Mass Spectrometer-based Flow Cytometer, Methods and Applications

StemSpecs

Volume 1, Issue 2 **November, 2005**

Welcome to the second issue of StemSpecs, the on-line newsletter of "Mass Spectrometer-based Flow Cytometer, Methods and Applications", a Genome Canada project launched in February 2005. This issue includes a biography of Scott Tanner - the leader of the instrumentation team, before and after photos of the new AIMS (Advanced Institute for Molecular Structure) facility and an introduction to some of our collaborators. We've also included photos from our recent meetings.

Our website, launched in June 2005, is intended to inform readers of the goals and structure of our project, and to introduce you to the multidisciplinary team that is doing this work. If you haven't seen the site, please have a look, starting from our homepage: http://www.uhnres.utoronto.ca/studies/stemspec/index.html. We hope that you will find the website informative, and that you'll check back to follow our progress.

Since our last issue, we have had meetings with both our international Scientific Advisory Council and our Canadian Scientific Advisory Group. Both meetings were very productive, and we are all grateful to all of our advisors for the time, effort and travel that they have dedicated to our project.

Welcome to StemSpecs; see you again soon.
Sincerely,
The Editors

Editor-in-Chief: John Dick
Contributing Editors: Scott Tanner, Amy Dambrowitz

Inside this issue:

This project was funded by Genome Canada through the Ontario Genomics Institute.
Complementary funding is gratefully acknowledged from:
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Biography: Scott Tanner

Dr. Scott Tanner, leader of the Stemspec mass spectrometry group at the University of Toronto wants to build an inductively coupled plasma mass spectrometer (ICP-MS) with cytometric capabilities. Scott and his team are creating an instrument which will combine flow cytometry with elemental analysis, and will enable the identification of rare cells in complex samples by simultaneously analyzing multiple (50+) protein and small molecule markers that are specific to and characteristic of a selected cell type. Dr. Tanner's team predicts that this technology will enable cell biologists to find "a needle (the cell) in a thousand haystacks (a cohort of unsorted cells)".

Confidence in camaraderie – (From left to right) Dmitry Bandura, Scott Tanner and Vladimir Baranov do the wave at the project kick-off party (April 2005).

Dr. Tanner, a recent addition to the Institute of Biomaterials and Biomedical Engineering (IBBME) at the University of Toronto, grew up in St. Catharines, in the heart of Ontario's Niagara Region. He bought his first chemistry set at the age of six (second hand, from his older brother, for five dollars). Six years later, in 1965, Scott made his first attempt to reproduce Rutherford's experiment (alpha particle penetration of gold atoms). After coating the inside of the family oven with melted Plexiglas and covering most of the surfaces of the laundry room with shattered glass and zinc sulphide, Scott was encouraged by his family to accept a small laboratory space offered to him at the newly founded Brock University. Under the auspices of Dr. E.A. Cherniak and Dr. F.P. Koffyberg, Scott used his space to build cloud chambers, experiment with vacuum systems and eventually conclude (in 1970) that he was not going to observe alpha particles on a scintillator; in Scott's words "it was a good lesson for a future instrument developer".

After he completed his B.Sc. (Honours) at York University in 1976, Scott, a nationally ranked gymnast, was forced to make a decision. Scott could either continue to train for competition at the 1980 Olympic Games, or pursue a Ph.D. Scott chose to focus on Chemistry and began his doctoral research, co-directed by Dr. John Goodings, a pioneer of gas-based ion chemistry in flames, and Dr. Diethard Bohme, one of the world's preeminent ion molecule chemists. It was a fortunate choice; Scott graduated with his Ph.D. in Physical Chemistry in 1980, the same year that Canada joined the US-led boycott of the Summer Olympics in Moscow. Dr. Bohme, a Canada Research Chair in Physi-

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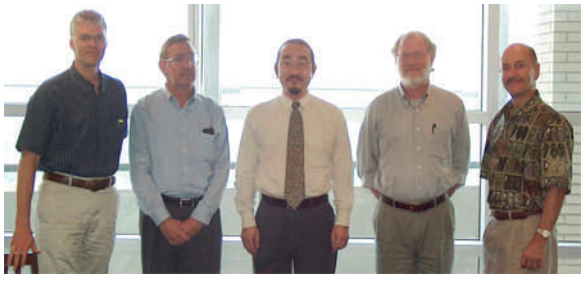
International Support



