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Editorial

# Proteases in the limelight: Both ordinary digestive enzymes and smart signaling pathway regulators

## 1. Introduction

In the recent past two special issues of Biochimie have been dedicated to the wide and diverse field of proteolytic enzymes. Both issues, viz. “Cellular Proteolysis” (Vol. 90(2), February 2008) and “Protease Inhibitors and Biological Control” (Vol. 92(11), November 2010) were satisfactorily received, enjoyed a good impact, and were well cited. Following the success of these issues the editors decided that the time was ripe for a third special issue devoted to proteolysis. It has been my pleasure to invite distinguished colleagues to contribute and to supervise as Guest Editor this collection of articles written by some of the world's best experts in the discipline.

The main purpose of this “digestive medley” is to offer a broad panorama of the most important and recent advances related to proteases. In a first part, original articles and reviews are dedicated to innovative tools that allow the scientific community to progress in the areas of molecular targeting of proteases, proteomics, bioinformatic analysis, substrate libraries and *in cellulo* or *in vivo* imaging. The second part presents reviews that update our knowledge of several proteases and inhibitors of major interest, as well as original articles on the biological and/or pathophysiological roles of proteolytic enzymes. The final part of the issue deals with new emerging functions of proteases, with a focus on the growing importance of proteases in the regulation or activation of various signaling pathways.

Newly developed chemical and biological tools and innovative analytical methods may help to clarify the mechanisms underlying proteolytic networks as well dysfunctions that occur during pathophysiological events. Ultimately, 5–10% of proteases may come to be considered as valid therapeutic targets, and a better understanding of their roles may lead to more effective and potent drugs, and the identification of more specific biomarkers of diseases.

## 2. Original contributions

This special issue presents a compilation of twenty-eight articles written by specialists in the domain and comprises three chapters.

### *2.1. Innovative tool boxes as appetizers: imaging, probes, substrate libraries, synthetic inhibitors, proteomics*

The opening article, written by Neil D. Rawlings from The Wellcome Trust Sanger Institute and the EMBL-European Bioinformatics Institute (Hinxton, United Kingdom), is an *ad hoc* introduction that presents the cleavage site collection in the MEROPS database (website: http://merops.sanger.ac.uk/) and discusses the potential exploitation and physiological relevance of these data. I add a grateful thought to Alan J. Barrett, who with Neil D. Rawlings in 1996 set up this valuable online resource and still contributes to its update.

In the next contribution, Marcin Drag and colleagues (Wroclaw, Poland) report the design of a library of fluorogenic substrates containing encoded as well as uncoded and synthetic amino acids to reliably measure the enzymatic activity of aminopeptidases. In contrast to assays using single substrates, this library fingerprint may be used to identify individual aminopeptidases and to measure their activity in cell lysates (Byzia et al.).

Rhomboid proteases, first identified in *Drosophila melanogaster*, form one widespread family of intramembrane proteases. Eliane V. Wolf (Freising, Germany) and Steven H.L. Verhelst (Dortmund, Germany) provide a didactic overview of the major milestones in rhomboid inhibitor research, including activity-based probes (ABPs) for functional characterization of rhomboids.

Christopher J. Scott and collaborators (Belfast, United Kingdom) present an elegant discussion of the development and application of molecular tools to detect cysteine cathepsin activity; they highlight the creative approaches being applied to probe design to overcome the challenges associated with *in vivo* imaging, the ultimate goal being to bring imaging tools into the clinic where they can report on molecular events in real-time, in a non-invasive manner (Caroline S. Hugues et al.).

Olga Vasiljeva and coworkers in collaboration with Charles S. Craik (San Francisco, CA, USA) describe the benefits of an innovative technology developed by CytomX Therapeutics, Inc (San Francisco, CA, USA). Probody therapeutics are recombinant, proteolytically-activated antibody prodrugs. They contain a masking peptide fused to the N-terminus of the light chain of the antibody through a protease-cleavable linker peptide engineered to remain inert until activated locally by tumor-associated proteases. In the diseased environment, the consequent release of the masking peptide results in a fully active antibody capable of binding to its target antigen.

Pathomimetic avatars for cancer developed by Bonnie Sloane and her group (Detroit, MI, USA) represent a powerful modality enabling study of complex co-cultures, long-term analysis of

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proteolysis and secreted factors (e.g., cytokines), and have the potential to be useful as a drug screening platform. Kyungmin Ji et al. offer a comprehensive review demonstrating the usefulness of these pathomimetic avatars for live-cell imaging and quantifying changes in protease activity in real-time (4D).

The next four contributions rely on proteomics analysis with a focus on degradomic methodologies. In a concise and comprehensible review article, Marko Fonovic, Boris Turk and colleagues (Ljubljana, Slovenia) draw up a general picture of the mass spectrometry-based approaches for determining the proteolytic events in complex biological samples. Methodologies for substrate identification and the determination of protease specificity are discussed, with a special focus on N- and C-terminomic strategies.

Oliver Schilling and his colleagues (Freiburg, Germany) describe an investigation of how cathepsin B may shape the secreted proteome of murine polyoma virus middle T oncogene (PyMT) breast cancers by employing a novel strategy to harvest tumor interstitial fluid in combination with chemical stable isotope tagging for quantitative proteomics. They identified a large number of shed ectodomains, which emphasize the importance of tumor cell surface proteolysis and complex proteolytic networks in the breast cancer secretome (Alejandro Gomez-Auli et al.).

