JAN 2014

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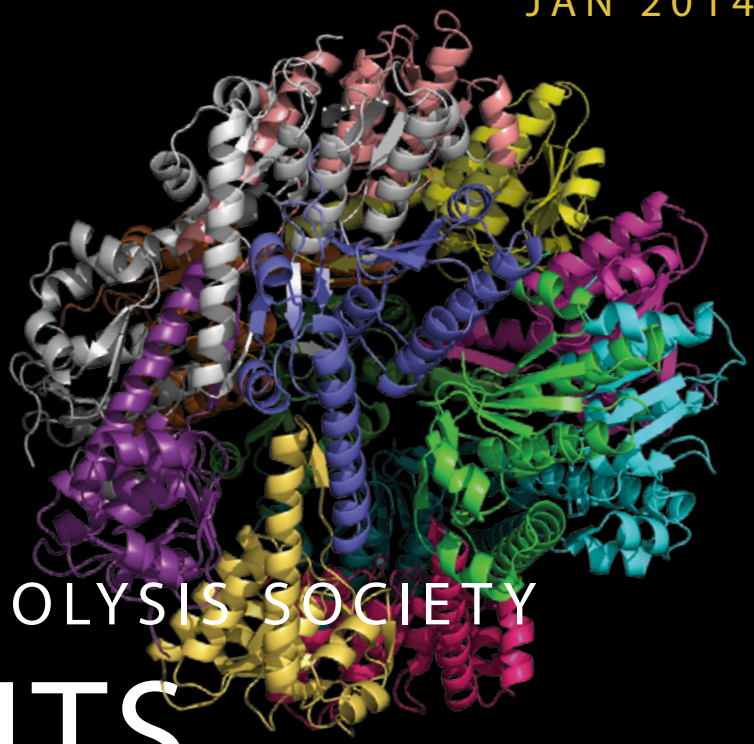

INTERNATIONAL PROTEOLYSIS SOCIETY

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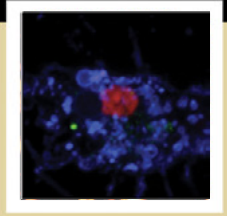

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FOR ALL YOUR IMPORTANT PROTEASE QUESTIONS

COUNCIL OF THE INTERNATIONAL PROTEOLYSIS SOCIETY

A Message From the President:

I would like to welcome all new IPS members into the Society! We look forward to your participation and engagement in years to come. I also formally welcome all our new Council members and thank our outgoing council members for their service.

In October , we held our 8th General Meeting at the Spier Estate near Cape Town, South Africa. This was the first IPS meeting held in Africa and a huge success both scientifically and socially. I would like to congratulate Ed Sturrock, the conference organizer, and his team who planned and ran a fantastic meeting. There is a great synopsis of the meeting in this issue, which will also be highlighted in a special issue of Biological Chemistry.

It was fitting that we inducted Clive Dennison as a new Lifetime Member of the Society, since he has contributed so much to protease research in South Africa. Please take a look at Clive's thoughts on receiving this award. Unfortunately we lost a Lifetime Member with the passing of Professor Nobuhiko Katunuma in November, who is also remembered here.

The 9th General Meeting of the Society will be organized by Judith Clements and held in Penang, Malaysia from October 4th-8th, 2015. In the time between there are many meetings of interest to IPS members. We always try to highlight these here and on our website (www.protease.org).

Please send suggestions or feedback to any of the IPS officers or councilors, especially as we update the website. We welcome your input. This is your society - the more active you are - the more successful it will be!

Bob Lazarus, IPS President Email: laz@gene.com

Eighth General Meeting of the International Proteolysis Society

During the period of 20-24 October 2013, delegates from 22 countries converged on the Spier Wine Estate outside Cape Town, South Africa for the 8th General Meeting of the IPS. Three IPS training workshops on "Protease Kinetics," "Imaging" and "Structural Biology" preceded the meeting and provided 35 members-in-training with hands-on, protease-specific trainings. The Meeting was organized by Ed Sturrock (IDM, South Africa) and included excellent scientific talks, stimulating poster sessions and discussions, and many networking opportunities. Delegates were treated to a unique African experience that encompassed the botanical gardens of Kirstenbosch, the Cape Point reserve and, for the more adventurous, diving with great white sharks!

Spier Wine Estate, Stellenbosch, South Africa

The 8th General Meeting of the IPS started on Sunday evening with an outstanding overview by the keynote speaker Vishva Dixit (Genentech, Inc, USA) of inflammatory caspases. He showed that the canonical TLR4 ligand lipopolysaccharide (LPS) can also signal intracellularly through a noncanonical inflammasome complex involving caspase-11, leading to pyroptosis and sepsis. Mice lacking caspase-11 were resistant to LPS-induced lethality, even in the presence of TLR4.

The Monday morning session on "Proteases in Immunity," chaired by Bonnie Sloane and Guy Salvesen, revealed unexpected roles for proteases in unexpected compartments. Chris Overall (Univ. British Columbia, Canada) showed that matrix metalloproteinase-12 (MMP-12) binds a promoter in the nuclei of virally infected cells and modulates IFN-γ expression. Promisingly, cell impermeable inhibitors of MMP-12 were antiviral in a mouse model. Phil Bird (Monash Univ., Australia) revealed that the presumed intracellular protease, granzyme B, has extracellular roles involving the cleavage of extracellular substrates; this novel role could affect the trafficking of cytotoxic lymphocytes. Gabor Pal (Eötvös Loránd Univ., Hungary) used compelling kinetic and inhibitory data to disprove a long-held assumption that MASP-2 was solely responsible for the activation of the lectin pathway of complement, implicating MASP-1 and 2 in this pathway. Rob Pike (Monash Univ., Australia) provided the first glimpses into the role of the MASP-3 protease from the same pathway. He presented the first structure of the enzyme carrying a mutation associated with the developmental disorder, 3MC syndrome and described detailed kinetic analyses that provided mechanistic insight into the disease. Thomas Reinheckel (Univ. of Freiburg, Germany) showed that intracellular cathepsins, especially cathepsin K, lead to the degradation of phagocytosed *Mtaphylococcus aureus*"in macrophages. The resulting bacterial peptides were recognised by TLRs and induced inflammatory cytokine production. Finally, Jack Lin (Genentech Inc., USA) showed remarkable structural and biochemical data on how neutrophil serine protease-4 (PRSS57) binds and cleaves its P1 Arg substrates, despite having a shallow S1 pocket similar to a typical elastase-like enzyme.

