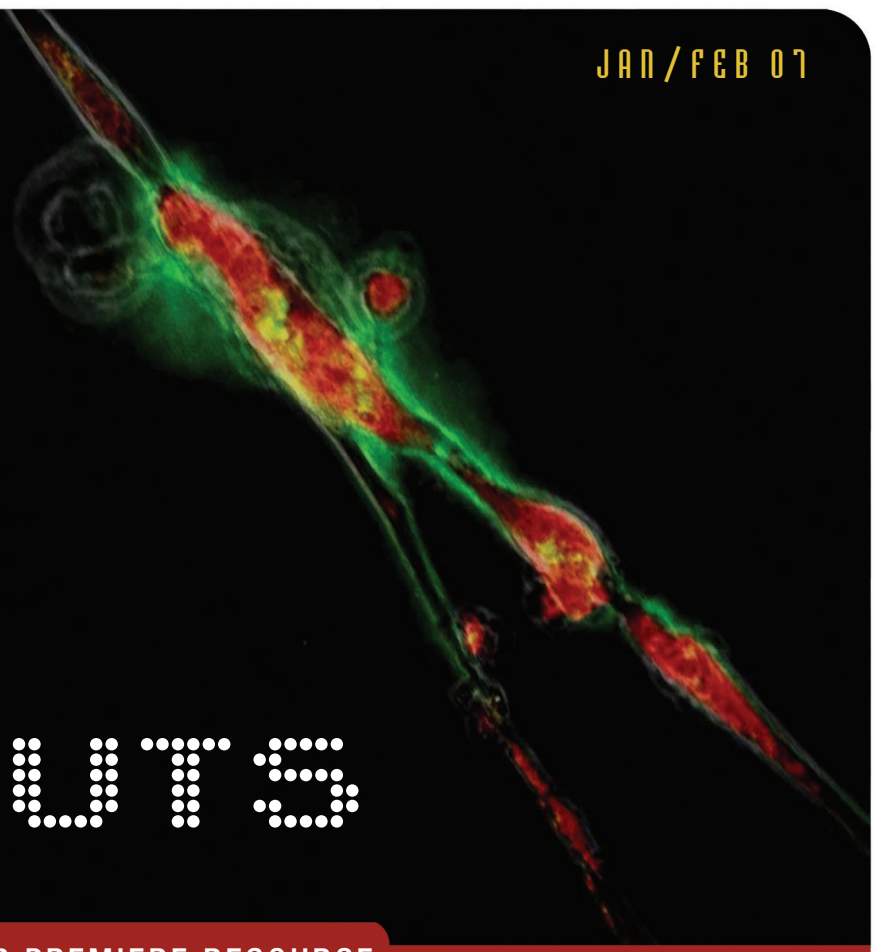
JAN/FEB 07

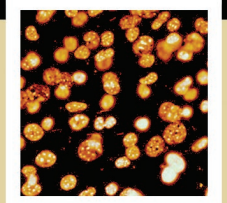
IN THIS ISSUE:

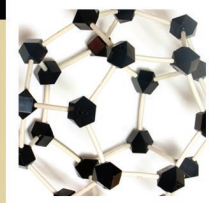




INTERNATIONAL PROTEOLYSIS SOCIETY

# QUICKCUTS





YOUR PREMIERE RESOURCE
FOR ALL OF YOUR IMPORTANT PROTEASE QUESTIONS

## A Message From the President:

Well, let's be honest and say that this issue of QuickCuts has been a long time in coming! The Council is determined to reinvigorate our use of this important mechanism of communication and so you will see more of these than ever before in the near future.

2005 ended for the Society with the 4th General Meeting in Quebec City, Canada, organized by Guy Tremblay and colleagues. A great meeting was had by all in a beautiful setting, even if the weather didn't always cooperate as well as it might have. As one of the people on the walking tour, I can certainly vouch for both the beauty and rain in Quebec City. The Society would like to thank Guy and his committee for their hospitality, although I won't be talking any further about the incident with the fiddle at the dinner!

A meeting report follows later in this issue, as do articles extolling the great careers of our two newest Lifetime Members, Jim Travis and Wolfram Bode. The meeting was also highlighted in two issues of Biological Chemistry, the journal with which our Society has a special relationship (website: www.deGruyter.de/journals/bc, see Vol. 387, issues 7 [July] and 8 [August]). It was good to see so many attendees taking up the offer to publish their science presented at the meeting in this peer-reviewed setting.

The next General Meeting of the Society will be held in Patras, Greece, October 20-24th, 2007. The organizing committee is chaired by Georgia Sotiropoulou and details of the venue etc can be found at: www.ips2007patras.gr. I hope that many of you will join us for this meeting in a beautiful setting. I would also like to remind you that joining the IPS before the meeting will bring you a substantial discount in the registration fee (more than the cost of joining) and therefore I urge you to renew your membership early this year when you are requested to do so by the Treasurer, Bruce Linebaugh.

Finally, I'd like to formally welcome all of our new Council members and remind you that your region is represented by four councillors (see left for the listing), who will be happy to hear from you if you have suggestions. Rob Pike, Council Chair, rob.pike@med.monash.edu.au

## COUNCIL OF THE INTERNATIONAL PROTEOLYSIS SOCIETY

**Rob Pike** - President of the Council
**Gillian Murphy** - Vice President of the Council
**Matt Bogyo** - Secretary
**Bruce Linebaugh** - Treasurer
**Georgia Sotiropoulou** – ex officio –
Chair of 5th IPS General Meeting

COUNCILLORS - EUROPE/AFRICA

Klaudia Brix
Christoph Peters
Gillian Murphy
Ed Sturrock

COUNCILLORS - ASIA/AUSTRALIA

Kohei Oda
Kenji Yamamoto
Shoichi Ishiura
Rob Pike

COUNCILLORS - Americas

Chris Overall
Bonnie Sloane
Jan Potempa
Matt Bogyo

Email addresses can be found on the IPS website: www.protease.org

# Latest News:

## IPS gets an image overhaul

Matthew Bogyo, IPS Secretary

As part of our efforts to revitalize the IPS and to encourage more communication among the protease community we have begun two significant projects in which we will improve the newsletter and website. In addition we hope that the increased use of the website and more regular mailing of a newsletter will help to continue to attract Sponsor Members in the coming years so that we can continue our efforts to make the General Meetings accessible to young protease scientists from around the world. As part of this initiative we have hired an outstanding design company (see www.purefusionmedia.com) that has helped us to establish a new template for our newsletter as well as a much more interactive website. I encourage you to check out the new site at the same old location (www.protease.org).



You will notice that much of the general content is unchanged but we have added new products and sponsor pages that highlight the companies that have decided to support the IPS with Sponsor Memberships. We will also include current job listings related to proteolysis. The most significant change is the addition of a blog module that will allow the secretary to take suggestions from the members for postings as topics on our site. These topics will then be open for discussion among members and will hopefully lead to interesting dialog and sharing of ideas. In addition this page can be used to post important findings or issues that should be made available to the protease community and for which people can make comments based on their own experiences.

For the new and improved newsletter you will see a number of additions including a recurring section where we will highlight recent papers published by IPS members. We plan to make this a central part of the newsletter as it helps to keep people up to date on the latest protease research. For this I will ask your help in identifying interesting papers (and don't be shy to point out your own papers!). Ideally we plan to send out a more formal newsletter with extended content 2-4 times a year but will send abbreviated e-mail style newsletters once a month to keep the lines of communication open.