In a collaborative work coordinated by Anthony J. O'Donoghue (San Francisco, San Francisco, CA, USA), a global analysis of peptidase activity in the excretory/secretory products of key *Schistosoma mansoni* developmental stages was conducted (Jan Dvorak et al.). It should be noted that the substrate specificity for peptidase activities was analyzed using the Multiplex Substrate Profiling by Mass Spectrometry (MSP-MS) strategy with some minor technical changes, compared to the seminal article published by the same group.

To conclude this first section, Christopher M. Overall and his collaborators propose an “off the beaten track” article that aims to make the link between proteomics and the global human proteome project, underpinning in the longer term the notion of personalized medicine. From a holistic and prospective point of view, Ulrich Eckhard et al. examine the interest of positional proteomics techniques, including TAILS (Terminal Amine Isotopic Labeling of Substrates) and COFRADIC (COmbined FRActional DIagonal Chromatography), as an essential component of emerging precision medicine initiatives.

### *2.2. Today's special: vegetarian dish, al dente microbial proteins or bloody proteases?*

The tobacco-related plant species *Nicotiana benthamiana* has emerged as a versatile expression platform for the rapid generation of recombinant biopharmaceuticals including monoclonal antibodies. Nevertheless, product yield and quality frequently suffer from unintended proteolysis involving papain-like enzymes. Lukas Mach and coworkers (Vienna, Austria) convincingly propose that downregulation of *N. benthamiana* cathepsin B could improve the performance of this plant-based factory.

Legumain, initially purified from germinating bean cotyledons, is also known as asparaginyl endopeptidase. Elfriede Dall and Hans Brandstetter (Salzburg, Austria) offer an exhaustive summary of the current knowledge of this fascinating cysteine protease (clan CD, family C13) that exhibits both endopeptidase and carboxypeptidase activities as well a peptide ligase activity.

Lung antimicrobial proteins and peptides (AMPs) are major sentinels of innate immunity, preventing microbial colonization and infection. Host proteases participate in concert with bacterial proteases in the degradation of key innate immunity peptides/proteins and thus may play immunomodulatory roles during chronic lung diseases. In this context, Pierre-Marie Andrault and his colleagues (Tours, France) summarize current knowledge and highlight recent discoveries concerning the ability of host enzymes to interact with AMPs, providing a better understanding of the role of human proteases in innate host defense (Lecaille et al.).

The calpains are associated with many diseases (muscular dystrophy, gastric ulcer, diabetes), also described as calpainopathies. Among human calpains, CAPN3 (a.k.a. p94, nCL-1, nCANP, and calpain-3) exhibits unusual features including an autolytic activity of such strength that it has been described as an ephemeral enzyme. Hiroyuki Sorimachi and his group (Tokyo, Japan) offer us a very comprehensive overview of this “eccentric” protease, in which defects caused by gene mutations are responsible for limb-girdle muscular dystrophy type 2A.

In an instructive review, Bruno Franzetti and collaborators (Grenoble, France) describe our current knowledge on M42 TET peptidases and discuss their possible physiological roles among prokaryotes. These metallo-enzymes that form 12-subunit hollow tetrahedral particles have revealed a unique mechanism of internal compartmentalization and peptide trafficking that distinguishes them from other oligomeric peptidases. Recent studies have shed light on the proteolytic activity of TET aminopeptidase, suggesting a functional regulation based on oligomerization control *in vivo* (Alexandre Appolaire et al.).

Chagasin was identified in the pathogenic protozoa *Trypanosoma cruzi* that is responsible for Chagas Disease. It is a thermostable, tight-binding inhibitor of cysteine cathepsins and papain-related endogenous parasite cysteine proteases, displaying an immunoglobulin-like fold. Orthologues of chagasin that are distributed among Protists, Bacteria and Archea are named Inhibitors of Cysteine Peptidases (ICP). Tatiana F. R. Costa and Ana Paula C. A. Lima (Rio de Janeiro, Brasil) cover in a compelling manner most of the findings on the structural and functional properties of chagasin-related inhibitors and overview the current knowledge of their roles in protozoa.

The two next original research articles relate to pathological events. Klaudia Brix and her colleagues (Bremen, Germany) report enhanced levels and altered subcellular localization of cysteine cathepsins in adenocarcinoma tissue and reveal the unexpected presence of cathepsin L in the nucleus. They provide convincing data and hypothesize that nuclear cathepsin L accelerates cell cycle progression, thereby supporting the notion that cysteine cathepsins may play significant roles in carcinogenesis due to deregulated trafficking (Tripti Tamhane et al.).

Human neuronal ceroid lipofuscinoses are inherited, neurodegenerative diseases. These lysosomal storage diseases lead to dramatic deterioration of sight followed by mental retardation and epilepsy. Thomas Reinheckel and his group (Freiburg, Germany) established a conditional cathepsin D knockout mouse model and demonstrated that cells of neuroectodermal origin initiated neuronal ceroid lipofuscinosis type 10. Finally, the authors suggest that their results provide proof-of-concept data on how the conditional cathepsin D knockout mouse will provide further insight in the cell-type specific pathogenesis of complex physiological and pathological processes (Anett Ketscher et al.).

The two last original contributions of this second part are devoted to thrombin, a key serine protease involved in the blood coagulation cascade by converting fibrinogen to insoluble fibrin. Recent evidence indicates that cleavage sites located within α-helices can also be cleaved by proteases. To address the question of how a protease cleaves within an alpha helix Robert N. Pike (Melbourne, Australia), Stephen P. Bottomley (Clayton, Australia) and their coworkers designed a model substrate, in which the optimal cleavage site for thrombin was placed within the alpha helix of the protein.