The next session on "Inhibitors for Therapeutic Intervention," chaired by Bob Lazarus and Henning Stennicke, began with Jim McKerrow (UCSF, USA) describing promising preclinical data for a protease inhibitor that targets a cathepsin L-like protease produced by the major pathogen *Trypanosoma cruzi*. He also discussed how protease inhibitors may be excellent anti-parasitic drugs, since parasite and vertebrate proteases are undergoing divergent evolution. Two talks addressed the challenging question of how to target a specific activities within a multifunctional protease. Dieter Brömme (Univ. British Columbia, Canada) used high-throughput screening to identify compounds that selectively impair cathepsin K collagenase and/or elastase activities and modulate bone resorption, while Niehls Behrendt (Copenhagen Biocentre, Denmark) developed a monoclonal antibody that inhibits MMP2 activation by the cancer-associated protease MT1-MMP. These studies highlight the feasibility of developing ultraspecific protease inhibitors that block certain pathways while leaving other protease functions intact. Mario Ehlers (Immune Tolerance Network, USA) described a promising phase II clinical trial that uses alpha-1 antitrypsin (AAT) to reduce Type 1 Diabetes progression via modulation potentially of inflammatory and anti-apoptotic pathways. Maria Luiza Oliva (Univ. Federal de Sao Paulo, Brazil) presented crystal structures of free Kunitz proteinase inhibitor EcTI and EcTI in complex with bovine trypsin. She also presented data indicating that this plant inhibitor may prevent thrombosis. Frederic Cumin (Novartis, Switzerland) described an integrated approach of high throughput screening, NMR and X-ray structural analyses, and *in silico* studies to identify human renin inhibitors that can counteract rat models of hyptertension.

The following session on "Cell Signaling and Metabolism" was chaired by Jean-Bernard Denault and Carminita Frost. Klaudia Brix (Jacobs Univ. Bremen, Germany) highlighted the importance of cysteine cathepsin-mediated proteolysis of the

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pro-hormone thyroglobulin on thyroid hormone homeostasis and metabolism. The ability of recently discovered thyroid hormones, the thyronamines, to trigger specific G-protein coupled receptors (TAARs) was also discussed. Nigel Bunnett (Monash Univ., Melbourne, Australia) described the role of protease-activated receptors (PAR) in nociception upon chronic inflammation of the colon. He explained how cathepsin S, a classical endo-lysosomal protease, undergoes secretion into the gut lumen during colitis and can process PAR2 at non-canonical sites through a "biased agonist" mechanism. Philip Keegan, a graduate student from Georgia Tech and Emory Univ., USA, explained a new methodological approach (multiplex cathepsin zymography) for analyzing cysteine cathepsin activities in a mouse model of sickle cell disease. He showed that biomechanical stimulation of large vessels can result in increased cathepsin activities, suggesting that low shear forces may regulate protease activation in a number of cardiovascular diseases. Annik Prat (Clinical Research Institute Montreal, Canada) described studies of the proprotein convertase PCSK9 in regulating LDL-receptor trafficking in severe hypocholesterolemia. Astonishingly, loss of PCSK9 in mice affected transport pathways of the LDL-receptor quite differently in males and females, suggesting that estrogen may influence receptor distribution in a PCSK9-dependent manner. Gilles Lalmanach (INSERM, France) highlighted the role of cathepsin B and the endogenous cysteine peptidase inhibitor cystatin C in modulating pulmonary fibrosis. Myofibroblast differentiation was shown to be dependent on cathepsin B activity, while the extracellular proteolytic activities of myofibroblasts was affected by cystatin C secretion rates via TGF-β signaling. John Creemers (K.U. Leuven, Belgium) concluded this session discussing how glucose intolerance without the development of insulin resistance is related to the levels of furin in beta cells in Type 2 diabetes. The mouse model showed symptoms of mild hyperglycemia due to insufficient insulin.

IPS evening reception - Spier Estate

Tuesday began with a session on "Cancer and Metastasis," chaired by Bill Bachovchin and Judith Clements. Johanna Joyce (Memorial Sloan Kettering, USA) described the use of a dual species-specific microarray to determine that cathepsin S plays a critical role in regulating breast-to-brain metastasis through its processing of junctional adhesion molecule (JAM)-B at the blood-brain barrier. Notably, small molecule inhibitors of cathepsin S reduced brain metastasis. Christoph Peters (Univ. Freiburg, Germany) further explored the role of cathepsins in regulating tumor-stromal cell interactions. His studies of cathepsins B and Z in mammary cancer indicate that these proteases regulate tumor invasion and metastasis remodeling the extracellular matrix and interfering with signaling molecules. A series of talks examined how matrix metalloprotease activity can modulate metastasis. Sharon Stack (Univ. Notre Dame, USA) explained how ascites accumulation increases the stiffness of mesothelium and enhances MMP-14-mediated cleavage of matrix and cell surface substrates. These changes promote intra-peritoneal metastasis during ovarian cancer. Beatrice Bachmeier (Univ. Munich, Germany) presented data indicating that plant-derived polyphenol curcumin, commonly found in turmeric, is a potent MMP inhibitor with anti-tumor activity. Curcumin reduces MMP expression by inhibiting AP-1 and NF-KB signaling. Conversely, Achim Krüger (Technical Univ. Munich, Germany) showed that pathological levels of the tissue inhibitor of metalloproteinases (TIMP)-1 actually promote a pre-metastatic niche by increasing neutrophil recruitment, which in turn facilitates tumor cells dissemination. Several talks provided novel mechanistic insight into proteases implicated in the regulation of metastasis. Jana Hachmann (Sanford Burnham Medical Research Institute, USA) used positional scanning substrate libraries to determine that MALT1's paracaspase domain cleaves strictly after arginines. These findings may faciltiate the development of MALT1 inhibitors that could be effective therapeutics against lymphoma progression. Adrian Herington (Queensland Univ. Technology, Australia) described a novel pathway in which kallikrein-like peptidase 4 (KLK4) proteolytically regulates EphB4 kinase activity in prostate cancers and tumor angiogenesis. Oliver Schilling (Univ. Freiburg, Germany) showed how Terminal Amine Isotopic Labeling of Substrates (TAILS) identified novel substrates for the cancer-associated fibroblast activation protease (FAP) and implicate FAP in shaping the tumor microenvironment.