I want to point out once again that this is your society and to make it great we need your participation. I am dedicated to increasing member input and communications between members and will be sending newsletters and other requests for content on a regular basis. Please take the time to make suggestions and get involved. I think that we will all benefit from the great society of protease scientists in the IPS!

## Post-doctoral fellowships in 2007

### Oxidative Stress and Matrix Metalloproteinase (MMP) Biology

We are seeking a post-doctoral fellow to do basic biomedical research projects (also translational) related to oxidative stress and matrix metalloproteinase (MMP) biology. Novel targets and actions of MMPs, opening a new avenue in our understanding of these proteases.

Excellent supportive lab environment within a dynamic support group (CIHR Group grants, CFI infrastructure, CV Research Group) at the University of Alberta. Excellent salary for the right candidate. Prefer someone who is eligible for fellowship awards (ie. not more than 3 years post-PhD). Techniques not important but experience in molecular biology, proteomics, biochemistry, in vitro/vivo physiology/pharmacology, imaging an asset.

Univ. of Alberta was selected in a world-wide survey as one of the best places to be a PDF. Edmonton is a million person city, warm sunny dry winters, less rain, thriving arts scene, 3.5 hrs from the Rocky Mountains, excellent outdoor recreational activities as well. Our lab is in the heart of campus which is in the heart of the city. Cost of living very reasonable.

Since 2000 our lab has published papers in Circulation Research, Circulation, FASEB J, Cardiovascular Research, British Journal of Pharmacology, Annual Reviews of Pharmacology & Toxicology on our research.

**CONTACT: by email to: richard.schulz@ualberta.ca**
**For info on the Univ. of Alberta see: www.ualberta.ca**

### Matrix Regulation and Growth Factor Signaling

A Postdoctoral Fellowship position is available for study of extracellular regulatory proteins that govern development and homeostasis via effects on matrix formation and the activation of growth factors. Molecular biology, cell biology, knockout and conditional knockout mouse approaches and zebrafish-based analyses are all currently being employed towards these studies in the lab. Focuses are presently on extracellular proteinases, inhibition and activation of TGF-beta growth factor superfamily members, novel extracellular regulatory proteins, and growth factor-matrix interactions. This is an opportunity to join a highly productive laboratory at one of the foremost biological research campuses in the U.S., at the University of Wisconsin-Madison.

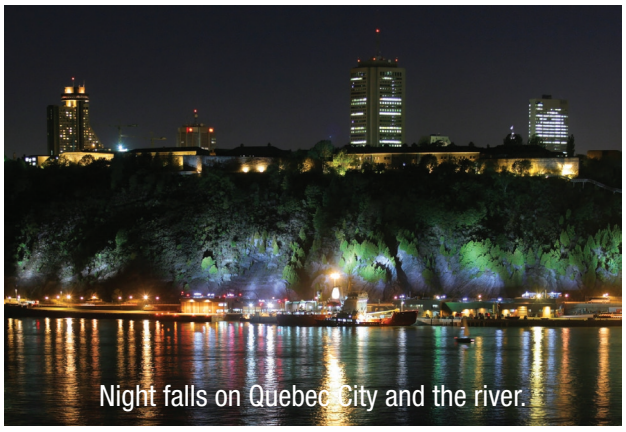
Suitable Candidates will have a Ph. D. or equivalent, and must have demonstrated skills in relevant techniques and fields of study. Previous publication activity will be taken as an important indicator for position suitability. Support will be from the PI's current R01s. However, applicants will also be expected to apply for extramural support.

**Contact: Daniel S. Greenspan, Ph.D. Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison 1300 University Avenue Madison, WI 53706 dsgreens@wisc.edu**

# Report on the 4th General Meeting of the IPS, Quebec City, Canada, October 2005

Rob Pike, IPS President

The 4th General Meeting of the IPS, associated with the International Conference on Protease Inhibitors, was held in beautiful Quebec City, Canada from the 15-19th of October 2005. The local committee, led by Guy Tremblay, did a tremendous job in organizing a very successful conference which was enjoyed by the approximately 400 delegates attending from all over the world.



Night falls on Quebec City and the river.

The opening lecture by Michael James gave a thorough overview and introduction to the structures of novel peptidases from fungi and viruses. These enzymes represent valuable therapeutic targets given their importance in disease. The tour de force presented by Michael was also an excellent advertisement for the high quality science being conducted in the protease area in Canada. The opening lecture and welcoming addresses by Guy Tremblay and Ben Dunn, President of the IPS, were followed by a cocktail party which led to further lively interactions at the local bars in the area, a reoccurring event for the rest of the meeting!

The first full day of the conference (Sunday) opened with excellent sessions, including a session on cutting edge technologies that are currently being developed to identify novel substrates, explore protease genomics (or degradomics) and uncover novel therapeutic targets or drugs. A session followed on apoptosis, in which a surprising variety of proteases and their inhibitors were shown to be involved. In the next session on protein processing, topics included processing of proteins both inside and outside cells, the latter focusing on the shedding of proteins from cell surfaces, a process now recognized to be important in many contexts. This session in particular highlighted the role of proteases as more than simple degradative molecules that act as "switches" that modulate important processes throughout normal physiology.

Monday's session was dedicated to structure-based drug design. As might be expected, a number of presenters were from commercial entities, allowing delegates to see the big-budget operation at work in the development of the latest therapeutics targeted at proteolytic enzymes. The academic contributors certainly held their own in this session and overall the audience was treated to important insights into the "state-of-the-art" of drug design. A session on the involvement of proteases in tumors and their microenvironment followed. It was amazing to see how sophisticated the work in this area has become. A variety of different approaches were presented, ranging from novel visualization techniques to detailed insights using genetically manipulated organisms. This theme was further expanded in the next session on "Proteolysis in development and diseases".

