COST-STSM-P9-03183
(Dr Adam Hunniford visit to CERN)
Antiproton irradiation of plasmid DNA

Purpose of the visit:
Funds were requested to enable a visit by the applicant to CERN in October 2007 in order for the applicant to:

1) Assist in dosimetric measurement of the antiproton beam produced by the AD. This information will inform irradiation times and beam parameters necessary to produce the energy deposition profile required for irradiation of biological materials
2) Assist in the preparation of cellular samples which will be irradiated in an experiment connected to the DNA irradiation.
3) Produce plasmid DNA embedded within an agarose gel matrix with specific gel density.
4) Process the irradiated gel in preparation for return to QUB for extraction of DNA from the gel and electrophoretic analysis.

The work was carried out under the Antiproton Cell Experiment (ACE) collaboration involving a multinational team of researchers. The main participants in the described work were Dr Hunniford, Dr Timson, Prof. McCullough (All from QUB) hosted by Professor Helge Knudsen (University of Aarhus, Denmark) at CERN.

Description of work carried out during the visit:
All radiotherapies work by causing unrepairable damage to cellular DNA leading to cell death. These mechanisms are well-studied at the molecular and cellular level for conventional therapies involving photons or electrons. More recently, ion beams have been utilised for radiotherapeutic purposes making use of the 'Bragg Peak', a well defined spatial region in which the ionising effects (and thus biologically relevant effects) are maximised. An additional biological effectiveness is believed to be possible through the use of antimatter, such as antiprotons, which will deposit energy as a proton but will also annihilate depositing even more energy within the vicinity of the Bragg peak. A recent study has shown that they have greater cell killing potential than protons at the same dose and kinetic energy [2]. It is hypothesised that this arises due to increased incidence of dsb and mdsb damage to cellular DNA.

We have recently completed a study on the irradiation of plasmid DNA with C^{+} and C^{2+} ions [1]. Plasmid DNA is a good model system for these studies as it is a covalently closed circular molecule whose electrophoretic properties depend upon its conformation in solution. It is thus possible to distinguish between (and quantify) single strand breaks (ssb), double strand breaks (dsb) and multiple double strand breaks (mdsb). This basic technique was also utilised in the present study with antiprotons.

The experiment built on the irradiation protocols used in studies on mammalian cells previously carried out by the host group [2]. Supercoiled plasmid DNA (prepared prior to the visit at QUB) was embedded into a low-melting point agarose matrix inside a plastic tube (approximate dimensions 150 mm by 5 mm). Antiprotons from the antiproton decelerator (AD) at CERN were used to irradiate the tube of DNA

along the long axis (see figure 1). Following irradiation, the gel matrix was extruded from the plastic tube and sliced into 2 mm sections. Sections from a control (non-irradiated) gel containing the same concentration of DNA were also cut. These gel sections have now been shipped back to the UK and frozen prior to analysis.

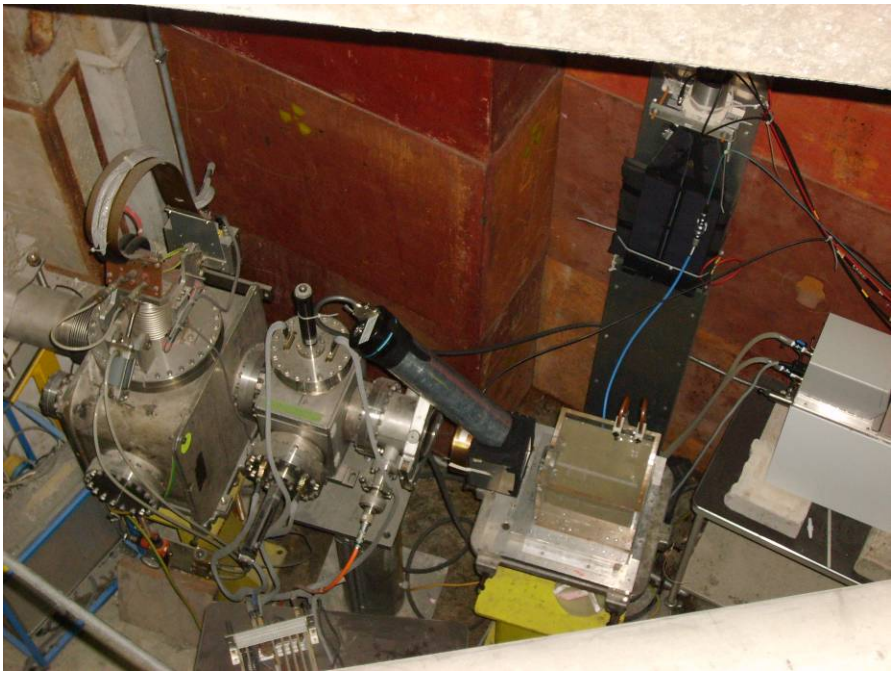

Figure 1: The apparatus used to irradiate plasmid DNA with antiprotons. Antiprotons enter from the left and irradiate the DNA which is embedded in agarose inside the white tube visible in the water bath. The same apparatus is used by the host group for their experiments with whole cells.

Description of the main results obtained

The analysis techniques used in our study of carbon ion irradiation on plasmid DNA [1]. In brief this will involve extracting the DNA from the gel sections (using commercially available kits) and running this DNA on agarose gels to determine the fractions of undamaged plasmid (supercoiled form), ssb (open circle form), dsb (linear form) and mdsb (loss of material compared to controls). Given the number of samples to be analysed (>150) and the need to run each sample more than once, this process is expected to take two to three months.

Future collaboration with the host institute

Negotiations are underway to enable Queen's University, Belfast to become a full member of the ACE collaboration. This will enable future, similar experiments to be carried out on DNA (and possibly other biopolymers) to examine the energy dependence of damage. If good results are obtained from this preliminary study, funding will be sought (from UK research councils, etc) to support QUB's participation in the collaboration.

Projected publications arising from the work

We would anticipate that the results of this work will be published in a peer reviewed journal, the choice of which will be decided together with the ACE collaboration and together with any complimentary results obtained therein.

Confirmation by host institute of the successful execution of the mission
Attached.

References
1. Hunniford CA, Timson DJ, Davies RJ, McCullough RW (2007) Damage to plasmid DNA induced by low energy carbon ions. *Phys Med Biol.* **52**:3729-40
2. Holzscheiter MH, Bassler N, Agazaryan N, Beyer G, Blackmore E, DeMarco JJ, Doser M, Durand RE, Hartley O, Iwamoto KS, Knudsen HV, Landua R, Maggiore C, McBride WH, Møller SP, Petersen J, Skarsgard LD, Smathers JB, Solberg TD, Uggerhøj UI, Vranjes S, Withers HR, Wong M, Wouters BG. (2006) The biological effectiveness of antiproton irradiation. *Radiother Oncol.* **81**:233-42