The next session on "Cell Death" was chaired by Agnes Noel and Boris Turk. Seamus Martin (Trinity College, Ireland) gave the EMBO Lecture on "Proteases and Cell Death," and discussed the inflammatory implications of apoptosis, necrosis, and necroptosis and the proteases that modulate inflammation during these cellular processes. Markus Grütter (Univ. Zurich, Switzerland) described the mechanism by which Designed Ankyrin Repeat Proteins (DARPins) inhibit specific caspases and showed that these DARPins can reduce apoptosis induced by multiple stimuli. Robert Smith (Univ. Oslo, Norway) provided insight into the mechanism of statin-induced myotoxicity by showing that simuvastatin inhibits legumain activity and glucose metabolism in skeletal muscle fibers. Boris Turk

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(Jozef Stefan Institute, Slovenia) discussed the relationship between lysosomotropic compounds and cell death, suggesting that their mechanism of action may not be solely related to their lysomotropic activity).

Wines at the Spier Estate

The evening "Intracellular Proteolytic Systems" session was chaired by James Whisstock and Neil Rawlings. Guy Salvesen (Sanford-Burnham Medical Research Institute, USA) discussed how post-translational SUMO modifications regulate diverse cellular processes through dynamic cycles of conjugation and deconjugation. David Komander (Cambridge Univ., UK), gave the EMBO Young Investigator Lecture and revealed new roles for linear ubiquitin chains and the deubiquitinating protease OTULIN (which uses a substrate-assisted catalytic mechanism) in cell signaling. These talks highlighted the "Dubs and SUMOs" as exciting new therapeutic targets. Chris Scott (Queen's Univ., Northern Ireland) described a novel N-terminally truncated isoform of Ras converting enzyme, RCE1, identified in several mammalian species. Interestingly, both RCE1 isoforms 1 and 2 are required for proper function and correct H-Ras membrane localization. Yasuko Ono (Tokyo Metropolitan Instit. Medical Science, Japan) spoke about modulation of the skeletal-muscle-specific calpain-3 activity through its interaction with PLEIAD, providing exciting insight into limb-girdle muscular dystrophy. Hans Brandstetter (Univ. Salzburg, Austria) presented an elegant story on their structural studies of human legumain. Their work uncovered a hidden carboxypeptidase activity in legumain. Given legumain's pH-sensitivity, only the carboxypeptidase, but not the known endopeptidase, activity can explain legumain's nuclear and extracellular activities. Hao Li, a graduate student at Stanford University, USA, showed how the malarial parasite proteasome can be targeted to control parasite growth without causing host cell toxicity, suggesting that significant differences exist between host and parasite proteasomes.

Wednesday was opened by a session on "Proteases in Developmental Biology" chaired by Hiroyuki Sorimachi and Chris Overall. Walter Stöcker (Johannes Gutenberg Univ. Mainz, Germany) dissected the balancing act between the antagonists ovastacin and fetuin B in regulating mammalian fertilization. Their findings could form the basis of future infertility treatments. Atsuko Sehara (Univ. Kyoto, Japan) developed ADAM19-deficient zebrafish to determine that the protease regulates cranial nerve development through Schwann cell (glial cell) survival rather than myelination. Margaret Gall (Univ. Sydney, Australia) generated a gene knock-in mouse homozygous for a DPP9 catalytic Ser mutation to show that DPP9 enzyme activity is essential for early mouse neonate survival. Rafiq Ahmad (Université Paris Est – Crèteil, France) proposed that inhibition of maize cysteine proteases by endogenous cystatins under ozone stress conditions could be exploited to develop transgenic plants with higher tolerance to tropospheric ozone and other biotic and abiotic stresses. Elwyn Isaac (Univ. Leeds, UK) used a *Caenorhabditis elegans* aminopeptidase null mutant to show that the peptidase regulates meiotic progression in *C. elegans* by modulating DNA double-strand break repair proteins. Ulrich auf dem Keller (ETH Zurich, Switzerland) used TAILS to monitor MMP10-dependent proteolysis over time. Their results identify novel substrates of MMP10 and suggest that MMP10 regulates ectodomain shedding of growth factor receptors and initiates hemidesmosome breakdown.

Yasien Sayed (Univ. of the Witwatersrand, South Africa) opened the "Proteases and Pathogens" session (chaired by Anthony Turner and Edith Elliott) with a demonstration that the more dynamic hinge region of South African HIV-1 subtype C protease (C-SA PR) explains its reduced susceptibility to protease inhibitors. Tim Skern (Medical Univ. Vienna, Austria) designed more effective E-64-based inhibitors of the foot-and-mouth disease virus (FMDV) Leader protease (Lbpro). The crystal structure of of Lbpro in complex with one of these inhibitors identified residues that determine P1 and P1' specificity and exosite-regulated peptidase specificity. Manu Platt (Georgia Instit. Technology & Emory Univ., USA) used gelatin zymograms and immunocytochemistry to show that HIV proteins differentially regulate cathepsin activity in arterial cells, promoting proteolytic imbalance and atherogenic arterial remodeling. James Marsh (Queensland Univ. Technology, Australia) elucidated specific interactions in the *Chlamydia trachomatis* HtrA protease that are critical for the allosteric activation of proteolytic activity and independent of oligomerization. Eloïc Colombo (Universitè de Sherbrooke, Canada) showed that matriptase-mediated cleavage of the H1 subtype influenza virus haemagglutinin is essential for host cell infection. Their group designed matriptase inhibitors and showed that these inhibitors inhibit subtype H1 and H3 viral replication. Grzegorz Dubin (Jagiellonian Univ., Poland) presented structure-function analyses of the *Staphylococcus aureus* Spl proteases. They identified an unusual mechanism of protease activation, mapped unique substrate specificities of these proteases, and solved the co-crystal structures with newly designed inhibitors.

