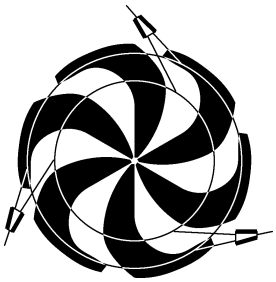
TRIUMF



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CANADA'S NATIONAL LABORATORY FOR PARTICLE AND NUCLEAR PHYSICS

OPERATED AS A JOINT VENTURE

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UNDER A CONTRIBUTION FROM THE
NATIONAL RESEARCH COUNCIL OF CANADA

DECEMBER 2006

The contributions on individual experiments in this report are outlines intended to demonstrate the extent of scientific activity at TRIUMF during the past year. The outlines are not publications and often contain preliminary results not intended, or not yet ready, for publication. Material from these reports should not be reproduced or quoted without permission from the authors.

LIFE SCIENCES

Experiment LS0
PET facilities
(K.R. Buckley, TRIUMF)

The Siemens HRRT (high resolution research tomograph) has been formally accepted and is now being used for new human research protocols. The Siemens microPET R4® scanner was used routinely for the past year and in November it was replaced with a new Siemens Focus 120 scanner. The ECAT 953B continues to be the most used scanner despite its advancing age though the number of scans did decrease again this year. The TR13 cyclotron continues to reliably produce radioisotopes for the varied projects undertaken by the Life Sciences program. Routine production of ^{18}F for the British Columbia Cancer Agency (BCCA) began in June.

Personnel

Paul Piccioni left the group at the beginning of the year. Wade English was hired in the spring to replace Paul and he brings an electrical/electronics skill set to the group. Kelly Crawford was also hired in the spring to assist with the ^{18}F production runs for the BCCA and help with tracer synthesis. Salma Jivan left TRIUMF in the summer to take up a position in the US. Jennifer Greene was hired to take over tracer synthesis.

BCCA

TRIUMF has entered into an agreement with BCCA where TRIUMF delivers up to 1 Ci of ^{18}F, once or twice a day, to BCCA for the synthesis of FDG. TRIUMF has constructed a facility in the TR13 area where BCCA personnel take possession of the ^{18}F and synthesize FDG. The routine production of FDG for patient use began near the end of June and over 160 irradiations have been performed for this project.

TR13 cyclotron

Usage of the TR13 cyclotron was down this year in both delivered beam and the number of irradiations. The total number of runs was 709 vs. 942 in total last year and delivered beam is 370,840 μA minutes vs. 454,438 μA minutes total for 2004.

Unscheduled downtime this year was quite minor. The rf amplifier tube had to be replaced in the fall. In the early summer a water leak inside target shield 1 caused a position sensing potentiometer on the target selector to fail. That in turn caused a ball joint coupling to break. An inflector power supply failed and was repaired by the TRIUMF Ion Source group.

One extraction foil change was required through the year and the fluoride production target was rebuilt twice. Three ion source filament changes have been done along with other miscellaneous service items.

Presently six target locations are occupied. These consist of

- one ^{18}O-O_2 gas target (aluminum body)
- one ^{18}O water target (niobium body)
- one ^{16}O water target (aluminum body)
- one N_2/H_2 gas target (niobium body)
- one ^{18}O water target (niobium body, not yet commissioned)
- one location used for various solid targets.

Very few irradiations took place for target development or research. The Canadian Nuclear Safety Commission took exception to the previous TRIUMF practice of allowing only internal reviews for experiments involving irradiating material not explicitly mentioned in our operating licence and instructed us to stop any such irradiations. A formal policy for the review of such irradiations was drafted (TRI-EHS-05-02) and approved by the CNSC and an amendment to the TRIUMF operating licence was sought, and recently granted. Research irradiations are just now resuming.

Prompted by the need to amend the TR13 operating licence to allow the production of larger amounts of ^{18}F, a shielding upgrade has taken place. This was accomplished with the placement of many concrete blocks around the cyclotron area and the addition of a fence outside the building. This brings the radiation fields around the cyclotron area periphery to the TRIUMF policy level of 10 μSv/h when operating dual beam on ^{18}O (the worst case for the shielding). In addition the TR13 Safety Analysis Report was updated to reflect present operating conditions and device descriptions. An amendment is being sought to remove irradiation time and isotope quantity restrictions from the licence. Beam current limits would still be in place.

ECAT tomograph

Gantry electronics are again the dominant failures for the camera this year. We received a collection of parts from the Hammersmith PET group and rather than take the time to troubleshoot errors or failures in the gantry, we are exchanging faulty boards with the spares received from Hammersmith. Generally the failures we observe manifest themselves as a detector channel that will not maintain a calibration. These faults are typically in the position/energy processor board or occasionally in the bucket controller.

A research protocol was started on the ECAT this year which intends to collect 8 year follow up data with this scanner.

HRRT

We are still learning our way around the HRRT scanner and interacting extensively with both CTI and other HRRT users to bring this system into routine use. We officially accepted the scanner from Siemens in June (Siemens bought out CTI in total this past spring) and are now on our warranty year. We are presently negotiating an extended warranty with Siemens. The funds for this extended warranty were part of the original CFI funding to purchase the scanner. The stability of the camera continues to be an issue with evidence of a drift in energy spectra for the blocks. The source of this drift has not been determined but is observed in all HRRTs that have looked for it. We again experienced one failure of the RAID this year though the routine backup of set-up data made this a less painful experience than the first time.

MicroPET

The microPET scanner was used routinely throughout the year until the fall when it was traded in on a new Focus 120 from Siemens (formerly Concorde). The Focus 120 offers slightly better image resolution and twice the sensitivity of the R4. There are, however, some issues with the software that are being worked out; dead time corrections are not being applied by the software to emission data. There is an error in the quickscan routine for the daily QC that only seems to be solved by rebooting the scanner and computer. We are somewhat puzzled by the existence of these troubles as the platform is the same for the R4 and the Focus.

Statistics

Table XVII. TR13 run statistics.

	2005	2004
Total runs conducted	709	942
Total runs lost	3	6
Total integrated charge delivered (μA min)	370,840	454,438
Delivered to – LS 3	203,558	310,309
– LS 4	13,464	63,925
– LS 8	12,170	78,518
– BCCA	98,241	–
– LS 33	3,263	–
– LS 39	20,543	–
– LS 56	3,757	1,210
– LS 66	8,667	–
– LS 75	3,834	–
– others	1,701	476

Table XVIII. ECAT scanning statistics.

