Reflections on the MPSA-Conferences: Development and Innovations of Protein and Peptide Structure Analysis in the Past 30 Years

Brigitte Wittmann-Liebold
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Summary

In 1975, the first MPSA conference was held in Boston. Subsequent workshops have taken place approximately every two years and have alternated between Europe, Japan and the United States. The focus of the early conferences dealt with new and advanced methods in sequence analysis of proteins and peptides, and the focus on sharing cutting edge techniques for protein analysis has remained. However, as methods for protein analysis increased in the early 1990s, the workshops expanded in scope and size to emphasize additional aspects of analysis of protein structure and function as well as of the chemistries related to primary sequence analysis. In 1999, the "International Association for Protein Structure Analysis and Proteomics" (IAPSAP) was founded, in large part to facilitate organizing future MPSA conferences. A personal reflection of the excitement of the past conferences over the years and a summary of the 14 Workshops/Conferences held to date are given below. Many of the new techniques for structural analysis of proteins and peptides were introduced to the public at these conferences and soon were spread among scientists world-wide. However, over the years the approaches for structural and functional analysis of polypeptides underwent drastic changes, and I noticed the loss of some good ideas with time. In this brief view of the past presentations and published articles, I hope to filter some new ideas out of the old innovations and technologies.

How it began

The MPSA conferences began in 1974 with a small workshop in Boston, MA, USA organized by Richard A. Laursen, Boston University, for the purpose of exchanging information on new approaches for sequencing proteins by removing amino acids from the amino terminus one at a time. This chemical scheme was developed principally by Pehr Edman at the Rockefeller Institute, the University of Lund, Sweden, and finally at the St. Vincent School of Medical Research in Melbourne, Australia.

Through a good friend, Konrad Beyreuther from Köln, I heard a rumour that Richard Laursen developed a solid-phase peptide sequencing method and constructed a solid-phase machine intended for sequencing peptides and proteins (Laursen, 1971). This happened shortly after the Edman spinning cup sequenator was invented (Edman & Begg, 1967), which became commercially available through Beckman Instruments in 1970. I persuaded Heinz-Günter Wittmann, the founder and director of the MPI of Molecular Genetics in Berlin, to contact Dr. Laursen at the International Biochemistry Congress in Lausanne in order to gain information on this machine. Richard's invention was of special interest for us, as we were fascinated by the idea of investigating the structure of the ribosome. At this time, primary structure analysis of proteins was undertaken with proteins available in at least gram amounts, such as myoglobin, hemoglobin or various enzymes, e.g. ribonuclease, chymotrypsin, lysozyme. However, sequencing of ribosomal proteins was beyond any scope of reality at that time; they were hard to purify and at best available in some milligram amounts. Hence, I was highly interested in any advanced manual or automatic peptide sequencing technique to analyze the hundreds of ribosomal peptides derived from these proteins in a reasonable time. I should

state that even our best colleagues and friends were extremely skeptical about our research goals in these years.

Richard Laursen was willing to help us; he gave us the blue prints of his machine. Indeed, the second solid-phase sequencer was constructed at our workshop in Berlin, and he came over to Berlin with his students Marcus Horn and Alex Bonner to demonstrate his new technique. He showed that lysine peptides could be sequenced well, though still amounts in the 200-400 nanomole range were required. The breakthrough of the technique came when Emil Schiltz from our group gave him a mixture of two peptides for sequencing, one containing an arginine while the other contained one lysine. The sequence of the lysine peptide clearly came out very well while the arginine peptide was not attached to the solid-phase support and did not sequence as expected. Attachment was made to the polystyrene resin by the so-called "lysine attachment method" employing *p*-phenylene diisothiocyanate-modified aminopolystyrene resin (Laursen et al., 1972). Immediately from this day on my students gave Richard their peptides for sequence analysis. The good success in our laboratory spread through Germany and Europe, and one after another, scientists came to Berlin to get his or her peptides sequenced on this machine. At that time, only some laboratories worked on the structure analysis of proteins, and only a few protein chemists were interested in sequence analysis of proteins available in only minute amounts.

With our new successes, we discussed holding a conference for the "insiders" who had problems with sequence analysis of minute peptide amounts. Finally, Richard obtained support from Pierce Chemical Company for organizing a meeting for the "people on the bench". So the first conference took place in Boston, named "Solid-phase Methods in Protein Sequence Analysis", the first MPSA Conference. This workshop was a great success, and it was decided to continue to organize this meeting every two years, alternating between Europe and The United States. Later this conference series was renamed "Methods in Protein Sequence Analysis" and more recently, "Methods in Protein Structure Analysis", as the field became broader and more and more participants attended the conference. The MPSA reflected the beginning of modern protein and peptide microsequence analysis! For all attendants, it became highly beneficial to learn the newest tricks and techniques in protein structure analysis directly from the experienced experts and to discuss the advantages and limitations of each method in a free and friendly atmosphere. As a result, the new techniques and innovations spread rapidly among all the insiders. The continuation of the conference series largely supported the rapid progress in this field during the past 30 years.

MPSA1975, Boston, Massachusetts, USA, May 21-23, 1975, 1st MPSA Conference.
Organizer: Richard A. Laursen, Boston University.
Proceedings Book: Solid Phase Methods in Protein Sequence Analysis (Proceedings of the First International Conference), R.A. Laursen (Ed.), Pierce Chemical Co. Press, Rockford, IL, 1975, 286 pp.