In a lively lunchtime session on "Industry and the Bioeconomy" Blanche Ting (Dept. Science and Technology, South Africa)

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presented an enlightening overview of South Africa's bioeconomy strategy (SABS), which seeks to develop a viable and sustainable biotech industry; address national imperatives such as job creation and the burden of disease; and build on existing competencies. Olga Vasiljeva described the Probody™ platform for identifying protease activity in tumour tissues developed by CytomX Therapeutics Inc, USA. Using a proprietary antibody-masking technology, CytomX targets Probodies to specifically bind antigens in diseased tissue. Marko Poglitsch (Attoquant Diagnostics GmbH, Austria) discussed the use of mass spectrometry to characterize the renin-angiotensin proteolytic cascade regulating peptide hormone metabolism. Limitations of the use of artificial substrates in the measurement of proteolytic enzyme activities in this and other systems were highlighted.

Poster Session at IPS

A diverse panel of studies ranging from novel roles of members of the ACE family to recent advances in structure/function studies on coagulation cascade proteases were covered in the session devoted to "Cardiovascular Disease" chaired by Christian Sommerhoff and Mark Gorrell. Ken Bernstein (Cedars-Sinai Medical Centre, USA) provided an entertaining overview of ACE's multiple functions in regulating physiological events from blood pressure to the immune response. Remarkably, overexpression of ACE in immune cells dramatically boosted immune responses in some cancer models, specific infections, and a mouse model of Alzheimer's disease, leading to decreased pathology. Likewise, Tony Turner (Univ. of Leeds, UK) highlighted multiple roles of the ACE homologue, ACE2, including its non-catalytic roles. Novel factors that regulate ACE2 expression were described, including some cytokines, the histone deacetylase SIRT1 and microRNAs. These new regulators may represent novel therapeutic targets for counterbalancing ACE activity. Jim Huntington (Cambridge Univ., UK) presented the structure of an IgA-thrombin complex that exhibited extensive interactions with exosite I of thrombin. The anti-thrombin IgA antibody, isolated from a patient, appears to inhibit thrombosis without causing bleeding, potentially providing the Holy Grail of thrombosis therapeutics. Galia Blum (Hebrew Univ., Jerusalem) described the development of a fluorescent probe that can visualize cathepsin activity and plaque progression in human atherosclerosis. The probe provides a valuable diagnostic tool for atherosclerosis. Ruby Law (Monash Univ., Australia) reported the structure of human plasminogen in its closed, activation-resistant conformation, highlighting distinct structural characteristics, and thus functions, of plasminogen in plasma. Finally, Jonas Emsley (Univ. Nottingham , UK) reported the first structures of the blood coagulation factor XII (FXII) protease domain in its glycosylated and deglycosylated forms, providing insight into the activation mechanism of the FXII zymogen.

The final day of the conference began with a session on "Membrane-Associated Proteolysis and Neurological Disorders" chaired by Sin Urban and Klaudia Brix. Bart de Strooper (VIB Centre for Biology of Disease, Belgium) gave an EMBO-sponsored lecture on Aß generation. He discussed how some destabilizing mutations in the Amyloid-beta Precursor Protein (APP) may explain the heterogeneity in Aß-peptides generated by γ-secretase. He also described how ß-arrestin signaling affects the association of specific members of the γ-secretase complex with lipid rafts and thus complex activity. Taisuke Tomita (Univ. Tokyo, Japan) dissected mechanisms for modulating γ-secretase functionality, particularly mechanisms regulating the subcellular localization of Aß-generation. He also described their use of photoaffinity labels to probe the active sites of presinilins (PS) and to identify small molecules allosteric activators of PS. Nabil Seidah (Clinical Research Institute of Montreal, Canada) described how iron levels can regulate the co-localization of the transferrin receptor TfR1 and proprotein convertase PC7, a regulator of transferrin receptor shedding. This interaction could be used to transfer drugs across the blood-brain-barrier. He also showed that BDNF in the amygdala and hippocampus is a natural substrate of PC7. Hans-Ulrich Demuth (Probiodrug & the Fraunhofer Instit. Cell Therapy & Immunology, Germany) discussed the subcellular localization and activities of the glutaminyl cyclases (QCs) and the development of QC inhibitors for treating Alzheimer's disease and other inflammatory diseases. These inhibitors may bypass the need to inhibit γ-secretase activity. Eliane Wolf, a young researcher from Technical Univ. Munich, Germany, screened a library of activity based probes and identified molecules targeting different prokaryotic and eukaryotic rhomboids. These molecules will be useful tools for studying these enigmatic intramembrane proteases. Seth Dickey, a graduate student at Johns Hopkins School of Medicine, USA, explored the molecular mechanism of intra-membrane cleavage and showed that transmembrane domains are very poor substrates for rhomboid enzymes; indeed, rhomboid proteases need 2.5 min to mediate a single cut! This surprising finding indicates that rhomboid-mediated proteolysis is a rate-driven process in which affinity has no physiological meaning!