	2005	2004
Total scans conducted	174	257
Total scans lost	50	64
Lost to – subject	33	31
– cyclotron	3	6
– chemistry	5	8
– scanner	0	12
– staff sick/away	9	7

Table XIX. HRRT scanning statistics.

	2005	2004
Total scans conducted	27	36
Total scans lost	3	9
Lost to – subject	–	–
– cyclotron	–	–
– chemistry	–	1
– scanner	3	8
– staff sick/away	–	–

Table XX. MicroPET scanning statistics.

	2005	2004
Total scans conducted	120	142
Total scans lost	17	19
Lost to – subject	8	12
– cyclotron	1	1
– chemistry	–	–
– scanner	3	4
– staff sick/away	–	–
– ECAT patient	–	2
– other	5	–

Experiment LS3
Synthesis of radiopharmaceuticals for positron emission tomography
(M.J. Adam, TRIUMF)

The PET group at TRIUMF continues to produce 2 to 3 radiopharmaceuticals on any given day despite the fact that we are still awaiting news of funding for our group grant and the departure this year of our senior (24 years at TRIUMF) production technologist, Ms. Salma Jivan. Fortunately, Salma's replacement, Jennifer Greene, has done an excellent job and is keeping production on track. Andrei Studenov, Dave Green and Kelly Crawford are also responsible for carrying out routine production of ^{11}C and ^{18}F radiopharmaceuticals.

Table XXI. Total number of deliveries for 2004 and 2005.

	2004	2005
UBC	323	238
Others*	57	167
Development runs	226	174

* Botany and Chemical Engineering Departments at UBC, PETScan Centre, BCCA-FDG.

Routine production

Table XXI summarizes the total number of deliveries to UBC hospital, other facilities and number of development runs.

Of the 238 deliveries sent to UBC, 9% were processed for the microPET scanner. ^{13}N deliveries for the Botany Department at UBC were reduced but still continue on demand. A decrease in development runs was due to the departure of Salma Jivan but the number of runs remains reasonably high.

We continue to use tetrabutylammonium fluoride, a base source in most of the ^{11}C methyl iodide reactions, to enhance and stabilize yields and lower the amount of precursor used. Most precursors are made in-house while some are purchased from ABX.

New development

Our two newest tracers, [F-18]FHBG and [F-18]FLT, continue to be developed and routinely produced for microPET scanning. FHBG is used as a reporter probe to image expression of herpes simplex virus type-1 thymidine kinase (HSV1-tk) reporter gene and FLT is used as a marker for cell proliferation in cancer cells. Development also continues in upgrading the methyliodide system and improving yields from targets.

Experiment LS4

Targetry

(T.J. Ruth, TRIUMF)

This project supports basic research in the production of radiotracers using the target systems on the TR13 cyclotron. The research aims to develop a fundamental understanding of the science involved in the use of fluid target systems at low energy and high beam current.

This year there were two interrelated studies that had significant results.

Interaction of the proton beam with the target chamber walls

Gas targets are the most common form of target used in the production of short-lived radioisotopes for positron emission tomography (PET). Many researchers, however, have reported a non-linear relationship between radioisotope production yield and particle beam current. This lowered yield has been attributed to several factors including the scattering of beam particles into the target body walls, radioactive species becoming trapped in the target body walls, and gas density reduction due to the deposition of heat from the incident ion beam. In this study we investigate the last factor. A 13 MeV proton beam from the TRIUMF TR13 cyclotron was used to measure the energy of scattered protons in a gas target. The average proton energy reaching the target body walls was determined by measuring the ratio of radioactivity of two simultaneously produced radioisotopes in a metal foil lining the wall of the target.

The production of a radioisotope can be expressed by the following equation:

$$A = \frac{dN}{dt} = nI\sigma(1 - e^{-\lambda t})$$

where

A is the radioactivity produced (disintegrations per second)
I is the proton flux (particles per second)
n is the number of target nuclei (per cm^2)
σ is the cross section (cm^2)
$(1-e^{-\lambda t})$ is the saturation factor (unitless) which takes into consideration the fact that the radioisotopes generated are decaying. There will be a point where the production and decay reach equilibrium.
λ the decay constant ($\ln 2$/half-life, per second)
t the time of irradiation in seconds.

The relationship between the ratio of radioactivities (production of ^{63}Zn/^{65}Zn from copper foils and ^{94}Tc/^{95m}Tc from molybdenum foils) and proton energy was determined using a stacked foil calibration technique. These experiments were compared to theory using a Monte Carlo program (SRIM) to model the interactions of a proton beam within a gas target.

$$\begin{aligned} \text{Activity Ratio} &= \frac{A_1}{A_2} \\ &= \frac{nI\sigma_1(1 - e^{-\lambda_1 t})}{nI\sigma_2(1 - e^{-\lambda_2 t})} = \frac{\sigma_1(1 - e^{-\lambda_1 t})}{\sigma_2(1 - e^{-\lambda_2 t})}. \end{aligned}$$

To date approximately 50 runs for 5 minutes each have been performed on the TR13 for this project.

Future experiments would include varying the target gas pressure and designing and building a new target with a larger radius, as well as including different particle energies and beam current regimes. We would also like to consider the question of why and how different radioactive species appear to be trapped in the target body walls.

Simple method for the determination of the energy of a low energy accelerator

Background Accelerators such as cyclotrons generate charged particles of various energies which are used in a number of applications including the production of radioisotopes for biomedical research and diagnostic medicine. The production cross sections are highly dependent upon the energy of the beam. Thus, having an accurate measure of the energy of the particle beam is very important.

The term cyclotron will be used to refer to any accelerator that produces charged particles, and proton also will serve as the generic charged particle which could be a deuteron or alpha beam.

Cyclotron manufacturers usually calibrate the energy of the beam (internally or extracted) through Rutherford scattering experiments. However, these experiments require sophisticated equipment and operational conditions that are not similar to the conditions under which the cyclotron would be used.

In addition there are circumstances under which the beam energy will be different than expected due, but not limited, to:

- Different stripper foil thickness on negative ion machines,
- Wrong radius of extraction,
- Wrong angle of incidence for extractor (azimuthal), and
- Wrong angle of beam emergence from the cyclotron to the target.

Thus a simple means of measuring the energy of the proton beam is important.

