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Welcome

Welcome to the Brazil-Canada Workshop on Advances in the Science of Prion and Prion-like Misfolding Diseases. The Alberta Prion Research Institute, the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) are excited to co-host this workshop in the beautiful town of Banff, Alberta, Canada. Finding solutions to the serious scientific and socioeconomic challenges associated with prion and prion-like diseases is a priority for both Brazil and Canada and specifically for Alberta. The Alberta Prion Research Institute, FAPESP and FAPERJ support research projects that take an innovative and inventive approach to solving the mysteries of prion and protein misfolding.

Innovation is driven not only by talent but also by collaboration and knowledge exchange. During this two-day meeting, 25 researchers from Canada and Brazil will present their recent findings in the fields of prion and protein-misfolding science, and there will be multiple opportunities for questions, discussion and networking. As well, 17 Albertan trainees will present posters.

We would like to thank attendees for their participation in the workshop and their dedication to the prevention, treatment and surveillance of prion and prion-like diseases. Your groundbreaking research is changing the fields of prion and protein misfolding science in Canada, Brazil and globally. This conference is just the first step in a scientific partnership between Alberta and Brazil, and there will be further collaborations in the future.

We hope you enjoy the Brazil-Canada workshop and your stay in Banff.

Sincerely,

Dr. Kevin Keough
Executive Director
Alberta Prion Research Institute

Professor Vilma Martins
AC Camargo Cancer Center

Professor Jerson Silva
Scientific Director
Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro

Schedule

Tuesday, March 18, 2014

Time	Event	Presenter(s)	Room
7:30 - 8:10	**Breakfast**		Hawthorn C
8:15 - 8:30	Welcome and Opening Remarks	Kevin Keough, Vilma Martins and Jerson Silva	Wildrose A
8:30 - 9:00	The role of exossomal secreted Stress Inducible protein 1 in PrP^C-dependent protection against injury *10-minute Q&A*	Vilma Martins, AC Camargo Cancer Center	
9:03 - 9:33	Characterization of prion and prion-like proteins *10-minute Q&A*	David Westaway, University of Alberta	
9:36 - 10:06	PrP^C in metabolic diseases *10-minute Q&A*	Glaucia Hajj, AC Camargo Cancer Center	
10:09 - 10:39	Prion systems biology using rat-adapted scrapie *10-minute Q&A*	Judd Aiken, University of Alberta	
10:39 - 11:10	**Networking and Posters**		Wildrose B
11:13 - 11:43	The prion organotypic slice culture assay revisited: application to new species, new brain regions, and therapeutic studies *10-minute Q&A*	Valerie Sim, University of Alberta	
11:46 - 12:16	Prion protein-STI1 engagement modulates proliferation and self-renewal of neural progenitor/stem cells from developing and adult nervous systems *10-minute Q&A*	Marilene Lopes, Universidade de São Paulo	
12:19 - 12:49	Prion infections: from molecular biology to therapy and prophylaxis *10-minute Q&A*	Hermann Schätzl, University of Calgary	
12:49 - 13:39	**Lunch and Posters**		Hawthorn C
13:44 - 14:14	Pathological, therapeutic, and functional implications of prion protein interaction with ligands *10-minute Q&A*	Yraima Cordeiro, Universidade Federal do Rio de Janeiro	
14:17 - 14:47	Propagated misfolding of SOD1 in ALS: a new prion-like disorder *10-minute Q&A*	Neil Cashman, University of British Columbia	
14:50 - 15:20	Microglia-derived TNF-a mediates depressive-like behavior induced by amyloid-b oligomers in a mouse model of Alzheimer's disease *10-minute Q&A*	Sergio Ferreira, Universidade Federal do Rio de Janeiro	
15:23 - 15:53	Identification of eukaryotic chaperones involved with protein disaggregation *10-minute Q&A*	Carlos Ramos, Universidade de Campinas	
15:53 - 16:23	**Networking and Posters**		Hawthorn C
16:26 - 16:56	PrP loss of function in zebrafish to understand conserved functions and assess conserved protein interactions in vivo *10-minute Q&A*	Ted Allison, University of Alberta	
16:59 - 17:29	Characterising misfolding in the prion protein PrP at the single-molecule level *10-minute Q&A*	Michael Woodside, University of Alberta	
	Daily Wrapup		
19:00 - 21:00	**Dinner**		Hawthorn C

Schedule

Wednesday, March 19, 2014

Time	Event	Presenter	
7:30 - 8:10	**Breakfast**		Hawthorn C
8:15 - 8:20	Housekeeping	Kevin Keough	Wildrose A
8:20 - 8:50	Prion-like aggregation of p53 in human cancer: new targets for antitumoral drugs *10-minute Q&A*	Jerson Silva, Universidade Federal do Rio de Janeiro	
8:53 - 9:23	A hybrid approach towards the structure of the infectious prion protein *10-minute Q&A*	Holger Wille, University of Alberta	
9:26 - 9:56	Structural aspects of the interaction between prion protein and heparin: importance for health and disease *10-minute Q&A*	Tuane Vieira, Universidade Federal do Rio de Janeiro	
9:56 - 10:26	**Networking and Posters**		Hawthorn C
10:29 - 10:59	Determining the diversity of CWD strains *10-minute Q&A*	Debbie McKenzie, University of Alberta	Wildrose A
11:02 - 11:32	Defective insulin signaling and inflammation as common molecular denominators connecting type 2 diabetes to Alzheimer's disease *10-minute Q&A*	Fernanda De Felice, Universidade Federal do Rio de Janeiro	
11:35 - 12:05	Peptide aptamers as therapeutic and analytic tools in prion research *10-minute Q&A*	Sabine Gilch, University of Calgary	
12:05 - 12:55	**Lunch**		Hawthorn C
13:00 - 13:30	PrPC modulates the secretion of extracellular vesicles by astrocytes *10-minute Q&A*	Marco Salles Dias, AC Camargo Cancer Center	Wildrose A
13:33 - 14:03	The challenge of atypical BSE *10-minute Q&A*	Stefanie Czub, Canadian Food Inspection Agency and University of Calgary	
14:06 - 14:21	Surveillance of human prion diseases in Brazil from 2005 to 2013 *5-minute Q&A*	Michele Landemberger, AC Camargo Cancer Center	
14:24 - 14:39	Prion diseases: perspective from the bedside *5-minute Q&A*	Jerusa Smid, Universidade de São Paulo	
14:39 - 15:09	**Networking and Posters**		Wildrose B
15:12 - 15:42	Biodegradation of infectious prions in compost *10-minute Q&A*	Tim McAllister, Agriculture and Agri-Food Canada	Wildrose A
15:45 - 16:15	The role of microglia and neutrophils in TTR-related amyloidoses *10-minute Q&A*	Debora Foguel, Universidade Federal do Rio de Janeiro	
16:15 - 16:30	Closing Remarks	Kevin Keough, Vilma Martins and Jerson Silva	

Presenter Biographies

Judd Aiken

Dr. Aiken's primary research interest is the role of the environment in the dissemination of prion diseases. He received his undergraduate training at the University of Wisconsin in Stevens Point, his MS degree at the University of Wisconsin in LaCrosse and his PhD in Medical Biochemistry at the University of Calgary under the direction of Professor Gordon H. Dixon. Following a short postdoctoral stint at North Carolina State University in Raleigh, Dr. Aiken joined Dr. Richard Marsh's lab working on the molecular genetics of prion disease. He then joined the faculty in the Department of Animal Health and Biomedical Sciences at the University of Wisconsin at Madison. After 20 years at UW-Madison, Dr. Aiken was recruited to the Centre for Prions and Protein Folding Diseases at the University of Alberta. Dr. Aiken's prion research is interdisciplinary, focusing on: 1) impact of soil/soil microparticles on prion infectivity; 2) biochemical analysis of the infectious agent; 3) inter-species transmission of the CWD agent; 4) contamination of the environment by the CWD agent (soil, landfills, wastewater treatment plants) and 5) development of biomarkers for disease as potential ante-mortem diagnostics.

Ted Allison

Dr. Allison is an Assistant Professor at the University of Alberta in the Department of Biological Sciences as well as an Intramural Faculty Member at the Centre for Prions and Protein Folding Diseases. At the Centre for Prions and Protein Folding Diseases, Dr. Allison is developing zebrafish as a model of neurodegenerative diseases, such as BSE, CWD and Alzheimer's disease. He is a member of the Team to Prevent Blindness, the Centre for Neuroscience and the Women's and Children's Health Research Institute.

Neil Cashman

Dr. Cashman is a neurologist-neuroscientist at the University of British Columbia and Vancouver Coastal Health Research Institute working in neurodegeneration and neuroimmunology. His special areas of work are the amyloid encephalopathies, such as the prion illnesses, Alzheimer's disease and motor neuron diseases, particularly amyotrophic lateral sclerosis (also known as Lou Gehrig's disease). He joined the McGill Neurology and Immunology faculties in 1986 and accepted the Diener Professorship of Neurodegenerative Diseases at the University of Toronto Department of Medicine (Neurology) in 1998. In July 2005, he was appointed Professor of Medicine at the University of British Columbia, where he holds the Canada Research Chair in Neurodegeneration and Protein Misfolding Diseases and has laboratories in the renowned Brain Research Centre. He is Founder and Chief Scientific Officer of Amorfix Life Sciences, a public company developing therapeutics and diagnostics for protein misfolding diseases. He is the author of over 300 publications and provides expertise and guidance to numerous scientific, medical and governmental committees related to his research interests. Special honours recently received include the Jonas Salk Prize in 2000 for "outstanding contributions to basic biomedical research", his election to the Canadian Academy of Health Sciences in 2008 and the Genome BC Scientific Excellence Award in 2012.

Yraima Cordeiro

Dr. Cordeiro obtained her PhD in Biological Chemistry in 2005 at the Federal University of Rio de Janeiro, Brazil. Since August 2006, she has been an Associate Professor at the Pharmacy School from the Federal University of Rio de Janeiro. Her current research interests are to understand the mechanisms of prion protein conversion into pathological species using cellular biology and spectroscopic tools and to screen and evaluate the mechanism of action of anti-prion compounds.

Stefanie Czub

Dr. Czub is a DVM and has a PhD in neuropathology from the Veterinary School of the Free University in Berlin, Germany. She was a postdoctoral fellow at the Rocky Mountain Laboratory, (NIH), Hamilton, Montana, USA and senior research scientist at the Pathology Institute of the Julius-Maximilians University in Würzburg, Germany. Joining the Canadian Food Inspection Agency in 2001, she currently manages the pathology, virology and wildlife disease sections. The Pathology Section also has the Canadian National Reference Laboratory status and the World Organization for Animal Health Reference Laboratory (OIE) status for bovine spongiform encephalopathy (BSE). All Canadian BSE cases have been confirmed by Dr. Czub. She is also a member of the TSE Expert Advisory Group, the Canadian Association of Veterinary Pathologists, the College of Veterinarians of Ontario, an Alberta Prion Research Institute Scholar and Adjunct Professor at the Veterinary School of the University of Calgary, Alberta. Dr. Czub's research is focused on neurodegenerative diseases; in the last years, predominantly on prion diseases including BSE, CWD and scrapie.

Marcos Vinicios Salles Dias

Dr. Dias graduated in Biology at the Federal University of Alfenas (2003) and received his MSc (2006) and PhD (2010) in Cellular and Molecular Biology at the University of São Paulo. Currently he is a post-doctoral fellow under Dr. Vilma Martins' supervision in the Molecular and Cell Biology Group at the AC Camargo Cancer Center in São Paulo, Brazil. Dr. Dias' research focuses on the role of PrP^{C} protein in cellular trafficking and extracellular vesicles biogenesis and secretion.

Fernanda G. De Felice

Dr. De Felice graduated in Biology in 1994, obtained her MSc (1994-1997) and PhD degrees (1997-2002) in Biological Chemistry at the Federal University of Rio de Janeiro. In 2005, she was awarded a Research Fellowship by two prestigious International Programs, namely the Human Frontier Science Program (HFSP) and the Pew Latin American Fellows Program in the Biomedical Sciences, to obtain postdoctoral training in the United States. She chose the HFSP fellowship and spent two years and eight months as a post-doc in Dr. William Klein's laboratory at Northwestern University, where she acquired advanced training in new approaches in Alzheimer's disease research. After returning to Brazil at the end of 2007, she was recognized as an independent researcher (Head of Laboratory) in her Institute and she currently directs the Laboratory of Alzheimer's Disease, now as an Associate Professor. In 2008, she received the "3rd Young Scientist Prize" in Medical and Health Sciences, conferred by the "Academy of Sciences for the Developing World" (TWAS), was elected an Affiliated Member of the "Brazilian Academy of Sciences" and also elected "TWAS Affiliated Member" in Neurosciences. In 2009, she was appointed a Fellow of the John Simon Guggenheim Memorial Foundation. Dr. De Felice has supervised six PhD thesis and eight MSc dissertations and eight postdoctoral fellows. She has authored 45 scientific articles and reviews and four book chapters.