At Boston, the new solid-phase principle was presented. The technique employed a polystyrene support to which peptides were attached; the support filled a column through which reagents and solvents for Edman degradation were washed. The degraded PTC-amino acids were collected in a fraction collector. An advantage was that the reagents and solvents needed no extreme purification as was the case for the spinning cup sequencer, and the wash-out of short peptide was circumvented. Several European peptide scientists among the 70 to 80 participants presented their results with the new solid-phase sequencing method, e.g. Marie-Anne Coletti-Previero, Montpellier; Emil Schiltz, Berlin; Werner Machleidt and Elmar

Wachter, München; Christian Birr, Heidelberg; Peter Fietzek, München; Joseph Reinbolt, Strasbourg; and myself. The sequence analysis of lysine-peptides advanced to less than 200 nanomoles. On the other hand, sequencing short arginine peptides or peptides without C-terminal basic groups was difficult to perform; this was thoroughly discussed during the meeting. Aldo Previero, Montpellier, summarized principles of peptide synthesis and their application to peptide sequencing techniques. Furthermore, the first comparison of the spinning cup liquid-phase machine versus the solid-phase sequencer was made, demonstrating advantages and the implied limitations of each technique.

The organisation of the meeting was excellent, and it was the beginning of many scientific and social contacts between protein chemists worldwide. Richard Laursen, with the sponsoring of Pierce, provided a fantastic buffet which we, at least from Germany, had never experienced before. The conference was a first highlight in exchanging methodology among the young "experts". Therefore, the offer of repeating the conference in Europe by Marie-Anne Coletti-Previero and Aldo Previero at the University of Montpellier in France was enthusiastically greeted. The final decision was to organize this conference every two years, alternating between North America and Europe; later the venue was extended to any suitable location worldwide.

MPSA1977, Montpellier, France, September 21-23, 1977, 2nd MPSA Conference.
Organizer: M. A. Coletti-Previero, Unite de Recherches, INSERM, Montpellier, France.
Proceedings Book: Solid Phase Methods in Protein Sequence Analysis (Proceedings of the 2nd International Conference on Solid Phase Methods in Protein Sequence Analysis), A. Previero and M. A. Coletti-Previero (Eds.), Elsevier/North Holland Biomedical Press, Amsterdam, 1977, 298 pp.

In Montpellier, under the perfect organisation and guidance of the eloquent and elegant Marie-Anne, about 200 participants attended, among them about 30 scientists from the USA who became aware of the usefulness of the solid-phase sequencing method. At this occasion, we mourned the unexpected death of Pehr Victor Edman, whose enormous contribution to protein sequence analysis was already obvious at this time, since the structures of known proteins increased dramatically from year to year, thanks to the PITC degradation technique (Edman 1950a, 1950b; Ilse & Edman, 1963). It must be stated that the fantastic progress protein structure analysis made in this time and in the following decades was based largely on many, many years of the personal, experimental and technical effort Pehr Edman had taken to establish a reliable sequencing method. Even more astonishing is the fact that the Edman degradation method with the phenylisothiocyanate reagent was never effectively replaced by any other chemical principles until three decades later, when mass spectrometry came to the forefront. All the protein experts expected Pehr Edman to be awarded the Nobel Prize together with Geoffrey Begg, who spent all his efforts in the construction of the sequencer. But unfortunately, at that time, "simply the development of a technique" was not regarded suitable for such high honours by the Nobel Prize Committee.

Pehr Edman died in March of 1977 at the age of 61. At the conference, Gerhard Braunitzer, Martinsried, gave a reflection on his tremendous personality and his intense scientific work (Braunitzer, 1977). At the conference, his wife, Agnes Henschen-Edman, München, referred to strategies and tactics in protein sequence determinations (Edman & Henschen, 1975; Henschen-Edman, 1977). These chapters gave a basic description of the strategy and methodology of the Edman degradation and provided clear guidance for practical work by the protein chemistry community for many years.

The topics of the MPSA meeting were “Chemistry of the Solid-phase Method” and “Strategies of Sequence Determination”. Several papers were presented on the chemical basis of C-terminal sequencing, a rather severe problem difficult to solve and discussed at so many subsequent meetings. Technical problems encountered with the commercial spinning cup liquid-phase sequencer also were discussed. Whereas Edman himself with his home-made sequencer could establish 50 degradation steps of a fibrinogen peptide in one run, with the commercial Beckman machine, barely 20 to 30 steps were possible (Wittmann-Liebold, 1973;Wittmann-Liebold et al., 1975). Besides the technical problems, the sequencer showed considerable protein and peptide wash-out and low repetitive sequencing yields; these problems led to the application of polybrene (Capra et al., 1977; Klapper et al., 1978; Tarr, 1975; Tarr et al., 1978). For small peptides, the solid-phase method turned out to be superior to the liquid sequencing mode, especially when porous glass beads (CPG), modified as aminopropyl glass (APG) or DITC-glass, were introduced for the attachment of peptides (Wachter et al., 1973; Wachter & Werhahn, 1977). During the meeting, various papers dealt with the various attachment methods of peptides (Laursen, 1977a, 1977b; Machleidt & Machleidt, 1977; Beyreuther, 1977; Inman et al., 1977).

At this time in both sequencer types, the released PTC-amino acid derivatives had to be collected in a fraction collector and converted manually into the more stable PTH-amino acid derivatives for their identification. Typically the PTH-derivatives were analyzed by silica-gel thin-layer chromatography using 2 to 3 ascending chromatography buffer systems one after the other (Reinbolt et al., 1977; Appella et al., 1977; Beyreuther, 1977; Horn & Bonner, 1977). Already, however, HPLC separations and quantification of the PTH-amino acids were described in several presentations (Appella et al., 1977; Capra et al., 1977; Ericsson et al., 1977; Champlin & Hawk, 1977; Frank and Strubert, 1973; Zimmermann et al., 1973) and this was an important advance in polypeptide sequencing. An alternative for analysing sequences was back-hydrolysis of the released PTH-amino acids, which had been proposed years earlier (Smithies et al., 1971). To increase the sensitivity in the solid-phase machine, Bridgen and Waxdal (1977) employed ^{35}S-PITC for quantification; another contribution used ^{14}C-labelled protein (Walker et al., 1977). In the group of Friedrich Weygand in Munich, bromophenylisothiocyanate was used to increase sensitivity and easy mass spectrometric identification of the double labelled PTH-amino acids (Tschesche & Wachter, 1970).