The next session on "New Approaches to the Design of Proteolytic Inhibitors" got off to an interesting start with chair

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Matt Bogyo introducing a unique time alert – Metallica's song 'Exit Light'. This prompted some of our speakers to linger over their talks - indeed session speakers Irit Sagi and Malte Gersch were quite happy to boogie to the music when their time was up. The session was heavily influenced by key questions to inhibitor design – specificity and selectivity. Do we want it, need it and how do we get it? Irit Sagi (Weizmann Institute of Science, Israel) presented a mechanistic approach to deciphering the selective nature of extracellular matrix proteolytic mechanisms of mechanisms whilst Marcin Drag (Wroclaw Univ. Technology, Poland) explained their approach to profiling endoprotease substrate specificity by use of a combinatorial hybrid substrate library. Malte Gersch, a graduate student at Technical Univ. Munich, Germany, investigated the ClpP protease's complex assembly showed that dissociation of its hexameric "donut" stacking prevents ClpP activity. Agents that interfere with ClpP complex assembly therefore have anti-microbial activity. Vincent Dive (Commissariat á l'Energie Atomique, France) discussed the use of halogen X-bonding in FRET substrates to design selective inhibitors for the MMP family. The introduction of a single halogen to the P1' of MMP FRET substrates significantly altered the protease's catalytic efficiency. Ravi Acharya (Univ. Bath, UK) and Jan Konvalinka (Academy Science Czech Republic, Praha) demonstrated how structural and molecular biology can dissect drug targets and allow the development of potent inhibitors. Ravi explained recent advances in understanding the role of the two catalytic domains of ACE and in the design of 'domain-specific' inhibitors, while Jan's structure-activity study of Glutamate Carboxypeptidase II identified potent inhibitors. By covalently linking these inhibitors to macromolecular nanocarriers, they could efficiently target GCPII-expressing cells.

Bob Lazarus (IPS President) presenting lifetime achievement award to Clive Dennison

The "Pathogens and Protease 2" session chaired by Rob Pike and Jim McKerrow contained several talks focused on developing inhibitors against parasite pathogens. Sheena McGowan (Monash Univ., Australia) described the hunt for inhibitors of aminopeptidases critical for the viability of the malaria parasite *Plasmodium falciparum* using structural and kinetic data combined with high throughput screening approaches *in vitro* and *in silico*. Kelly Chibale (Univ. Cape Town, RSA) discussed similar efforts to find inhibitors of malaria parasite cysteine proteases in the context of recently established drug design centers in South Africa. Edgar Deu (MRC National Institute for Medical Research, UK) described the development of inhibitors targeting *Plasmodium* dipeptidyl aminopeptidases, which regulate critical processes during the parasite life cycle. Matt Bogyo (Stanford Univ., USA) next showed how focused protease inhibitor screens against the parasite *Toxoplasma gondii* unexpectedly identified an inactive cysteine protease that functions as a reactive oxygen species sensor in the parasite, and a palmitoyl protein thioesterase-1 that appears to regulate parasite invasion and motility. Matt's approach was particularly appreciated by Czech colleagues for his recognition of studies conducted at their university! Isaura Simões (Centre for Neuroscience and Cell Biology and Biotechnology Innovation Centre, Portugal) presented a talk on aspartic proteases from the Rickettsiae with potential biotechnology applications. In the final talk of the conference, Michael Kotsyfakis (Academy Science Czech Republic, Praha) showed that a range of inhibitors from the saliva of disease-causing ticks had specific effects on a large range of host proteases involved in critical homeostatic mechanisms. Not only does this shed light on the mechanisms of pathogenesis by these arthropods, it also identified molecules that could be used to control proteases in a wide range of human diseases.

After this stimulating series of talks and poster sessions, the conference culminated with the awards banquet at Moyo in a magical setting under a canopy of Bedouin tents amidst the ancient oaks of the Spier Wine Farm.

IPS 2013 Trainee Awards

Leila Akkari	Memorial Sloan Kettering Cancer Centre, USA
Yael Ben-Nun	The Hebrew University, Israel
Seth Dickey	Johns Hopkins University School of Medicine, USA
Catherine Duclos	Université de Sherbrooke, Canada
Danuta Florczyk	Jagiellonian University, Poland
Margaret Gall	University of Sydney, Australia
Martina Gansz	Albert-Ludwigs-University, Germany
Malte Gersch	Technische Universität München , Germany
Maresa Grundhuber	Ludwig-Maximilians-University, Germany
Elizabeth Hamson	University of Sydney, Australia
Tomasz Kantyka	Jagiellonian University, Poland
Philip M. Keegan	Georgia Institute of Technology, USA
Maria Koczorowska	Albert-Ludwigs-University, Germany
Hao Li	Stanford University, USA
James Marsh	University of Technology, Australia
Cyrielle Martini	Université de Sherbrooke, Canada
Andreas Maurer	Interfaculty Institute of Biochemistry, Germany
Oakley Olson	Memorial Sloan Kettering Cancer Centre, USA
Karolina Plaza	Jagiellonian University, Poland
Marcin Poreba	Wroclaw University of Technology, Poland
Fabio Sabino	Institute of Molecular Health Sciences, ETH Zurich
Robert Smith	University of Oslo, Norway
Tripti Tamhane	Jacobs University of Bremen, Germany
Stefan Tholen	University Freiburg, Germany
Yannick Waumans	University of Antwerp, Belgium
Catera Wilder	Georgia Institute of Technology, USA
Elaine Wolf	Technical University Munich, Germany
Awards Declined	
Ivana Parker	Georgia Institute of Technology, USA
Keon-Young	Georgia Institute of Technology, USA
Jack Lin	Genentech, USA

1st Prize Biochemical Journal Poster Award
Yael Ben-Nun, Hebrew Univ., Israel

1st Prize Biological Chemistry Poster Award
Alexandre Desroches, Univ. Sherbrooke, Canada

2nd Prize Biological Chemistry Poster Award
Anna Byzia (Wroclaw Univ.) and Fabio Sabino (ETH Zurich)

IPS 2013 Trainee Travel Awardees

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IPS Lifetime Achievement Award 2013

Clive Dennison – A Sketch of Me and Some of My Work

I was born in Durban and raised on a farm in the midlands of Natal (now known as KwaZulu-Natal). On a farm one has to be largely self-reliant, and fortunately my father was very practical. I learnt the basics of mechanicking at his knee, pulling engines out of our cars and motorbikes and overhauling them. I was driven by curiosity and always wanted to know how things worked and to understand how and why things were as they were.