The process described below only requires foils of the appropriate material, a timing device accurate to 1 second and a γ-ray spectrometry calibrated for energy and efficiency.

Approach As described above, the production of radioisotopes is, in general, highly energy dependent. By the choice of foil to irradiate one can generate one or more radioisotopes in the foil, simultaneously. If two radioisotopes are generated and their production cross sections have a different energy dependence, the ratio of the amount produced can be used to determine the energy of the beam.

From this relationship it can be seen that the only variables in the ratio equation are the cross sections (energy dependent) and the time of irradiation. This ratio will have a distinct energy dependence. Note that target isotopic abundances must be taken into account.

The radioactivities are measured using the calibrated γ-ray spectrometer or equivalent device. The n term must reflect the abundance of the corresponding target nuclei in the foil.

An example of such a ratio is for the production of ^{63}Zn and ^{65}Zn from copper foils of natural abundance.

A curve was generated based on the reactions $^{\text{nat}}\text{Cu}(p,n)^{65}\text{Zn}$ and $^{\text{nat}}\text{Cu}(p,2n)^{63}\text{Zn}$. By bombarding a copper foil with protons of unknown energy between the values of 14 and 20 MeV and measuring the induced amounts of ^{63}Zn and ^{65}Zn, their ratio will provide an accurate measure of the incident proton energy. There is no need for an independent measure of the beam current or the size/amount of target material. The irradiation time is only important for calculating the saturation factor, thus the degree of accuracy will depend upon the relationship of the half-life to actual irradiation time. Thus in most cases the precision of the time is minimal.

Limits This technique is only limited by the selection of foil material and the potential radionuclides thus generated by a charge particle beam. An array of foils (reactions, including bombarding particles) can be envisioned that would span the range of energies required for the accelerator of interest. Thus the approach would not be limited to protons or cyclotrons.

Heat transfer Efforts continue in the design of gas targets to optimize the transfer of induced thermal energy to the target walls which can then release the heat to the cooling water. Preliminary results indicate that increasing the turbulence within the target improves the cooling capacity of the system.

Experiment LS8
Radiotracers
(T.J. Ruth, TRIUMF)

This project over the years has worked to support a number of other projects both on site at TRIUMF as well as at member institutions where the use of radiotracers will enhance the investigators' ability to obtain meaningful data for their experiments. The most significant contribution has been in the form of ^{13}N for the Prof. Tony Glass Group in the Botany Department at UBC. This particular project has been separated into its own project (LS76) and a progress report will be submitted separately.

The following projects have benefited from this resource:

LS39 – Positron emission profiling (PEP) for pulp and paper fluid dynamics studies (^{18}F)

LS60 – The physiological role of copper in marine phytoplankton (^{64,67}Cu)

LS71 – Investigation of salt stress in rice plants (requesting sodium isotopes)

LS73 - Production and evaluation of high specific activity ^{186}Re

LS76 – Studies of nitrate uptake in plants and fungi

Expt. 995 – An alternative approach to radioactive beam production for volatile elements ($^{11}CO_2$)

In addition, the 8π group at ISAC has received a number of calibration sources for their detector system that were produced on the TR13 cyclotron.

The possibility of using radiotracers in research continues to attract new potential users and the coming year promises to maintain this involvement in a broad spectrum of activities.

Experiment LS35
Development of ^{18}F labelled nitroimidazole PET imaging agents for tissue hypoxia
(M.J. Adam, TRIUMF; D. Yapp, BCCA)

Hypoxia in cells and tissues is an important component of various pathological states (e.g. ischemia and stroke). Hypoxic tumour cells are extremely important within cancer treatment because they are more likely to survive radiation and chemotherapy, leading to an increase in tumour resistance to treatment. More recent evidence suggests that hypoxia is related to the aggressiveness of disease. Such studies employed a microelectrode, used in many centres, but were limited because of invasiveness and requirement for an accessible tumour.

Derivatives of 2-nitroimidazole are used extensively as hypoxia markers. The 2-nitroimidazoles are not metabolized in oxygenated tissues, but bind to macromolecular proteins after reduction in hypoxic cells. This permits detection by a variety of techniques. For example, the products of such binding for the (pentafluoropropyl)acetamide (EF5) and (trifluoropropyl)acetamide (EF3) derivatives of 2-nitroimidazole can be detected by specific fluorescent antibodies.

The synthesis of ^{18}F-EF5 has now been developed and was achieved by preparing the allyl precursor and fluorinating it with ^{18}F elemental fluorine in trifluoroacetic acid. The radiochemical yield is 17% after HPLC purification.

Submission of a clinical trial application (CTA) with Health Canada in 2005 for a pilot study to examine hypoxia in resectable lung cancer tumours was delayed again while Health Canada finalized its regulations on the human use of positron emitting radiopharmaceuticals (PERs). Thus, our original plan to carry out human PET imaging was delayed despite our hope for submitting an application in the second quarter of 2005.

However, Health Canada has released its final guidance document for submitting applications for PERs (December, 2005); in addition, the first human subject was imaged with ^{18}F-EF5 in the US (Glioblastoma, Cam Koch *et al.*). We are currently in discussion with the Koch group and Varian (who has the licensing rights for ^{18}F-EF5) on the best route forward with this application. Drs. Laskin and Gelmon are still the primary clinicians for this pilot study and will be preparing the application for Health Canada. As part of the preparations for this application, all pyrogen and sterility testing has been completed and all QC and production data are available so the Quality Information Summary (QIS) portion of the application can be finalized. At present we have asked Health Canada for a pre-submission meeting to discuss potential issues in preparing a CTA for a joint radiolabelled and non-labelled study for EF5 in humans.

Experiment LS39
Positron emission profiling (PEP) for pulp and paper fluid dynamic studies
(D.M. Martinez, UBC)

It is widely known that pulp suspensions do not flow until a certain critical shear stress (or yield stress) is exceeded. With traditional papermaking, the papermaking suspension is "fluidized" by turbulence created locally from a sudden expansion in flow area. In this case, the fibre network is broken down into smaller flocs and single fibres with weakly correlated velocities. Characterizing this event is difficult as these suspensions are opaque.

