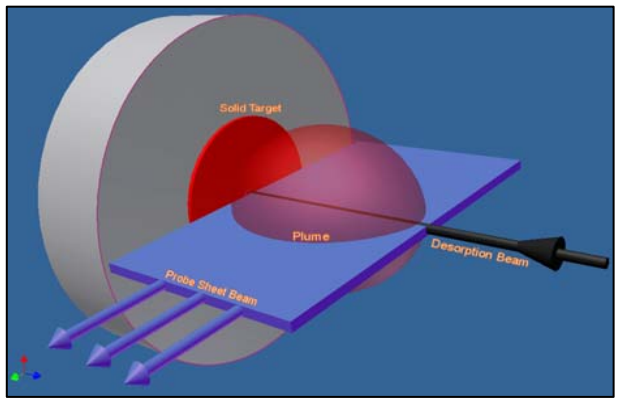
- MALDI – Matrix Assisted Laser Desorption and Ionisation
  - spectroscopic technique to analyse heavy molecules



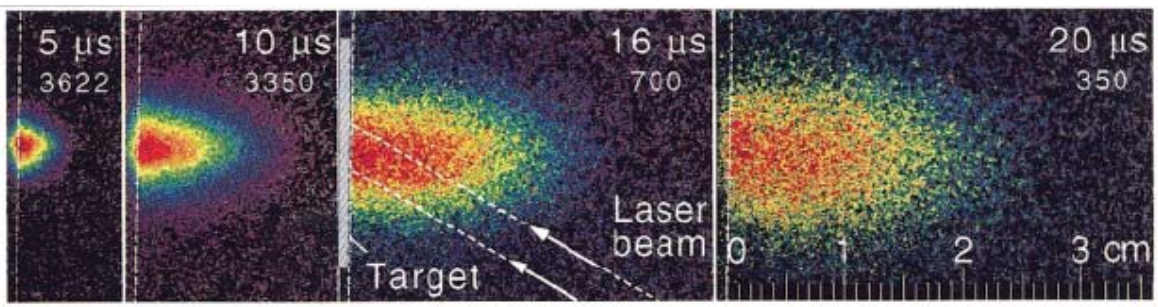
  - Embedded in matrix which absorbs laser energy and desorbs entraining the heavy molecules
  - In spite of being used for the ions, many more neutrals are produced (~10^5 more neutrals than ions for an HPA desorbant)

MALDI plume

- Probed using laser induced fluorescence (LIF)
  - Molecules absorb at a specific wavelength and then re-emit, this emitted light is then detected (typically using an ICCD camera)



- Example of a matrix plume (Puretzky et al., Phys. Rev. Lett. 83, 444 - 447 (1999))