We had horses and motorbikes, and I was fascinated by the similarities and differences between them. While I understood how the bikes worked, I had no idea how the horses' "engines" worked. I was also interested in flight and built countless model aeroplanes, first from kits and the rest from my own designs, usually to test some idea. One can't see the air, of course, and to understand flight requires the development of the "mind's eye" – something that is necessary in science. Birds and their amazing flying abilities – to land on a wire, for example – especially impressed me and, again, I wondered what it was about life that was so special? My model aircraft engines ran on ether or methanol – but what were these, besides names that I could rattle off? The structures were made of balsa wood, covered with tissue and painted with "dope" - but what was dope?

When I was 13-years-old, my brother's fiancée, Marie, became ill with liver cancer. As a family we did the conventional things, like mega-praying, and the medics did what they could, but to no avail, and Marie died. This impressed me greatly, and I concluded it could only be because the medics did not know enough. From my experience with mechanics and model aircraft I knew that one cannot fix something if you don't know how it works, and my observations on horses, birds and death-by-cancer all led me in the direction of wanting to know, at a fundamental level, how life works.

When it came time to go to college, I did some thought experiments. Should I pursue business, with the goal of becoming rich? Nah, I thought, what if I was rich, what would I do? Probably buy bigger and better toys. But that didn't sound fundamentally satisfying. Should I do medicine? Nah, the medics couldn't fix Marie, so they obviously didn't know what they were doing. What should I do to get a fundamental understanding of how life works?

At that time, students of medicine, veterinary science and agriculture all did the same subjects in first year. Knowing no better, I registered for these. One of the subjects was chemistry, and I was delighted to learn what ether and methanol really were. And I finally discovered what "dope" was: cellulose acetate, dissolved in a volatile organic solvent. Talking to fellow students I discovered a subject called biochemistry that seemed to hold the prospect of answering my questions. "But it is only for real boffins," my informants told me. That made it even more attractive to me, as I have always had a tendency to 'swim upstream.' So, biochemistry it was, and wow, what a satisfying subject to study. It was all so interesting that it didn't seem like work at all.

I won a scholarship for Masters' study and was assigned to isolate toxic proteins in legume seeds (beans). My supervisor was trying to formulate an inexpensive but balanced food to combat malnutrition in indigent children, or "kwashiorkor." One of the 'toxins' in beans is a trypsin inhibitor, and my very first experiments involved measuring trypsin activity using benzoyl-arginine-ethyl-ester (BAEE) as a substrate. However, my project proved to be exceptionally challenging for three main reasons:

i) Seed proteins have an intrinsic tendency to aggregate. Osmotic pressure is a colligative property that depends on the number of particles in a given volume. In order to pack seed proteins into storage granules, they need to aggregate so that the granule will not burst by osmosis. Thus, every time I tried to concentrate a protein isolate it would aggregate!

ii) Toxicity is not a specific activity. Two slightly toxic proteins might be more toxic in combination than in isolation. So, as I proceeded to isolate the proteins, the activity (toxicity) went down instead of up!

iii) The assay for toxicity, an LD_{50} assay using mice, was not economical and nearly all of the protein isolated was consumed in the assay.

Nevertheless, it was a good training in how to go about protein isolation – and how important it is to have a specific and economical assay.

One day a fellow student was trying to make up a phosphate buffer following a 'recipe': "make up 1L of 0.1 M NaH_2PO_4 and 1L of 0.1 M Na_2HPO_4 and titrate to the correct pH." But which way to titrate? The student exhausted his litre, and then made up another and another, etc. "Hang on," I thought, "something's not right here. Why do we need to make two solutions? How can we know which is the right way to titrate?" After some thought, I came up with a much better understanding and a much simpler way of making buffers that only requires one solution to be made up accurately (Biochemical Education 16 (4), 210–211, 1988).

I continued my study on bean toxins through to my Ph.D. and later as a Research Officer in the Department of Agriculture. One of my extra duties included managing an amino acid analysis facility. As I was familiarizing myself with the analyzer I observed that its output was in the form of a tracing on a log-scale strip chart, with peaks whose areas were proportional to the concentrations of the various amino acids. The baseline of the tracing was not always on zero, so to compensate, the technician had a clear plastic ruler to measure the peak height,

Clive Dennison – A Sketch of Me and Some of My Work continued...

with its zero point aligned with the baseline of the tracing. I wondered, "could this be right?" So I did an experiment of injecting a constant amount of a standard sample and measuring the peaks, with the baseline in different places. This showed that the use of the ruler was incorrect and that the peak height should be read directly off the chart scale, regardless of the baseline . The implication was that the past 5 years of results from the amino acid analyzer were all nonsense! Again the moral was that if you don't understand what you are doing, you can go astray.

This is me piloting the Trike taking someone for a flip earlier in the year.

In 1990 I joined the University of Natal, and in 1994 I did my first sabbatical with Dr Irv Liener (an examiner of my Ph.D. thesis) in Minnesota. Dr Liener was working on beans, but when I told him I was interested in cancer, he assigned me a project to find tumor specific proteins in cancerous rat pancreases. The trypsin inhibitor in beans, if not completely destroyed by heating, leads to pancreatic hypertrophy and, if coupled with exposure to aflatoxins, lead to pancreatic cancer. So, Dr Liener gave me three cancerous rat pancreases about the size of a fingernail.

I thought 2D electrophoresis would be the way to go. However, I discovered that upon homogenization of pancreatic tissue, all the inactive pro-enzymes were activated, leading to runaway proteolysis. This could not be prevented by homogenization in the presence of protease inhibitors. In the time available I did not solve this problem, although a solution later presented itself (the use of t-BuOH in the buffer, Methods in Protein Biochemistry, H. Tschesche). However, my sabbatical gave me the uninterrupted time to think of how to approach the problem of cancer back home.