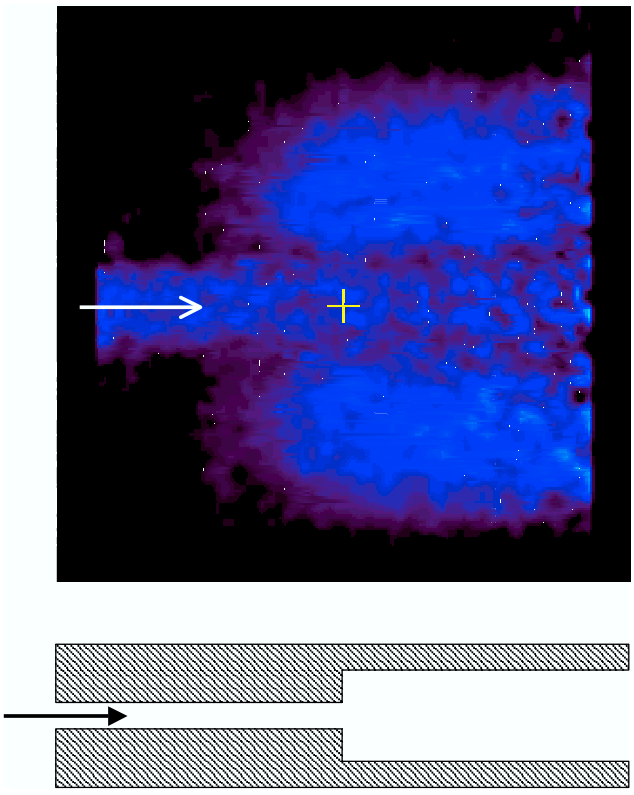

Fig. 214. The activity profile for a dilute SBK pulp suspension travelling through a 1:5 sudden expansion. The upstream velocity of the suspension is 0.9 m/s.

Positron emission tomography was used to investigate the dynamics of a 0.4% (wt) fibre suspension flowing through an axisymmetric 1:5 sudden expansion. Six scans were conducted in which both the upstream velocity and the size of tracer particles labelled were varied. Images were taken upstream and downstream of the expansion plane with the upstream Reynolds number being varied from 7000 to 14000. Both an asymmetry in the flow and a water annulus surrounding the core plug are clearly visible downstream of the expansion (see Fig. 214). We consider these to be the most significant findings in this work and are currently trying to develop a mechanistic understanding of these phenomena.

Experiment LS50
Antisense imaging agents for Parkinson's disease
(H. Dougan, TRIUMF)

This project has been directed at antisense based imaging of Parkinson's disease and tumours. Antisense is a therapy in which small DNA molecules (antisense) are introduced to bind a target messenger RNA ("sense") leading to the destruction or inactivation of the target mRNA. Earlier work at the Pacific Parkinson's Research Centre at UBC was based on natural DNA molecules. In the past, oligos have been infused directly into brain tissue from a narrow-gauge hypodermic cannula. We have gone on to label the same oligonucleotides ("oligos") with radioisotopes ^{125}I and ^{18}F using a small organic molecule termed the "Synthon". In this work non-radioactive control oligos (incorporating ^{127}I and ^{19}F) are assayed for physiological activity.

One concern is toxicity associated with the synthetic oligos, especially when introduced into brain tissue. No results have been obtained with the oligos, *in vitro*, in tissue culture assays, or imaging. Recent work was therefore aimed at the elementary problem of assaying and removing the toxicity. An assay for toxicity was developed based on PC3 human prostate cancer cells. A variety of biochemical techniques were used to remove toxic materials from the oligonucleotides. For example, dialysis of the oligos against pure water was effective in removing the cell-killing effects of oligo directed towards PC3 cells. One question is whether antisense therapy functions when the Synthon is present. Antisense activity was demonstrated in PC3 tissue culture with purified anti-clusterin oligos conjugated to the Synthon. Real-Time Polymerase Chain Reaction, an assay technology highly sensitive to mRNA, was used to detect antisense activity against clusterin mRNA in PC3 cells. Successful *in vitro* hybridization results were also obtained with cancer-related oligos (labelled with Synthon) at the BC Cancer Control Agency.

A major problem with antisense oligos is inefficient entry into cells, or across the blood brain barrier. We have therefore been developing protein technology to carry oligos across the blood-brain-barrier. Radiolabelling of peptide nucleic acid oligos was developed to provide a suitable nucleic acid component to be carried by the protein. Progress was also made with the synthesis of the protein portion, but it has not been thoroughly tested.

Experiment LS53
Synthesis of ^{99m}Tc and ^{186,188}Re sugar derivatives
(M.J. Adam, TRIUMF; C. Orvig, UBC)

An NSERC strategic grant was awarded (October, 2001) (M.J. Adam PI, $78,200/year for 3 years) to carry out research on the synthesis of technetium and rhenium labelled carbohydrates for use in nuclear medicine imaging and therapy. An extension to September 30, 2005 was granted. Dr. Adam is collaborating with Dr. Orvig in the UBC Chemistry Dept., AnorMED (M. Abrams, CEO) and MDS Nordion (Brian Abeysekera). Post doctoral fellows (Dr. Simon Bayly and Nathaniel Lim) and two graduate students (Cara Fisher and Charles Ewart) have also been working on this project for approximately four years. Cara Fisher was awarded an NSERC IPS scholarship for two years, starting in September, 2004, with MDS Nordion as the sponsoring company.

Radiolabelled carbohydrates have been of significant interest to nuclear medicine due to the success of 2-^{18}F-fluoro-2-deoxy-glucose (FDG) as an imaging agent in positron emission tomography (PET). This success has naturally raised the question of whether a single-photon emitting glucose analogue with similar properties to FDG can be developed for use with single-photon emission computed tomography (SPECT). In fact, recently another group demonstrated that a glucosamine Tc complex does indeed get taken up into tumours. The mechanism for this uptake is as yet unknown. Because of the relatively short half life of ^{18}F (110 min) its use is limited to facilities that have an accelerator in close proximity to chemistry laboratories and medical facilities. ^{99m}Tc is the most widely used isotope in SPECT due to the fact that it is a generator produced, commercial isotope which makes it convenient to use and relatively inexpensive. It also has ideal physical properties for imaging. The drawback to this isotope is that it must be attached to the molecule via a chelate or organo-metal conjugate, which may perturb the system being studied. A SPECT analogue based on a widely available isotope such as ^{99m}Tc would make

these agents available to the broader medical community. Among elements of the same series as Tc, the isotopes ^{186}Re and ^{188}Re show promise in the development of therapeutic strategies. For a β^- emitting radioelement to be therapeutically useful, a half-life of between 12 hours and 5 days is preferred; moreover, for a 1 MeV β^- particle, the depth of penetration into tissue is approximately 5 mm. Furthermore, if some of the disintegrations are accompanied by 100–300 keV gamma photon, the behaviour of the radioelement can be conveniently followed by using a gamma camera. The nuclear properties of ^{186}Re and ^{188}Re are optimal for these purposes.