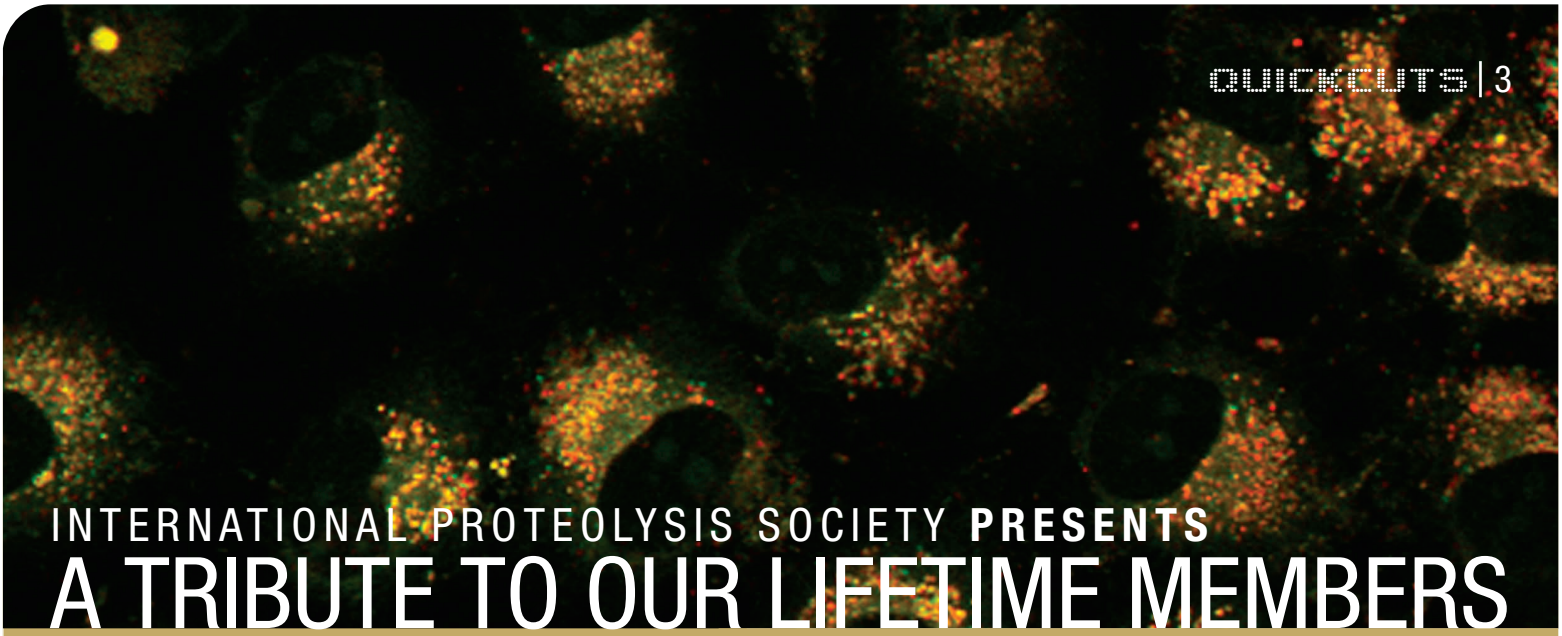
Tuesday's program was more diverse, starting with a session on the "Structure and evolution of proteolytic enzymes". As might have been expected, delegates were treated to insights into how our favorite proteases have evolved using a variety of different examples. This was followed by an excellent session on the "Control of Proteolysis in Plant Systems". Plants obviously are important in their own right as major contributors to the Earth's biomass but the session was a timely reminder to even the devout mammalian biologists that there is excellent science being conducted in this area. This was followed by the weird and wonderful world of "Virulence Factors and host-pathogen interactions" with examples from bacteria, fungi and parasites presented to provide examples of the complex interaction between host and pathogen.

The last day of the conference (Wednesday) was kicked off with a session on the often controversial area of "Membrane-associated proteolysis and signal transduction". As expected, this session treated the audience to some excellent science in one of the most frontier areas of proteolysis, while including some healthy debate over the work presented. The second to last session on "Chronic and degenerative diseases" provided more lively debate and an interesting cross section of talks across a diverse set of topics. The final session on the "Ubiquitin-proteasome pathway" included excellent talks to finish off the conference in this "still-hot" area of cell science.

In addition to oral presentations, a diverse range of excellent posters were presented on each day of the conference apart from Wednesday. The sessions were well attended and gave many of our members in training the valuable experience of presenting their work to experts in the field.

Finally, the conference finished on a high note with the Farewell Dinner, at which travel awards were presented to our members in training. Honorary life memberships, the highest awards of the Society, were bestowed on James Travis (University of Georgia) and Wolfram Bode (Max-Planck, Martinsried). I'm sure most IPS members would agree with the choices for these prestigious memberships as both Jim and Wolfram are two major contributors to protease science as it exists today. The dinner was highlighted by excellent food and entertainment, the latter to the embarrassment of many of the Council members!

On behalf of the IPS Council, I would like to once again thank Guy Tremblay and his local organizing committee and scientific advisory committee for their excellent work and we now look forward to the 2007 conference to be held in Patras, Greece in October of that year. See you in Greece!!



INTERNATIONAL PROTEOLYSIS SOCIETY **PRESENTS**

# A TRIBUTE TO OUR LIFETIME MEMBERS

## Wolfram Bode:
### A King of Proteases

A central figure in the research on proteases and their inhibitors for more than three decades – this has been and more than ever is our colleague Wolfram Bode who contributed exceptionally to the field by elucidating the tertiary structures of numerous proteases and protease inhibitors and clarifying their activity, functions and interactions.

Wolfram joined Robert Huber at the Max-Planck-Institute for Biochemistry in Martinsried near Munich in 1972. He was also appointed as docent at the Faculty for Chemistry and Pharmacy in 1983 and subsequently as Professor for Physical Biochemistry at the Ludwig-Maximilians-University (LMU) of Munich. Wolfram entered the world of proteinases by contributing to Robert's trypsin-BPTI complex structure, which for the first time showed the substrate-like interaction of a 'canonical' proteinase inhibitor with a trypsin-like serine proteinase. Shortly thereafter, Wolfram solved the structure of the zymogen trypsinogen and, together with Robert, formulated the concept of the 'activation domain': This allosteric subdomain of serine proteinases exists in a disordered (zymogen-like) or alternatively, after activation cleavage and unmasking of an Ile-Val-Gly-Gly-like amino terminus, a structured (active) state that creates a functional oxyanion hole and specificity pocket.

Subsequently, Wolfram turned his attention to more specific serine proteinases of industrial (e.g. subtilisin Carlsberg) and medical interest, such as kallikreins, human leukocyte elastase, cathepsin G, chymase, the granzymes, and the first snake venom serine proteinase, TSV-PA. Simultaneously, he solved the structures of various proteinaceous serine proteinase inhibitors, both serpins such as α1-proteinase inhibitor, α1-antichymotrypsin, and horse leukocyte elastase inhibitor, as well as canonically binding inhibitors, e.g. the Kazal-type ovomucoid inhibitors, eglin c, secretory leukocyte protease inhibitor (SLPI/HUSI-I/MPI), and bdellastasin. Martinsried/Munich became one of the leading centers in proteinase and proteinase inhibitor research.

Among the numerous structures (and mechanisms) solved by Wolfram's team together with collaborating colleagues are e.g. (i) the structure of kumamolysin that has a subtilisin-like fold, but carries a Ser-Glu-Asp active-site triad; (ii) the crystal structures of several human mast cell α- and β-tryptases which form cage-like quaternary oligomers that explain most of their unusual properties; (iii) the first structure of a cystatin and its mode of interaction with cysteine proteinases; (iv) the structure of cathepsin B, a cysteine proteinase that deviates most from the other papain-like cathepsins; (v) the first full-length structure of human m-calpain, a calcium-dependent intracellular cysteine proteinase whose catalytic domain is distantly related to papain but disrupted in the absence of calcium and must fuse in its presence to become active (Wolfram's 'electrostatic switch' hypothesis stimulated discussions worldwide on calcium activation); (vi) the structure of Arg-gingipain that unexpectedly displays a fold and active-site features similar to caspases; and (vii) the structure of pro-caspase-7 that provides the structural basis for the understanding of the inactivity of procaspases and the structural changes occurring during the activation of apoptosis-related proteinases.

Since the second half of the '80s, Wolfram has been interested in coagulation proteinases and the coagulation cascade. A milestone in his career has been the structure of α-thrombin, which clarified the conformation of specificity determining and active-site shaping insertion loops and exosites. By studying the binding of lead compounds to thrombin, he and his colleagues laid the groundwork for the rational design and the worldwide search for specific thrombin inhibitors (and anticoagulants in general). Wolfram also determined the structures of α-thrombin in complex with a number of protein-type inhibitors, for example, those derived from blood sucking animals that often display unusual bifunctional and/or exosite binding mechanisms. The structure of thrombin in complex with an active thrombomodulin fragment explains the loss of thrombin's procoagulant specificity upon thrombomodulin binding and suggests that thrombomodulin acts as a substrate presenter rather than as an active-site modulator. To expand his blood coagulation gallery, Wolfram's team determined the first crystal structures of the coagulation factors Xa, IXa, VIIa, activated protein C and the membrane-associating C2

domain of cofactor Va, thereby not only making these structures available, but also fostering various studies of the interactions of these proteinases in the coagulation cascade.