Many of the labile amino acids were difficult to detect due to their destruction at the time of release in the sequencer. Even with 5-7 mg of protein and about 200 nanomoles of peptides, the detection of some labile amino acids was not possible. Therefore, an automatic conversion device for the solid-phase machine was proposed by the Laursen group for the Sequemat Sequencer by Horn & Bonner (1977), Schiltz et al. (1977), Birr and Frank (1975), and by Machleidt & Machleidt (1977) in their self-constructed solid-phase sequencers. The relative recoveries of the amino acid derivatives were analysed for the manual and automatic conversion. For the liquid phase sequencer an appropriate conversion device was installed (Wittmann-Liebold et al., 1976). The use of both types of sequencers and, in addition, the manual degradation by the Dansyl-Edman degradation was demonstrated for newly sequenced proteins (Reinbolt et al., 1977; Hitz et al, 1977; Wittmann-Liebold et al., 1975, 1977).The highly efficient manual Dansyl-Edman degradation technique had been invented years ago by Gray and Hartley (Gray & Hartley, 1963; Gray, 1967; Hartley, 1970) and for many years was the main manual degradation techniques in many laboratories at that time.

The Uppsala group of Lars Rydén isolated cysteine peptides from proteins by solid-phase techniques based on thiol-disulfide exchange (Carlsson et al., 1977). Other work dealt with

modified amino acids or analysis of mutations (Darbre & Chauhan, 1977; Zabin et al., 1977; Gross, 1977). P.E. Hare, Washington, DC, reported fluorescence detection of peptides at the picomole level. C-terminal attachment via the peptide carboxyl group through activation by various carbodiimides was reported by B. Kassell et al. (1977), by A. Previero and Coletti-Previero (1977) and by G. C. Barrett et al. (1977). B. Keil discussed the use of various enzymes to yield large fragments for sequence determinations (Keil, 1977). Laursen provided a method of enzymatic splitting of the polypeptide chain after attachment to solid-phase (Laursen, 1977b). Further, new resins on the basis of macroporous polystyrene and a new polar gel-phase, respectively, were proposed for solid-phase attachment (Appella et al., 1977; Birr & Garoff, 1977).

In his summary of the conference, Richard Perham, Cambridge, UK, made the statement that DNA-sequencing stood at the horizon: “what impact the development of the astonishingly rapid technique of DNA sequencing will have on protein chemistry” and “where the genetic problems are simpler, sooner or later, perhaps sooner rather than later, the impact could be considerable” (Perham, 1977). Lively discussions and an excursion to one of the famous caves in the area, Grotte Exsurgence du Colombier, organized by Marie-Anne Coletti-Previero, served well to bring the protein chemists together and did much to make this meeting communicative and unforgettable.

MPSA1979, Heidelberg, Germany, October 1-4, 1979, 3rd MPSA Conference.
Organizer: Christian Birr, Max-Planck-Institut für Medizinische Forschung, Heidelberg, Germany.
Proceedings Book: Methods in Peptide and Protein Sequence Analysis. (Proceedings of the 3rd International Conference on Solid Phase Methods in Protein Sequence Analysis), C. Birr (Ed.), Elsevier/N.Holland Biomedical Press, Amsterdam, 1980, 531 pp.

The third meeting was organized by Christian Birr in Heidelberg, and again about 200 young scientists attended. Richard N. Perham gave a lecture in memoriam of J. Ieuan Harris from the MRC laboratory in Cambridge, who also left us much too early. He had obtained experience with the “paper-strip sequencing method” in California with C.H. Li and H. Fraenkel-Conrat (Fraenkel-Conrat et al., 1955). The method degraded peptides by PITC on paper strips in the vapour phase of a beaker or desiccator, a method widely used for peptide sequencing in the early times. In Cambridge, Harris organized courses demonstrating the Dansyl-Edman degradation. The method follows the usual PITC degradation, but at each step an aliquot of the newly-generated test peptide also was removed for dansylation. The dansylated peptide was hydrolyzed, and the N-terminal dansyl-amino acid was extracted and identified by 2D thin-layer chromatography. This method enhanced the sensitivity of peptide sequencing considerably and was the first sensitive manual sequencing method used in early protein sequence analysis.

Elmar Wachter and Robert Wehrhahn employed the cyanogen bromide (CNBr)-cleavage of proteins (Gross & Witkop, 1961) to split the polypeptide chains at methionines, oxidizing the resultant C-terminal homoserine peptides to their lactones and attaching these to glass beads with high coupling yields (of better than 90%). The authors showed nice GC traces of degradation cycles for Trp-lactone attached melittin, Tyr-lactone attached myoglobin peptides and for His-lactone fragments. Erhard Gross showed possibilities for sequencing peptides with alpha-, beta-unsaturated and related amino acids (Gross, 1977). Werner Machleidt and H. Hofer (1981) demonstrated the automatic conversion of PTH-amino acids in their solid-phase sequencer and presented the yields in the low nanomole range of the released amino

acids. The carbodiimide activation of the C-terminal carboxyl group allowed sequencing of arginine peptides and peptides without C-terminal arginine or lysine as summarized (Horn and Bonner, 1980). These authors also employed insoluble carbodiimides as supports for the attachment of these peptides.

