1. SUMMARY

Career focus. *Combining molecular and biophysical methods to understand the biological roles of molybdenum and flavin-containing electron-transfer enzymes*. My focus is to seek an atomic-resolution understanding of how electron-transfer enzymes are assembled, how they catalyze important reactions in biology, and how they impact human health.

Fundamental biological questions. (*i*) *How are electron-transfer enzymes assembled and targeted to their final cellular locations?* (*ii*) *How do their cofactors function in electron-transfer and catalysis?* (*iii*) *How do these enzymes contribute to bacterial metabolic diversity?* I am answering these questions using two types of model system, each containing distinct electron-transferring cofactors: (*i*) enzymes containing molybdenum, including the respiratory nitrate reductase (NarGHI) and the sulfite oxidase homolog YedY; and (*ii*) enzymes containing flavins, specifically two closely-related examples - succinate dehydrogenase and fumarate reductase. These model systems are critically-important: (*i*) *molybdoenzymes play roles in human health, bacterial metabolism, agriculture, and geochemical sulfur and nitrogen cycles; and (ii) functional succinate dehydrogenase is essential to human health and aerobic metabolism.*

Worldwide impact. My 1998 discovery (see attached CCV) of how fully-folded cofactor-containing enzymes are translocated across biological membranes spawned an entirely new field of research. My expertise in protein overexpression and purification led to the determination of the structure of NarGHI in 2003 (with Natalie Strynadka, UBC), providing an atomic-resolution framework for understanding long-range electron-transfer. My work on the pyranopterin component of the molybdenum cofactor challenged the paradigm that only the immediate metal environment is critical in defining catalysis. My work has been cited over 3,800 times in international scientific journals.

Expertise and experience. I have embraced emerging techniques over almost 40 years as an independent investigator. These include, in historical order of application: use of bacterial plasmids (1970s); DNA cloning, sequencing, and overexpression (1980s); enzyme tagging, affinity chromatography, protein purification and crystallography (1990-); spectroscopic and kinetic techniques such as electron paramagnetic resonance (EPR) and stopped flow kinetics (1980-); and "informatics"and "omics" driven endeavors (2000-).

Collaborations. I created and was the first Director of the prestigious CIHR Molecular Biology of Membranes Research Group. Other initiatives included collaborative grants from NATO, the Human Frontiers Science Program, and the National Institutes of Health. Ongoing collaborations include: with Natalie Strynadka (UBC; determination of the structures of NarGHI and YedY); with Martin Kirk (University of New Mexico; determination of the role of pyranopterin conformation in defining molybdoenzyme catalytic diversity); and with Fraser Armstrong (Oxford; application of novel electrochemical methods to molybdo- and flavo-enzymes).

Vision of Proposed Research Program. *To obtain an atomic-resolution understanding of the role of electron-transfer enzymes in biology and apply this understanding to problems in human health. My vision is of basic science discovery. "It's simple: If there is no basic research upstream, there is no application and therefore no added value downstream." Dr. Alain Beaudet, CIHR President, June 2013.*

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2A. LEADERSHIP

Recognition – from international conference presentations to the Royal Society of Canada. I have presented >100 talks at international conferences and other universities, including at Gordon Conferences and at European Bioenergetics Conferences. I have had a leadership role in the organization of 21 conferences, most recently being the chair of the International Meeting on Molybdoenzymes at the University of Alberta in 2011. Recent awards recognizing my accomplishments include a Canada Research Chair in Membrane Biochemistry (2001-2008), a Killam Annual Professorship (2005-2006), an IUBMB Distinguished Service Award (2006), and election as a Fellow of the Royal Society of Canada in 2011 (for others, see CCV).

Leadership - from institutional to international. Catalyzing rapid scientific progress requires collaborations between individual lab members, and more importantly, the construction of multidisciplinary synergies between research groups. Consequently, in 1990 I formed the CIHR Membrane Protein Research Group, initially composed of five research teams at the University of Alberta, and this group immediately became an incubator that matured a large number of initial collaborations into prestigious publications. The international collaborations I led (funded by NATO, Human Frontiers Science Program, and NIH) fostered highly-productive multidisciplinary efforts to understand the structure and function of electron-transfer enzymes. I also served as Associate Dean for Research in the Faculty of Medicine between 1993 and 2005, and this appointment enabled me to encourage the wider university community to enter into collaborations from the institutional to international levels.

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2B. SIGNIFICANCE OF CONTRIBUTIONS

Advancement of knowledge. I have had a huge impact on our understanding of the assembly, expression, and structure of membrane-bound electron-transfer enzymes, using a range of molybdo- and flavo-enzymes as model systems. Each has required the development of protein expression, purification, and characterization systems. Seemingly intractable problems of membrane protein overexpression and purification have often had to be addressed before work leading to “pinnacle” publications could even be contemplated. Over my career, the methods I developed have been applied in many research groups around the world. My work has been continuously funded by the MRC and then the CIHR since 1977. The significance of my work is such that I was awarded two concurrent CIHR operating grants over the last two funding cycles. I obtained training awards for summer students from the Alberta Heritage Foundation and then Alberta Innovates every year since the early 1980s, and I set up and obtained CFI/AHFMR equipment funding for the UofA's electron paramagnetic resonance facility.