More recently Wolfram also turned to the proteinases of the fibrinolytic system. His team determined the structure of the catalytic domain of tissue-type plasminogen activator (tPA) and of the related vampire bat plasminogen activator. The later zymogen lacks a typical activation cleavage site, but is intrinsically active because the side chain of a lysine residue takes over the function of the amino terminus (normally generated by activation cleavage) to stabilize a functional active site. By elucidating the structure of the ternary microplasmin-staphylokinase-microplasmin complex, the Martinsried group not only clarified the structure of the catalytic domain of plasmin, but also the principle of plasminogen activation by staphylokinase: The staphylokinase 'cofactor' does not affect the active-site geometry of the plasmin 'enzyme', but instead modifies its subsite specificity by providing additional docking sites for enhanced presentation of the plasminogen 'substrate' to the 'enzymes' active site. This first structure of a productive proteinase-cofactor-macromolecular substrate complex thus demonstrates how cofactors such as those involved in the coagulation cascades might work.

In late 80s, Wolfram became interested in zinc-containing metalloproteinases. He started with the elucidation of the structure of astacin, a digestive enzyme from the sweet water crayfish, *A. astacus*. Astacin for the first time showed the zinc environment and the fold of a large protein(ase) family that Wolfram and colleagues coined 'metzincins'. Astacin not only became the structural prototype of the 'astacins' (a proteinase family also comprising the procollagen C-peptidase alias bone morphogenetic protein 1 and meprin), but also of other metalloproteinases such as the serralysins, the matrix metalloproteinases (MMPs) and the ADAMs/adamalysins. To the latter family, he contributed the structure of a zinc endopeptidase from the venom of the snake *Adamanteus crotalus*, adamalysin II, the prototype of the snake venom metalloproteinases and the ADAM family whose members are associated with shedding, growth factor activation and cell fusion. More recently, with the structure of the catalytic domain of the TNF$\alpha$-converting enzyme (TACE), Wolfram's team determined the first ADAM structure of an enzyme involved in growth factor activation and presumably also in receptor shedding, another highlight in Wolfram's search for targets of rational drug design.

Wolfram's team was the only one from academia that published the first structure of the catalytic domain structure of an MMP in 1994. By determining the structures of MMPs in complex with synthetic inhibitors, Wolfram contributed both to rational drug design in this cancer- and arthritis-related field and to a better understanding of the catalytic mechanism of the metzincins in general. Subsequently, Wolfram's team determined the first structure of a 'tissue inhibitor of metalloproteinases' (TIMP) and elucidated its mode of interaction with cognate MMPs. This was complemented by the structure elucidation of the complex of TIMP-2 with membrane-type-1 MMP (MT1-MMP), which forms a cell surface located 'receptor' involved in pro-MMP-2 activation. These structures formed the basis for rational design of more specific TIMPs. Meanwhile, Wolfram's group added a number of other MMP structures, such as that of MMP-12 (metallo-elastase), MMP-16, and pro-MMP-1, and also entered the zinc exopeptidase field by solving the structures of mammalian and pest carboxypeptidases, as well as of a dinuclear zinc aminopeptidase.

Overall, Wolfram has not simply collected structures of proteins that became available, but has utilized them to uncover the mysteries of an important and ubiquitous class of enzymes, the proteinases, and their inhibitors. His work spans almost the whole range of the proteinase world and has stimulated and spawned worldwide efforts in biochemistry and drug design. He has been able to do this enormous amount of work due to the extraordinary technical and scientific environment provided by Robert and the proteinase-related Munich-based SFBs, the close collaboration with numerous colleagues from all over the world, and – last, but not least - due to the invaluable contribution of a large number of highly motivated and skilled young students and post docs, most of whom now have their own careers.

Unbelievably, Wolfram still finds time to take care of his family and his hobbies - classical music (he is a good violinist), biking, skiing, mountaineering - when he is not writing papers, modeling and designing structures, discussing molecular mechanisms with colleagues, preparing and presenting lectures, or strolling somewhere in the world with his science.

Contributed by Hans Fritz and Christian Sommerhoff (University of Munich, Munich Germany)
Addendum: For a more detailed description of Wolfram's scientific achievements see e.g. Biol. Chem. 383, 1031-1034 (2002)

# James Travis:

## Life-long love affair with proteolytic enzymes and their inhibitors

Professor James Travis, or simply Jim to his countless friends and colleagues, is one of the founding fathers of modern research on proteolytic enzymes and their inhibitors. For more than one generation of scientists in this field, he is a symbol of a successful researcher, merging basic science with practical applications.

According to Jim, he was not driven by science in his adolescence. Instead, his parents made him finish college and get a decent job at the local brewery in Manitoba, Canada. We can bet Jim would now be retiring as the CEO of Anheuser-Busch breweries if his duties as a supervisor had not included the cleaning of fermentation vessels. This first encounter with biotechnology made him realize that continuing his education was not such a bad idea, so he returned to school in Manitoba to study vitamins and obtained an M.S. degree in 1960. Fortunately, Jim was then recruited by Dr. Ervin E. Liener, a somehow forgotten forefather of protease research, to study for a Ph.D. degree in his lab. This was a turning point in Jim's scientific career and initiated a lifelong romance with proteolytic enzymes and, by default, their inhibitors. Jim only once betrayed his love for proteases. Fed up with severe winters in Manitoba, he moved south to Baltimore, Maryland, in 1964 to do a post-doc with Dr. W.D. McElroy at the John

CONTINUED NEXT PAGE ▶

# A TRIBUTE TO OUR LIFETIME MEMBERS

## James Travis: [continued]

Hopkins University, where for two years he studied the structure and function of firefly luciferase. Thankfully for the protease/protease inhibitor enthusiasts, the luciferase affair lasted only two years. In 1967, after the short stop at the University of Maryland, Jim moved further south and landed at the University of Georgia, in Athens, which would become his hometown for forty years. Here, in a typical southern college town where cotton and peaches ruled the land, Jim started his splendid scientific career. He was promptly promoted to the position of Associate Professor (1973), then Professor (1976) and finally he advanced to the rank of Research Professor in 1986. His career is peppered with milestone achievements that firmly secure his position in the proteolytic hall of fame.

From the perspective of scientists trained in the era of "-omes", like genome, transcriptome, proteome, degradome, etc., some of Jim's ground-breaking research may appear unimposing. However, all things were different during the dawn of modern chromatography, which is where Jim made his mark. Purification was not just Jim's work, it was his mantra. We wonder how many young scientists could manage to purify six human pancreatic proteases from tissue extracts, just as many human plasma inhibitors, as well as myeloperoxidase, neutrophil elastase and cathepsin G from neutrophil granules, all without the help of FPLC's, plate readers, or synthetic substrates, much less His-tags, FLAG-tags, etc. In the process of plasma protein purification, Jim developed Blue Sepharose for removal of albumin from plasma and used this matrix for purification of several different proteins. The next time you see a column filled with the blue stuff, please think about Jim.