For the last four years we have been developing two synthetic approaches to the preparation of sugar-metal derivatives because of the known avidity of glucose to tumours. One approach forms compounds containing a Cp-M-tricarbonyl moiety (M = Tc or Re) and the other forms chelate compounds with *fac*-$[M(H_2O)_3(CO)_3]^+$ metal cores. With the first approach several ferrocenyl sugars have been synthesized. Attempts to synthesize the corresponding Cp-M-tricarbonyl products have been successful and we are now employing a single ligand transfer reaction. As a side project we have observed that several of these ferrocenyl sugar conjugates have shown cytotoxicity towards mouse lymphoma cells with IC_{50} values less than 20 μm. They are now being tested for cytotoxicity towards human breast cancer cells and are being examimed for antimalarial activity. We are collaborating with groups in London and South Africa to test their antimalarial properties. So far two of these ferrocenyl sugar derivatives have shown moderate antimalarial activity and further testing continues. The second approach involving sugar-pendent ligands has been even more productive and we have now synthesized and characterized several new glucose and glucosamine Tc-99m and Re-186 complexes. The first of these was published last year in Bioconjugate Chemistry [Bayly *et al.*, **15**, 923 (2004)]. These complexes with Tc and Re radionuclides were formed by using the *fac*-$[M(H_2O)_3(CO)_3]^+$ aqueous reagent originally developed by Alberto and co-workers. Since this first glucosamine compound, we have synthesized several pyridinone, diamime and bipyridyl sugar chelate complexes with ^{99m}Tc and ^{186}Re and are now focusing on developing other tridentate and Cp-tricarbonyl ligand compounds. These are expected to be the most stable and thus, more likely to be better candidates as therapy and imaging agents. Several of these chelate ligands have also been complexed with positron emitting Cu-61,64 and Ga-66. Complexes with these isotopes are structurally different than the Tc or Re tricaronyl derivatives giving us a broader range of agents to evaluate. With this combination of SPECT and PET agents, coupled with the recent findings that similar agents are indeed taken up into tumours, we feel these compounds have significant potential as therapy and imaging in oncology. This year we started the *in vivo* animal imaging of some of our Tc and Cu compounds. One of the Tc compounds appears to be taken up into the mouse tumour but further investigation is under way. This year 4 more papers were published on the chemistry.

Experiment LS56
Synthesis of radiolabelled nucleotides and oligonucleotides
(M.J. Adam, T. Ruth, H. Dougan, TRIUMF; D. Perrin, UBC)

This is a progress report on the development of chemistry for the F-18 labelling of oligonucleotides. One of the strategies that we are pursuing is to develop a general method to label the phosphorous atom directly with nca F-18 fluoride so that there is little structural change to the molecule. Thus, the biological activity of the oligo will be preserved. Model compound systems were developed to determine if P(III) and P(V) containing derivatives could be labelled with nca fluoride. We were able to successfully incorporate F-18 into both P(III) and P(V) model compounds. Another approach undertaken by Dr. Perrin's group in UBC Chemistry, makes use of the fact that fluoride anion reacts readily with boron and silicon containing derivatives of oligonucleotides. A grant application to the CIHR to support this work was successful.

Significant progress was made this year on both of these routes. The P-F chemistry was finished to a point where we were able to publish a paper on this chemistry. We will start to apply this chemistry to molecules of interest early in 2006. The work with Perrin *et al.* has proceeded extremely well with a successful proof of principle publication being accepted in the Journal of the American Chemical Society [J. Am. Chem. Soc. **127**, 13094 (2005)]. Application of this novel labelling chemistry is now under way.

Experiment LS57
Quantitative imaging with the Concorde microPET R4®
(V. Sossi, UBC)

This year we traded the microPET R4® for a Focus 120. This had some impact on the physics and methodology studies addressed in this proposal, and had a major impact on LS69. Major achievements were the definition of scanning protocols and acquiring experience in performing accurate comparisons between PET and post-mortem data.

The simulation of the transmission scan on the mi-

croPET using GATE was successfully implemented, thus providing a tool required to develop a scatter correction method for the transmission data. The importance of incorporating positron decay physics into the simulation was demonstrated.

Further studies on the effect of anesthesia on VMAT2 and DAT binding were performed demonstrating most reliable results when a mixture of isofluorane was used.

Two papers related to this work were published [Sossi *et al.*, Phys. Med. Biol. **50**, 2859 (2005); Sossi and Ruth, J. Neural Trans. **112(3)**, 319 (2005)]. Two more are in preparation. Two papers were presented at the IEEE Medical Imaging Conference in Farjardo, Puerto Rico, and one paper was presented at the Academy of Molecular Imaging Meeting, Orlando, Florida.

For the following year we are planning to further develop an accurate attenuation correction method while further efforts will be devoted to data analysis.

Experiment LS59
Mechanisms underlying therapeutic benefit from retinal pigmented epithelial cell implantation for Parkinson's disease
(D.J. Doudet, UBC)

We have little to report on the *in vivo* microPET studies. Our pilot studies in a few rats had shown that the effect of raclopride binding (decrease) in the implanted striatum was transient and we were not seeing an effect on DTBZ binding (increase in implanted side was expected if VMAT2 was functional in RPE cells).

This lack of significant effect could be due to the relatively low resolution of the R4 to image a small change in the physiology of the implanted striatum (rats receive less than 30,000 epithelial cells) but could also be due to a lack of survival of the cells. We elected to focus our interest on this aspect of the research while continuing to collaborate with Dr. Sossi on determining appropriate scanning reconstruction parameters for rats [Sossi *et al., Effect of anesthesia on DTBZ and MP binding as evaluated by a Concord-CTI microPET R4® imaging and post-mortem measures*, Proc. AMI, Orlando, 2005]. Our initial postmortem studies led to data which needed replication and optimization of the immunohistochemical methods and we achieved that aim [Flores *et al., Definitive characterization of long-term implants of human retinal pigment epithelial cells attached to gelatin microcarriers in Parkinsonian rats*, Proc. 16th Int. Congress on Parkinson's Disease and Related Disorders, Berlin, 2005; Cepeda *et al., Intrastriatal implantation of microcarrier attached human retinal pigment epithelial cells ameliorates motor deficits in a rat model of Parkinson's disease, ibid.*; Flores *et al., Magnetic resonance labeling for human retinal pigment epithelial cell implantation: in vitro development of a potential method for in vivo follow up*, Soc. Neuroscience, 2005].