With a combination of the new inventions, it became possible to establish the complete primary structure of ribosomal proteins in picomole quantities. Methods employed were i) the technically improved liquid-phase sequencing in combination with the automated flask-like conversion device for the spinning cup sequencer (Wittmann-Liebold et al., 1976), ii) the solid-phase techniques for small peptides as given above, iii) the newly established thin-layer separation of peptides on cellulose sheets derived from small protein quantities (Hitz et al., 1977), and iv) the manually performed DABITC-double coupling method (Chang et al., 1978) by which manual sequencing of peptides recovered from the thin-layer plates became routine. With these methods, 36 out of the 55 *E. coli* ribosomal proteins and/or their mutants were entirely sequenced in the years between 1972 and 1978. Without such enhancements in methodology, these results would not have been feasible!

Many more new protein sequences were demonstrated in Heidelberg, e.g. of yeast aspartyl-tRNA-synthetase (Potier et al., 1980); lactose permease (Beyreuther & Ehring, 1980); fragments of myosin (Elzinga & Trus, 1980); tubulin (Ponstingl et al., 1980), and the adenovirus hexon protein (Jörnvall et al., 1980). Clearly protein analysis and enhanced sequencing techniques had become much more effective in these years. The first “Atlas of Protein Sequence and Structure” was edited by Margaret Dayhoff et al. (1965) at the National Biomedical Research Foundation, Georgetown University Medical Center. By 1978, already about 1,100 protein sequences with approximately 120,000 amino acid residues were collected and annotated.

Dancing through the night and into the early morning by the “active members” of the conference is still well remembered!

MPSA1981, Brookhaven, New York, USA, September 21-25, 1981, 4th MPSA Conference. **Organizer**: Marshall Elzinga, Brookhaven National Laboratory, Upton, NY USA. **Proceedings Book**: Methods in Protein Sequence Analysis. (Proceedings of the 4th International Conference on Methods in Protein Sequence Analysis), M. Elzinga (Ed.), Humana Press, Clifton, NJ, USA, 1982, 589 pp.

The 1981 MPSA-meeting, organized by Marshall Elzinga at the Brookhaven National Laboratory on Long Island, USA, was a conference full of new techniques. Again, a severe loss in the peptide field foreshadowed the meeting, the unexpected tragic death of Erhard Gross. A brief memoriam was given by Christian Birr. Together with B. Witkop, Erhard Gross had invented the cyanogen bromide cleavage of proteins (see above). This chemical cleavage method became highly suitable to generate larger fragments for liquid- and solid-phase sequencing in order to align shorter fragments to the entire polypeptide chain. The method is still widely used for fragment isolation of larger proteins for subsequent sequence analysis. More recently, it was employed to establish domain structures of big proteins and for immunoglobulin verifications.

A new type of microsequencer using chemicals in the gas-phase was introduced by R. M. Hewick and Mike Hunkapiller (Hewick et al., 1981; Hunkapiller et al., 1982; Hunkapiller et al., 1983). It was equipped with a newly designed cartridge as the reaction chamber

(Hunkapiller & Hood, 1980; Hewick et al., 1981); further, it contained new types of dead-volume-free valves introduced for the spinning cup sequencer (Wittmann-Liebold, 1973; Wittmann-Liebold et al., 1976; Wittmann-Liebold and Ashman, 1985). Yet, in the first years this sequencer was not equipped with an on-line conversion device. The sequencer became commercially available through the newly founded company Applied Biosystems, Foster City, CA. It revolutionized protein sequencing by allowing sequencing in the low picomole range. This machine, with some enhancements (e.g. of the detector system), is still routinely used in laboratories; later it was complemented by DNA-sequencers and solid-phase peptide synthesizers from Applied Biosystems.

As Panel Lecturer, Russell Doolittle, La Jolla, CA, gave an anecdotal account of the history of the peptide stepwise degradation procedure (Doolittle, 1982). He told a story about the financial commitment the NIH had to face for purchasing such a sequencer. He stated: “Why not go with one of the elegant manual procedures like DABITC?” But his colleagues absolutely wanted the machine!

Wolf Brandt, Capetown, South Africa, had experienced the non-specific cleavage of peptide bonds during long sequencer degradations, which made long runs cumbersome (Brandt et al., 1982). Ken Walsh, Seattle, WA, discussed new possibilities for protein and DNA sequencing, and mentioned mass spectrometry as a coming “future wave” (Walsh, 1982). Howard Morris, London, UK, gave a talk on protein sequencing by fast atom bombardment mass spectrometry (Eckart, 1976; Morris, 1979; Morris et al, 1982). He also discussed the strategies for extended sequencing as well as for N-terminally blocked proteins, which was a frequently encountered difficult problem in protein structure analysis. Several other papers dealt with mass spectrometric protein sequencing, as presented by Steven Carr and V. N. Reinhold, Boston (1982); Yasutsugu Shimonishi, Osaka (1982); and Klaus Biemann, Cambridge, MA (1982).

John Walker, Cambridge, UK, had started to analyse the *E. coli* ATP-synthase complex (Walker et al., 1982), while the Ovchinnikov laboratory, Moscow, Russia, had begun studies of RNA polymerase (Lipkin et al., 1982). Most impressive at this meeting was the fast adaptation of the new high performance liquid chromatography method (HPLC), using reversed phase columns, for the purification of peptides, proteins and amino acids in the picomole range. An overwhelming number of papers showed the progress possible in protein analysis with this straightforward and fast technique (Bahr-Lindstrom et al., 1982; Bradley et al., 1982; Fullmer & Wassermann, 1982; Heinemann & Ozols, 1982; Henderson et al., 1982; Kratzin et al., 1982; Klapper, 1982; L´Italien & Laursen, 1982; McKean & Bell, 1982; Shively et al.,1982; Takahashi et al., 1982; Taylor et al., 1982; Tripier, 1982; Williams et al., 1982; Wilson et al., 1982;). Immediately it became obvious that any older paper- or thin-layer separation methods for peptide purification could be replaced by the HPLC methodology. However, we all also became aware that protein analysis was no longer a cheap way to investigate polypeptides as it was before. Can the young people today imagine that the investment for a protein laboratory in the fifties and sixties was just a few chromatography setups and simple electrophoresis tanks in addition to some columns and fraction collectors, still without flow-through detectors? The amino acid analyzer and a sequencer then became mandatory for protein centres, but in addition, HPLC techniques in various modes were on the market for separating peptides and proteins and for analysing amino acid derivatives. HPLC began its conquest!