Having masterminded plasma protein purification, Jim went on to their characterization. He determined the reactive site sequences of five serine protease inhibitors. Later, together with Robin Carrell, he coined the term Serpin (SERine Proteinase INhibitor) to describe these proteins and showed that mutation of the P-1 reactive site residue altered inhibitory specificity. His most cherished serpin was $\alpha$1-antitrypsin ($\alpha$1AT). However, as a biochemist, it infuriated Jim to no end that this name did not reflect the inhibitor specificity or its physiological target. He reasoned the name should be changed and thus, he began referring to it as $\alpha$1-protease inhibitor ($\alpha$1PI) in all of his subsequent publications. Over the years, this name has been universally adopted by all scientists who work on the protein, yet, someone must have forgotten to tell the clinicians who still insist on calling it antitrypsin for unknown reasons. Nonetheless, $\alpha$1PI was his muse and his research efforts on this inhibitor resulted in a 25 year lung grant….one of the longest continuously funded investigator grants in the history of the NIH.

We owe Jim for being one of the first to investigate the physiological role of serpins. Together with Jo Bieth, he determined target enzymes for most plasma inhibitors using kinetic analysis. Next, he showed that oxidation of human $\alpha$1PI abrogated its inhibitory activity towards human neutrophil elastase, thus providing evidence for the oxidation hypothesis in the development of pulmonary emphysema. On the other hand, by showing that prokaryotic proteinases are not inhibited by $\alpha$1PI, but instead cleave this important regulatory protein, Jim pointed to possible clinical implications of microbial proteases in pathologies of infectious diseases. This observation, that the reactive loop can be cleaved and inactivated by non-target eukaryotic and prokaryotic proteinases, was later extended to all serpins. His work on serpins was crowned by the first comprehensive review (with Guy Salvesen) in Annual Reviews in Biochemistry (1983), which has been cited more than 1600 times and can be considered a classic on how a good review article should be written. Later, Jim investigated the role of cytokines in the upregulation of plasma proteinase inhibitor synthesis during acute phase conditions and, working in collaboration with Wolfram Bode and Robert Huber (MPI, Martinsried, Germany), Jim determined the primary and three dimensional structures of human neutrophil elastase and cathepsin G, including carbohydrate side chain structures.

While Jim is an excellent scientist in his own right, part of his success is that he surrounds himself with good people and has formed extensive, life-long collaborations. This is most apparent with the case of Polish scientists, when in 1986 a beach-head was taken in Athens, GA by Wieslaw Watorek and Jan Potempa and shortly thereafter, the lab was overrun with Polish scientists. At one time, nine Poles were working in Jim's lab, a fact which was even noticed by an NIH study section reviewing Jim's grant. In a grant evaluation, the panel blessed Jim for achieving so much for so little money spent on Polish scientists! Being surrounded by Poles, Jim never learned to speak Polish, but in our judgment, he is the only person who never had problems understanding Pinglish. Due to Jim's scientific achievements and strong scientific connection to Polish scientists, whom he voluntarily, or involuntarily, supplied with tens of thousands of dollars in supplies that ended up in Polish laboratories, he earned the Medal of Honor from the University of Wroclaw, Poland (1995) and was crowned Doctor Honoris Causa from the Jagiellonian University, Krakow, Poland (2001), the oldest university in the Central-Eastern part of Europe (established in 1364).

The Polish invasion had one more important consequence; it had infected Jim with the importance of bacterial proteases in microbial infections. In the late 1980's, it was already accepted that proteolytic enzymes of the periodontopathogenic bacterium *Porphyromonas gingivalis* were important in disease development, but none of these proteases had been characterized. In a short time, Jim rigorously characterized several individual proteases of *P. gingivalis*. Together with Jan Potempa, he coined the acronym, gingipain (*P. gingivalis* + papain), to refer to major cysteine proteases and virulence factors produced by *P. gingivalis*. Seminal work has been carried out in Jim's laboratory on the effect of gingipains on serpins, antimicrobial peptides, coagulation pathway, complement cascade, release kinins from kininogens, cell surface receptors, and cytokines which laid the foundation to the understanding how these proteases can deregulate host defense processes and contribute to pathogenesis of periodontal diseases. Jim also pioneered studies on the protective effect of anti-gingipain antibodies and inhibitors against P. gingivalis infections. Additionally, again in collaboration with Wolfram Bode and Robert Huber, he solved the crystal structure of Arg-gingipain. Thanks to these investigations, gingipains are now considered legitimate targets to develop therapeutic inhibitors and prophylactic vaccines. More recently, Jim's research has begun to look at proteases secreted by

CONTINUED NEXT PAGE ▶

# LIFETIME MEMBERS

## James Travis: [continued]

*Staphylococcus aureus* with a major achievement being the finding that proteases of this pathogen can produce kinins indicative of a role for these enzymes in septic shock due to infection by this organism.

During Jim's distinguished career, he graduated more than 20 Ph.D. students and trained numerous post-doctoral associates, some of which then advanced to the top ranks in academic and corporate research. These include, but are certainly not limited to, Jan Potempa, Guy Salvesen, Takahisa Imamura, Rob Pike, Adam Dubin, Joanna Cichy, Wieslaw Watorek, Dave Johnson, Dave Farley, Daniel Nelson, Andrez Kozik, Maria Rapalla-Kozik, Dennis Bangarozzi, Jason Goldstein, Suhanto Sinha, Ralph Pannell, Tomasz Kordula, Duke Virca, and Chris Reilly. Jim always emphasizes that he owes his achievements to his brilliant students, research associates and collaborators. The list of collaborators he feels indebted to is long and includes Jim Powers, Hans Fritz, Jo Bieth, Wolfram Bode, Robert Huber, Robin Carroll, Vito Turk, Hideaki Nagase, Bud Ryan, Michael Laskowski, Aleksander Koj, Tadeusz Wilusz, Antoni Polanowski, Adam Dubin and Jan Potempa. For Jim, all these people are more than co-workers or collaborators; they are his close friends, and both of us who wrote this letter treasure very much his friendship. For us, Jim was even more than friend; he treated us like his own children. Everybody who has passed through Jim's lab remember times with Jim as an unforgettable experience. He inspired us to pursue our own ideas and helped us and many others to develop into independent researchers.

The lab was always vibrant with laughter and the best ideas were often born at lab parties, cookouts, camping trips, or on the golf course. Experiments were often delayed if the weather was nice and Jim wanted to golf. However, do not get the wrong impression. When we worked, we worked very, very hard. Jim was never as pleased as when he drove by the lab late at night or on weekends and the only lights on in the building were coming from his laboratory. "Work hard, play hard", was more than just a logo we printed on customized Travis lab golf balls, it was a lifestyle in the Travis lab. Perhaps Jim's greatest attribute is his sense of humor, even if it came at his own expense. In one prolific year, the laboratory had more than 30 peer review, original research papers, which Jim swore could not be done. To commemorate the event, Jim agreed to let us dye his hair blue at the Tiers Winter School, perhaps reminiscing of his days in the cold room with Blue Sepharose! This is an example of how playful and friendly relations in Jim's lab generated an exceptionally creative environment to perform first class research. This we owe to Jim!

Jim's life has come full circle, which means he has returned to his Canadian roots, at least partially. During the hot summer months, he lives on an island off the Vancouver coast, no doubt slicing Travis lab golf balls into the forests. For the cool winter months, he remains in Athens, where he still keeps an eye on the remaining people in the lab. Although Jim is author and co-author of more than 340 research papers, few people are aware that he writes poetry and now, he tells us, he is writing a great epic novel. We wish him luck as he devotes himself to the passions he never had time for while he was a full-time researcher.