Experiment LS60
The physiological role of copper in marine phytoplankton
(M.T. Maldonado, UBC)

The short-lived radionuclide ^{64}Cu/^{67}Cu was requested in the fall of 2003 to investigate the physiological role of copper in Fe-limited phytoplankton. Physiological research during the last two years in our laboratory suggests that Fe-limited diatoms have a higher demand for Cu than Fe-sufficient cells, and that Cu limitation impairs their high-affinity Fe transport system. These results are the first to demonstrate Cu limitation of marine phytoplankton growth and a role for Cu in phytoplankton Fe uptake. During 2004, after spending almost half a year on method development, Amber Annett determined the intracellular Cu concentrations of one coastal and one oceanic species of diatom, using the radioisotope ^{64}Cu provided by TRIUMF. Her results have shown that the oceanic species has a unique Cu requirement which we believe is associated with the photosynthetic apparatus (over and above the Cu demand associated with their high-affinity Fe transport system). Given the novelty of our findings, we have decided to extend the project to survey 4 additional species to have a more robust data set for publication. Ms. Annett will graduate in May, 2006 and will have the publication submitted by then.

In our original proposal we envisioned investigating the kinetics of Cu transport in marine phytoplankton during year 3. However, we started this work last summer (ahead of schedule) and have made excellent progress thus far. We have already determined the first rates of Cu uptake by Cu-sufficient and Cu-limited phytoplankton, and demonstrated a novel interaction between Fe and Cu transport in these organisms. We are presently investigating the kinetic parameters of Cu uptake in Fe and/or Cu-limited cells (i.e. maximum transport velocity and substrate affinities). This work will be presented at the International ASLO Ocean Sciences meeting in Hawaii in February, 2006 [Maldonado *et al.*, *Copper acquisition mechanisms in centric diatoms of the genus Thalassiosira*], and we expect to submit an article sometime in 2006.

In the coming year, a new Ph.D. student (J. Guo) and a M.Sc. student (L. Moccia) will start working in this project group and will focus their research on the substitution of Cu for Fe in various biochemical pathways of Fe-limited phytoplankton. This work will utilize a new multi-dimensional protein purification sys-

tem to identify all of the Cu and Fe containing proteins expressed by cells.

Experiment LS61
***In vivo* PET study of the effect of striatal implantation of retinal pigment epithelial cells in a primate model of Parkinson's disease**
(D.J. Doudet, UBC)

Due to the delays with the HRRT, we have little to report on this study. We have not performed cell implantation in new monkeys and the HRRT data acquired in 2 animals last year are only being reconstructed now.

Experiment LS62
The effects of electroconvulsive therapy in an animal model of Parkinson's disease: mechanisms of a potential adjunct treatment
(D.J. Doudet, UBC)

Work on trying to understand the mechanism of action of electroconvulsive therapy (ECT) as a therapeutic treatment for Parkinson's disease (PD) has continued in the lab. Pilot studies to develop methods for phosphor imaging autoradiography vs. microPET vs. behavioural assessment were done [Strome *et al., In vivo and in vitro imaging in rats with [11C](±) dihydro-tetrabenazine: measuring the integrity of the dopamine system with microPET vs. phosphor imaging*, Proc. AMI, Orlando, 2005; *ibid.*, Proc. BrainPET05, Amsterdam, 2005]. We have not, however, performed many microPET studies on this project. Because of the time and financial investment needed, we elected to wait for the Focus scanner and to select the tracers to use using first *in vitro* autoradiography.

In the 6-OHDA rat model of PD, using tritiated tracers for the dopamine D1 receptors, we have shown that ECT increases binding to D1 receptors in many regions of the brain that receive dopaminergic innervation in both the lesioned and non-lesioned hemispheres. A manuscript is being written.

We also initiated a collaboration with Dr. Robert Mach at Washington University in St. Louis who has given us some of his newly developed tritiated D3 ligand. We are in the process of using this more specific tracer to replicate our very preliminary data suggesting that the dopamine D3 receptor is upregulated in the brain region where it is most dense; the nucleus accumbens only in the lesioned hemisphere and that effect is enhanced by ECT treatment.

These same animals that show increased dopamine D1 receptors after ECT also show improvements in hindlimb motor function as measured by the tapered beam test.

Another on going study suggests that ECS also specifically increases some neurotrophic factors such as BDNF and FGF2 but not GDNF in the striatum.

We have also been developing an *in vitro* Scatchard technique using phosphor imaging autoradiography and the D1 receptor [11C]Sch23390 and D2 receptor tracer [11C]raclopride. We hope to translate these methods to microPET studies to allow us to monitor changes in the density and affinity of receptors *in vivo* over time.

Experiment LS63
Non-invasive monitoring of tumour progression in the Shionogi tumour model for prostate cancer
(M.J. Adam, TRIUMF; D. Yapp, BCCA)

The original project was based on literature reports suggesting that androgen ablation in prostate cancer was associated with initial oxygenation of the tumour/prostate, but that as the tumour became androgen independent it became more hypoxic. The change in hypoxic fraction in the tumour would have implications for treatment since radiotherapy is increasingly being used with hormonal withdrawal therapy.

The work carried out in this project, however, was to examine 1) whether hypoxia increased in a tumour model for prostate cancer following androgen ablation and 2) whether this change in hypoxia could be detected with EF5 using immunohistochemistry and ^{18}F-EF5/PET. The main goals of the research project, funded by the PCRFC, were completed in 2005; we showed that tumour hypoxia in the Shionogi tumour model, before castration, during regression of the tumour and after the tumour re-grows in an androgen-independent manner, can be imaged with ^{18}F-EF5 and PET. The activity of the radiotracer correlated with flow cytometry analysis of the tissue. Perfusion in the Shionogi tumours was also examined using MRI, and there did not appear to be a correlation with hypoxia.