The first annual meeting of our international Scientific Advisory Council was held on July 18 at the Bahen Centre for Information Technology at the University of Toronto. Council members include: (From L to R) Guy Sauvageau (Universite de Montreal), Diether Recktenwald (BD Biosciences), Hiro Suga (University of Tokyo), Ger van den Engh (Institute for Systems Biology/Cytopeia) and Gary Hieftje (Indiana University). The council listened critically and provided us with plenty of encouragement, positive feedback and thoughtful advice. We're looking forward to next year's meeting and to all of our interim conversations with council members.

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cal Chemistry, has remained an inspiring mentor and good friend to Scott and his team members.

Upon completing his Ph.D., Scott joined the growing team at Sciex (now MDS Sciex) as a Senior Scientist. His early research

Parallel goals – For Scott, a part of the attraction to York University was that athletes competing for the York Yeomen trained with the coach of the Canadian national gymnastics team.

interests included the development of mass spectrometric methods for the detection of explosives and contraband, detection of chemical and biological warfare agents, determination of dioxins in soil, food and water, and the identification and tracking of volatile organic compounds (air pollutants).

In 1994, Scott became the leader of the ICP-MS research group at MDS Sciex. Vladimir Baranov joined the group in 1996 and Dmitry Bandura in 1998. In 1997, Scott and Vlad invented the Dynamic Reaction Cell (DRC) for ICP-MS. The DRC is a highly efficient on-line chemical reaction filter which acts as a mass bandpass filter to remove isobaric interferences before analytes enter the MS detector. The DRC won the gold medal for the best new product at PittCon in 1999, and in 2001, Scott and Vlad were awarded the Manning Innovation Foundation Award of Distinction for the invention of the DRC. That same year, Scott was promoted to Principal Scientist at MDS Sciex. Scott, the author of over fifty publications, coeditor of four books and coauthor of 10 US patents and patent applications, was further honoured in 2003 with the W.A.E. McBryde Medal for young analytical scientists in Canada (44 years after the purchase of that fabled used chemistry set).

Meanwhile, the ICP-MS group at MDS Sciex had begun work in 2000 on an element-tagged immunoassay (an immunoassay designed to be compatible with an ICP-MS detection system). The team had realized that it was time to introduce the strengths of inorganic mass spectrometry to the arena of bioanalytical chemistry. ICP-MS instruments feature large dynamic range, high sensitivity and absolute quantification, which is a powerful combination for the analysis of biological systems.

In 2002, Scott and his team met Dr. John Dick and Dr. Mark Minden, two leukemia researchers. John Dick's research had demonstrated that acute myeloid leukemia (AML) is a stem cell disease. John and his coworkers had found that AML development is sustained, not by the majority of cells that comprise the tumour's bulk, but by the few cancer stem cells at its root. These leukemic stem cells (LSC), representing far less than one percent of the cells in a tumour (approximately one in a million), drive the tumour's growth, immortality and malignancy. In order to understand how the LSC differ from normal, healthy hematopoietic (blood) stem cells and to be able to detect the LSC in samples from patients, John and Mark needed new tools. This was the intersection where Scott's inorganic analytical chemistry met John and Mark's biological chemistry and medicine.

The group agreed that it is reasonable to expect that a cell of interest has certain protein "markers" that distinguish that type of cell from other cells in the sample. They constructed a scheme to detect those markers using a radically modified ICP-MS instrument and novel reagents. As Scott describes it:

"An affinity product (antibody or aptamer) that has been tagged with a specific element binds to cellular protein marker. When the cell is introduced into the ICP, it is atomized and ionized. The elemental composition of the cell, including the tagged affinity products that are bound to the protein markers, is measured.

Multi-tasking – Scott ran six marathons (and defended his Ph.D.) between 1978 and 1981 – his best time was 2:47:13.