Cancer has two dimensions – a loss of control of cell division, and invasion and metastasis formation (characteristic of malignant tumors). I realized that most people were working on the cell division aspect, so in order to compete, we should work in a less-populated niche area. I determined to explore the proteolytic enzymes involved in cancer invasion.

One of the deficiencies in my training as a biochemist was that we were never told where things happened in a cell. I decided to make good this deficiency by looking at where the various proteases acted in normal and cancerous cells. So my overall plan was i) isolate candidate proteases, ii) raise antibodies to these, and, iii) use the antibodies to determine where the proteases were, using immunocytochemistry. Luckily, I was very fortunate to recruit an exceptional cohort of postgraduate students, three of whom (Theresa Coetzer, Edith Elliott and Rob Pike) attended the recent 8th meeting of the Proteolysis Society and one of whom (Rob Pike) was a one time President of the Society.

We set out to identify proteases involved in cancer invasion. This was an open question, with different research groups backing their own "favorite". I thought cathepsin L looked a likely candidate, but it seemed to be ruled out by the fact that it was apparently not active at physiological pH. However, I remembered a paper where the pH/activity profile of a cysteine cathepsin was determined using acetate, phosphate and Tris buffers, of constant molarity, across the pH spectrum. Intriguingly, there was a big step in activity at each change of buffer. But why?

To cut a long story short, we discovered that cysteine cathepsins are sensitive to ionic strength and pH. Cathepsin L was originally shown to be inactive at physiological pH, when phosphate buffers of constant molarity were used across the pH spectrum. However, if the molarity of a phosphate buffer is kept constant, the ionic strength increases with pH in a sigmoidal manner. This increase in ionic strength suppresses cathepsin L activity at high pH, giving an erroneously low apparent pH optimum and no apparent activity at physiological pH. If the ionic strength is kept constant, however, the apparent pH optimum is much higher and the enzyme is substantially active at physiological pH. The ionic strength may be kept constant by using acetate-MES-Tris (AMT) buffers (Methods Enzymol. 1982; 87: 405-26). Again, if you don't fully understand what you are doing, you can go astray.

Although my work didn't (or doesn't) end there, I would like to end with a take-home message for young scientists. *Do not approach your work with a superficial, glib attitude. Don't be derivative; think your own thoughts and take the trouble to really understand what you are doing. Don't take the word of "authorities" from famous labs and don't follow recipes and kits, blindly. Really understand what you are doing so that the results you get will give you confidence that you are correct and – unlikely, but possible – that the "authorities" are wrong.* Most scientists contribute only one or two "pixels" to the whole picture that makes up their field. Sometimes, if you really understand what you are doing, you can change the phase of all the pixels to give an entirely new picture.

In closing, I will mention that I switched from model aeroplanes to actual flying in 1970. I have kept at it since and now fly a Trike "microlight". I find it fascinating to view the world from an aerial perspective down to an electron microscope view. Flying is not intrinsically dangerous but it is unforgiving. If you don't really know what you are doing and make a single mistake, it can be fatal. Although flying can give one an adrenaline rush, it is nothing compared to the rush one gets when you gain a new insight in science and realize that you are the first person – ever – to have gained that particular new understanding.

In Memorium – Prof Nobuhiko Katunuma (1926-2013)

"The Kendo Fighter Biochemist"

by Hiroshi Kido, Vito Turk and Hans Fritz

This article celebrates the life of Professor Nobuhiko Katunuma, a pioneer in the field who contributed tremendously to the understanding of intracellular proteolysis, with a particular focus on and lysosomal cysteine proteases and their natural and synthetic inhibitors. He will be remembered not only for his many scientific achievements but also as a great mentor, respected scientist, colleague and loyal friend. Professor Katunuma was an honorary lifetime member of the International Proteolysis Society.

Prof Katunuma was instilled with a keen interest in medical and biomedical research from his great uncle who told a young Nobuhiko "Biochemists working at Medical Schools should contribute in the fight against diseases of man". Professor Katunuma went on to do exactly that, graduating from the School of Medicine in 1953 at Nagoya University, and then joining the laboratory of Prof. Theorell at the Nobel Institute in Stockholm. In 1959, he returned to Japan as an Associate Professor at Institute for Protein Research as Osaka University. In 1963, he moved to his favorite place Tokushima, where he took up a Professorship at the School of Medicine at Tokushima University. He later served as Dean and finally as Director of the Institute for Enzyme Research at the same university. In 1992, he retired from Tokushima University and then moved to the Institute for Health Sciences, at Tokushima Bunri University. From 2000 to 2006, he was appointed as the President of Health Sciences at Tokushima Bunri University.

Outside science Prof Katunuma had many interests including music, hiking mountains and sword fencing known as 'kendo'. Indeed, Prof Katunuma was a master of kendo, having earned his 7th masters degree in the sport. Friends and colleagues affectionately called him a 'kendo fanatic' and enjoyed his excellent performances during international conferences. Prof Katunuma enjoyed international conferences, particularly ones that took him to mountains or coastal areas. Prof Vito Turk (pictured above-with Prof Katunuma) remembers a trip to Slovenia where the two biochemistry professors spent the afternoon enjoying the view over the blue sea of the Adriatic coast.

During his thirty-odd years at Tokushima University, he studied the enzymes involved in vitamin B-6 metabolism. His discovery of mitochondrial glutamic-oxalacetic transaminase, studies on the regulation of the urea cycles, and on glutaminase isozymes and their role in carcinogenesis in livers cells, all constitute important contributions to biochemistry. Important too were his studies on the metabolism of pyroxidal enzymes and their limited proteolysis by serine proteases under in vivo conditions. Prof Katunuma and co-workers found the acceleration of protein turnover rates of pyridoxal enzymes in vitamin B-6 deficient animals and discovered the protease, which inactivates the apo-proteins of these pyridoxal enzymes by limited proteolysis. He suggested that apo-protein formation is an initial step in the degradation of these enzymes in vitro and in vivo. His 'second career' at Tokushima Bunri University saw Prof Katunuma actively involved in the development of new synthetic cysteine protease inhibitor, the derivatives of E-64 and the CLIK inhibitors.