Training of highly qualified personnel (HQPs). The key to generating successful HQPs is to provide a truly multidisciplinary training environment, and this has been one of my key achievements. Because of this, many former trainees have gone on to enjoy outstanding success as independent investigators, including Drs. Bernard Lemire (UofA), Peter Bilous (Eastern Washington University), Russell Bishop (McMaster University), Raymond Turner (University of Calgary), John Robinson (Memorial University), and Damaraju Sambasivarao (UofA). Many others have achieved success in medicine, the biotechnology industry, in technology commercialization, or in university administration.

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2C. PRODUCTIVITY

I have published over 200 papers (*h* index 42) and hold 4 patents describing protein translocation and export technologies. I focus on two related research directions: the maturation and membrane translocation of fully-folded molybdoenzymes; and how molybdoenzymes and flavoenzymes function at atomic resolution (see CCV). My productivity can be summarized as follows. **O*verexpression and assembly of complex membrane proteins***: I overcame the problems of membrane protein overexpression, enabling characterization of number of electron-transfer enzymes. I discovered the twin-arginine translocase, revolutionizing our understanding of how fully-folded cofactor containing proteins are translocated across membranes. I discovered a novel protein export system in *E. coli* that transports proteins of interest into the bacterial growth medium. ***Characterization of electron-transfer enzymes***: I use a multidisciplinary approach (e.g. EPR, fluorescence) to address electron-transfer and catalytic mechanisms in a range of molybdo- and flavo- enzymes. ***Determination of enzyme structures*:** I collaborated with Natalie Strynadka (UBC) to solve the structures of two important molybdoenzymes – the large membrane-bound nitrate reductase and a soluble sulfite oxidase homolog known as YedY. ***The role of the pyranopterin component of the molybdenum cofactor***: I collaborated with Martin Kirk (University of New Mexico) to address the fundamental reason for the structural complexity of the molybdenum cofactor, demonstrating that additional redox states are available to its pyranopterin component. My work has been published in *Cell*, *J. Mol. Biol.*, *Nature* (*Struct. Biol., Biotechnol.*, and *Chem. Biol.*), *Proc. Natl. Acad. Sci. USA*, *J. Biol. Chem.*, and *Biochemistry*.

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3. VISION AND PROGRAM DIRECTION

Vision: understanding the role of electron-transfer enzymes in biology and human disease. Aerobic organisms depend on electron-transfer enzymes located in mitochondria for their survival. Electrons are transferred from reduced substrates to oxygen in a process that generates ATP. *Electron-transfer is thus essential to higher organisms, including man*. It also plays roles in global geochemical cycles: in the agriculturally-significant reduction of nitrate to nitrite and the environmentally-significant oxidation of sulfite to sulfate. Electron-transfer enzymes also play roles in human disease: point mutations can have deleterious consequences (e.g. neurodegeneration) and have been implicated in tumorigenesis (*e.g.* paragangliomas and pheochromocytomas); and point mutations in human sulfite oxidase can be embryonic lethal. I will continue to advance our knowledge of a critically-important family of enzymes. *Biological electron-transfer is critical to human health, either directly through enzyme-related disease states; or indirectly, through its impact on environmentally pivotal global geochemical cycles. My program represents a logical career progression, continuing the application of decades of innovative approaches to critical biological questions.*

Tractable bacterial model systems. Genetic testing has linked disease states to point mutations that generate dysfunctional electron-transfer enzymes. The recent explosion of available protein sequence data has revealed bacterial homologs for many enzymes implicated in human disease. These homologs can be expressed and purified in batches approaching tens to hundreds of milligrams, enabling spectroscopic, kinetic, and structural analyses that are impossible with patient biopsy samples. The advantages of bacterial protein overexpression extend to those electron-transfer enzymes important in global geochemical cycles. In all cases, variants can be rapidly designed, over-expressed, purified and characterized. The use of bacterial model systems will continue to be the foundation of my research program. *I will continue to use a range of Escherichia coli model systems so that hypothesis testing is not shackled to an individual system*.

Understanding redox-active cofactors. These cofactors either form the sites of catalysis (e.g. flavin and molybdenum cofactor), or form stepping stones in electron-transfer relays ("molecular wires") that can extend to ~100Å in length (e.g. hemes and iron-sulfur clusters). My focus is to delineate the mechanism of the electrochemical steps enabling catalysis, and to understand how disease-inducing variants impact catalytic efficiency. *How has evolution enabled effective electrochemistry and electron-transfer? How do the cofactors contribute mechanistically to catalysis? What are the consequences of disease-mimicking variants on catalysis? How does the biological membrane impact catalysis? How are cofactor insertion and subunit localization orchestrated to generate active mature enzyme? Can enzyme variants mimicking those implicated in human disease provide insights into possible therapies?*

Impact of advancement of knowledge. My overarching theme is to understand biological electron-transfer and its impact on disease states and global geochemical cycles at atomic resolution. My program will provide excellent opportunities for research trainees to address basic science problems, which can ultimately be translated into understanding disease states and possible therapies.

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