In summarizing the outcome of the conference, B. Wittmann-Liebold spoke of the fantastic new HPLC tools that allowed rapid purification of proteins, their peptides and amino acids. The meeting was happily concluded by Kenneth J. Wilson, Foster City, CA, with his famous

statement: “All - protein isolation, peptide purification, amino acid analysis - could be done in one day”, which impressed me greatly. Purification and sequencing of picomole quantities became a fact at this time. For the first time, Ken Walsh, Seattle, WA, envisioned: “Sequence analysis will be done in the femtomole range”. Although still a dream for us all at that time, indeed he was right. It really became true in a few years!

Remarkable at this conference was the excursion to the “Sunken Forest” at the Fire Island National Seashore of Long Island. Unfortunately, this icon was later damaged by several storms, but this precious natural treasure remains to be enjoyed and explored, perhaps at a future MPSA meeting.

MPSA1984, Cambridge, U. K., July 29 – August 2, 1984, 5th MPSA Conference.
Organizer: John Walker, Medical Research Council, Cambridge, U.K.

The conference took place at beautiful Churchill College, again a splendid site for meeting and discussions. Many lectures dealt with new protein structures derived from gas-phase and solid-phase sequencing. Direct methods for amino acid sequence analysis by radio-sequencing (by Nisse Kalkinnen, Helsinki) and approaches for subpicomole sequencing (Stephen Kent, Pasadena, CA) were presented. D. H. Williams and Howard Morris, London, UK, discussed new mass spectrometric applications for protein structure analysis. At that time and at later conferences, we intensely discussed incorporating the Edman degradation technique directly into the ionic source of the mass spectrometer - a fantastic idea brought up by Howard Morris but of course hard to realize.

Protein isolation was the topic of several talks; large scale preparation (Anthony Atkinson, Salisbury, UK) and the increasing possibilities to employ HPLC also for proteins, which was limited by low recoveries, were discussed (Kenneth J. Wilson, Foster City, CA). Already, sensitive antigen and antibody purification (Neil Barclay, Oxford, UK) and DNA sequencing and synthesis (B. G. Barrell & M. J. Gait, Cambridge, UK) were raised. Protein engineering (Greg P. Winter, Cambridge, UK; Alan R. Fersht, Lensfield, UK; and J.H. Richards, Pasadena, CA) and the design of peptides as immunogens (Christian Birr, Heidelberg) became new topics for the conference. Others included the analysis and role of membrane proteins (John BC Findlay, Leeds, UK; Dieter Oesterhelt, Martinsried; Jörg Rosenbusch, Heidelberg), discussions about hormone action, the structure and function of blood kinases, the human complement system and the structure-function relationship in fribrinogens (Philip Cohen, Dundee, Scotland; Staffan Magnusson, Aarhus; Kenneth BM Reid, Oxford, UK; and Agnes Henschen, Martinsried). In addition, an analysis of multiple subunit systems, including the proton translocating ATPase (J.E. Walker, Cambridge, UK), the pyruvate dehydrogenase complex (Richard N. Perham, Cambridge, UK) and the structure and evolution of ribosomal proteins (Wittmann-Liebold, 1980; and 1981), were presented. Ken Walsh, Seattle, WA, summarized the conference in “Tactics Used in Sequence Analysis of Protein Kinases”.

An excursion was organized to the famous and reconstructed cathedral of Ely, to Wicken Fen, and the beautiful site of the famous Colleges at the river Cam, where the younger attendees enjoyed punting on the river.

MPSA1986, Seattle, WA, USA, August 17-21, 1986, 6th MPSA Conference.
Organizer: Kenneth A. Walsh, University of Washington, Seattle, USA.
Proceedings Book: Methods in Protein Sequence Analysis - 1986 (Proceedings of the 6th

International MPSA Conference, Seattle, WA), Kenneth A. Walsh (Ed.), Humana Press, Clifton, New Jersey, 1987, 658 pp.

Ken Walsh made the 6th MPSA conference a great event. For the first time about 500 participants attended the conference, which now emphasized micro-analytical techniques, the merits and limitations of DNA sequencing and the role of mass spectrometry for protein analysis. Even very distinguished protein chemists, such as Hans Neurath, attended the meeting. He presented the opening lecture and, joined by his wife, led the excursion to Mount Helena; they climbed the mountain as actively as ever. The mountain still was visible as a beautifully snowy full cone before it exploded in an earthquake the following year.

Hans Neurath gave his opening lecture on "New Ways to Look at Old Proteins" in which he stressed our changing views of proteins, the role of gene structure and protein function, their folding and their evolution. He discussed the maturation and lifetime of proteins and the role of newly discovered proteins and protein systems. He spoke "On New Approaches - the Renaissance in Methodology" (Neurath, 1987).

For the first time, DNA sequencers and DNA synthesizers became available through Applied Biosystems. The interface between protein chemistry and molecular biology made rapid progress. Presentations by Leroy Hood, Caltech, Pasadena; Hugh Niall, Genentech, San Francisco; Donald Capra, Dallas, TX; Frank Robey, Bethesda, MD; R. Mattaliano Cambridge, MA) on protein and DNA technology, e.g. automation in DNA-sequencing and recombinant protein production, enhanced the impact of the conference. The growing role of mass spectrometry was detailed by Klaus Biemann, Cambridge, MA, and Peter Roepstorff, Odense, Denmark. Problems, such as the structure analysis of surface receptors, sequence analysis of large proteins and membrane proteins were dealt with in the conference by J. Ramachandran et al., Genentech, San Francisco; Koiti Titani, Seattle, WA, and Yuri Ovchinnikov et al., Moscow.