The presence and intensity of the signals corresponding to the tags

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indicates the relative expression of the biomarkers on that cell. The distribution and concomitance of those signals can be used to identify the cell type."

In other words, if the protein markers could be element-tagged, Scott and his team could provide the means to quantitate those markers on individual cells, and discern the patterns that are unique to LSC. John and Mark could use such an instrument for two important purposes. First, to determine the molecular changes that occur to change a healthy stem cell into an LSC and second, to determine what types of cells are present in patient samples. These applications would be important both to understanding the fundamental biology of AML and to giving individual patients individualized diagnoses. In 2003, John and Scott, along with Mark and Dr. Yingfu Li, submitted a project proposal to build a Mass Spectrometer-based Flow Cytometer to the Genome Canada Applied Health Competition.

The proposal was funded in 2004, with great enthusiasm from the Genome Canada review panel. Unfortunately, in the intervening months, MDS Sciex had informed the ICP-MS team that the project was outside the business mandate of the company. Scott, Vlad and Dmitry were faced with a conundrum. They wanted to pursue their new project, but they had been very productive at MDS Sciex: they were able to work without applying for research funding, outstanding colleagues surrounded them in a state-of-the-art facility, and Sciex wanted them to stay and add to their impressive

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The All Participants Meeting

Attendees met their collaborators, heard up-to-the-minute research updates from each group involved in the project, engaged in a lively cross-disciplinary discussion, and consumed an impressive amount of pizza.

On September 14, we had our first All Participants Project Meeting at the Princess Margaret Hospital. All of the project researchers were invited to attend, and one member of each research group was asked speak about the latest work in their lab. Introductions, colour commentary, photography services and wrap-up were ably provided by Drs. John Dick and Scott Tanner. An attentive, interdisciplinary crowd of thirty-five kept the speakers busy with insightful questions and continued the discussion around the refreshments table after our official wrap-up.

Dr. Olga Ornatsky, from the Tanner lab (University of Toronto) outlined her latest results in the analysis of biomolecules with element tagged reagents. Olga has been testing the signals produced by a number of affinity reagents using both conventional flow cytometry and bulk ICP-MS analysis. Her results are promising, and confirm our assumption that, in order to produce the best possible detection results for single cell analysis by ICP-MS, the primary affinity reagents will need to be directly labeled with the element tags.

Olga Ornatsky discusses the analysis of biomolecules with element tagged reagents

The element tags and the linkers that will be used to attach them to affinity products are being developed in the labs of two collaborators: Drs. Mitch Winnik and Mark Nitz of the Chemistry Department at the University of Toronto. Representatives from both labs attended the meeting and explained the proposed structure of the reagents. Dr. Xudong Lu, a collaborator from the Winnik lab,

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Xudong Lu describes the synthesis of lanthanide-containing functional copolymers

Naveen Kumar outlines the generation of DNA aptamers for the detection of cancer-related markers

John Dick clarifies a point about AML biomarkers

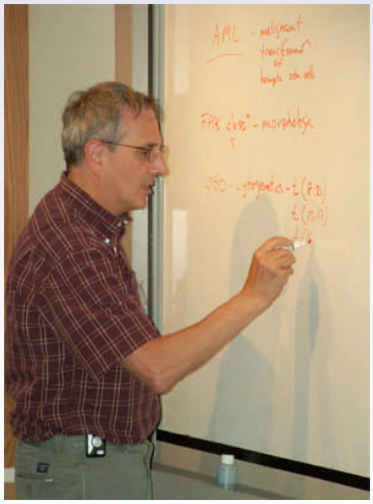
described the synthesis of the functional copolymers that will be used to link element tags to our affinity reagents. Mark Nitz followed Xudong and explained the structure of the lanthanide chelators (our element tags) that will be connected to the polymer linkers.

Dr. Naveen Kumar from the Li lab (McMaster University) described the generation of DNA aptamers specific for AML-related targets. Naveen explained a number of different approaches that the Li group is employing to select the aptamers, including CE-SELEX and the selection of aptamers against whole cells. In collaboration with Mark Nitz, the Li lab is also labeling oligonucleotides with the lanthanide chelators; Naveen outlined their synthesis scheme.