Prof Katunuma (left) with Profs Jim Travers & Hiroshi Kido and colleagues in Austria

Prof Katunuma was a dedicated mentor and valued supervisor. He was always happy with a laboratory full of young active researchers. To date, more than 30 researchers who had worked with Prof Katunuma in the past have established their own laboratories both in Japan and overseas. His philosophy of science and love for scientific research were passed on to the next generation of young researchers.

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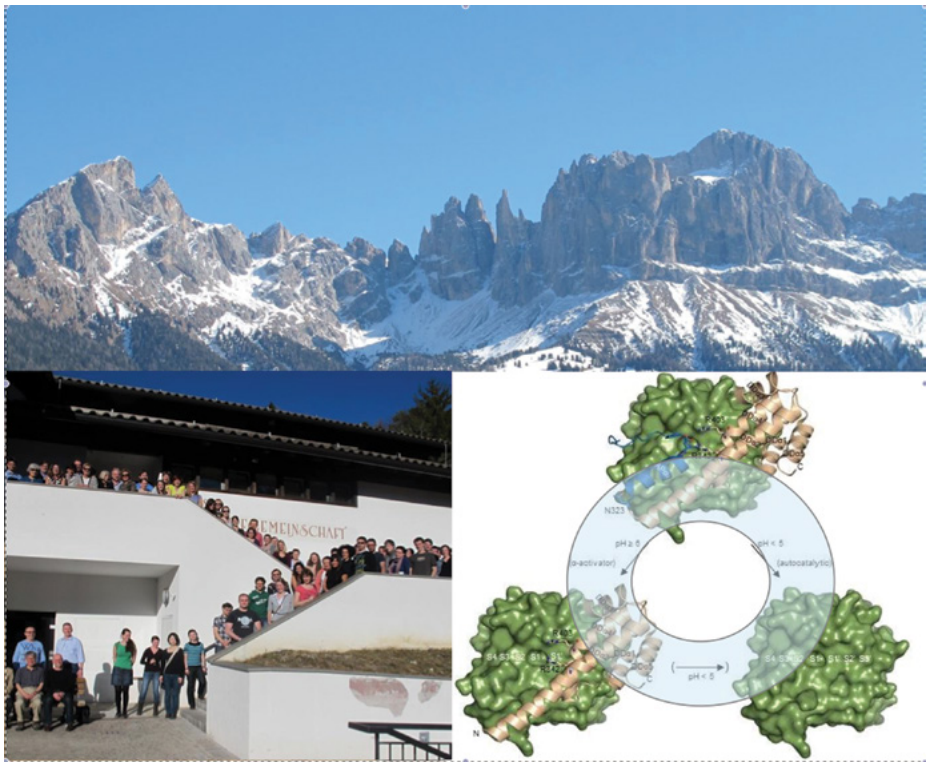

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Feb 26 - March 2, 2014 at Tiers, South Tyrol, Italy.

http://www.uni-salzburg.at/index.php?id=25444

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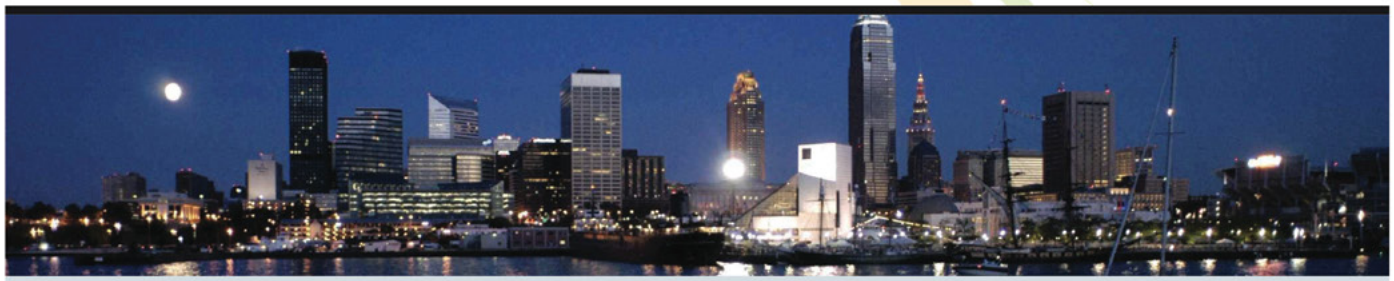

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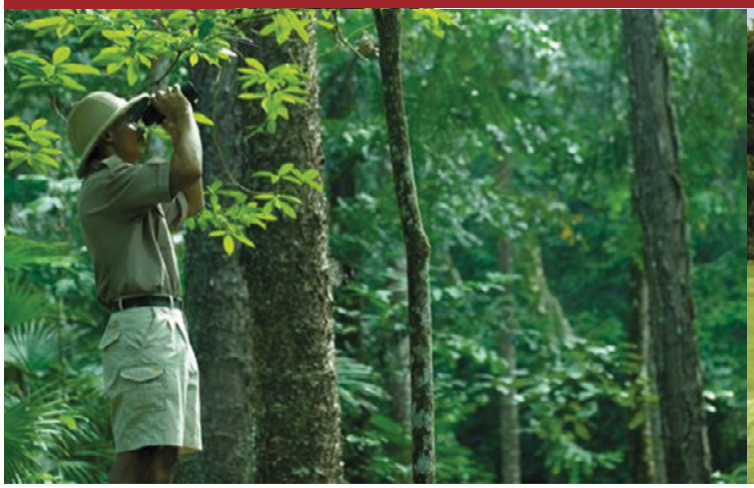

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