New microbore HPLC methods in sequence analysis were introduced by Richard Simpson, Melbourne, Australia and Roland Niece, Madison, WI, while submicrogram amino acid analysis techniques were presented by Brian Bidlingmeyer, Waters Chromatography Divion, Milford, MA. Fluorescent Edman-type reagents were described by Johann Salnikow et al., Berlin, and reliable methods for sequencing electroblotted proteins were evaluated by Joel Vandekerckhove, Ghent; Belgium, Terence Kirley, Cincinnati, OH, and Thomas Bergman and Hans Jörnvall, Stockholm (see also Towbin et al., 1979; Masudaira, 1987). New two-dimensional gel techniques in combination with recovery of proteins for analysis were presented by Ruedi Aebersold, Pasadena, CA, and James D. Pearson, Upjohn Company, Kalamazoo, MI. Critical topics, such as analysis of phosphorylated proteins were discussed by Bradford Gibson et al., San Francisco, CA and Yuan Wang et al., Indiapolis, IN, and proteins with other posttranslational modifications, especially the difficult task of glycosylated proteins, described by Steven Carr and G. D. Roberts, Swedeland, PA; and R. Paxton et al., Duarte, CA, were exciting topics.

The question of the secondary and tertiary analysis of protein structure by NMR and X-ray crystal analysis were raised. Rachel E. Klevit, Seattle, and B. Bersch, Grenoble, communicated on secondary and tertiary structure determination of protein in solution by NMR; discussed were the manner of protein isolation, the type of label requested and size of the protein (up to 40 kDa were possible), and which structural and dynamic information would be obtainable. Another aspect of the conference was the comparison of protein structures as an important tool in the field, and identification and interpretation of potential

domains in proteins by L. Hunt and W. Barker et al., Washington, DC, and David Speicher, Philadelphia, PA.

Concluding remarks were provided by Agnes Henschen, Martinsried (Henschen-Edman 1987). She summarized the present protein methodologies and stressed the fact that the alternative methods of DNA-sequencing, the increase in the number of scientists involved in protein structure analysis and many other factors influence the speed of sequence analysis. During the past 30 years, amino acid sequencing had continued to be performed by the Edman sequencing method; in 1960, the structure analysis of haemoglobin, with 2 chains each of about 150 residues, took four years; fibrinogen with some 1500 residues was finished in 1970, and the sequence of the von Willibrand factor with 2050 amino acids was established over 2 years in 1986. Although DNA-sequencing and mass spectrometry would now add much speed and sensitivity to protein analysis, she reminded us that the complete information about protein structure and function could only be achieved by examining the protein itself, a statement that is still valid.

MPSA1988, Berlin, Germany, July 3-8, 1988, 7th MPSA Conference.
Organizer: Brigitte Wittmann-Liebold, Max-Planck-Institut für Molekulare Genetik, Berlin, Germany.
Proceedings Book: Methods in Protein Sequence Analysis (Proceedings of the 7th International Conference, Berlin, July 3-8, 1988), B. Wittmann-Liebold, Johann Salnikow, Volker A. Erdmann (Eds.), Springer-Verlag, Berlin, 1989, 575 pp.
Pehr Edman Award (First): Richard A. Laursen, Boston University, Boston, Massachusetts, USA; for contributions to solid-phase protein sequencing; Sponsor: Milligen Division of the Millipore Corporation; the inauguration of the first award was by Hubert Köster and the laudation to Richard Laursen was presented by Hans Salnikow.

A memoriam lecture for Yuri A. Ovchinnikov, the young director of the famous Shemyakin laboratory in Moscow, who died much too early in the prime of his life and scientific activities, was given by Valery M. Lipkin (MPSA Proceedings, 1989, pp. XXXII-XXXV).

Sessions of the conference were: Advanced Edman Chemistry; New Instrumentation; C-terminal Sequence Analysis and Alternative Techniques to the Stepwise Edman Degradation; Purification of Polypeptides for Microsequence Analysis and Recovery from Gels; A Critical Evaluation of Sensitive Amino Acid Analysis; New Approaches in Mass Spectrometry of Biopoylmers; New Strategies for Protein and Peptide Characterization; Protein Folding and Three-dimensional Structure Elucidation; Recombinant DNA-Technology; Domain Structure Analysis and Interaction of Biomolecules; Immunological Recognition and Strategies; Analysis of Membrane Proteins; Panel and General Discussions. The conference hosted 550 attendants.

The Plenary Lecture was presented by Leroy E. Hood, Pasadena on “How Sequencing of the Human Genome Will Impact Protein Chemistry”. In “Future Views”, he summarized the striking advances in biotechnology including the development of recombinant DNA techniques, the advances in monoclonal antibody methods and the design of novel instrumentation (Hood, 1989). Whereas direct protein sequencing would be required in the future to define the purity of individual proteins and their amino- and carboxy-terminals, partial amino acid sequence analysis would be valuable to verify by DNA sequences. Post-translational modifications should be determined from the proteins for investigating regulation and function. Also, subtle protein changes in complex biological systems would

have to be analysed directly in proteins. This would require a high resolution of complex protein mixtures, as achieved by advanced 2-dimensional gel electrophoresis; of course, optimization of image analysis of the 2-dimensional gels would have to come. These improvements would enable subtractive analysis at the protein level of cells before and after undergoing a particular physiologic transition (the word “Proteomics” was not yet invented!). So far, direct sequence analysis of human proteins at the nanomole level was possible for only about 100 different proteins. It became obvious that sequencing at the low femtomole level would be needed, and many more proteins would need to be examined. Hood stated: “As a consequence of sequencing the human genome, the DNA diagnosis of human genetic diseases will be enormously facilitated by all these new techniques”.