John Dick's lab (University Health Network) was represented by Dr. Liqing Jin. Liqing described the characterization of CD123, a potential marker of leukemic stem cells. The Dick lab is currently analyzing the function of a number of oncogenes, all of which may be potential targets for affinity product development during the course of the project.

Dr. Mark Minden (Ontario Cancer Institute) was our final speaker. Mark explained the generally accepted prognostic subclassification systems for acute myeloid leukemias and outlined the chemotherapy treatment that is used for many AML patients. The disease is currently classified using morphologic and cytogenetic methods. Mark believes that our flow cytometer-based ICP-MS will present a unique opportunity to analyze the characteristics of these leukemias at the molecular level, and give us further in-

Liqing Jin discusses CD123, a marker of AML stem cells



Mark Minden explains the medical subclassifications of acute myeloid leukemia

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sights into patients' disease states, especially those whose disease falls outside the current classification.

The meeting provided a great opportunity to have scientists from a wide range of disciplines in the same room, talking about their shared goals, which gave all of the attendees a sense of the scope of the project. We're already looking forward to next year.

Extreme Lab Makeover

The renovations of the new, purpose-built mass spectrometry facility in the Chemistry Department of the University of Toronto were completed in late June 2005. The Tanner group moved in and got to work immediately. The facility, which will be shared with members of the Chemistry Department, was named AIMS (the Advanced Instrumentation for Molecular Structure) and a grand opening was held September 29, 2005.

Before (taken in early May 2005 – with Vladimir Baranov inspecting the duct work)

After (taken June 29, 2005)

Scott Tanner Biography (Cont)

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track record of new inventions. However, the team believed that the project was of sufficient importance for them to leave MDS Sciex and pursue their goals in a new place. The search for that "new place" ensued, and with the help of their friend and colleague, Dr. Javad Mostaghimi, they found appointments at the IBBME at the University of Toronto. With funding from Genome Canada and the Department of Chemistry at the University of Toronto, they also found a new physical home in a purpose-built laboratory in the heart of campus.

In early 2005, Scott, Vlad and Dmitry parted ways with their colleagues at MDS Sciex and accepted positions at the University of Toronto. It was hard to leave the people of Sciex behind, but the team was looking ahead to their new challenge. When asked about the risks, Scott put it simply:

"The only reason that I'm willing to take a risk on this project is my confidence in the quality and camaraderie of my colleagues."

Not a bad opening line for a new chapter in Scott's biography.

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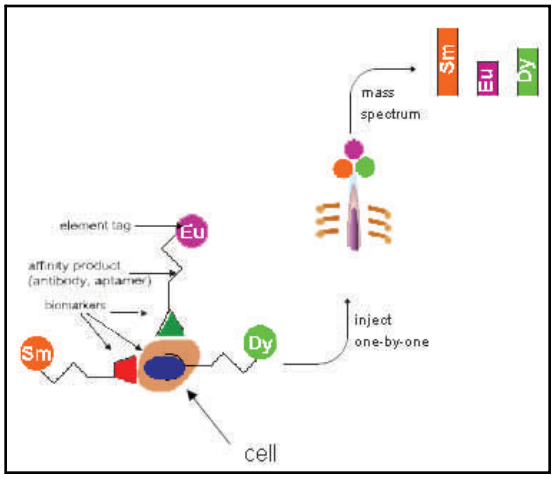
Attaching The Element Tags

Detection of the biomarkers on our cells of interest will be achieved by:

1. Labeling those biomarkers with affinity products that have element tags attached
2. Quantitating the element tags by ICP-MS.

The Stemspec group is excited to be working with two collaborators in the Department of Chemistry at the University of Toronto to create a suite of universal element tags that can be attached to any affinity product that is needed for a particular detection protocol. Dr. Mark Nitz will be directing the synthesis of lanthanide chelators (the lanthanides will be the element part of the element tags). Dr. Mitch Winnik's lab will synthesize the functional copolymers that will be used to link the chelators to our affinity products.



Mark Nitz

For more information about Mark Nitz, please visit: http://www.chem.utoronto.ca/peoples/facult_profile.php?id=80

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