Sergio Ferreira

Dr. Ferreira graduated in Chemistry in 1985 and obtained his MSc and PhD degrees in 1987 and 1989, respectively, from the Federal University of Rio de Janeiro (UFRJ). Since 1999, he has been Professor of Biochemistry at UFRJ and from 1998-2007 he held an additional position as Associate Investigator at the Brazilian National Synchrotron Light Laboratory (Campinas, State of Sao Paulo). In 2006, he created the Laboratory for Neurodegenerative Diseases, which he presently directs at the Federal University of Rio de Janeiro. Dr. Ferreira has served on editorial boards for a number of publications, which currently include the *Biochemical Journal*, *Journal of Neurochemistry* and *Dementia & Neuropsychologia.* He has received a number of national and international awards, including being twice appointed a Howard Hughes Medical Institute International Research Scholar (1997-2001; 2002-2006), Fellow of the John Simon Guggenheim Memorial Foundation (2001), election to the Brazilian Academy of Sciences (2000) and admission into the National Order for Scientific Merit of the Republic of Brazil (2004). He was Vice-Chairman (2003-2005), Chairman (2006-2008) and is currently Immediate Past-President of the Pan-American Association for Biochemistry and Molecular Biology (PABMB). Dr. Ferreira has supervised 39 thesis and dissertations and 13 postdoctoral fellows. He has authored or co-authored 140 scientific articles, reviews and book chapters.

Debra Foguel

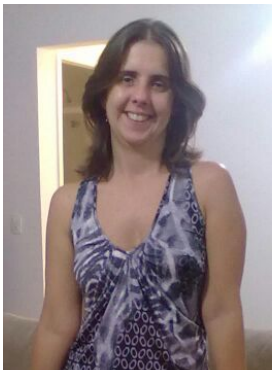
Sabine Gilch

Dr. Gilch obtained her PhD in Molecular Biotechnology in 2009 from the Technical University of Munich, Germany. She undertook postdoctoral studies on the molecular and cellular biology of prion infection at the Technical University of Munich and the University of Wyoming in Laramie, USA. In 2013, Dr. Gilch joined the UCVM Faculty as Assistant Professor and Tier II Canada Research Chair. Dr. Gilch's studies primarily involve using cell cultures and animal models to study the molecular and cellular biology of prion infection in order to identify new targets for treatment of prion diseases. In particular she looks at how prion infection interferes with neuronal metabolism and how this might lead to neurodegeneration and she uses peptide aptamers to interfere with prion conversion *in vitro* and *in vivo*. Another focus of her research is CWD. Dr. Gilch aims to improve diagnosis of CWD in fecal samples and to study the molecular basis of the distribution and shedding of CWD prions.

Glaucia Hajj

Dr. Hajj received her degree in Biological Sciences (2000) and her PhD in Biochemistry (2004) from the University of São Paulo. In 2000, she joined Dr. Vilma Martins and Prof. Ricardo Brentani in their quest to find the physiological functions of the cellular prion protein, at the Ludwig Institute for Cancer Research. For many years she investigated PrP^C and its functions in neurons and non-neuronal cells, identifying ligands that induce neuritogenesis, neuroprotection and memory formation and consolidation. She also explored the influence of PrP^C and its ligands in the control of mRNAs translation, leading to the differential expression of proteins that regulate cell proliferation, differentiation and death. Together with Dr. Marco Prado and Dr. Vilma Martins, Dr. Hajj explored the properties of PrP^C ligands against neurotoxicity in Alzheimer's cellular models. She has published over 20 articles in highly qualified journals including *PNAS*, *EMBO Journal*, *Journal of Neuroscience*, among others. Dr. Hajj received the Highly Qualified Personnel Award from PrioNet Canada (2010) and the Young Investigator Award from the International Society for Neurochemistry (2011) among many prizes in scientific meetings. Currently, Dr. Hajj is at the AC Camargo Cancer Center and is interested in the mechanisms by which mRNAs are differentially translated in the nervous system and in brain tumors.

Michele Christine Landemberger



Dr. Landemberger graduated in Pharmacy and Biochemistry at the Universidade Paulista - UNIP (2002) and received her PhD (2006) in Sciences, with a focus in Oncology, at the Fundação Antonio Prudente. Dr. Landemberger is Scientific Researcher at the AC Camargo Cancer Center, São Paulo, Brazil and Titular Professor at the UNIP, São Paulo, Brazil. Currently, she is responsible for the genetic analysis of the suspected cases of prion disease in Brazil.

Marilene Hohmuth Lopes

Dr. Lopes received her undergraduate degree in Biological Sciences at University Bandeirante of Sao Paulo, Brazil (1996-1999). In 2004, she obtained her PhD in Sciences from the Fundação Antonio Prudente of AC Camargo Cancer Center - Ludwig Institute for Cancer Research where she studied the role of prion protein (PrP) in neuron differentiation and characterized new ligands for PrP under the supervision of Dr. Vilma Martins. She continued her postdoctoral fellowship at Ludwig Institute for Cancer Research where she focused on PrP and its partners' functions in tumorigenesis of central nervous systems. In 2008 she joined Dr. Martins's group as Junior Investigator at Ludwig Institute for Cancer Research and continued to work with PrP and its partners in brain tumors. Currently, she is Assistant Professor of the Institute of Biomedical Sciences at University of Sao Paulo, Brazil, and leads a group of two technicians, four PhD students and four undergraduate students focused on investigating the role of PrP and its partners in embryonic, neural and tumor stem cells biology. Dr. Lopes is a member of the scientific advisory for research projects submitted to national and international funding agencies including FAPESP, CNPq, Medical Research Council (MRC) and The Portuguese Foundation for Science and Technology (FTC). Her articles have been published in such journals as *EMBO, Journal of Neuroscience, Journal of Neurochemistry, Prion and Stem Cells.*

Vilma Regina Martins

Dr. Martins graduated in Pharmacy at the University of São Paulo (1984), and obtained her MSc (1989) and PhD (1993) in Biological Sciences (Biochemistry and Molecular Biology) at the University of São Paulo. She is currently the Scientific Director and Head of the Molecular and Cell Biology Group of the AC Camargo Cancer Center in São Paulo, Brazil. Dr. Martins coordinates scientific research in molecular and cellular aspects of neurodegenerative diseases, particularly prion diseases. Presently, her research interests focus on the molecular and cellular aspects of the secretion of extracellular vesicles and their role both in astrocyte-neuron communication and neuroprotection/neurodegeneration as well as in tumor microenvironment and metastasis. The potential role of the extracellular vesicles to carry disease biomarkers is also being explored. Her previous positions include Assistant (2002-2008) and Associate (2008-2011) Member of the Ludwig Institute for Cancer Research, São Paulo Branch, International fellow of the Howard Hughes Medical Institute (2005-2010), Council Member of the International Society for Neurochemistry (2010-2013), Chair of the Committee for Aid and Education in Neurochemistry (CAEN) (2011-2013) from the International Society for Neurochemistry and President (2008-2010) and (2012-2014) Vice-President of the Brazilian Society for Cell Biology. Dr. Martins is also a member of the Editorial Board of the *Journal of Neurochemistry* and the *Brazilian Journal of Medical and Biological Research*. She has published 82 articles in journals including *Nature Medicine*, *EMBO J.*, *PNAS* and *J. Neurosc*.

Tim McAllister

Dr. McAllister is a principal research scientist with Agriculture and Agri-Food Canada and is located at the Research Centre in Lethbridge, Alberta. His research focuses on microbiology, nutrition and beef production and on food and environmental safety issues related to livestock production. His work in the area of prion science has involved examining the use of composting for the biodegradation of prions. He has also conducted studies to assess the ability of plants to uptake prions from the environment. Dr. McAllister has over 400 peer-reviewed publications and provides expertise and guidance to numerous scientific, medical and governmental committees related to his research interests.

Debbie McKenzie

Dr. McKenzie received her BSc degree from Simon Fraser University and her PhD in Medical Biochemistry from the University of Calgary. Following postdoctoral training at the University of Wisconsin-Madison with Dr. James Dahlberg, she joined the Veterinary Science/Animal Health and Biomedical Sciences department as a scientist. She has been researching prion diseases since 1988, focusing primarily on the etiology of infection. Her prion research interests include the role of prion protein genetics in disease resistance and susceptibility, the role of metals in pathology as well as inter and intra-species transmission.

Carlos Ramos

Dr. Ramos majored in Biochemistry at the University of Minas Gerais-Brazil in 1991, and received his PhD in Biochemistry in 1996 for work using molecular biology and protein biochemistry at the University of São Paulo (USP)-Brazil. Realizing the importance of folding and stability to the understanding of protein function, he performed postdoctoral work with Prof. Robert Baldwin at Stanford University from 1996-1998. He was nominated a PEW Fellow in 1997, received the Title of Privat-dozent in Biochemistry from the Institute of Chemistry from USP in 2005 and in 2006 received a Fogart International Research Collaboration Award from the NIH. He is the Chair of the Organic Chemistry Department from University of Campinas (UNICAMP) since 2011 and Full Professor since 2013. Dr. Ramos' contributions to science have generated 85 publications and are mainly related to the folding/unfolding and misfolding of myoglobin, a model for globular proteins, on the stability of protein domains, on the relationship between structure and function in molecular chaperones from high eukaryotes and on studies focused on protein homeostasis. Presently, he coordinates scientific projects focused on the structure, function and interaction of Hsp90s and disaggregases from human and plants.

Hermann Schätzl

Dr. Schätzl obtained his MD at the Ludwig-Maximilans-University of Munich, Germany, where he also received a Dr.med./PhD degree (summa cum laude; retrovirology). He was a postdoctoral fellow with Dr. Stanley. B. Prusiner at UCSF from 1993-1995 in prion research before establishing his own lab as Assistant Professor at the Max von Pettenkofer-Institute and the GeneCenter Munich in 1996. In 2002 he was appointed Professor of Clinical Virology (with tenure) and Head of the Clinical Virology Unit at the Technical University of Munich, where he also headed an accredited diagnostic virology lab. In 2010 he moved to the University of Wyoming as Wyoming Endowed Excellence Chair in Prion Biology and in 2013 he joined the University of Calgary as Professor of Prion Biology and Immunology. Dr. Schätzl has participated in research and grant review activities worldwide, has trained over 50 students and 25 researchers and has published 95 research articles, 18 reviews, 12 book chapters and co-edited two textbooks.

Jerson Silva

Dr. Silva received his degree of Medical Doctor from the Federal University of Rio de Janeiro (UFRJ) in 1984 and finished his PhD in Biophysics in 1987 (Institute of Biophysics, UFRJ). He received postdoctoral training in the laboratory of Professor Gregorio Weber at the University of Illinois, specializing in methodologies of fluorescence spectroscopy and high pressure applied to biological systems. Currently Dr. Silva is a Full Professor at the Institute of Medical Biochemistry (UFRJ) and Director of the Jiri Jonas National Center for Nuclear Magnetic Resonance (CNRMN - UFRJ). His main research interest is the study of the basic factors responsible for protein folding, protein-nucleic acid interactions and the formation of biological assemblages such as viruses and amyloid aggregates. Dr. Silva has published more than 140 full papers, and has been a recipient of many awards. Honours include International Fellow of the Howard Hughes Medical Institute (1997-2002), Brazilian Order of Scientific Merit (Command in 2002, Great-Cross in 2009), TWAS Prize in Biology (2005) and FCW Prize in General Science from Fundação Conrado Wessel (2010). Dr. Silva is member of the Brazilian Academy of Sciences and Fellow of the Academy of Sciences for the Developing World (TWAS). Dr. Silva is the Coordinator of the National Institute for Science and Technology for Structural Biology and Biomaging and Scientific Director of the Rio de Janeiro State Funding Agency.

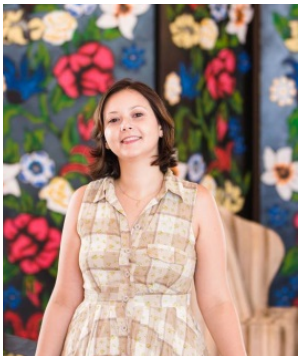
Valerie Sim

Dr. Sim joined the University of Alberta in January 2009 as Assistant Professor in the Department of Medicine, Division of Neurology. Her research focuses on prion disease pathogenesis, the role of infectious oligomers in this process and how the abnormal structure of prion protein relates to the infectious properties of these diseases. As a clinician scientist in neurology, 75 per cent of her time is dedicated to basic science research while the remainder is spent doing clinical neurology. Dr. Sim received her BSc Hon. at the University of Calgary in 1996, majoring in Cellular, Molecular and Microbial Biology. She graduated from medical school at the University of Calgary in 1999 and then completed a Neurology residency at the University of Ottawa in 2004. With funding from the Alberta Heritage Foundation for Medical Research, she pursued a postdoctoral fellowship on the basic science of prion disease at Rocky Mountain Laboratories, NIAID, NIH, in Montana.