Data from this project were presented at the 5th ISR (Whistler) and at the American Association for Cancer Research 2004 and 2005 meetings (Orlando and Anaheim, respectively). A manuscript is in preparation for submission to the British Journal of Urology, and the data generated in this pilot project will be used in future applications.

Experiment LS65
***In vivo* study of the direct and indirect striatal output pathways in mild and severe primate models of Parkinson's disease**
(D.J. Doudet, UBC)

Because of the necessary on-going studies done on the HRRT scanner to test and validate acquisition and reconstruction softwares, and because we had not been able to analyze the already acquired data from the first 6 monkeys, we elected to suspend the study until we

have a better grasp of the preliminary result. The kinetic data are being reconstructed and we expect to be able to have a look at them early in 2006. At that time, the decision will be made to continue the study or not, and what animals need to be scanned first.

Experiment LS69
***In vivo* studies on regulation of dopamine turnover using a Parkinson's disease rat model and a microPET**
(V. Sossi, UBC)

The studies described in this research proposal are now funded by CIHR. The start, however, has been very seriously delayed due to the fact that we have been successfully negotiating the trade of the microPET R4® with the Focus 120 starting in March with final Focus delivery in October. The reason justifying the delay was that data obtained on the Focus could not be accurately compared to those obtained on the microPET R4®.

In preparation for this study, however, we have addressed several methodological issues such as comparison between binding obtained with PET and autoradiography and accurate placement of regions of interest (ROIs). Co-registration of PET data with a brain atlas was found to be crucial to an accurate ROI delineation. Furthermore we are currently performing systematic mass effect studies on the Focus 120. The information gathered from these data will be used in the planning of the studies related to this project.

Experiment LS70
Quantification of high resolution brain imaging
(V. Sossi, UBC)

The following progress has been accomplished this year in the studies related to the high resolution research tomograph (HRRT).

1. The multi-centre study was accomplished.
2. A scatter-correction method for list mode reconstruction was developed.
3. Systematic artifacts related to the scanner geometry and emission/normalization mismatch were defined.
4. The quantitative aspect of image reconstruction was implemented and tested with non-human primate studies.
5. The Polaris motion coordinates are being interfaced with the list mode data.

Six HRRT related abstracts were presented at the 2005 IEEE Medical Imaging Conference. A paper entitled "Statistical dynamic image reconstruction in state-of-the-art high resolution" has been published [Rahmim *et al.*, Phys. Med. Biol. **50**, 4887 (2005)]. Two more papers are in preparation.

Current and future studies

1. Development of a hybrid image reconstruction scheme.
2. Full implementation of motion correction.
3. Evaluation of the quantification corrections on parametric imaging on the HRRT.
4. Implementation of an efficient data archival procedure.

Experiment LS73
Production and evaluation of high specific activity ^{186}Re
(S. Lapi, SFU)

Therapeutic radiopharmaceuticals are radiolabelled molecules which are designed to deliver therapeutic doses of ionizing radiation to specific disease sites (most often cancerous tumours) with high specificity in the body. Recently much work had been conducted into the radiolabelling of monoclonal antibodies to test the efficacy of radioimmunotherapy. For site-specific therapy high specific activity is desirable. If an excess of "cold" labelled antibodies are present they may saturate the binding sites on the target (cancer) cells and hence the dose delivered to the cells will not be sufficient to induce apoptosis. While this fact is mentioned repeatedly in the literature, no in depth studies into the effect of specific activity on *in vitro* cell kill has been conducted.

The goal of this research project is to compare the therapeutic efficacy of cyclotron produced high specific activity (HSA) ^{186}Re with commercially available reactor produced low specific activity (LSA) ^{186}Re using radioimmunotherapy. The high specific activity ^{186}Re will be produced via the ^{186}W(p,n) reaction at TRIUMF using the TR13 cyclotron. A chemistry method for the separation of trace rhenium from tungsten targets has been developed using a dry distillation technique. Currently we are optimizing the antibody labelling chemistry of antibodies with rhenium using ^{188}Re, which is readily available from a ^{188}W generator. Antibodies will be labelled with HSA and LSA ^{186}Re and incubated with cancer cell lines available from the BC Cancer Agency. Cell mortality will be determined by traditional methods.

Experiment LS75
Cell proliferation imaged with FLT
(D. Green, BCCA)

FLT (3′-deoxy-3′-[^{18}F]-fluorothymidine) is being studied as a marker of cellular proliferation for tumours using positron emission tomography (PET). The principle behind this theory is that FLT is an analogue of thymidine, a nucleoside required for DNA synthesis and cell proliferation. Thymidine and FLT are both

transported into proliferating cells. The phosphorylation of FLT by thymidine kinase 1 (cytoplasmic enzyme which is upregulated during the S phase of DNA synthesis) creates a charged molecule which remains trapped in the proliferating cell.

Cell proliferation occurs at a higher rate in tumour tissue compared to normal tissue. With this in mind, FLT uptake is expected to be increased in tumour cells. PET imaging with FLT could help determine the fraction of proliferating cells in the tumour. Determining the proliferative state of the tumour will better direct treatment regimes for the tumour in terms of chemotherapy and/or dose of radiation therapy.

WiDr (human colorectal carcinoma) xenografts in non-obese diabetic severe combined immunodeficiency (NOD SCID) mice were used to follow FLT uptake. Mice were injected with $60\,\mu$Ci FLT, anesthetized (isofluorane), and scanned for a 2 hour period to observe FLT uptake from a time-activity curve. Tumour uptake of FLT was similar to that of muscle, and future scans could be followed from as early as 30 minutes post-injection. Scanning occurred 17 days post-implant and a large portion of the tumour consisted of a central necrotic mass. Future work will focus on scanning in the 10–14 day period following implants where less necrotic and more viable tissue should be available.

Experiment LS76
Studies of nitrate uptake in plants and fungi

(A.D.M. Glass, UBC)

Increased nitrogen uptake

(M.Y. Siddiqi, W. Li, UBC; J. Vidmar, Alberta Agriculture Council)

Our goal of increasing nitrate uptake in tobacco plants by over-expressing the high-affinity NO_3^- transporter gene (*AtNrt2.1*) has moved from the laboratory to greenhouse trials. We have developed strains of tobacco over expressing both the *AtNRT2.1* gene and the *AtNAR 2* gene. Using $^{13}NO_3^-$ to measure nitrate uptake, we have obtained increased nitrate uptake and these lines are currently being tested by agronomists in Alberta. We are presently developing canola lines that we hope will also exhibit improved nitrate uptake and yield using the same methodology.

