COST-STSM-P9-03150
(Dr David J Timson 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 colleagues from the School of Mathematics and Physics at Queen's University, Belfast in carrying out a preliminary experiment to determine the effects of antiprotons on plasmid DNA
2. Provide advice on the biological aspects of the project
3. Enhance his knowledge of advanced radiation sources and antiproton physics
4. Obtain preliminary data which is likely to be publishable in its own right and may lead to further grant applications and publications

The work was a collaboration between physicists (Prof RW McCullough, Dr Adam Hunniford) and a biochemist (the applicant) at Queen's University, Belfast and the ACE (Antiproton Cell Experiment) Collaboration at CERN (contact person Dr Helge Knudsen of the University of Aarhus, Denmark who acted as the named host for this application).

Description of work carried out during the visit:
All radiotherapies work by causing unrepairable damage to cellular DNA which leads to cell death. These mechanisms are well-studied at the molecular and cellular level for conventional therapies involving photons or electrons, but less is known about the effects of ion beams. 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).

We are seeking to extend this work by looking at the effects of antiprotons on plasmid DNA. The enhanced energy deposition of antiprotons due to the annihilation event makes them an attractive candidate for the use in novel radiotherapies. 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. However, this has not been shown experimentally.

A preliminary study into the effects of antiproton irradiation of DNA at the antiproton source based at CERN, Geneva was carried out. 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.

In addition, the applicant was able to observe other experiments being carried out at the AD (principally the continuing cell irradiation studies of the ACE collaboration and the work of the ASACUSA collaboration).

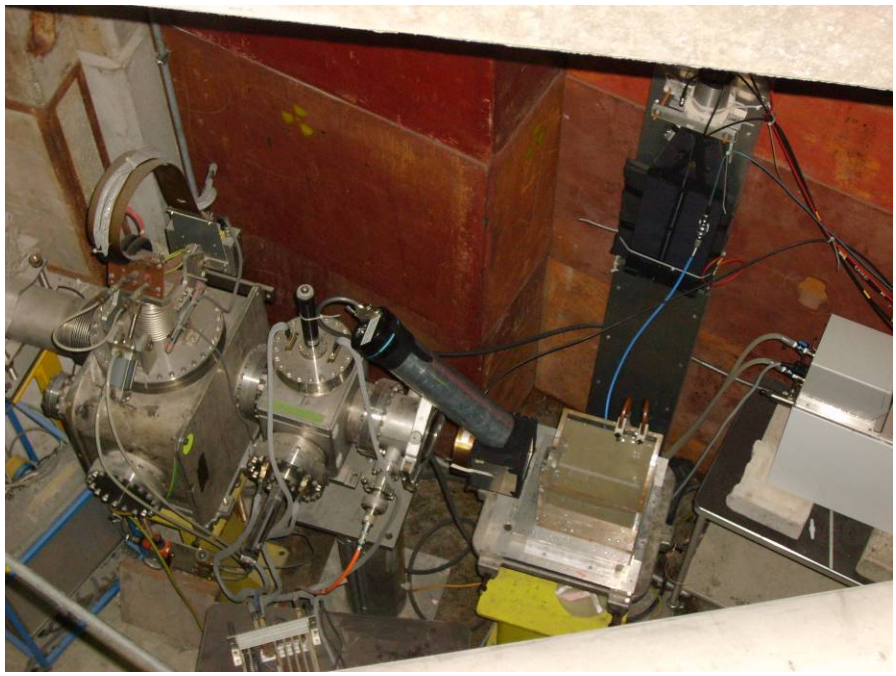

Figure 1: The apparatus used to irradiate plasmid DNA with antiprotons. Antiprotons enter from the left and irradiate the DNA which is embedded in agarose inside the white tube visible in the water bath. The same apparatus is used by the host group for their experiments with whole cells.

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
Assuming that the analysis of the DNA damage is successful, we would anticipate publishing the results. The choice of journal will be made based on the quality and interest of the results obtained.

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

From: hk@phys.au.dk [mailto:hk@phys.au.dk]
Sent: 31 October 2007 06:55
To: David Julian Timson
Cc: hk@phys.au.dk
Subject: work at CERN

Dr. David Julian Timson
QUB
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Dear David,

This is to confirm that your visit to participate in the ACE run 2007 at CERN
took place and was successfull. I also agree in the contents of your report.

Yours sincerely,

Helge Knudsen
Prof.
Group leader ACE
CERN