Jerusa Smid

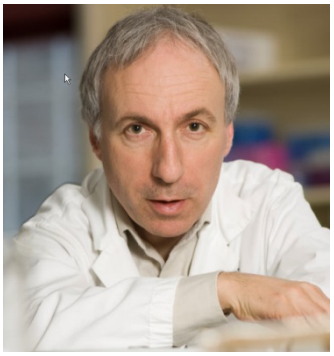
Dr. Smid graduated in Medicine at São Paulo University (2000) and in Neurology at Hospital das Clínicas, Medicine School, São Paulo University (2004). She received her PhD (2011) in Sciences, Neurology at São Paulo University. Since 2004, Dr. Smid has been a Neurologist at the Cognitive and Behavioral Neurology Unit at Hospital das Clínicas, Medicine School, São Paulo University. Dr. Smid has also been a Neurologist at Emilio Ribas Institute of Infectious Disease since 2004. She is a scientific journal referee for *Clinics*, *Dementia & Neuropsychologia* and *Annals of Medicine*. A Fellow of the Brazilian Academy of Neurology, Dr. Smid has published 20 articles in journals including *PLoS Neglected Tropical Diseases*, *Virus Research*, *Journal of Alzheimer's Disease*, *Scandinavian Journal of Immunology*.

Tuane Vieira



Dr. Vieira graduated in Biology at the Federal University of Rio de Janeiro (UFRJ) in 2004. She received her Master's degree in 2005 and PhD degree in 2009, both in Chemical Biology at UFRJ and did a post doctorate at the National Institute of Allergy and Infectious Diseases (NIAID/NIH) in 2012. From 2000-2005 her research was in the area of glicobiology and from 2005 to now she has been devoting her research to structure biology, focusing on biological systems of medical importance. Nowadays she is a postdoctoral fellow in the laboratory of Professor Jerson Silva at UFRJ working on prion biology, investigating PrP-polysaccharide interactions and its importance for prion conversion, at the molecular level and systems biology level.

David Westaway



Dr. Westaway is a molecular biologist with a special interest in the use of genetically engineered mice to recreate and understand human neurological disorders. He earned a degree in Biochemistry from the University of Sussex and a PhD in Biochemistry at the University of London, in England. He completed postdoctoral training at the University of California. Before becoming Director of the Centre for Prions and Protein Folding Diseases at the University of Alberta, Dr. Westaway was appointed as Professor in the Centre for Research in Neurodegenerative Disease at the University of Toronto. There he focused on the cellular prion protein and two other related proteins, as well as creating lab models of Alzheimer's disease. Dr. Westaway has won a number of awards for his research, including a Premier's Research Excellence Award, a Canadian Institutes of Health Research Investigator award and a Zenith Scholar award from the Alzheimer's Association (USA).

Holger Wille

Dr. Wille graduated from the University of Hamburg in Germany. He conducted the experimental parts for both his Master's and PhD theses at the Max-Planck Unit for Structural Molecular Biology under the supervision of Dr. Eckhard Mandelkow. At the Max-Planck group, he studied the structure and aggregation of the microtubule-associated proteins Tau and MAP2. Upon being awarded his doctorate, Dr. Wille joined the laboratory of Dr. Stanley B. Prusiner at the University of California, San Francisco (UCSF) as a postdoctoral fellow. While at UCSF, he focused on the structure and aggregation of the infectious prion protein. At the end of his postdoctoral fellowship, Dr. Wille became a faculty member of the Department of Neurology at UCSF, continuing his studies on the structure of the infectious prion protein. In 2012, Dr. Wille joined the Faculty of the Department of Biochemistry at the University of Alberta in Edmonton, Alberta, Canada. Dr. Wille's laboratory is located in the Centre for Prions and Protein Folding Diseases and focuses on the structure of amyloids and other misfolded proteins. In particular, the structure of infectious prions and the structure-function relationship underlying their infectious nature is being investigated.

Michael Woodside

Dr. Woodside is an Associate Professor of Physics at the University of Alberta, a Senior Research Officer at the National Institute for Nanotechnology and the iCORE Chair in Biophysics. His research focuses on understanding the microscopic mechanisms of structure formation in biological macromolecules, with a particular emphasis on behaviour at the level of single molecules. His lab is currently engaged in probing the misfolding and aggregation of single molecules of disease-related proteins such as PrP, alpha-synuclein and SOD, as well as studying the folding and dynamics of mRNAs as they relate to gene regulatory function.

Presenter Abstracts

Prion systems biology using rat-adapted scrapie

Judd Aiken,* Danielle Gushue, Debbie McKenzie, Lingjun Li and Allen Herbst

Centre for Prions and Protein Folding Diseases, University of Alberta; Edmonton, Canada

*Presenting author; Email: judd.aiken@ualberta.ca

The BSE and variant CJD (vCJD) outbreaks have prompted the need for rapid, reliable and inexpensive screening methods for diagnosing prion diseases at both symptomatic and pre-symptomatic stages. The focus of these studies is to examine brain tissue (genomics) and cerebral spinal fluid (CSF; proteomics) during preclinical stages of prion disease to identify ante-mortem biomarkers. Our recently developed rat prion disease model is an excellent model as the rat genome is sequenced providing a complement to mouse genomic analyses and significant volumes of CSF are obtainable from the rat facilitating proteomic studies. We have analyzed genomic (brain) and proteomic (CSF) changes in rats at the clinical stage of prion infection. At the genomic level, we have found that only ~30 per cent of the differentially expressed RNAs were in common between mouse and rat suggesting that molecular pathology observed in the mouse may not be directly applicable to other species. For the proteomics analyses, CSF from clinically affected rats (compared to mock-infected rats) was analyzed by mass spectrometry. A number of proteins were more abundant in CSF of clinically affected rats, including CJD biomarkers 14-3-3s and neuron specific enolase.

PrP loss of function in zebrafish to understand conserved functions and assess conserved protein interactions *in vivo*

Patricia L.A. Leighton,[1,2] and W. Ted Allison[1,2,3]*

[1]Centre for Prions and Protein Folding Diseases, University of Alberta; Edmonton, Canada; [2]Department of Biological Sciences, University of Alberta; Edmonton, Canada; [3]Department of Medical Genetics, University of Alberta; Edmonton, Canada

*Presenting author; Email: ted.allison@ualberta.ca

Background/Introduction. With a long-term view towards the development of therapeutics for prion and Alzheimer's disease, our immediate goal is to understand the normal biological role of the prion protein. Any phenotypes that are revealed by loss-of-function approaches in zebrafish will suggest ancient/important functions of PRNP. Zebrafish possesses two homologs of PRNP (prp1 and prp2). These can be replaced by mammalian PRNP, suggesting a conserved function. An ancient role for PrP in the biology of Alzheimer's-related proteins is supported by our recent demonstration of a genetic interdependence between zebrafish homologs of PRNP and APP.
Materials and Methods. We have engineered zebrafish with a knockout of the gene prp2. Here we explore for phenotypes in these fish using behavioural assays, characterizing gene expression and developing novel imaging approaches to post-larval brain development.
Results. In sharp contradistinction to phenotypes observed when prp1 is disrupted by morpholino gene knockdown (including dramatic effects on early embryonic and later CNS development), it has been challenging to identify phenotypes in our prp2-/- mutant zebrafish. We will review the possible explanations for these disparate outcomes that may be related to the timing of gene expression and/or gene sub-functionalization. Behavioural and molecular assays following application of a convulsant

suggest that prp2-/- mutant zebrafish are susceptible to seizures. Progress towards knockout of additional genes will be described.
Conclusions. These phenotypes in our prp2-/- zebrafish will allow us to dissect which portions of PrP^C are required for its role in regulating neuronal excitability *in vivo*. The data argues for an ancient/important role for PrP in seizure susceptibility and neuronal hyper-excitability. Our prp2-/- zebrafish have phenotypes similar to PRNP knockout mice, but divergent from the result from knockdown of zebrafish prp1. The results provide a promising path forward to understand the role of PrP in Alzheimer disease etiology, especially regarding excitotoxicity.

Propagated misfolding of SOD1 in ALS: a new prion-like disorder

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Key words: amyotrophic lateral sclerosis, copper-zinc superoxide dismutase, prion-like mechanisms

Approximately 10 per cent of ALS cases are familial, with ~20 per cent of these due to mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1), a ubiquitous free-radical defense enzyme. We sought to molecularly dissect the effects of intracellular obligately misfolded SOD1 mutant proteins on natively structured wild-type SOD1. Expression of the enzymatically inactive, natural familial ALS SOD1 mutations G127X and G85R in human mesenchymal and neural cell lines induced misfolding of wild-type natively-structured SOD1, as indicated by: 1) acquisition of immunoreactivity with SOD1 misfolding-specific monoclonal antibodies; 2) markedly enhanced protease sensitivity suggestive of structural loosening; and 3) non-native disulfide-linked oligomer and multimer formation. Cytosolic mislocalizing mutations of FUS and TDP43, two proteins implicated in familial and sporadic ALS, also triggered SOD1 misfolding. Expression of G127X and G85R in mouse cell lines did not induce misfolding of murine wtSOD1 and a species restriction element for human wtSOD1 conversion was mapped to a region of sequence divergence in loop II and beta-strand 3 of the SOD1 beta-barrel (residues 24-36), then further refined surprisingly to a single tryptophan residue at codon 32 in human SOD1. Culture medium from cells transiently transfected with wild-type or mutant SOD1 induced misfolding of endogenous SOD1 when added to native cell cultures and this process was stably propagated in serial passage. Nonspecific uptake of misfolded SOD1 was excluded by siRNA knockdown of SOD1 in the fresh recipient cells, indicating a requirement for endogenously expressed SOD1 as a substrate. The agent responsible for induction of misfolding was determined to be a misfolded SOD1 aggregate which pelleted by ultracentrifugation of 100,000 X g for 1 hr. Transmission of SOD1 misfolding *in vitro* was abrogated by extracellular pan- and misfolding-specific SOD1 antibodies. G37R Tg mice treated with misfolding-specific SOD1 antibodies displayed prolonged survival of ~11 days ($p < 0.001$). On quantitative immunoprecipitation, misfolded wtSOD1 was found to constitute ~5 per cent of total SOD1 in spinal cord samples from SOD1 familial as well as sporadic ALS. SOD1 misfolding and toxicity can propagate within and between cells, prompting novel targeted therapies for all forms of ALS. ALS now joins company with Alzheimer's, Parkinson's, and other neurodegenerative and systemic diseases as a "prion-like" disorder that transmits from cell to cell in the CNS.

Pathological, therapeutic and functional implications of prion protein interaction with ligands

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Transmissible spongiform encephalopathies (TSEs) are infectious neurodegenerative disorders that do not have a symptomatic, curative or prophylactic treatment. Conversion of the cellular prion protein (PrP^{C}), which is soluble, into the scrapie isoform (PrP^{Sc}) is the hallmark of these disorders. Although many compounds have been effective *in vitro,* they were poorly active *in vivo*, mainly because the inability to cross the blood-brain-barrier and to high toxicity. Herein we employed *in vitro* and *in silico* methodologies for selecting effective anti-prion compounds. Screening in prion-infected cell culture (ScN2a) revealed active compounds and *in silico* pharmacokinetic and pharmacodynamics predictions indicated the most promising ones for future *in vivo* evaluation. We are also interested in investigating PrP interaction with different macromolecular ligands, such as nucleic acids (possibly involved in TSE pathogenesis) and proteins (related to the PrP^{C} physiological function). We showed that nucleic acids catalyze the misfolding of PrP^{C} into a scrapie-like conformer. Our group characterized the interaction of PrP with DNA showing that it is non-specific and that the PrP-nucleic acid complex can be toxic to cultured cells. By the use of spectroscopic and cell biology techniques, we found that rPrP:DNA interactions lead to different aggregated species, depending on the sequence and size of the oligonucleotide tested. Regarding the still non-elucidated PrP^{C} physiological role, we proposed that PrP^{C} serves as a cell surface scaffold protein for a variety of signaling modules. There are evidences for allosteric functions of PrP^{C}, which constitute a common property of scaffold proteins. Our goal is to prove that the main function of PrP is to act as a scaffolding protein that is allosterically regulated, by investigating its interaction with previously identified and validated protein ligands. We are using molecular simulation techniques such as molecular modeling and dynamics, generalized simulated annealing and docking, to initially generate conformational ensembles for PrP containing the flexible N-terminus, and afterwards, to stabilize structural models of PrP bound to other ligands. These interactions will be validated by spectroscopic and calorimetric techniques. This study may shed light on both physiological roles of PrP^{C}, as well as on the pathogenesis of the TSEs.