$^{13}NO_3$ influx in the fungus *Aspergillus nidulans*: a structure function study of the *NRTA* gene sequence and NO_3^- uptake

(S. Unkles, J. Kinghorn, St. Andrews, Scotland; A.D.M. Glass, M.Y. Siddiqi, W. Ye, UBC)

In plants, unlike *Aspergillus*, high-affinity nitrate uptake requires expression of two distinct families of genes, namely the *NRT2* group (mainly *NRT2.1 and NRT2.2*) and *NRT3.1* family (formerly named *NAR2*). There are a total of 7 genes in the NRT2 family as well as other plant genes involved in low-affinity nitrate uptake, making it difficult to characterize the gene(s) thought to be responsible for high-affinity nitrate transport. In *Aspergillus* there are only two nitrate transporter genes and they belong to the *NRT2* group. Also we have *Aspergillus* mutants disrupted in both of these genes and hence incapable of growth on nitrate. In trying to express the plant gene in these *Aspergillus* mutants, we were successful only when we replaced the short plant cytoplasmic loop between transmembrane regions 6 and 7 by the long fungal cytoplasmic loop. Using this modified plant gene, efficient nitrate uptake occurred in the fungus without the necessity of co-expressing *NRT3.1*. We have been able to characterize the plant gene with respect to its kinetic properties (Km and Vmax), its capacity to absorb nitrite as well as nitrate, and its sensitivity to inhibition by ammonium ions.

Thus we hypothesize that in *Aspergillus* the long cytoplasmic loop serves the same function as the *NRT3.1* gene of higher plants. To test this hypothesis we have transformed a plant mutant disrupted in *NRT3.1*, with the loop-modified plant gene. Our hypothesis is that the plant gene should now no longer need *NRT3.1*. We are presently generating homozygous strains of this genetically modified line which will be tested for nitrate uptake using $^{13}NO_3^-$.

We are continuing to probe gene structure/function by replacing putative phosphorylation sites (serine and other amino acid residues) of the *Aspergillus* nitrate transporter. We believe that these phosphorylated amino acid residues are responsible for regulating nitrate uptake. The genetically modified fungal strains will be tested for nitrate uptake using $^{13}NO_3^-$.

Role of *NRT3.1* (formerly *NAR 2* gene family)

(A.D.M. Glass, UBC)

Our submitted paper on this work (see last year's Annual Report) was rejected because reports based upon single T-DNA mutation are now no longer sufficient for publication in top journals. We isolated a second mutant disrupted in the ORF region of the gene, rather than in the promoter, and this mutant proved to be more intensely blocked in nitrate influx (96% inhibition). Interestingly, the constitutive influx (influx in plants previously deprived of nitrate) was also blocked in this mutant. Thus this gene (*NRT3.1*) is essential for normal function of constitutive and inducible high-affinity transport by plant roots. Using molecular methods we have evidence that the two proteins are associated in the plasma membrane for normal function. We have been frustrated in our biochemical attempts to co-purify the two proteins and this work is ongoing.

Anticipated need for ^{13}N in 2005

Increased nitrate uptake by tobacco We have overexpressed the *NRT2.1* gene (encoding the high-affinity transporter) and the *NRT3.1* gene and obtained increased nitrate uptake rates of 17% and 7%, respectively, in separate experiments which unfortunately were not statistically significant differences. Nevertheless, although seemingly a small increase, in agronomic terms this increase is large. Our first trials in Alberta gave statistically significant growth yields only when the replicate numbers were large because of large heterogeneity among individual plants. We must return to our $^{13}NO_3^-$ fluxes using larger numbers of replicates.

Gene structure and physiological function: A large portion (perhaps >50%) of applied nitrogen fertilizer is lost from soils. One significant proportion of this loss is attributed to ammonium blocking nitrate uptake. Using the fungus *Aspergillus* as a model system we have been studying the mechanism of this effect. The effect is rapid and due to ammonium *per se* not to its metabolic product, e.g. glutamine. We hypothesize that the *NRT3.1* association with *NRT2.1* described above may serve to shut off nitrate influx when the two proteins move apart, perhaps following protein phosphorylation. We are presently generating lines of Arabidopsis, our model plant in which the two proteins are labelled with different fluors. Using FRET it may be possible to visualize spatial intramembrane movements of these two proteins in response to treatments such as ammonium. We have established a link to collaborate with FRET enthusiasts at Wageningen who have agreed to assist us in these methods. We are simultaneously exploring the use of antibodies directed against phosphorylated residues to investigate the possible role of phosphorylation in regulating *NRT2/NRT3* function.

Nitrite transport Although nitrite is not typically present in large amounts in the environment, it does accumulate in waterlogged and anaerobic soils. Using net uptake and NO_2^-: $^{13}NO_3^-$ competition studies with *Aspergillus* as a model organism, Wang Ye has shown that both of the *Aspergillus* nitrate transporters are also nitrite transporters. Their affinity for nitrite is actually greater than that for nitrate. In the double mutant (lacking both nitrate transporters) there is still very significant nitrite transport but no nitrate transport. This means that there is an additional nitrite transporter. Our Scottish collaborators have identified a putative nitrite transport mutant and now successfully cloned the nitrite transporter (nitA) and we have completed characterizing nitrite influx in wild type *Aspergillus* strain using $^{13}NO_2^-$. The nitrite transporter has a very low Km for nitrite, ~4 μM. This compares to ~10 and 100 μM, respectively, for the two nitrate transporters.

This summer Profs. Kinghorn and Unkles will travel to Vancouver to undertake a series of $^{13}NO_2^-$ flux analyses using mutants blocked in nitA and with specific amino acid residues replaced to establish a structure function relationship for this gene.

Ammonium transport in Arabidopsis Earlier work from this laboratory going back to the 1990's examined $^{13}NH_4^+$ influx in rice and then later in Arabidopsis. We were able to demonstrate that in Arabidopsis *AMT1.1* was responsible for only ~30% of high-affinity ammonium influx. Subsequent work revealed as many as 6 members of the AMT family and, working at Adelaide, Brent Kaiser has obtained mutants and various crosses among the mutants to enable us to analyze the roles of all of the AMT1 family of genes. Beginning in April, Dr. Kaiser's graduate students and PDFs will travel to Vancouver to begin flux analysis with these mutants.