



# International Society for Enzymology (ISE)

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Editor: David M. Goldberg

### ENZYMES MEET PROTEOMICS: An Informal Report

Which University is the oldest in Europe — Bologna or Padua? It is only to the alumni of these two institutions that the issue is one worth fighting about. For those of us whose academic pedigree is much more recent, their origins are shrouded in the mists of such prodigious and timeless achievements that the question holds as much significance as that posing the priority between the chicken and the egg. At the opposite biological extreme to that represented by these pinnacles of human civilization, the futility of attempting to make fine distinctions between near equals was brilliantly epitomized by the great English writer and humorist, Dr Samuel Johnson, when he asked the famous rhetorical question: “What is the point in settling the precedence between a louse and a flea?”

It was the latter of these two primeval seats of learning that played host to the above one-day conference, specifically, the state-of-the art Lecture Theatre of Padua's University Hospital, also known as the Aula Morgagni in honour of the founding father of Pathology and Laboratory Medicine. The conference itself, held on Friday 29<sup>th</sup> October 2004, was designed to pay homage to another founding father: Angelo Burlina, to whom the International Society of Enzymology owes its creation. In this very institution, he worked and died, having attained the very peak of his profession at the end of a journey crowned with accomplishments and distinctions that few could equal. It started modestly enough in the peaceful little town of Conegliano to the North of Venice, and continued in the more romantic milieu of Verona, but it was as Morgagni's successor to the Chair of Pathology and Laboratory Medicine at Padua that he achieved his ultimate apotheosis. The organizer of the conference was his most illustrious former student, Mario Plebani, President of the Society, who as his successor in Padua, uses his office, sits in his Chair, and draws his salary that is hopefully a little larger than it was in Angelo Burlina's time.

The conference opened with an eclectic and anecdotal **History of Clinical Enzymology**, attributed to Zelig, the hero of a Woody Allen movie of the same name, and delivered by **David Goldberg** (*Toronto, Canada*). In it, he traced the origins of what should have, but failed to, become a major clinical discipline, fertilizing so many other divisions of Laboratory Medicine during its 50-year

life span prior to its death and cremation. He characterized Proteomics as the Phoenix that arose from these ashes, and counseled the audience to be mindful of the errors that led to this demise so that Proteomics could avoid sharing the same fate.

**In Proteomics: The Next Revolution in Laboratory Medicine?** our host, **Mario Plebani**, presented a fluent and convincing case for the affirmative, amplified by many visually-stunning illustrations. Describing the challenge facing proteomics as that of identifying uniquely characteristic genetic alterations and protein profiles associated with specific disease states, he charted the astounding progress already made in conditions such as cancer, cardiovascular and neuromuscular diseases. Much of this progress could be attributed to advances in technology as the tools of proteomics expanded from two-dimensional gel electrophoresis to sophisticated mass spectrometric applications. He concluded that the main obstacles to translating these successes into procedures that could be routinely employed by diagnostic laboratories were the problems related to standardization, and the legitimacy of comparing data obtained by different laboratories.

A more basic agenda was spelled out by **Piero Pucci** (*Naples, Italy*) in his lecture entitled **Functional Proteomics**. This seeks to establish the function of proteins or protein sequences whose structure is known, whereas what they actually do is a total mystery. A further objective is to define cellular mechanisms at the molecular level through the analysis of multi-protein complexes. To accomplish these goals, affinity-based procedures have been developed comparable to the use of bait to catch fish. These techniques, that include histidine tails and biotinyl tags, can identify interacting proteins in stable complexes that are then characterized by mass spectrometric methods. The association of an unknown protein with those of previously defined function can help in elucidating its biological role, presumably on the principle that a person can be judged by the company he keeps. The drawback is that disruption of intracellular compartments and altered protein molar ratios may provide misleading information. Nevertheless, the approach seems well worth pursuing, since viable alternative options hardly exist.

The clinical laboratory was once again the focus of the next presentation, **Lights and Shadows of Quantitative Proteomics in Two-Dimensional Electrophoresis**, given by **Annalisa Castagna** (*Verona, Italy*). In effect, Dr Righetti rephrased the original title to pose the questionable role of the clinical laboratory as *Myth or Reality?* She cited recent examples coming from advanced research in the subject: the association of protein phosphorylation with advancement in the malignancy of a specific cancer cell line; the discovery of a new protein named 14-3-3 that confers upon human cervical cancer cells chemoresistance to cis-platin therapy; the presence of the same protein in the cerebrospinal fluid of subjects with Creutzfeld-Jacob disease and its absence from patients with other forms of dementia. Attempts to exploit human serum for diagnostic purposes have not so far been successful, but the development of immuno-subtractive procedures that allow detection of proteins in concentrations lower than 10ng/mL hold out hope for more rewarding discoveries in the future.