The challenge of atypical BSE

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After the initial detection and identification of atypical bovine spongiform encephalopathy (BSE) in 2003, more than 60 L-type and H-type BSE cases have been reported worldwide, including Europe, Japan, North and South America. Although there is a relatively low incidence of atypical BSE reported at present, the disease poses a challenge for current surveillance and control programs and potentially for free trade. The programs' goal is to identify and accurately distinguish classical from atypical BSE; and to have appropriate consumer and animal health protection measures in place, most notably preventing consumption of specified risk materials (SRMs).

In our research activities, we focus on uncertainties of detection methods and control measures against atypical BSE. We were able to demonstrate that standard BSE rapid tests show sufficient analytical sensitivity for detection of PrP^{Sc} in atypical BSE which is supporting epidemiological evidence (Gray et al. 2012). However, most tests are unable to determine the type directly; it requires western blot analysis to identify the type-specific shifting of the glycosylation pattern to clearly differentiate between the three different BSE types.
Oral and intra-cerebral challenges have been performed for classical and atypical BSE to identify clinical features, distribution pathways and location of PrP^{Sc} in cattle. At this time, however, only intra-cerebral challenges have successfully induced infection and resulted in clinical disease. In our second research focus, we are determining the potential of atypical BSE transmission into cattle by an oral challenge. The goal is to provide a clear-cut analysis of clinical disease and distribution pattern of PrP^{Sc} in cattle infected with atypical BSE. This information will allow a better on-farm identification of diseased animals and exclusion of tissues from human or animal consumption to prevent spread of atypical BSE. In a related experiment, we are investigating the existence of an age-related susceptibility of cattle to BSE infection. Results from these experiments are presented.

Defective insulin signaling and inflammation as common molecular denominators connecting type 2 diabetes to Alzheimer's disease

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Compelling preclinical and clinical evidence supports a pathophysiological connection between Alzheimer's disease (AD) and diabetes. Altered metabolism, inflammation and insulin resistance are key pathological features of both diseases. For many years, it was generally considered that the brain was insensitive to insulin, but it is now accepted that this hormone has central neuromodulatory functions, including roles in learning and memory, that are impaired in AD. However, until recently the molecular mechanisms accounting for brain insulin resistance in AD have remained elusive. We investigated the role of synaptotoxic Aβ oligomers in AD-associated defective insulin signaling and memory impairment using mice models of AD and macaques receiving Aβ oligomer infusions. We found that Aβ oligomers activate the stress sensitive kinases PKR and JNK in a TNF-α-dependent manner, resulting in eIF2α-P, neuronal insulin receptor substrate (IRS-1) inhibition, synapse loss and memory impairment. Bolstering insulin signaling protected the brain from the deleterious effects of Aβ oligomers in mice and macaques. Development of disease-modifying therapeutics for AD has been hampered by the difficulty in translating therapies that work in rodents to humans. Since human and macaque brains share significant similarities in terms of overall architecture and functional networks, we have been engaged in generating a reliable macaque model of AD, which is likely to enable development of new AD therapies.

PrPC modulates the secretion of extracellular vesicles by astrocytes

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Extracellular vesicles (EVs) are membrane surrounded structures released by cells in normal physiologic conditions and function in signaling and in transfer of membrane and/or cargo molecules. The majority of EVs are intracellular luminal vesicles (50–90 nm), formed in endocytic compartments called multivesicular bodies (MVBs) by inward budding of their limiting membrane. After endocytosis, proteins and lipids that are destined for lysosomal degradation are first incorporated into intraluminal vesicles (ILVs) of MVBs and delivered to lysosomes for digestion. Alternatively, MVBs can directly fuse with the plasma membrane, which leads to release of the vesicles to the extracellular environment. How proteins and lipids are sorted to these subsets of vesicles directed either for lysosomal degradation or for secretion is unknown. We have previously demonstrated that *Prnp*$^{0/0}$ astrocytes have a defect in producing soluble factors that support neuronal survival and differentiation. Additionally, the protein content in pellets from ultracentrifugation of the conditioned medium (CM) from wild-type astrocytes is much higher than in CM from *Prnp*$^{0/0}$ cells and the distribution of STI1 (a EV marker) and lipids by gel chromatography demonstrate that in CM from *Prnp*$^{0/0}$ astrocytes, STI1 is presented mainly as a soluble form and the amount of lipids were reduced approximately 20 fold. Thus, the aim of this work was to determine if PrP^{C} regulates the EVs biogenesis pathway in astrocytes. We have quantified the amount of EVs secreted by astrocytes by nanoparticle-tracking analysis. The results confirm a reduction in the number of EVs in CM from *Prnp*$^{0/0}$ astrocytes when compared to CM from wild-type cells. Conversely, in astrocytes overexpressing PrP^{C} (from TG20 mice) the number of vesicles in CM is considerably higher than that from wild-type cells. Similar results were obtained in CM from embryonic fibroblasts and in plasma from these animals. Accordingly, the re-expression of PrP^{C} expression in knockout cells can restore the EV secretion. We also found that the absence of PrP^{C} leads to a defect in the endocytosis of both lipid rafts and the EGF receptor, two phenotypes associated with the failure of MVB formation. Additionally, electron microscopy experiments demonstrated that *Prnp*$^{0/0}$ cells have MVBs without intraluminal vesicles and interestingly, these cells have an increased number of autophagosomes. These results were confirmed by immunofluorescence, in which *Prnp*$^{0/0}$ cells shown a decrease of LBPA (an intra-luminal vesicle marker) and an increase of LAMP1 (autophagosome/lysosome marker) staining. In addition, autophagy induction by several ways (serum deprivation, glucose deprivation and rapamicyn treatment) decreased the EVs secretion. Thus, these results suggest that in the absence of PrP^{C}, MVB intraluminal vesicles are directed to the autophagy pathway with consequent inhibition in EVs release. This unpredicted function of PrP^{C} might have important implications upon neuroprotection, neuronal differentiation and also in prion diseases, where the release of PrP^{Sc} in EVs, particularly in exosomes is important for prion spread.

Microglia-derived TNF-α mediates depressive-like behaviour induced by amyloid-β oligomers in a mouse model of Alzheimer's disease

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Depression is one of the most common psychiatric symptoms in Alzheimer's disease (AD) and considerable evidence indicates that major depressive disorder increases the risk of AD. To date, however, the molecular mechanisms underlying the association between depression and AD have remained elusive. Soluble oligomers of the amyloid-β peptide (AβOs) accumulate in the brains of AD patients and are increasingly recognized as the proximal neurotoxins responsible for synapse failure and memory deficits in AD. We recently demonstrated that a single intracerebroventricular (i.c.v.) injection

of 10 pmol AβOs induces cognitive deficits[1] and depressive-like behaviour in mice (Ledo et al., Mol. Psychiatry 2013). Interestingly, the antidepressant fluoxetine rescued both depressive-like behaviour and cognitive deficit in AβO-injected mice. Behavioural and cognitive alterations induced by AβOs correlated with an inflammatory process, with increased brain cytokine levels and recruitment of microglia and astrocytes. More recently, we found that AβOs instigate microglial activation and that microglial-derived TNF-α plays a key role in mediating behavioural alterations induced by oligomers. Current findings establish that AβO-induced microglial activation and TNF-α release link memory impairment and depressive-like behaviour in mice, providing molecular mechanistic support to clinical evidence connecting AD and depressive disorder and suggest a mechanism by which elevated brain levels of AβOs may be linked to changes in cognition and mood in AD.

References
1. Figueiredo et al. J. Neurosci. 2013.

The role of microglia and neutrophils in TTR-related amyloidoses

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Neutrophil extracellular traps (NETs) are key players in a death mechanism in which neutrophils release DNA traps decorated with proteins such as elastase and histones to entangle pathogens. We asked whether NETs are triggered by amyloid fibrils, reasoning that since proteases are present in NETs, protease digestion of amyloid may generate soluble, cytotoxic species. We show that amyloid fibrils from three different sources (α-synuclein, Sup35 and transthyretin) induced NADPH oxidase-dependent NETs in vitro from human neutrophils. Surprisingly, NET-associated elastase digested amyloid fibrils into short species that were cytotoxic for BHK-21 and HepG2 cells. We observed in situ, NETs in amyloidotic deposits from amyloidotic patients which co-localized with amyloid deposits. These data reveal that NETs, so far described to be elicited by pathogens, can also be triggered by amyloid fibrils. Moreover, the involvement of NETs in amyloidoses might be crucial for the production of toxic species derived from fibril fragmentation.
In a second approach, we have investigated the pathogenic mechanism behind oculoleptomeningeal amyloidosis (OA) caused by fibrillation of A25T. We have showed that fibrils of A25T activate microglia leading to secretion of TNF-α, IL-6 and nitric oxide. We further found that A25T amyloid fibrils induce Akt activation, culminating in NFκB translocation to the nucleus of microglia. While A25T fibrils are not directly toxic to neurons, exposure of neuronal cultures to conditioned media of fibril-activated microglia causes synapse loss culminating in extensive neuronal death via apoptosis. Finally, intracerebroventricular (i.c.v.) injection of A25T fibrils caused microgliosis, increased brain TNF-α and IL-6 levels and cognitive deficits in mice, which could be prevented by minocycline. These results indicate that A25T fibrils act as inflammatory agents in OA, activating microglia and causing neuronal damage through inflammation.

Peptide aptamers as therapeutic and analytic tools in prion research

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Prion diseases are fatal and neurodegenerative disorders caused by the accumulation of a misfolded isoform (PrP^{Sc}) of the cellular prion protein PrP^{C} for which neither therapy nor prophylaxis is available. Upon direct interaction of the two isoforms, PrP^{C} adopts the conformation of PrP^{Sc}. In humans, prion diseases include Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD). Examples of animal prion diseases are scrapie in sheep, bovine spongiform encephalopathy (BSE) and chronic wasting disease (CWD) in cervids. Interestingly, prions can exist as different prion strains which are thought to consist of variable PrP^{Sc} conformers despite an identical primary structure. Prion strains are characterized by different pathologies in the same host and currently it is not known how a variety of conformations can be formed from the identical PrP^{C} primary structure.
We have described peptide aptamers (PAs) as a class of novel anti-prion compounds which interfere with prion propagation by inhibiting PrP^{C}-PrP^{Sc} interaction. PAs consist of a random peptide moiety presented by a constant scaffold protein. For the first time, we were able to demonstrate that PAs can be expressed in the secretory pathway and retain the binding affinity for their target protein. Furthermore, we use PrP peptide-grafted aptamers to characterize the PrP^{C}-PrP^{Sc} interface and possible strain differences thereof. We have found that N-terminal PrP- peptides presented by the scaffold protein interfere with 22L but not RML prion propagation in persistently infected cell cultures. Assuming competitive binding of PrP peptide-grafted aptamers and PrP^{Sc} with PrP^{C}, this data indicates a differential binding site of PrP^{C} to RML and 22L PrP^{Sc}, respectively.
In summary, our approach to use peptide aptamers will result in novel therapeutic and prophylactic molecules to combat prion diseases. Furthermore, using PrP peptide-grafted aptamers we will gain new insights into prion strain biology.

PrP^{C} in metabolic diseases

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The cellular prion protein or PrP^{C} is a cell surface molecule responsible for triggering several signal transduction cascades and mediating many physiological processes. Given this range of effects and the possible proximity of the insulin receptor and PrP^{C} in lipid rafts, we decided to analyze its influence on the insulin signaling pathway. We evaluated the blood glucose and insulin control in wild-type animals (wt), PrP^{C} deficient mice (KO) from two independent strains and mice overexpressing PrP^{C} (TG20) that were fed chows containing different amounts of fat. The normal response to the introduction of the high fat diet is that animals lose the control of blood glucose on fasting and post prandial situations. Strikingly, in KO animals this regulation is lost in an earlier age than wt animals and TG20 retain the ability of regulating blood glucose until an older age. Despite the loss in the ability of regulating blood glucose, insulin levels were much higher in KO animals when compared to wt, characterizing insulin resistance in KO animals. Interestingly, KO animals also presented higher weight gain than the wt. TG20 animals presented lower blood insulin and lower weight gain when compared to the wt. Furthermore, histological sections of tissues affected by insulin signaling were made and we observed a significantly

higher level of hepatic steatosis and increased adipocyte size in the KO group when compared to wt, characterizing metabolic syndrome in KO animals. To check which pathway could be responsible for this metabolic alteration, we treated primary MEF cells derived from KO mice, wt and TG20 with insulin. We observed that similar to the mice, glucose uptake by MEFs is impaired in KO cells. However, we observed no difference in the phosphorylation of P-IRS – 1(Ser636/639), IRS-1, P-IR and IR. We observed a difference in AKT phosphorylation (T308), but how this difference reflects on blood glucose regulation is still to be determined. These results show us that PrP^C regulates glucose metabolism and animals that do not have PrP^C present insulin resistance and metabolic syndrome.