Fondly contributed by Jan Potempa (Athens, GA & Jagiellonian University, Krakow, Poland) with Pinglish translation by Daniel Nelson (New York, NY).

# IMPORTANT **PROTEASE** PAPERS I

## Research Publications

**Acuff HB, Sinnamon M, Fingleton B, Boone B, Levy SE, Chen X, Pozzi A, Carbone DP, Schwartz DR, Moin K, Sloane BF, Matrisian LM.**

Analysis of host- and tumor-derived proteinases using a custom dual species microarray reveals a protective role for stromal matrix metalloproteinase-12 in non-small cell lung cancer.

Cancer Res. 2006 Aug 15;66(16):7968-75.

---

**Szabo R, Molinolo A, List K, Bugge TH.**

Matriptase inhibition by hepatocyte growth factor activator inhibitor-1 is essential for placental development.

Oncogene. 2006 Sep 18; [epub ahead of print]

---

**Warren EH, Vigneron NJ, Gavin MA, Coulie PG, Stroobant V, Dalet A, Tykodi SS, Xuereb SM, Mito JK, Riddell SR, Van den Eynde BJ.**

An antigen produced by splicing of noncontiguous peptides in the reverse order.

Science. 2006 Sep 8;313(5792):1444-7.

---

**Mueller-Steiner S, Zhou Y, Arai H, Roberson ED, Sun B, Chen J, Wang X, Yu G, Esposito L, Mucke L, Gan L.**

Antiamyloidogenic and neuroprotective functions of cathepsin B: implications for Alzheimer's disease.

Neuron. 2006 Sep 21;51(6):703-14.

---

**Wang Y, Zhang Y, Ha Y.**

Crystal structure of a rhomboid family intramembrane protease

Nature. 2006 Oct 11;:1-5.

---

**Wu Z, Yan N, Feng L, Oberstein A, Yan H, Baker RP, Gu L, Jeffrey PD, Urban S, Shi Y.**

Structural analysis of a rhomboid family intramembrane protease reveals a gating mechanism for substrate entry

Nat Struct Mol Biol. 2006 Dec;13(12):1084-91.

---

**Shen Y, Joachimiak A, Rosner MR, Tang WJ.**

Structures of human insulin-degrading enzyme reveal a new substrate recognition mechanism

Nature. 2006 Oct 19;443(7113):870-4.

---

**Bieniossek C, Schalch T, Bumann M, Meister M, Meier R, Baumann U.**

The molecular architecture of the metalloprotease FtsH

Proc Natl Acad Sci U S A. 2006 Feb 28;103(9):3066-71.

---

**Leeuwenburgh, MA, Geurink, PP, Klein, T, Kauffman, HF, van der Marel, GA, Bischoff, R, Overkleeft, HS**

Solid-Phase Synthesis of Succinylhydroxamate Peptides: Functionalized Matrix Metalloproteinase Inhibitors.

Org. Lett. 2006, 8, 1705-1708.

---

**Watermeyer JM, Sewell BT, Schwager SL, Natesh R, Corradi HR, Acharya KR, Sturrock ED**

Structure of Testis ACE Glycosylation Mutants and Evidence for Conserved Domain Movement.

Biochemistry. 2006 Oct 24;45(42):12654-63.

---

**Frottin F, Martinez A, Peynot P, Mitra S, Holz RC, Giglione C, Meinnel T**

The proteomics of N-terminal methionine cleavage.

Mol Cell Proteomics. 2006 Sep 8; [Epub ahead of print]

---

**Martin, MM, Jean, F**

Single-cell resolution imaging of membrane-anchored Hepatitis C virus NS3/4A protease activity.

Biological Chemistry. 2006, 387:1075-1080.

---

**Hamill, P, Hudson, D, Kao, R, Chow, P, Xu, H, Richer, M, and Jean, F**

Development of a red-shifted fluorescence-based assay for SARS coronavirus 3CL protease: Application to identify an anti-SARS compound from the marine sponge Axinella corrugata.

Biological Chemistry. 2006, 387: 1063-1074.

---

**Fluhrer R, Grammer G, Israel L, Condron MM, Haffner C, Friedmann E, Bohland C, Imhof A, Martoglio B, Teplow DB, Haass C**

A gamma-secretase-like intramembrane cleavage of TNFalpha by the GxGD aspartyl protease SPPL2b.

Nat Cell Biol. 2006 Aug;8(8):894-6.

---

**Friedmann E, Hauben E, Maylandt K, Schleeger S, Vreugde S, Lichtenthaler SF, Kuhn PH, Stauffer D, Rovelli G, Martoglio B**

SPPL2a and SPPL2b promote intramembrane proteolysis of TNFalpha in activated dendritic cells to trigger IL-12 production.

Nat Cell Biol. 2006 Aug;8(8):843-8.

CONTINUED NEXT PAGE ▶

# IMPORTANT **PROTEASE** PAPERS II

**Banaszynski LA, Chen LC, Maynard-Smith LA, Ooi AG, Wandless TJ**

A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules.

Cell. 2006 Sep 8;126(5):995-1004.

---

**Chersi A, Ferracuti S, Falasca G, Butler RH, Fruci D**

Assembly and selective "in synthesis" labeling of quenched fluorogenic protease substrates.

Anal Biochem. 2006 Oct 15;357(2):194-9.

---

**Clarke EE, Churcher I, Ellis S, Wrigley JD, Lewis HD, Harrison T, Shearman MS, Beher D**

Intra- or inter-complex binding to the gamma -secretase enzyme: A model to differentiate inhibitor classes.

J Biol Chem. 2006 Oct 20;281(42):31279-89.

---

**Guo H, Wlodawer A, Nakayama T, Xu Q, Guo H**

Catalytic role of proton transfers in the formation of a tetrahedral adduct in a serine carboxyl peptidase.

Biochemistry 2006 Aug 1;45(30):9129-37

---

**Kramerova I, Kudryashova E, Wu B, Spencer MJ**

Regulation of M-cadherin -{beta}-catenin complex by calpain 3 during terminal stages of myogenic differentiation

Mol Cell Biol. 2006 Nov;26(22):8437-47.

---

**Kumar S, Dare L, Vasko-Moser JA, James IE, Blake SM, Rickard DJ, Hwang SM, Tomaszek T, Yamashita DS, Marquis RW, Oh H, Jeong JU, Veber DF, Gowen M, Lark MW, Stroup G**

A highly potent inhibitor of cathepsin K (relacatib) reduces biomarkers of bone resorption both in vitro and in an acute model of elevated bone turnover in vivo in monkeys.

Bone. 2006 Sep 6; [Epub ahead of print]

---

**Freije, JR, Klein, T, Ooms, JA, Franke, JP, Bischoff, R**

Activity-Based Matrix Metallo-Protease Enrichment Using Automated, Inhibitor Affinity Extractions.

J. Proteome. Res. 2006, 5, 1186-1194.

---

**Falkevall A, Alikhani N, Bhushan S, Pavlov PF, Busch K, Johnson KA, Eneqvist T, Tjernberg L, Ankarcrona M, Glaser E**

Degradation of the amyloid beta-protein by the novel mitochondrial peptidasome, PreP.

J Biol Chem. 2006 Sep 29;281(39):29096-104.

---

**Denault JB, Bekes M, Scott FL, Sexton KM, Bogyo M, Salvesen GS.**

Engineered hybrid dimers: tracking the activation pathway of caspase-7.