After a break that refreshed the audience with a generous assortment of cold beverages and traditional Italian biscotti, as well as those miniature cups of steaming black espresso coffee that most North Americans consider to be both delicious and incomprehensible, **Daniel Chan** (*Baltimore, USA*) spoke on **Application of Clinical Proteomics to Cancer**, building his presentation around a case study of ovarian cancer. He was followed by **Andreas Scorilas** (*Athens, Greece*), whose title was **Kallikreins: A Newly Recognized Family of Genes and Enzymes Involved in Cancer**. Beginning with a broad outline of the kallikrein family of which 15 genes have now been cloned, and the responsiveness of most kallikreins to steroid hormones, he went on to describe some of their clinical associations: hK6, hK8 and hK10 with ovarian carcinoma; hK5 and hK14 with both ovarian and breast cancer; hK13 as a protective factor *against* ovarian cancer. He focused the rest of the talk on the role of hK11 in prostate cancer, and while he made a spirited case for its utility, the evidence presented was not entirely convincing. Although it is detectable in 65% of prostate cancer cases, its specificity in discriminating between cancer and benign hypertrophy of the prostate is only 49%. More detrimental is the significant negative correlation between hK11 and the Gleason score (an index of prostate cancer severity), as well as its lack of correlation with prostate-specific antigen concentration.

A sumptuous lunch featuring the finest wines and gastronomic specialties of the region was then served. The trouble was that the restaurant was more than a kilometer away by foot from the conference venue and the rain was descending in sheets. Padua is one of the few cities where this inclemency can be overcome without the need for an umbrella. Most of the streets are

lined by pillared arcades that offer shelter to the pedestrian, and the majority of the conference participants were able to make the journey back and forth without too much suffering.

The afternoon session began with a title that, by its length, might have been expected to cause postprandial indigestion: **Lysozyme—A Paradigmatic Molecule for the Investigation of Structure, Function and Misfolding in Amyloid Disease**. Mercifully, **Gianpaolo Merlini** (*Pavia, Italy*) managed to create a dish more reminiscent of a light dessert soufflé than a beefsteak main-dish. In the course of a relaxed and beautifully illustrated lecture, he described how human lysozyme is one of many polypeptides that can be assembled in antiparallel beta-strands to form amyloid fibrils that are a feature of several major chronic disease states. In addition to non-pathogenic polymorphisms, lysozyme is subject to four mutational events that may precipitate amyloid formation. Comparison of the detailed structural properties of native and amyloidogenic lysozyme may permit the development of drugs to stabilize the amyloid precursors and prevent amyloid formation.

This was followed by **Pancreatic Cancer-Associated Diabetes: An Open Field for Proteomic Applications** presented by **Daniela Basso** (*Padua, Italy*), once again illustrating the scientific strength of the group that Mario Plebani has built up in his institution. An intriguing tale was narrated concerning the search for a factor that might be incriminated as the cause of diabetes in subjects with pancreatic cancer. The detective (no relation of Sherlock Holmes or Maigret) credited with discovering the culprit went by the unlikely name of MALDI-TOF (Matrix-Assisted Laser Desorption Ionization Time-of-Flight). Studies of cultured pancreatic cancer cells and their conditioned media, and of sera from pancreatic cancer patients, normal controls, and patients with chronic pancreatitis uncovered a peptide with 14 amino acids (1.5 kDa) sharing homology with an S100 calcium-binding protein that was found only in material associated with pancreatic cancer. It was charged with being the cancer-associated diabetogenic agent, but a legal conviction will require clearer proof of its ability to alter glucose and insulin homeostasis in animal or human experiments. Meantime, the jury is still out.