Lectures on quantitative N-terminal analysis of polypeptides, new Edman-type reagents, a new manual gas-phase sequencing method (Wolf Brandt, Cape Town & G. Frank, Zürich), and improvements of the sequencers and the peptide synthesis technique (Richard Laursen et al., Boston; James Coull et al., Millipore, Burlington, MA; Rudi Aebersold, et al., Pasadena, CA) were presented. The use of DABITC, the Chang reagent, and further the use of DNSAPITC, FITC and BAMPITC were evaluated by Jui-Yoa Chang, Basel, Shan-Wei Jin, Shanghai, China, and Hisashi Hirano, Tsukuba City, Japan. Important for the selection of an appropriate Edman-type reagent is the necessity for flexibility in order to ensure a complete coupling reaction; e.g. dansylamino-phenylisothiocyanate is superior to dansyl-phenylisothiocyanate. The most sensitive manual Edman method was developed by Wolf Brandt, Capetown. Richard Laursen et al., Boston, presented a new second generation solid-phase sequencer and DITC-PVDF-membranes for attachment. Ruedi Aebersold et al., Pasadena, CA, accelerated highly sensitive sequencing with the DABITC-double coupling method in combination with an immobilized chemically modified glass-fiber disc for solid-phase sequence analysis when a few picomoles to 500 femtomole of sequenceable peptide material were applied to the sequencer.

A new modular sequencer, to be commercialized by Herbert Knauer GmbH, Berlin and financed by the German Government with a cooperative grant given to Brigitte Wittmann-Liebold and Harald Tschesche, was presented by Stefan Fischer and Frank Reimann (Fischer et al., 1988, Reinmann et al., 1988). Although the technical improvements were advanced and a cross-flow reactor was incorporated for pulsed-liquid-phase micro-sequencing, the sensitivity was not optimised due to the limited HPLC-system incorporated. After my move to the Max-Delbrück-Center (former Academy of Science Institute of the DDR in East-Berlin), an updated version of this machine employing better HPLC columns was in routine service for several years (Herfurth et al., 1991). Improvements to the commercially available Applied Biosystems sequencer were proposed by Geoffrey Begg (Begg, 1980) and by Richard Simpson, Melbourne, Australia, who chose a more reliable injection system allowing 100% of the released PTH-amino acids to be injected. Unfortunately, these improvements were never installed in the commercialized sequencer. Other updates included on-board isocratic HPLC-detection by R. J. Mattaliano et al., Foster City, CA, and a highly improved isocratic separation through application of ionic detergents by Gerhard Frank, Zürich.

“The long search for a viable method of C-terminal sequencing” was summarized by George E. Tarr, Boston, MA, who made the statements: 1) Don´t rely on carboxypeptidases; 2) don´t waste your time with cyanamide degradation; 3) listen to Shively and Inglis on the thiocyanate method, but be skeptical; 4) don´t wait for a new reagent-based method; 5) cheat by using the so-called good methods that incidentally yield C-terminal information; 6) consider physical methods, namely MS, for instance with chemical methods. How right he was! In spite of this, Adam Inglis, Parkville, Victoria, Australia, discussed the Schlack-

Kumpf Thiocyanate Chemistry (Schlack & Kumpf, 1926) and presented 6 and 9 clear degradation steps of leucine-enkephaline and of a synthetic decapeptide; however he used 15 and 30 nanomoles, respectively! Shively applied phosphorylisothiocyanate to yield phosphoanhydrides that released isothiocyanate, which in turn reacted with the activated carboxyl group. An additional prior activation was not necessary.

Alternative proposals to attain C-terminal sequence information were i) purify C-terminal peptides with a labelled amino acid (Christophe Carles & Bruno Ribadeau-Dumas, Jouy-en-Josas, France); ii) use immobilized anhydrotrypsin-agarose as a specific affinity adsorbent for lysine and arginine peptides (Shin-ichi Ishii & Takashi Kumazaki, Hokkaido, Japan); iii) activate the C-terminal carboxyl group with ethyl diethylaminopropyl carbodiimide and attach a labeled dipeptide amide (D.W. Hawke et al., Foster City, CA); iv) reinvestigation of the BNPS-skatole cleavage and C-terminal sequencing of the cleavage products (Wang Yu et al., Shanghai, China).

A critical evaluation of “Sensitive Amino Acid Analysis” was presented by P. E. Hare, Washington, DC; and by Graham Hughes and Séverine Frutiger, Geneva, Switzerland. The FMOC-chloride reaction versus the ninhydrin or phenylthiocarbamyl (PTC) chemistries were discussed by Peter Földi, Leonberg, and by Alan Smith, Davis, CA. Jui-Yoa Chang, Basel, used his DABITC reagent to quantify N-terminal residues of polypeptides. As more and more sensitivity was demanded due to the limited protein amounts available for most studies, the new pre- and post-column derivatization methods employing HPLC-techniques and reactions with fluorescent reagents were discussed with their advantages and disadvantages summarized. Problematic are contaminations, the destruction of labile amino acids and incomplete hydrolysis, which made high sensitivity, quantitative compositional amino acid analysis cumbersome. With low picomole quantities of proteins, amino acid analysis became less favourable than Edman sequencing. In the end, it turned out that with scarce protein amounts, micro-sequencing is superior to amino acid analysis and yielded more information, starting with the same small amount.