Surveillance of human prion diseases in Brazil from 2005 to 2013

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Global surveillance of vCJD and other forms of CJD was recommended from the WHO for a better understanding of potential causes of iatrogenic CJD, as well as the distribution of various hereditary forms. Prion diseases have been under compulsory notification in Brazil since 2005. From 2005 to 2013, we received 320 blood samples from notified cases of suspected CJD. Blood samples were analyzed by direct genomic sequencing to identify mutations and polymorphisms in the PRNP gene. Cases with mutation in direct sequencing were cloned to confirm results. The presence of 14.3.3 protein in cerebrospinal fluid (CSF) was evaluated using immunoblotting and brain tissue obtained by autopsy or biopsy was analyzed by imunohistochemistry for the presence of spongiosis and proteinase K resistant PrP. The average age of our patients was 56.8 years with a median of 60 years (range 13-90 years), males representing 48 per cent of the cases. PRNP polymorphisms analysis showed that 53 per cent of the cases were homozygous for methionine at codon 129 (M129M), 29 per cent were heterozygous (M129V) and 18 per cent were homozygous for valine (V129V). The silent polymorphism at codon 117 was detected in nine per cent of the patients and four per cent had deletion at the octarepeat. E200K mutation at PRNP was found in seven unrelated patients and all of them presented methionine at codon 129 in the mutated allele. We found two patients with GSS syndrome with a P102L mutation. We also described the first case in America of insomnia familial fatal with a punctual mutation in 178 codon (N178+M129).
Brain tissue of 32 patients was available, 28 (87 per cent) of them had spongiosis and were positive for proteinase K resistant PrP. After clinical evaluation, imaging exams, 14.3.3 protein presence, genetic and immunohistochemical analysis, notified cases were classified according to the WHO criteria. This study provides the first epidemiologic data about human prion diseases in Brazil. Similar to any other country the availability of brain tissue from these patients is a limiting factor to confirm the diagnosis of prion diseases. This study also represents an important tool for prion-prevention policies and is of great importance for future implementation of clinical trials.

Prion protein-STI1 engagement modulates proliferation and self-renewal of neural progenitor/stem cells from developing and adult nervous systems

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Prion protein (PrP^C), when associated with the secreted form of the stress inducible protein 1 (STI1), plays an important role in neural survival, neuritogenesis and memory formation. However, the role of the PrP^C-STI1 complex in neural progenitor/stem cells (NSCs) biology is unknown. Here, we show that PrP^C and STI1 are directly involved in NSCs maintenance derived from fetal and adult nervous tissue. Neurospheres cultured from fetal forebrain of wild-type (*Prnp*$^{+/+}$) and PrP^C-null (*Prnp*$^{0/0}$) mice were maintained for several passages without the loss of self-renewal or multipotentiality, as assessed by their continued capacity to generate neurons, astrocytes and oligodendrocytes. The homogeneous expression and co-localization of STI1 and PrP^C suggests that they may associate and function as a complex in neurosphere-derived stem cells. The formation of neurospheres from *Prnp*$^{0/0}$ mice was reduced significantly compared to their wild-type counterparts. In addition, blockade of secreted STI1, as well as PrP^C, with specific antibodies, impaired *Prnp*$^{+/+}$ neurosphere formation. The STI1-PrP^C interaction was able to stimulate cell proliferation in the neurosphere-forming assay. The current data provides additional support for the idea that STI1 can be considered a neurotrophin owing to its indubitable properties in NSCs behaviour in complex with PrP^C during brain development. In addition, the involvement of the PrP^C-STI1 complex in adult NSCs self-renewal may help build the rationale for novel therapeutic strategies for the treatment of acute brain injuries, such as ischemia and trauma, as well as for the treatment of chronic neurodegenerative illnesses, such as Alzheimer's disease and prion diseases.

The role of exossomal secreted stress inducible protein 1 in protection against injury

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Stress-inducible phosphoprotein 1 (STI1) is part of the chaperone machinery, but it also functions as an extracellular ligand for the prion protein (PrP). The physiological relevance of these STI1 activities *in vivo* is unknown. The absence of STI1 is embryonic lethal by E10.5 with mutant mice showing increased caspase-3 activation, 50 per cent impairment in cellular proliferation, placental disruption and lack of cellular viability. STI1 haploinsufficient mice showed increased vulnerability to ischemic insult. Extracellular STI1 prevented ischemia-mediated neuronal death in a PrP-dependent way. In addition, specific binding of AβOs (Aβ oligomers) to PrP is efficiently inhibited by STI. Treatment with STI1 prevented AβO-induced synaptic loss and neuronal death in cultured neurons and long-term potentiation inhibition in mouse hippocampal slices. Interestingly, STI1-haploinsufficient neurons were more sensitive to AβO-induced cell death and could be rescued by treatment with recombinant STI1. Thus, indicating the essential roles for intracellular and extracellular STI1 in cellular resilience. STI1 is released by astrocytes, however it lacks a signal peptide and does not follow a classical secretion mechanism. Diverse biochemical and electronic microscopy approaches demonstrated its secretion by exosome-like vesicles. STI1 partially co-localized with Rab5 and Rab7 in endosomal compartments and a dominant-negative for vacuolar protein sorting 4A (VPS4A), required for formation of multivesicular bodies (MVBs), impaired EV and STI1 release. Flow cytometry and PK digestion demonstrated that STI1 localized to the outer leaflet of EVs and its association with EVs greatly increased STI1 activity upon PrP-dependent neuronal signaling. Thus, indicating that the interaction between EVs and neuronal surface components enhances STI1-PrP signaling preventing neuronal injury by ischemia and AβO toxicity.

Biodegradation of infectious prions in compost

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Key words: prion, specified risk materials, composting, biodegradation, western blotting, protein misfolding cyclic amplification, bioassay

Background/Introduction. Composting may serve as a practical and economical means of disposing of specified risk materials (SRM) or animal mortalities potentially infected with prion diseases (transmissible spongiform encephalopathy, TSE). Our study investigated the degradation of prions associated with scrapie (PrP^{263K}), chronic waste disease (PrP^{CWD}) and bovine spongiform encephalopathy (PrP^{BSE}) in laboratory-scale composters and PrP^{263K} in field-scale compost piles.
Materials and Methods. Laboratory-scale composting was conducted over 28 days using manure matrices with or without poultry feathers. Compost was mixed at day 14 to generate a second heating cycle. Degradation of PrP^{263K}, PrP^{CWD} and PrP^{BSE} was measured using western blotting (WB) and with additional analysis on PrP^{263K} and PrP^{CWD} using protein misfolding cyclic amplification (PMCA). For field-scale composting, duplicate biocontained composters containing PrP^{263K} adhered to stainless steel beads and cattle mortalities were used. Degradation of PrP^{263K} was assessed at 0, 14, 56, 112 and 230 days using composted beads in a Syrian golden hamster bioassay.
Results. Analysis of WB indicated that PrP^{263K}, PrP^{CWD} and PrP^{BSE} were reduced by at least 2 $\log_{10}$, 1-2 $\log_{10}$ and 1 $\log_{10}$ after two cycles of laboratory-scale composting, respectively. Further analysis using PMCA confirmed a reduction of 2 $\log_{10}$ in PrP^{263K} and 3 $\log_{10}$ in PrP^{CWD}. Enrichment for proteolytic microorganisms through the addition of feather keratin to compost enhanced degradation of PrP^{263K} and PrP^{CWD}. Field-scale composting effectively reduced TSE transmission in intracranially implanted hamsters with beads composted for 14, 56 and 112 days. After 230 days of composting, only one in five hamsters succumbed to TSE disease.
Conclusions. Estimates of infectious titre reduction based on incubation period suggest that composting achieved at least a 4.8 $\log_{10}$ reduction in PrP^{263K} infectivity. Our findings demonstrate that composting significantly reduces PrP^{TSE} and with consideration for overall risk, may be a viable method for the disposal of SRM.

Determining the diversity of CWD strains

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (prion disease) affecting cervid species in North America and Korea. The disease spreads readily amongst the different cervid populations, currently infecting elk, white-tailed deer, mule deer and moose. Transmission likely occurs by both direct (animal to animal) and indirect routes with the environment serving as a reservoir of infection. Genetic polymorphisms in the prion gene (Prnp) affect disease progression in most species affected by prion disease, including cervids. We have focused on two specific polymorphisms in white-tailed deer, a glutamine to histidine change at amino acid 95 and a glycine to serine change at position 96. Oral experimental infections of deer demonstrated the 95Q/96G allele (wild-type) result in shorter incubation periods than infections of animals lacking this wild-type allele. Biochemical analysis of the disease-associated PrP (PrP^{CWD}) suggests that alleles encoding 95H/96S proteins result in generation of PrP^{CWD} with a different conformation than observed with wild-type prion proteins. The PrP^{CWD} resulting from these amino acid polymorphisms differ in their biochemical properties including resistance to proteinase K. Differences in the isolates are also observed in scrapie cell assay. Subsequent transmission of these deer isolates into transgenic mice expressing either 96G or 96S alleles of cervid PrP identified a novel strain of CWD which results in clinical infection in the normally resistant 96S mice. Characterization of these novel strains is ongoing and includes serial passages in transgenic mice expressing the different polymorphic alleles of cervid PrP, biochemical analysis; prion organotypic slice culture assays, infection of primary neuron/astrocyte cultures and myotubes. The properties of these CWD agents underscore the importance of identifying and characterizing CWD strains; the different biochemical, biological and biophysical properties emphasize the importance of defining host range.

Identification of eukaryotic chaperones involved with protein disaggregation

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In the cell, the quality control of protein structure is maintained by a sophisticated system of protein chaperones that, if disrupted, can lead to improperly folded proteins, which not only lose their appropriate function, but can also aggregate to form insoluble oligomers. These oligomers can be toxic and are implicated in a number of different pathologies. In many organisms, specialized chaperones called disaggregases eliminate these aggregates either by proteolytic degradation, or by facilitating their dissociation into monomers. The chaperone family involved with disaggregation cooperates with the proteasome system and other chaperone, called foldases and holdases, to form the PQC (Protein Quality Control) system. Foldases, holdases and disaggregases have been reported in many organelles, such as mitochondria, and in all organisms. However, no single disaggregase has been described in metazoans and one emerging possibility seems to be that metazoans may have multiple systems that degrade protein aggregates, with weak activities but high specificities. Therefore, it is important to search for proteins with intrinsic disaggregase activities in high eukaryotes. We will present results on potential proteins from sugarcane and humans that have similar functions to that of a disaggregase chaperone. The cDNAs were cloned and their function characterized by *in vitro* and *in vivo* assays and in the present stage our results indicate that we have found potential candidates in sugarcane and human, as the proteins behave similarly to Hsp104, the archetype disaggregase from yeast. For instance, the cloned cDNAs were capable to complement Δ*hsp104* phenotypes in yeast: they protected against heat stress and, when overexpressed, were able to cure the [PSI+] prion phenotype but not [RNQ1+]. Further

results and the implication of the findings will be discussed.

Prion infections: from molecular biology to therapy and prophylaxis

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The long-term goal of my group is to develop therapeutic and prophylactic anti-prion strategies. The overall objective we have is to study the cellular and molecular biology of prion infections and to use gained understanding for delineating novel targets for intervention. We have focused our attempts on two main strategies. One is the endogenous cellular clearance capacity for prions which we want to induce to a level which can halt prion infections. The other one is to target PrP^C expression of which is an obligatory prerequisite for prion conversion and execution of neurodegeneration. We are addressing existing cellular programs like autophagy and ER/post-ER quality control which we manipulate in a way that this either disfavors the formations of prions or enhances their degradation. A clearly translational project is our attempt to develop an active vaccine approach against CWD by overcoming self-tolerance against PrP^C. A new approach is to interfere with prion disease by down-regulating the PrP promoter, thereby reducing the substrate for prion conversion. We are establishing a high-throughput screen, which will allow us to identify and characterize natural and chemical compounds, in order to effectively interfere with *Prnp* expression. We use primary cells from a novel reporter mouse line Dr. F. Jirik's group has engineered that expresses GFP and luciferase under the control of the murine *Prnp* promoter. This allows us to test hit compounds in reporter mice by *in vivo* bioluminescence imaging, thereby validating which candidates would be promising *in vivo*. This work will also provide mechanistic insights into pathways involved in regulation of PrP expression in various cell types *in vitro* and *in vivo*. Taken together, our work will provide mechanistic insights into basic mechanisms which are relevant for neurodegenerative diseases and will result in novel targets for rational therapy against prion diseases and protein misfolding disorders.