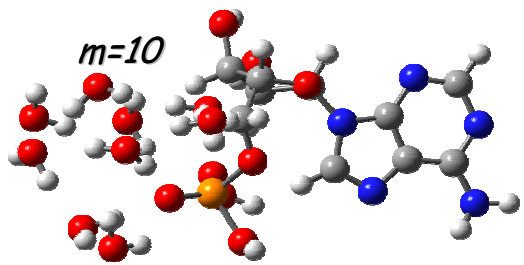


Improvements for ion interaction studied

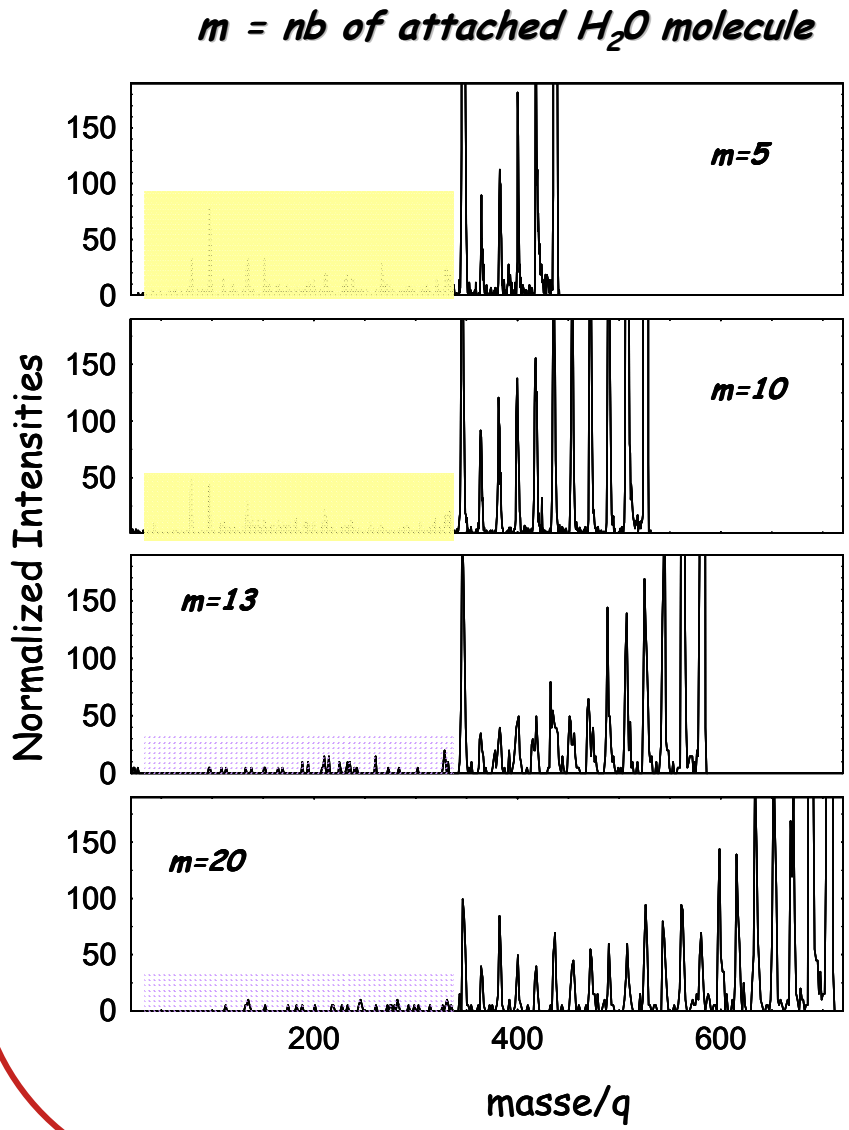
- MALDI cannot be used due to matrix contamination
- Instead use SALD or DOS
- 1 or 3 micron light to provide a matrix free laser desorption
  - LIF imaging to obtain beam profile and density
- Once target is characterised, it will be crossed with an ion beam and combined with a TOF mass spectrometer
  - Fragment species can be extracted and analysed for site specific damage etc.

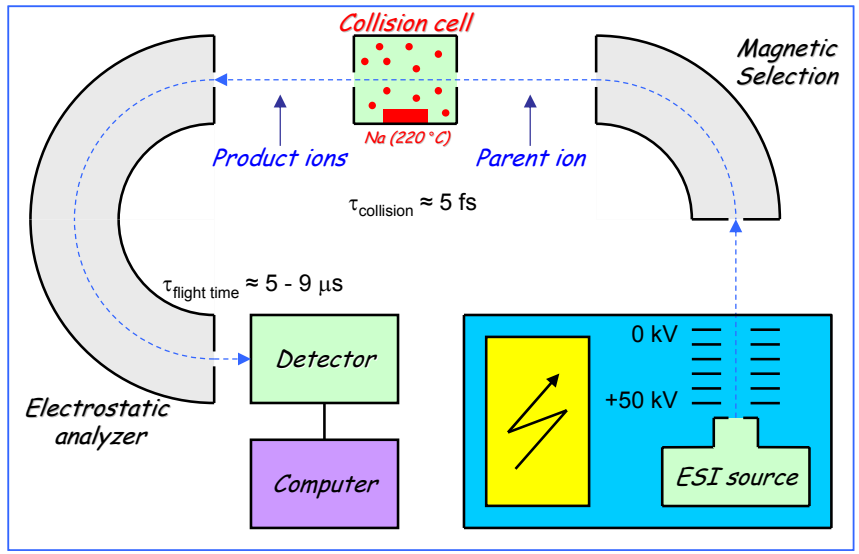
Clusters

- Attempts to address the problem presented by the lack of water
- Biomolecules (as yet relatively small) are produced embedded in a water matrix
  - What is the overall structure of the water??
- Essentially a gaseous technique
  - Target clean
  - Can be well characterised
  - Accurate and sensitive analysis



Nano-solvated AMP





- If m≤10, the AMP fragmentation pattern does not vary with the increasing water coverage
- Whereas if m>10, only the loss of water molecules is observed
  - When m is larger than ~13, the AMP molecule seems fully protected by the surrounding water molecules

Summary

- Large biomolecular studies further bridge the gap between physics and biology
- Currently several ways of studying large biomolecules
  - Condensed phase
  - Gas phase
  - Clustered molecules with water
- Each technique has distinct advantages and disadvantages