**Claudio Belluco** (*Padua, Italy*) continued this display of local talent with an equally fascinating topic: **Kinase Substrate Protein Microarray Analysis of Human Colon Cancer and Hepatic Metastasis**. Her conceptually exciting presentation described a comparison between the profile of phosphoproteins (protein kinase substrates) in samples of primary and metastatic colon cancer. Reverse phase protein microarray analysis was among several techniques employed in these studies that showed quite marked differences in profile between the two sets of samples,

even when both were derived from the same patient. These were taken to be indicative of different signaling pathways induced by the different microenvironments to which primary and secondary cancer cells are exposed. Dr Belluco suggested that molecular targeting of metastatic cancers based only upon the characteristics of the primary may prove to be ineffective, and she persuasively argued the need for analysis of metastatic tumors as well. As only 20,000 cells are required, these procedures may not be beyond the limit of feasibility. This excellent presentation was followed by a coffee break, necessarily abbreviated by comparison with the morning equivalent by virtue of the amplitude of the luncheon that had concluded a mere 90 minutes earlier.

The fourth member of the Padua group and the penultimate speaker was **Annunziata Lapolla** whose topic was **Glycooxidation in Diabetes and Related Diseases**. This was another coherent and convincing performance, again marked by visually superb illustrations. After reviewing the evidence that advanced glycosylated end-products (AGE) are generated as a consequence of damage to the mitochondrial transport chain by superoxide that is induced by hyperglycemia, Dr Lapolla went on to describe the range of techniques employed in the identification of AGEs and their receptor (RAGE) in diabetic atherosclerosis and other conditions such as mere aging, especially in early and intermediate phases of these processes. These include a formidable army of acronyms such as MALDI (defined above), LC/ESI – MS/MS (liquid chromatography – electrospray ionization – tandem mass spectrometry), and FTMS (Fourier Transform Mass Spectrometry).

The curtain was brought down on the conference by **Giorgio Federici** (*Rome, Italy*) with a lecture entitled **Perspectives of Proteomics in Neurodegenerative and Neoplastic Diseases**. In the record of the conference proceedings that appears as a Special Issue of *Clinica Chimica Acta* edited by its Chairman, **Mario Plebani**, the following presentation, with full text and Figures, is attributed to Dr. Federici: **Analytical Assessment of MALDI – TOF Imaging Mass Spectrometry on Thin Histological Samples. An Insight into Proteome Investigation**. However, the aspiring reader who is anxious to share in the ample cornucopia of truth and knowledge that was revealed to an audience of registrants approaching 500 in number in the miraculously short space of five and one-half hours during a rainy day in Padua may be reassured: there is for all the other contributions some genuine resemblance between what is printed in the pages of the journal and what was actually said in the Aula Morgagni on Friday 29<sup>th</sup> October, 2004.

Sponsorship of the conference was generously provided by the following companies: Abbott, Adaltis, Alfa Wassermann, Becton Dickinson, Biomerieux, Bio – Rad Laboratories, Bouty, Celbio, Chematil, Dianoema, Euroimmun, Helena Laboratories, Medical Systems, Olympus, Ortho – Clinical Diagnostics, Pantec, Pharmacia, Polymed, Radim, Sebia and Sentinel.

As with all Italian scientific meetings, the hospitality and the social program were outstanding. A reception was held at the **Caffe Petrocchi**, known for more than a century-and-a-half as one of the intellectual centres of the city, a gathering place for artists, intellectuals and revolutionaries. Nowadays, business is presumably bad and they have to put up with the patronage of doctors and scientists, but the management succeeded in providing a delightful assortment of those mouth-watering appetizers at which Italian cuisine excels, washed down by liberal glasses of that charming bubbly wine, Prosecco, one of the enological delights of the Veneto region. The long walk from the University Hospital to the Caffe had to be accomplished in a continuous downpour of rain, but, as already mentioned, Padua is one of the few cities in the world where this does not present an immediate threat to health. The broad arcades that grace its streets protect the pedestrian while also allowing a glimpse of the remarkable and venerable architecture through which one passes. Although this is very much an academic town, the University is so inextricably integrated with the historic centre that you hardly ever notice it. The only tell-tale marks are the inevitable graffiti scrawled on the walls, or the cluster of evil-looking young ruffians of both sexes entering or exiting the buildings. Students are instantly recognizable no matter where one is in the world.

Following the reception, a further walk was necessary to reach the **Hall of the Giants**, one of the principle auditoria of the University, as impressive a piece of Baroque architecture as its name implies. Here, a special concert was performed for the conference guests and registrants by the **Concentus Musicus Patavinus**, as melodious an ensemble of musicians as one could wish to hear at the end of a busy day, with a program that was well suited to the taste of the audience, beginning with Benjamin Britten's "Simple Symphony" and continuing with two works of Mozart. At the end of it all, one could only shout, *a propos* the day as a whole: "Bravo Mario!" and "Encore!"

**David M Goldberg,**  
**Editor, ISE Newsletter.**