Prion-like aggregation of p53 in human cancer: new targets for antitumoral drugs

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p53 is a master regulatory protein that participates in cellular processes such as apoptosis, DNA repair and cell cycle control. p53 is a tetrameric protein in which each subunit consists of 393 amino acid residues. The function of this tumor suppressor protein is lost in more than 50 per cent of human cancers.[1] Recent studies have suggested that the formation of prion-like aggregates of mutant p53 is associated with loss-of-function, dominant-negative and gain-of-function (GoF) effects.[2-8] Studies from our group have shown that p53 aggregation in a mixture of oligomers and fibrils that sequestrates the native protein into an inactive conformation, typical of a prionoid behavior.[2] These aggregates are

present in tissue biopsies of breast cancer especially in more aggressive ones.[2, 9] More recently, we have evaluated whether p53 aggregates may be also present in human glioblastoma cells and participate in the tumorigenic process. Our data shows great colocalization between p53 and oligomers as well as amyloid fibers in glioblastoma cells. The prionoid properties of p53 aggregates are considered potential targets for drug development. The prion-like properties of nucleation, templating, multiplication and spread have been already considered potential new targets for the development of neurodegenerative disease therapies.[10, 11] We have evaluated whether PRIMA-1, a classical drug described to stabilize mutant p53 structure and function, exerts its effect on aggregated mutant p53. Also, 2-methylene-3-quinuclidinone hydrate (MQ) has been shown to inhibit WT and mutant recombinant p53 central core domain (p53C) aggregation at 37oC. The WT form has been protected in a lower degree. MQ has also been shown to inhibit the seeding promoted by mutant p53 cellular extracts fractions enriched in oligomers on WTp53C. The same seeding inhibition effect was observed for extracts from cells treated with PRIMA-1.

References

1. Muller, et al. Nat. Cell Biol 2013; 15:2-8.
2. Ano Bom, et al. J. Biol. Chem. 2012; 287:28152-62.
3. Silva, et al. Acc. Chem. Res. 2010; 43:271-9.
4. Ishimaru, et al. Biochemistry 2003; 42:9022-7.
5. Silva, et al. Biosci. Rep. 2013; 33:593-603.
6. Xu, et al. Nat. Chem. Biol. 2011; 7:285-95.
7. Forget, et al. PLoS One. 2013; 8:e69242.
8. Lasagna-Reeves, et al. Biochem. Biophys. Res. Commun. 2013; 430:963-8.
9. Levy, et al. Int. J. Biochem. Cell. Biol. 2011; 43:60-4.
10. Soto. Cell. 2012; 149:968-77.
11. Prusiner. Science. 2012; 336:1511-3.

The prion organotypic slice culture assay revisited: application to new species, new brain regions and therapeutic studies

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The prion organotypic cerebellar slice culture assay (POSCA) allows the propagation of rodent-adapted scrapie strains in cerebellar slice cultures prepared from 10 to 12 day old tga20 mice that overexpress mouse PrP^{C} by six fold. These slices replicate PrP^{Sc} within 21 days of infection and recapitulate aspects of prion disease pathology within 40 days. As such, it is a powerful tool for studying disease pathogenesis and treatment. We have now adapted POSCA for use with transgenic mice expressing deer PrP^{C} and have successfully infected it with deer chronic wasting disease. In addition, we have been able to replicate prions in organotypic cultures from other brain regions. I will discuss these adaptations of POSCA, in addition to data on prion inhibition studies we are conducting in this system.

Prion diseases: perspective from the bedside

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Prion diseases are rare conditions often included in the differential diagnosis of rapidly progressive dementias (RPDs). In several series, Creutzfeldt-Jakob disease (CJD) was one of the most frequent causes of RPD. However, there are other causes of RPD, many of them potentially reversible when the diagnosis is made not too late to allow adequate treatment. To misdiagnose a case of CJD as another type of dementia is an error; but to misdiagnose a reversible disease as a prion disease is a serious error. Neurologists should use all available data to reach the correct diagnosis on time. They need to confirm CJD as well as to exclude potentially reversible conditions. The diagnosis of CJD relies on the clinical features, electroencephalogram (EEG), presence of abnormal concentrations of proteins in the cerebrospinal fluid (CSF) and neuroimaging. In spite of the initial expectations on characteristic patterns of CJD in the EEG, which shows short periodic activity, it is now clear that this pattern does not occur in many cases of CJD and it may be found in other diseases. The same is true for the presence of high levels of 14-3-3 and tau proteins. Recently, high sign in the cortical ribbon in diffusion-weighted magnetic resonance imaging, previously considered to be the most typical finding of CJD, has been reported in RPDs caused by autoimmune and mitochondrial encephalopathies. Other diagnostic tools for CJD are needed in clinical practice, such as the detection of the pathological prion proteins in the CSF or an equivalent to amyloid PET for prion diseases.

Structural aspects of the interaction between prion protein and heparin: importance for health and disease

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The conversion of cellular prion protein (PrP^{C}) into scrapie PrP (PrP^{Sc}) is a central event in prion diseases. The conversion requires contact between both isoforms and probably also requires a cellular factor, such as a nucleic acid or a glycosaminoglycan (GAG). GAGs exhibit a paradoxical effect, as they convert PrP into PrP-res but also exert protective activity. Our group has been investigating the structural features implicit in GAG-PrP interaction. We reported that low molecular weight heparin (LMWHep) does not induce recombinant PrP conversion. The interaction between these two molecules induced local conformational changes in the protein, resulting in decreased solvent accessibility, which led to oligomerization and aggregation. However, this aggregation was transient and followed by a stabilization process; after reaching equilibrium, the soluble forms of the protein exhibited a tertiary structure very similar to that of the free protein. We also showed that the PrP octarepeat region, a highly conserved region in the PrP N-terminal domain, is important for the PrP-LMWHep interaction at neutral pHs and suggested the existence of a second binding site in the C-terminal domain, near helices 2 and 3, at acidic pHs. In addition, we showed that LMWHep protects rPrP23-231 from aggregation induced by RNA molecules, increases thermal PrP stability, and affects not only the extent of PrP fibrillization but also its kinetics. These results shed light on the role of GAGs in PrP conversion. The transient aggregation of PrP may explain why some GAGs have been reported to induce the conversion into the misfolded, scrapie conformation, whereas others are thought to protect against conversion. Our findings explain the protective effect of heparin in different models of prion and prion-like neurodegenerative diseases and establish the groundwork for the development of therapeutic strategies based on GAGs.

Pleiotropic *in vivo* effects of PrP octarepeat substitutions

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Introduction. PrP^{C}'s globular domain is preceded by a flexible N-terminus that can bind copper via four histidine-containing octarepeats. Alternative conformations of the PrP^{C} N-terminal octarepeat region (OR), denoted components 1, 2 and 3, occur in the presence of different concentrations of copper ions.
Hypothesis. Since conformation is a crucial parameter in prion disease we hypothesized that different N-terminal PrP conformations might be associated with different phenotypic properties.
Work Plan. Reiterative peptide mutagenesis was performed to produce octarepeat variants with discrete, component 1 or 3 copper-binding geometry with respective binding stochiometries at saturation of 4 and 1 copper ions per molecule. DNA sequences encoding tandem variant OR's with "geometry-locked" conformations were engineered into a full-length mouse PrP DNA coding region. We created PrP constructs with component 1 PrP geometry (TgS1), component 3 geometry (TgS3) and control Tg wt mice bearing identical 5' untranslated region leader sequences. Construct DNAs were injected in $Prnp^{0/0}$ oocytes (*Zrch1* null allele).
Results. Independent TgS1 and TgS3 mice lines were obtained with expression levels of full-length PrP no greater than 2.5-fold those of the endogenous wt locus. None of the Tg lines succumbed to spontaneous neurologic disease with observation periods of up to 600 days of age. Allelic differences were noted in the ability to rescue the peripheral neuropathy present in uninfected aged Zrch1. $Prnp^{0/0}$ mice. Brain PrP^{C} in uninfected S3 mice, but not in S1 mice, had an increased propensity to C2 cleavage adjacent to the main Aβ binding region. Both S1 and S3 PrP alleles supported replication of the RML isolate of mouse-adapted scrapie prions, with TgS3 mice exhibiting incubation periods of under 100 days.
Conclusion. Within *in vivo* contexts, our data establishes that i) different conformations of the PrP OR can be associated with different phenotypic attributes, ii) conformational plasticity of the ORs has an unexpected relationship to PrP endoproteolysis and iii) that C2 PrP deriving from cleavage of full-length PrP - rather than a gene construct with translation initiating at codon 90 - can support prion replication.

A hybrid approach towards the structure of the infectious prion protein

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The structure of the infectious prion protein (PrP^{Sc}) and its proteolytically truncated homologue (PrP 27-30) has eluded experimental determination due to their insolubility and propensity to aggregate. Molecular modeling has been used to predict their structure, but various modeling approaches produced mutually incompatible models indicating their limitations. In absence of a three-dimensional structure, a variety of experimental techniques have been used to gain insights into the fold of this isoform. Negative stain electron microscopy and X-ray fiber diffraction indicated that the β-sheets of the infectious prion protein are likely to form a β-helix or β-solenoid structure with a height of four β-strands (rungs) per molecule (= 19.2 Å).
Our current efforts to analyze the structures of delta-GPI PrP^{Sc} and PrP 27-30 employ a hybrid approach by combining the results from different experimental methodologies. In particular, the helical periodicity that is inherent to most amyloid fibrils can be used to reconstruct a three-dimensional structure from two-dimensional image data. Preliminary reconstructions of negatively stained PrP 27-30 fibrils showed repeating densities along the fibril axis spaced at ~40 Å (2x ~19.2 Å), in good agreement with the earlier X-ray fiber diffraction results. Higher-resolution structures require electron micrographs to be recorded using cryo low-dose techniques. Cryo low-dose electron micrographs of delta-GPI PrP 27-30 amyloid fibrils routinely exhibit a 4.8 Å spacing, confirming the presence of β-strands in a cross-β configuration. The three-dimensional reconstructions of individual fibrils show two protofilaments coiled around a common axis again with 4.8 Å striations perpendicular to the fibrils axis, suggesting that the β-strands of PrP^{Sc} are being visualized. Furthermore, single-particle analyses of 15,942 individual fibril segments show a repeating pattern of ~40 Å densities with ~20 Å sized subunits confirming earlier observations. Moreover, this finding suggests a head-to-head arrangement of the PrP^{Sc} subunits along the fibril axis.

Characterising misfolding in the prion protein PrP at the single-molecule level

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The mechanism by which PrP misfolds to produce infectious conformers remains a central question in prion science. We are investigating structural conversion in PrP by using mechanical tension to induce conformational fluctuations in individual molecules held in optical tweezers. By measuring the changes in length of the protein molecules as they change structure, we can map out the properties of the folding and misfolding pathways. Whereas isolated PrP molecules can form several types of misfolded structures but they are unstable and occupied only transiently, two PrP molecules linked to be in close proximity invariably misfold into a single, thermodynamically-stable non-native structure that is rich in beta-sheets. We characterised the energy landscape for the misfolding of PrP dimers, identifying a partially-folded intermediate involving interactions between the two monomer domains that played a crucial role in promoting misfolding.

Posters

Autophagy in prion diseases: friend or foe?

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Key words: prion, autophagy, prion clearance, prion fragmentation, disaggregase

Background. Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of fatal neurodegenerative disorders that affect both humans and animals. Prion diseases are characterized by conformational conversion of the normal cellular prion protein (PrP^{C}) into the pathologic isoform PrP^{Sc}. Accumulation of the latter leads to brain damage and the characteristic signs and symptoms of the disease. Recently, the role of autophagy in prion diseases has been addressed in several reports. Autophagy is a basic cellular degradation machinery which has been shown to play a role in several neurodegenerative diseases such as Alzheimer's and Huntington's diseases. Our group previously has shown that stimulation of autophagy by chemical compounds can have anti-prion effects by increasing PrP^{Sc} clearance *in vitro* and *in vivo*.
Objectives. We postulate that components of the autophagic flux represent the biological equivalent for disaggregase or fragmentation activity as postulated in prion biology. We are studying now how autophagy affects molecular and biophysical features of prions and how autophagosomes, late endosomes and the ERC/ESCRT machinery are interconnected with prion propagation at the cellular level.
Results and conclusions. Here, we demonstrate that a basal level of autophagy is required for PrP^{Sc} propagation. Our data in mouse embryonic fibroblasts showed that the homozygous knockout of Atg5 blocking autophagic flux results in inability to propagate PrP^{Sc} whereas wild-type and ATG5-reconstituted cells do so. Notably, all types of cells were able to uptake PrP^{Sc} and initial degradation was equal. Additionally, a stable knock-down of Beclin-1 (one of the autophagy regulating proteins) using shRNA-expressing lentiviruses resulted in decreased PrP^{Sc} propagation in neuronal cells (N2a) compared to wild-type cells. Overall, our studies will provide mechanistic insights into basic cellular and molecular mechanisms which are relevant for neurodegenerative diseases and will result in novel targets for therapy against prion diseases and protein misfolding disorders.