Mol Cell. 2006 Aug;23(4):523-33.

---

**Berger AB, Witte MD, Denault JB, Sadaghiani AM, Sexton KM, Salvesen GS, Bogyo M**

Identification of early intermediates of caspase activation using selective inhibitors and activity-based probes.

Mol Cell. 2006 Aug;23(4):509-21.

## REVIEWS

**Mohamed MM, Sloane BF**

Cysteine cathepsins: multifunctional enzymes in cancer.

Nat Rev Cancer. 2006 Oct;6(10):764-75.

---

**Turk B.**

Targeting proteases: successes, failures and future prospects.

Nat Rev Drug Discov. 2006 Sep;5(9):785-99.

---

**Meinnel T, Serero A, Giglione C**

Impact of the N-terminal amino acid on targeted protein degradation.

Biol Chem. 2006 Jul;387(7):839-51.

---

**Lah TT, Duran Alonso MB, Van Noorden CJ**

Antiprotease therapy in cancer: hot or not?

Expert Opin Biol Ther. 2006 Mar;6(3):257-79.

---

**Overall CM, Dean RA.**

Degradomics: systems biology of the protease web. Pleiotropic roles of MMPs in cancer.

Cancer Metastasis Rev. 2006 Mar;25(1):69-75.

# IN MEMORIAM Commemoration of Claudio Sampaio

by Hans Fritz

It was with great sadness that we noted the passing of our Brazilian colleague and friend, Claudio Sampaio, on January 16th, 2005.

In Claudio's passing, Brazil lost a highly respected University Professor, a very successful scientist and an esteemed high school teacher. His family lost a fabulous father and grandfather, and we, as a community, lost an unusually warm-hearted, wonderful person and close friend.

Claudio Sampaio was continuously in service with the University Federal de Sao Paulo and the Escola Paulista de Medicina for more then 40 years in total, and 35 years as assistant, associate, and full professor (since 1988) with numerous important tasks.

He was a member of the University Council, advisor to the Rector, and President of the UNESP evaluation committee, which estimates the quality ranking of all Brazilian Universities. He was engaged in a committee responsible for Graduate Molecular Biology Studies and in the committees of Federal Grant Agencies (CAPES, CNPq), where the scientific quality of projects and researchers are evaluated; Claudio maintained the highest ranking position in this respect.

First contacts between Claudio's group and our Department in Munich came about in the early 70s, when Claudio worked as a Post-doc - together with his wife Misako - in the Brookhaven National Laboratories. There he worked on kallikreins and kinins with Elliot Shaw, while Edwin Fink from our department worked in Lew Green's team on radio immunoassays; their apartments were located next door to each other.

Ten years later, in 1981, Claudio and Misako came to Munich for 2 years as Humboldt Scholars, together with their 3 girls. This marked the beginning of an intensive collaboration between our labs on kallikreins and kinins, and later on various proteases and their inhibitors. From this time onwards, Claudio often joined our department as a Visiting Professor, sometimes accompanied by students or colleagues seeking training in special techniques, for exchange of ideas and extended collaborations. All of these students, Isabel Batista, Cida Tanaka, Solange Serrano, Alexandre Lopez, and Guacyara da Motta have become well established professors in Sao Paulo. An especially close relationship developed between our department and Maria Luiza Oliva, which has now seen nearly 15 years of fruitful collaboration and friendship.

The scientific work of Claudio and his lab has been published in more than 100 papers. Focused originally on plasma and urinary kallikreins and kininases, it comprises (besides phospholipases):

*- numerous newly discovered serine, cysteine and metallo proteases and inhibitors of them from Brazilian plants and/or snake venoms;*

*- their biochemistry and structures,*

*- their pharmacological properties and/or therapeutic potential in inflammatory models,*

*- the search for natural substrates of these proteases and the design of synthetic substrates for structure-function studies and their specific identification, and*

*- also their molecular biology.*

The most recent collaborative project between Claudio's lab and our department had been focused on "protease inhibitors of the Brazilian flora as models for the design of substrates and inhibitors of proteolytic enzymes involved in the clotting/fibrinolysis cascades and in inflammatory and neoplastic diseases".

Claudio was a very popular participant in meetings and conferences due to - besides his lively presentations of special Brazilian scientific topics - his excellent capabilities as a moderator and his warm personal contributions, especially in social events. In 1993, Claudio organized, together with Misako, the International Kinin Conference in Guaruja near Sao Paulo, an unforgettable event, both scientifically and socially. He (and colleagues and students) also participated in many International Kinin Conferences, Gordon Research Conferences on Kinins and Proteases and their Inhibitors, the BRDO and Portoroz Conferences in Slovenia, the Conferences of the International Proteolysis Society in Mackinac (where Claudio was elected as IPS Board member), Munich and Nagoya, and last but not least, he has attended our Winter School 10 times.

We - and I guess some of you, too - are grateful that we could accompany his scientific and personal life for a long time, nearly 25 years, and enjoy his warm-hearted friendship on numerous occasions.

Claudio will live on in our memories and hearts.

## IPS Membership Reminder!!

Just a quick reminder to all our IPS members that the end of our dues cycle is upon us. As most of you know your membership dues cover a two year period which began in January 2005. This means that everyone is up for renewal this month. You will be notified by our Treasurer, Bruce Linebaugh, shortly with information on how to renew your membership. Alternatively, you can get membership forms on our website (www.protease.org). We are asking that you renew early to prevent the rush of applications just prior to our meeting in October 2007. Remember that your dues support the Society and provide you with:

- Reduced Registration Fees to our Meeting in Petras, Greece
- Access to Our New and Improved QuickCuts Mailings
- Access to Our Network of Protease Researchers Around the World Through our New and Improved Web Site

Please renew your membership now and also encourage your colleagues in the protease field to join/renew. WE NEED YOUR SUPPORT.

# Post-doctoral fellowships in 2007

## Developmental Biology and Biochemistry of Metalloproteases

Post-doctoral fellowships will be available in 2007 to study the biology and biochemistry of ADAMTS proteases. ADAMTS proteases have been implicated in arthritis, inherited connective tissue disorders, cell migration and angiogenesis. The overall goal of the laboratory is to understand the role of ADAMTS proteases in molecular networks.

The laboratory has characterized a number of ADAMTS proteases and ADAMTS-like molecules. Ongoing projects include the mechanisms of phenotypes in ADAMTS null mice, analysis of post-translational modification of ADAMTS proteases and ADAMTS-like molecules, proteomics for identification of substrates and intermolecular interactions, and interfaces with cell signaling mechanisms.

The laboratory will suit highly motivated new or recent PhD or MD/PhD graduates who are interested in augmenting or developing skills in mouse genetics, embryology, cell biology, enzymology and protein chemistry, including structural biology. The laboratory offers a stimulating and constructive environment for your professional development. The Lerner Research Institute has state of the art research facilities in a major clinical center, the Cleveland Clinic Foundation, and is affiliated with the adjacent Case Western Reserve University. Cleveland and its vicinity offer an affordable, high quality of life with outstanding recreational and cultural opportunities.

### RECENT PUBLICATIONS


**Koo, B-H, Longpre, J-M., Somerville, RPT, Alexander, J.P., Leduc, R.,Apte, SS.** Cell surface processing of pro-ADAMTS9 by furin. *J Biol Chem, 2006 281(18):12485-94*

**LeGoff C, Somerville, RPT, Kesteloot, F, Powell, K, Birk, D.E., Colige, A., Apte, S.S.** Regulation of procollagen amino-propeptide processing during mouse embryogenesis by specialization of homologous ADAMTS proteases; Insights on collagen biosynthesis and dermatosparaxis. *Development, 2006 133(8):1587-96*

**Oblander SA, Zhou Z, Galvez BG, Starcher B, Shannon JM, Durbeej M, Arroyo AG, Tryggvason K, Apte, SS.** Distinctive functions of membrane type 1 matrix-metalloprotease (MT1-MMP or MMP-14) in lung and submandibular gland development are independent of its role in pro-MMP-2 activation. *Dev Biol. 2005 ;277:255-69.*

**Somerville R P T, Longpre JM, Apel ED, Lewis RM, Wang LW, Sanes J, Leduc R, Apte, SS.** ADAMTS7B, the full-length product of the ADAMTS7 gene, is a chondroitin sulphate-proteoglycan containing a mucin domain. *J Biol Chem.2004 279; 35159-35175*



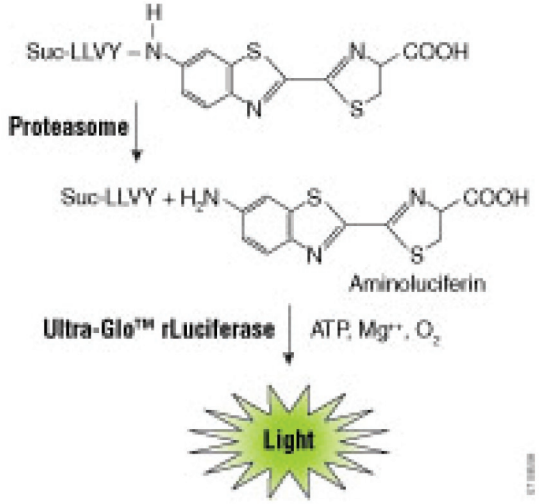
**Contact: Suneel S. APTE, MD, PhD (aptes@ccf.org)**


# Proteasome Assays Made Easy

Promega recently launched a series of assays for the detection of chymotrypsin-like protease activity of the proteasome complex in cultured cells (Proteasome-Glo™ Cell-Based Assay) and for the measurement of the three proteolytic activities associated with the proteasome in an enzyme-based format (Proteasome-Glo™ 3-Substrate System).

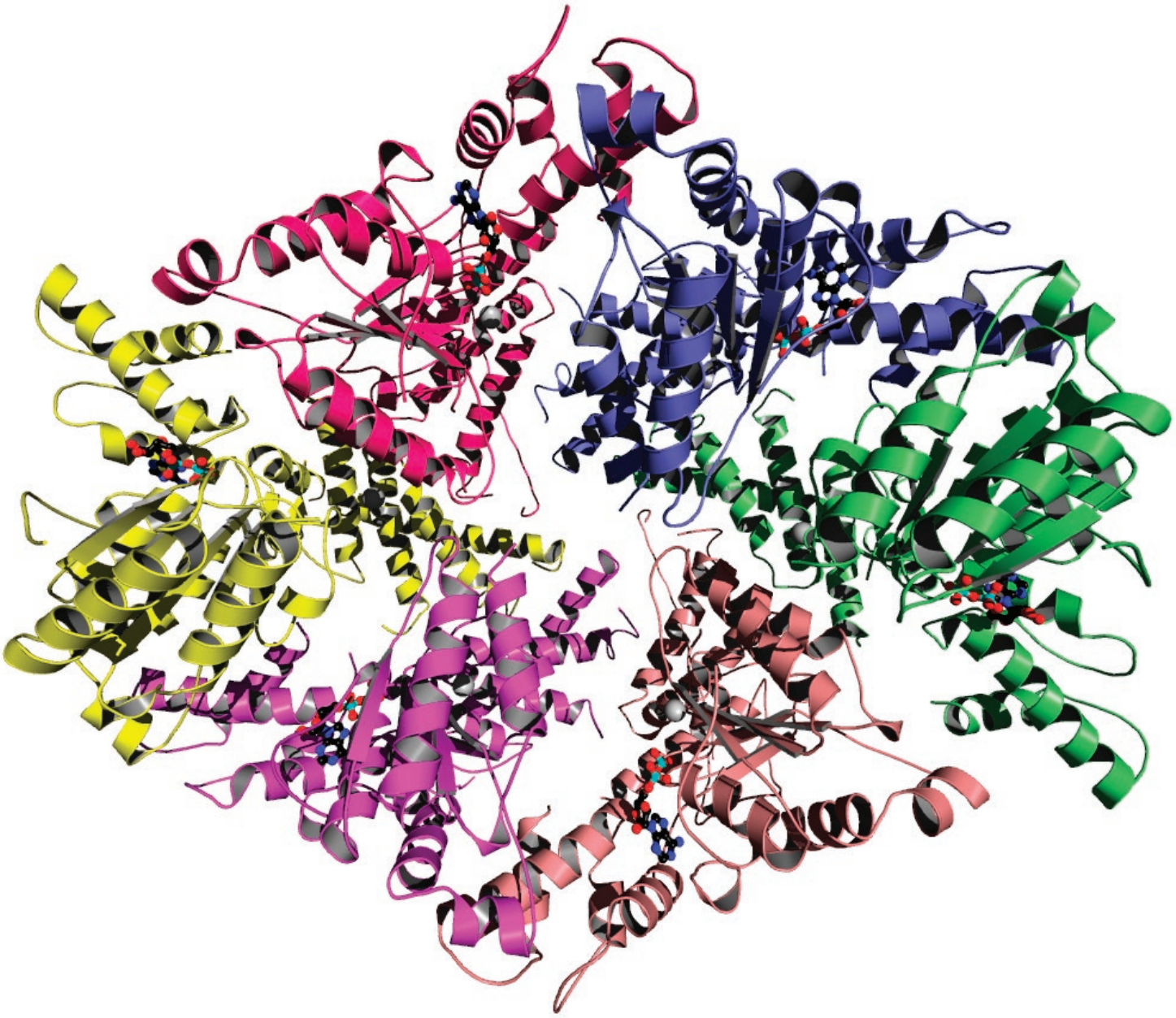
These homogeneous bioluminescent assays feature simple "add-mix-measure" protocols and greater sensitivity with linearity over 3 logs of cell number or 4 logs of proteasome concentration, and are ideally suited for measuring the chymotrypsin-like activity of the proteasome in cultured cells or screening of inhibitor libraries and monitoring proteasome-regulated protein degradation in cells.

Reaction Chemistry (chymotrypsin-like activity example):




| Assay | Substrate | Cell-Based Assay | Biochemical Assay |
|---|---|---|---|
| Proteasome-Glo™ Cell-Based Assay | Suc-LLVY-aminoluciferin | Yes | |
| Proteasome-Glo™ Chymotrypsin-Like Assay | Suc-LLVY-aminoluciferin | | Yes |
| Proteasome-Glo™ Tryptase-Like Assay | Z-LRR-aminoluciferin | | Yes |
| Proteasome-Glo™ Caspase-Like Assay | Z-nLPnLD-aminoluciferin | | Yes |

To learn more about the Proteasome-Glo Assays from Promega: **www.promega.com/drugdiscovery**



OUR LIST OF CORPORATE SPONSORS

Promega

MERCK
Research Laboratories

AMGEN®

INTERNATIONAL PROTEOLYSIS SOCIETY

QUICKCUTS