Human prions - distinguishing sporadic from familial forms via structure and function

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Human prion diseases present as sporadic, familial, infectious, or iatrogenic diseases. They include diseases such as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI). The wide range of phenotypic variation in human prion diseases is caused by aberrantly folded versions of the prion protein, termed PrP^{Sc}. Evidence indicates that the diversity of the PrP^{Sc} strains is mediated by variations in the PrP^{Sc} structure rather than by mutations in an agent-specific nucleic acid.
Using high-resolution electron microscopy, we will investigate the structural differences that may exist between sporadic and familial forms of human prions and the molecular bases for different types of prion aggregates. Preliminary data, obtained with mouse Δ-GPI PrP^{Sc}, which lacks the C-terminal glycophosphatidylinositol anchor, have revealed the basic architecture of the PrP^{Sc} amyloid fibril: helical assemblies consisting of pairs of PrP^{Sc} protofilaments. Fourier-transform analyses of the electron micrographs consistently show a 4.8 Å signal indicative of the cross-β structure of the PrP^{Sc} fibrils, consistent with the amyloid nature of these polymers. If a similar resolution can achieved with patient-derived human prion samples, then disease-specific differences between the various forms of CJD, GSS and FFI prions may be visualized. Ultimately, the results obtained from this study will improve our knowledge on the structural differences that determine the properties of sporadic and familial human prions.

A novel role for amyloid beta protein during hypoxia/ischemia

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Introduction. Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that is associated with both genetic and environmental risk factors, including stroke (ischemia).[1] The major characteristic of AD involves the formation of deposits of the protein amyloid beta (Aβ);[2] interestingly, basal levels of Aβ are up-regulated under hypoxic conditions through the stabilization of hypoxia-inducible factor (HIF-1α).[3] We hypothesize that Aβ up-regulation during hypoxia functions to protect brain tissue by interacting with the large pore channel pannexin-1 (Panx1), which is associated with ionic dysregulation and cell death under ischemic conditions.[4]
Materials and Methods. Whole cell patch clamp electrophysiology was used in hippocampal slices from rats, which were continuously perfused with hypoxic artificial cerebral spinal fluid in order to mimic ischemia and induce membrane depolarization and ionic dysregulation (known as the anoxic depolarization).[4] Various concentrations of Aβ protein were applied concurrently with hypoxia in order to determine if Aβ affects the severity of the anoxic depolarization. In a separate set of experiments, Panx1 antagonists will be added to observe if there are any additive neuroprotective effects of pharmacologically blocking Panx1 in the presence of Aβ.
Results. Physiological concentrations of Aβ dimers (pM to low nM range)[5] attenuated the severity of the anoxic depolarization. Interestingly, more pathological concentrations of Aβ (μM range)[5] exacerbated the anoxic depolarization.
Conclusions. These data suggest that Aβ modulates Panx1 over a large concentration range and give insight into the endogenous activity of Aβ during hypoxia/ischemia. If Aβ production is up-regulated to

act on Panx1 in a neuroprotective manner during hypoxia, this will provide better understanding of the underlying cause of stroke-induced AD.

References
1. Pluta, et al. Folia Neuropathol 2012; 50:101–9.
2. Chin, et al. J Neurosci 2007; 27:9262–9.
3. Sun, et al. PNAS 2006; 103:18727-32.
4. Weilinger, et al. J Neurosci 2012; 32:12579–88.
5. Bruggink, et al. Anal Biochem 2013; 433: 112–20.

Modulation of protein quality control pathways as a novel intervention strategy in prion diseases

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Key words: prion related diseases, quality control mechanisms, therapeutic strategies, ER stress, UPR

Background. Prion diseases comprise a family of fatal neurodegenerative disorders caused by the conformational re-arrangement of normal cellular prion protein, PrP^{C}, to an abnormal infectious isoform termed PrP^{Sc}. Increasing evidence suggests the involvement of several signalling pathways in prion pathogenesis, including proteasome dysfunction and unfolded protein response (UPR). Recently, our group has shown that endoplasmic reticulum (ER) stress and UPR impairment are critical events mediating PrP^{C} aggregation and PrP^{Sc} accumulation in persistently prion-infected cells which also positively affect the subcellular trafficking of PrP through the secretory pathway to the cell surface.[1] This finding provides a novel pathway which contributes to the prion propagation. Alteration of ER function triggers an adaptive stress response to restore homeostasis termed the UPR which is mediated by the up-regulation of ER-chaperones and folding enzymes playing a role in maintenance of PrP quality control (QC) in addition to an increasing in the efficiency of ERAD pathways. The UPR induces also QC proteins acting downstream the ER.
Objectives. Our central hypothesis here is that endogenous cellular quality control mechanisms can counteract prion conversion. Our main objective was to interfere with prion propagation by improving PrP quality in ER and post ER-compartments.
Methods. Depending on the subcellular location, different cellular QC mechanisms will be targeted. Using lentiviral mediated stable expression the effect of a collection of QC, folding and cargo sorting proteins in modulating prion protein aggregation and PrP^{Sc} biogenesis will be investigated by western blot and immunofluorescence.
Conclusion. Our preliminary data strongly suggests that stable expression of such proteins results in a long-term anti-prion effect in prion-infected cells. From *in vitro* studies proof of concept will be validated for a further utilisation of QC proteins as anti-prion strategy *in vivo*.
As the involvement of irreversible ER/UPR stress pathways has been reported in the pathogenesis of several neurodegenerative diseases, our studies are crucial and may ultimately serve as a basis for the development of rationally-designed therapeutic strategies for other neurodegenerative disorders such as Alzheimer's disease.

References

1. Nunziante, et al. J Biol Chem, 2011; 286(39):33942-53.

Detection of CWD prion in fecal samples by RT-QuIC

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Key words: chronic wasting disease, transmissible spongiform encephalopathy, PrP^{Sc}, urine, feces, RT-QuIC, caribou, *prnp*, wildlife disease management

Chronic wasting disease (CWD), a unique transmissible spongiform encephalopathy (TSE) of North American deer, moose and elk, is the only prion disease occurring in both captive and free-ranging animals. A hallmark of CWD is that infectious prions are shed into excreta such as saliva, urine and feces, which is thought to contribute to the facile horizontal transmission within cervid species. For controlling the disease spread, a potent method of diagnosis is highly desired; regarding this, several ultrasensitive assays have been developed. Real-time quaking-induced conversion assay (RT-QuIC) is one of the most promising *in vitro* amplification techniques. It gives an end point quantitation for detecting minute amount of misfolded prion protein (PrP^{Sc}) in infected samples upon various prion diseases. Since urine and feces may be practical specimens for CWD diagnosis concerning their accessibilities. Previously we employed RT-QuIC to detect CWD prions in urine and fecal extracts of orally infected white-tailed deer and mule deer. We have proven that urine can be used for preclinical diagnosis of CWD by RT-QuIC. The fecal extracts we prepared were able to seed RT-QuIC reaction, though there is space to improve the sensitivity. Our future works aim at trying diverse approaches to purify fecal extracts by applying different buffers as well as concentrations of detergents. PrP^{Sc} in feces will be concentrated by either employing ultracentrifugation or PTA precipitation. Once we improve feces seeding activity, the goal will be to analyze feces specimen from caribou, since they are susceptible to CWD and potential transmission is of huge concern. Finally, to understand the distribution of *prnp* polymorphisms that modulate susceptibility to CWD among caribou populations, we will extract DNA from caribou feces, amplify the PrP coding sequence by PCR and sequence the resulting products. This work will provide some clues about predicting the spread of CWD.

Peptide aptamers interfering with PrP^{C}-PrP^{Sc} interaction inhibit prion protein misfolding

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Key words: prion, peptide aptamer, misfolding

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases affecting both humans and animals. They include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cows, scrapie in small ruminants and chronic wasting disease (CWD) in cervids. The causative agent is the prion, a pathogen consisting of an abnormally folded isoform (PrP^{Sc}) of the

cellular prion protein (PrP^C). The steric interaction between these two isoforms leads to misfolding of the soluble and protease-sensitive PrP^C into an insoluble, protease-resistant form, which displays the same primary sequence but different secondary structure. This process is still poorly understood and no therapeutic or prophylactic tools are available yet.
In order to identify anti-prion compounds, we aim to inhibit prion conversion by interfering with the PrP^C-PrP^{Sc} interplay. We have previously described a peptide aptamer (PA8) with high affinity for PrP^C which reduces prion propagation in prion infected cultured cells. Peptide aptamers consist of a peptide inserted in a scaffold protein, here the *Escherichia coli* thioredoxin A (trxA). To improve binding affinity and anti-prionic activity of PA8, its binding to PrP^C has been modelled *in silico.* New mutant clones of PA8 with an expected higher binding affinity will be created by selected amino acid replacements in the peptide moiety. These new peptide aptamers are created by site directed mutagenesis, expressed in *E. coli*, purified via His_6-Tag and employed for treatment of prion-infected cells. An improvement of their binding property is expected, as well as an enhanced inhibition of the endogenous PrP^{Sc} conversion in treated cells. The anti-prion effects of the most promising candidates will be further evaluated in murine bioassays. In future, our results will be useful for identifying a compound by structure-based drug design with anti-prionic activity, able to counteract prion conversion *in vivo.*

Measuring the energy landscape for PrP misfolding in single molecules

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Protein folding involves a search for the minimum-energy state in a conformational free-energy landscape, with the landscape encapsulating the key molecular properties that determine how folding proceeds. Similarly, protein misfolding and aggregation, common features in many diseases, are a manifestation of free-energy landscapes for structure formation, hence mapping these landscapes should yield powerful insights. However, the inherent complexity of misfolding has heretofore precluded direct measurement of the related energy landscapes. We reconstructed the free-energy landscape for misfolding of the prion protein, ShaPrP(90-231), using single-molecule force spectroscopy to observe directly the misfolding of individual dimers into minimal aggregates. Two PrP monomers were joined end-to-end to form dimers and were repeatedly unfolded and refolded using optical tweezers. Remarkably, neither domain of the dimer ever formed the native structure and instead formed exclusively a non-native structure. The free-energy landscape of the PrP dimer was funneled into a thermodynamically-stable misfolded state via a single pathway containing multiple intermediates, one of which promoted misfolding by blocking native structure formation. These features contrast sharply with those observed for other protein oligomers studied at the single-molecule level, where misfolding was rare. This suggests that PrP is uniquely pre-disposed to conversion into specific misfolded structures through intermolecular interactions. These results demonstrate a quantitative approach to measuring misfolding landscapes and provide mechanistic insight into the formation of non-native structures in PrP.

Involvement of lysosome-associated degradation in fulminant neurodegeneration

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The misfolding, aggregation and accumulation of proteins is known to be the causal event in many neurodegenerative disorders. Under normal conditions a network of chaperones and proteolytic systems fight against the toxic effects of aberrant proteins. Multiple lines of evidence indicate that an imbalance between the production and clearance of proteins initiates a toxic sequence of events leading to protein misfolding, synaptic dysfunction, synaptic loss and neurodegeneration. Lysosomes are highly acidic organelles that contain hydrolytic enzymes that receive and clear macromolecules from the secretory, endocytotic and autophagic pathways. The link between synaptic loss and lysosomal function is an area that is not well investigated. To begin to assess the role of lysosome function in neurodegeneration, we used mice lacking the chaperone cysteine string protein (CSPα) as a model of neurodegeneration. CSPα deficient mice are normal at birth, but postnatally develop an impairment of synaptic function in an activity-dependent manner followed by a fulminant form of neurodegeneration, paralysis and early death. CSPα-null mice were injected intraperitoneally with either 5mg/kg chloroquine, a weak base that increases lysosomal pH and inhibits lysosome activity or 20mg/kg Z-Phe-Ala-diazomethylketone, which increases lysosomal activity by increasing lysosomal cathepsin B levels, or vehicle (saline/DMSO) every two days beginning at postnatal day five for two weeks and weight loss was monitored. Following treatment, synaptic fractions were isolated to determine the production and clearance of amyloid precursor protein and $A\beta_{1-42}$, a 42 residue long β amyloid protein as well as synaptic plasma membrane/lysosome markers. Our preliminary results indicate that Z-Phe-Ala-diazomethylketone slows the onset of neurodegeneration. This line of investigation will directly answer the question whether the progression of neurodegenerative disorders is altered by pharmaceutical agents that up-regulate or down-regulate lysosomal activity.

Transmissibility of chronic wasting disease allotypes in susceptible and resistant transgenic mice

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Transmission of chronic wasting disease (CWD), a fatal prion infection responsible for epizootics in free-ranging and farmed cervids is influenced by the primary amino acid sequence of the host prion protein (PrP^C). In white-tailed deer, *Prnp* alleles including Q95G96 (wild-type allele), Q95S96 (*S96*) and H95G96 (*H95*) encode PrP^C isoforms, which differentially impact susceptibility. Deer with at least one copy of *S96* or *H95* alleles exhibit longer survival times following CWD infection. Transgenic mice expressing cervid *Prnp* alleles recapitulate the differences in CWD susceptibility observed in deer. Considering the proteinaceous nature of prions, we hypothesize that, during CWD infection of deer with different *Prnp* genotypes, a mismatch between the prion protein sequence of the invading CWD agent and the host PrP^C would impact the biological properties of the adapted CWD prion. We tested this possibility by evaluating the transmissibility of CWD isolates derived from deer with different *Prnp* genotypes (CWD

allotypes). Inoculation of transgenic (tg) mice expressing deer *wt-Prnp* resulted in 100 per cent attack rates. Tg mice expressing the *S96* allele succumbed with prion disease only when inoculated with CWD allotypes containing H95 PrP. Our finding indicates that structural modifications associated with the deer *H95-Prnp* allele facilitated the adaptation of CWD prions into a resistant genotype, implicating *Prnp* genotype heterogeneity in the emergence of CWD agents with novel transmission properties.

Prion disease biomarkers: a systems approach

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Ante-mortem testing for human prion diseases is currently performed once clinical symptoms are present, during the end stage of disease. Surrogate markers are used in combination with other methods for differential diagnosis of Creutzfeldt-Jakob disease (CJD). Definitive diagnosis is based on post-mortem analysis of the diseased brain. The identification of pre-clinical disease markers would offer efficacy in diagnosis as well as provide further insight into mechanisms involved with neurodegeneration and drug efficacy regimes. To identify pre-clinical ante-mortem markers of prion disease, we adapted prion disease to rats, facilitating a comparative analysis between species. The rat prion infection allows us to assess the abundance of prion disease biomarkers at multiple time-points over the course of the disease. This contrasts with human samples, which are generally only available at the clinical stage. We are analyzing a readily available bio-fluid, cerebrospinal fluid (CSF), as the composition reflects the pathological processes of the brain. Proteomic analyses of clinically affected rats, compared with uninfected rats, revealed a number of proteins up-regulated and/or specific to prion disease, including CJD biomarkers 14-3-3s and neuron specific enolase. CSF was also analyzed via western blot for further validation. Biomarkers from rat CSF are similar to those detected in CJD, demonstrating the utility of using rat prion disease for biomarker identification. By tracking the progression of prion infection in rats, we may be able to further define and compare the pathophysiological changes associated with prion infection to better understand mechanisms of neurodegeneration and drug efficacy regimes. Identifying molecular biomarkers of prion disease during pre-clinical phase and clinical phase of disease may lead to the development of a pre-clinical ante-mortem screening test for prion diseases.

Propensity of translocation of anti-prion therapeutic agents across blood brain barrier: prediction by molecular theory of solvation

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Key words: neurodegenerative diseases, molecular theory of solvation, antibody functionalization

Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases with prion-like mechanism of pathology. In both AD and ALS, proteins misfolding and aggregation lead to death of neurons and possible prion-like propagation of these diseases at the cellular level. One of the most promising diagnostics and therapeutic methods for neurodegenerative diseases is the immunological approach.[1] Antibody binding to neurotoxic oligomers or misfolded proteins can inhibit prion-like propagation of misfolding.
A general problem in the immunological therapeutic approach is how to efficiently deliver the antibody across the blood-brain barrier (BBB) to the central nervous system (CNS). This blood-membrane (hydrophilic/hydrophobic) feature has been characterized by water-octanol transfer solvation free energies, which can be estimated from the experimental partition coefficient of a solute in water and in octanol.[2]
We developed a new protocol based on the 3D-RISM-KH molecular theory of solvation[3] to predict propensity of antibody crossing the BBB. The approach is validated based on a library of approved drugs and is applied to extended set of molecules with functional groups from known AD antibodies. Based on this new approach we provide suggestions on functionalization of antibodies against neurodegenerative diseases for their optimal delivery to CNS.

References
1. Roettger, et al. Nat. Rev. Neurol. 2013; 9:98-10.
2. Kolar, et al. J. Phys. Chem. B. 2013; 5950-62.
3. Kovalenko. Pure Applied Chem. 2013; 85:159-99.

Immunogens for containing chronic wasting disease

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Key words: prion, CWD, immunogen, vaccine

Chronic wasting disease (CWD) is a fatal prion disease of cervids. CWD is the most contagious prion disease with substantial lateral transmission and shedding of infectivity into the environment. Appearance of CWD in wild living and migrating animals makes it uncontrollable. There is a potential future risk to humans and other animals (other cervids and non-cervids). Therefore, it is mandatory to develop effective means for containing CWD. Our central aim is to interfere in peripheral prion infection by inducing autoantibodies against PrP^C by active vaccination, thereby also reducing shedding of prion infectivity. To achieve this goal we have to design an immunogen which can overcome self-tolerance against PrP^C. Our group has previously shown that a covalently linked dimer of mouse prion protein as immunogen induces robust humoral and cellular responses against murine PrP^C in wild-type mice without adverse side effects. Also, polyclonal auto-antibodies bound to surface-located PrP^C, thereby interfering with prion biogenesis. Here, our study focuses on developing cervid PrP immunogens and to determine their immunogenicity and safety when delivering them using oral delivery strategies in mice expressing cervid PrP. We prepared gene constructs encoding a monomer and dimer form of mule deer prion protein. These constructs were cloned in pQE30 vector and transformed in *E. coli* strain BL21. We are now expressing and purifying the monomer and dimer forms of mule deer prion protein. Purified

immunogens will be co-packaged with adjuvants into poly-lactide-co-glycolide (PLG) microspheres. We will then deliver microspheres orally into deer PrP-transgenic mice for studying immune responses and possible side effects. In a second approach we started expressing these immunogens using plant-based expression systems. Eventually our study will establish immunogens which can be used for oral vaccination for controlling CWD.

A new anti-prion strategy: modulation of *Prnp* transcriptional activity

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Key words: novel anti-prion strategy, novel transgenic reporter mouse, Prnp-EGFP-Luc2 transgenic mouse, high-through put screening, chemical compound library

Prions diseases are infectious fatal neurodegenerative disorders with no treatments or cures. The diseases occur when the normal cellular prion protein (PrP^{C}) converts into the infectious isoform PrP^{Sc}. It is our goal to develop anti-prion treatments by disrupting the prion conversion process. Interfering with PrP^{C} expression has no apparent side effects and genetically modified knockout animals are immune to the diseases. Therefore, we hypothesize that compounds that reduce PrP^{C} expression can be used as therapeutic or prophylactic treatments against prion diseases. We will develop targeted and high-throughput screens (HTS) of natural and chemical compounds using cells from a novel reporter mouse line. This line has been engineered to express GFP and luciferase under control of the *Prnp* promoter (Prnp-EGFP-Luc2). This novel strategy has the advantages of (1) no major side effects, since $PrP^{0/0}$ mice do not exhibit a pronounced phenotype, (2) this should be effective against different prion strains and (3) we can screen uninfected cells and animals. We will test luciferase expression of macrophages and primary neurons treated with growth factors and cytokines we have previously shown to alter PrP^{C} expression. We will develop a HTS using the INCell2000 imaging system to examine EGFP fluorescence in primary neurons and astrocytes treated with our in-house libraries of about 7000 compounds. Compounds causing a >2-fold decrease in *Prnp* expression will be evaluated *in vivo* by administering candidates to transgenic mice, then examining PrP^{C} expression in brain homogenates and spleen using immunoblot and qRT-PCR. Lastly, we will test candidate compounds on prion infected cell cultures to validate our hypothesis. We expect to identify novel candidates useful for treating prion diseases, gain insight into pathways controlling *Prnp* in various cell types and *in vivo* and the research may have relevance to Alzheimer's disease since PrP^{C} has been shown to transduce Aβ toxicity.

PrP^{C} modulates A-type K^{+} currents mediated by Kv4.2 complexes through Dipeptidyl Aminopeptidase-like protein 6

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Widely expressed in the adult central nervous system, the cellular prion protein (PrP^C) is implicated in a variety of processes including neuronal excitability. Dipeptidyl aminopeptidase-like protein 6 (DPP6) was first identified as a PrP^C interactor using *in vivo* formaldehyde crosslinking of wild-type (wt) mouse brain. This finding was confirmed in three cell lines and, as DPP6 directs the functional assembly of K^+ channels, we assessed the impact of wt and mutant PrP^C upon Kv4.2-based cell-surface macromolecular complexes. Whereas a Gerstmann-Sträussler-Scheinker (GSS) disease version of PrP with eight extra octarepeats was a loss-of-function both for complex formation and for modulation of Kv4.2 channels, wt PrP^C - in a DPP6-dependent manner - modulated Kv4.2 channel properties causing an increase in peak amplitude, a rightward shift of the voltage-dependent steady-state inactivation curve, a slower inactivation and a faster recovery from steady-state inactivation. Thus the net impact of wt PrP^C was one of enhancement, which plays a critical role in the down-regulation of neuronal membrane excitability and is associated with a decreased susceptibility to seizures. Insofar as previous work has established a requirement for wt PrP^C in the Aβ-dependent modulation of excitability in cholinergic basal forebrain neurons, our findings implicate PrP^C regulation of Kv4.2 channels as a mechanism contributing to the effects of oligomeric Aβ upon neuronal excitability and viability.

Biodegradation of infectious prions in compost

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Key words**:** prion, specified risk materials, composting, biodegradation, western blotting, protein misfolding cyclic amplification, bioassay

Background/Introduction. Composting may serve as a practical and economical means of disposing of specified risk materials (SRM) or animal mortalities potentially infected with prion diseases (transmissible spongiform encephalopathy, TSE). Our study investigated the degradation of prions associated with scrapie (PrP^{263K}), chronic waste disease (PrP^{CWD}) and bovine spongiform encephalopathy (PrP^{BSE}) in laboratory-scale composters and PrP^{263K} in field-scale compost piles.
Materials and Methods. Laboratory-scale composting was conducted over 28 days using manure matrices with or without poultry feathers. Compost was mixed at day 14 to generate a second heating cycle. Degradation of PrP^{263K}, PrP^{CWD} and PrP^{BSE} was measured using western blotting (WB), and with additional analysis on PrP^{263K} and PrP^{CWD} using protein misfolding cyclic amplification (PMCA). For field-scale composting, duplicate biocontained composters containing PrP^{263K} adhered to stainless steel beads and cattle mortalities were used. Degradation of PrP^{263K} was assessed at 0, 14, 56, 112 and 230 days using composted beads in a Syrian golden hamster bioassay.
Results. Analysis of WB indicated that PrP^{263K}, PrP^{CWD} and PrP^{BSE} were reduced by at least 2 $\log_{10}$, 1-2 $\log_{10}$ and 1 $\log_{10}$ after two cycles of laboratory-scale composting, respectively. Further analysis using PMCA confirmed a reduction of 2 $\log_{10}$ in PrP^{263K} and 3 $\log_{10}$ in PrP^{CWD}. Enrichment for proteolytic microorganisms through the addition of feather keratin to compost enhanced degradation of PrP^{263K} and PrP^{CWD}. Field-scale composting effectively reduced TSE transmission in intracranially implanted hamsters with beads composted for 14, 56 and 112 days. After 230 days of composting, only one in five hamsters succumbed to TSE disease.
Conclusions. Estimates of infectious titre reduction based on incubation period suggest that composting achieved at least a 4.8 $\log_{10}$ reduction in PrP^{263K} infectivity. Our findings demonstrate that composting significantly reduces PrP^{TSE} and with consideration for overall risk, may be a viable method for the disposal of SRM.

Detection and typing of PrP^{BSE} in formalin-fixed and formalin-fixed paraffin-embedded tissue using western blot analysis

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The mandate of a reference laboratory is to confirm and characterize BSE suspect cases. While IHC on paraffin-embedded brain tissue is one of the two confirmatory tests, molecular typing via western blot requires fresh/frozen brain. To handle a situation comparable to Brazil's first BSE case where fresh/frozen tissues were unavailable, we worked up a western blot protocol for detecting PrP^{BSE} in formalin-fixed (FF) and formalin-fixed paraffin-embedded tissues (FF-PET).
Sections of FF-PET tissue were submerged in Tris Buffer and deparaffinised using a series of boil and snap freeze cycles. Deparaffinised tissue was moved to clean tubes containing fresh buffer and homogenized. Formalin fixed (FF) tissues were removed from 10 per cent buffered-formalin solution, placed in 95 per cent ethanol to remove the formalin and then into PBS (pH 7.5) to remove the ethanol. Tissue was then homogenized in fresh buffer. Homogenates from both sample types were sonicated, digested under mild and stringent conditions, denatured for 45 minutes, separated by electrophoresis and transferred to a membrane for immuno-detection. Several PrP specific antibodies were used to facilitate BSE typing.

BSE was detected and typed in both FF and FF-PET tissue using this optimized method. Monoclonal antibody P4 could differentiate the H-type from C- and L-type BSE. The WB banding profiles of C- and L-type BSE were dissimilar enough to allow for identification of each type. The mild and stringent PK digestion did not help in identifying the BSE types which is likely related to the residual formalin cross links stabilizing the structures of atypical PrP^{BSE}.

Using this protocol to extract and detect PrP^{BSE}, we are able to determine the molecular characteristics of the misfolded BSE prions in FF and FF-PET samples. This provides the ability to use a WB assay as a diagnostic alternative when fresh tissues are limited or not available.