Most important was the session on new instrumentation and approaches in mass spectrometry (given by Klaus Biemann, Cambridge, MA; Donald Hunt, Charlottesville, VA; A. Benninghoven, Münster; Peter Roepstorf, Odense, Denmark; and Howard Morris, London). For the first time, Michael Karas and Frank Hillenkamp, Münster, introduced a mass spectrometer for mass analysis of proteins up to masses of >100 kDa by MALDI-mass spectrometry (Karas & Hilenkamp, 1988; Hillenkamp & Karas, 1990; Hillenkamp et al., 1991). Klaus Biemann discussed the fragmentation of small sized proteins by high performance tandem mass spectrometry in combination with computer-aided sequencing; he showed a collision-induced dissociation (CID) sequence spectrum derived from 5 picomoles of a short peptide (Biemann & Scople, 1987; Biemann, 1990). Donald Hunt et al.(1986) discussed protein sequence analysis by tandem quadrupole fourier transform mass spectrometry, obtained from 100 femtomole of a short 11-mer peptide; sequence analysis was by laser photodissociation on 10 picomole of a photoaffinity-labeled peptide methyl ester. Peter Roepstorff presented a strategy of plasma desorption, time-of-flight mass spectrometry in protein sequence determination and *in situ* peptide mapping (Hoejrup et al., 1987). Time course experiments with carboxypeptidase digestion were followed for selection of optimal conditions. Alfred Benninghoven´s talk explained time-of-flight secondary ion mass spectrometry (TOF-SIMS), a technique that allows monitoring of genetic polymorphisms and posttranslational modifications, as verified on apolipoprotein mutants and carbohydrate containing peptides. Howard Morris et al. analysed post-translational modification by high mass, fast atomic bombardment (FAB) mass spectrometry including the characterization of

N-terminally blocked groups. He also studied blocked C-termini, “ragged ends” and glycans by this technique.

Contributions in the session on “New Strategies of Protein and Peptide Purification for Micro-Sequence Analysis” were on HPLC-purification of proteins and peptides and on the recovery of proteins from gels by blotting, presented by Joel Vandekerckhove´s group, Ghent, Belgium, and by Paul Matsudaira, Cambridge, MA. Friedrich Lottspeich et al., Munich; Curt Horvath, New Haven; Jean Rivier, San Diego; Angelo Fontana, Padova; and Stephen Heinemann, San Diego, showed that HPLC methods became more and more advanced; they reported in detail on the various columns and their running conditions. Membrane supports for blotting also found much interest. New strategies for polypeptide characterization in the micro-scale were proposed, e.g. fluorescent labeling by 5-I-AEDANS (Jeff Gorman et al.), by the use of Asp-N-endoproteinase cleavage at aspartic acid residues, and at cysteic acid (Ken Wilson et al., Foster City, PA), by thermolysin hydrolysis (Angelo Fontana et al.), and by post-translational processing enzymes (Robert Mackin et al., San Diego, CA). Gonadotropin hormone-releasing factors (Jean Rivier et al., San Diego, CA) and brain nicotinic acetylcholine receptors and their mutants were analysed in physiological studies (Stephen Heinemann et al., San Diego). The biochemical pathways of N-alpha-acetylation of eukaryotic proteins was studied by Fang-J. Lee et al., Boston, MA.

Among the protein sequence databases, a collaboration between the National Biomedical Research Foundation in Georgetown, the Japanese group and the Heidelberg group was initialized through the CODATA task group (Committee for Data in Science and Technology of the International Council of Scientific Union), and their joint efforts were reported by Hans-Werner Mewes, Heidelberg, by David G. George and Winona C. Baker, Washington, DC; and by Akira Tsugita, Noda, Japan. It became obvious that the increasing production of protein data in different data banks should be coordinated between the three groups: PIR-NBRF (USA), JIPID (Japan), and MIPS (Germany). More data collecting banks were under development, e.g. that of a Biological Activity Database, a Variant Database, an NMR-Database, the Nucleic Acid Database, Genbank and DDBJ, and the International Carbohydrate Databank. In this context, an interesting computer-assisted investigation of cleavage sites in proteins was undertaken by Boris Keil and Nguyen.T. Tong, Paris. A panel Discussion on Protein Data Collection and Protein Evaluations were provided by H.-W. Mewes, Akira Tsugita, Andreas Köpke, B. Keil, and Richard J. Feldmann. The standardizing of the different databases and their software still remained a problem to be tackled in the future.

Further chapters of the conference were on “Protein Folding and 3D Structure Elucidation of Proteins” (Patrick Argos, Heidelberg; Kozo Nagano, Tokyo; Dimitros Tsernoglou, Heidelberg; Ada Yonath, Berlin & Hamburg); and of “Recombinant DNA-Technologies” (Werner Machleidt, München; Dirk Heinz, Basel; Bertrand Castro, Montpellier, France; Georgio Fassina, Bethesda, MA). Interestingly also “Domain Substructure Analysis and Interaction of Biomolecules” was a new topic (Ken Walsh, Seattle, WA; Donald Capra, Dallas, TX; Peter Mitchell, Berlin; Carl-Ivar Bränden, Uppsala, Sweden) and “Immunological Recognition and Strategies” were reviewed by D.E. Rivett, Parkville, Australia, Zouhair Atassi, Houston, TX, Ettore Appella, Bethesda, and Hans J. Fritz, Martinsried. Another difficult task, the “Analysis of Membrane Proteins” was described by Najmoutin G. Abdulaev, Moscow, John B.C. Findlay, Leeds, UK, Jon Gershoni, Rehovot, Israel, and Herwig Ponstingl, Heidelberg.

From the abstracts of the posters, oral presentations were selected as had been done in Seattle at MPSA1986. Also as in Seattle, a huge exhibition, with over 100 companies and publishers involved in protein and DNA biotechnology, accompanied the conference and added to the success of the meeting.

Concluding remarks were given by Mark Hermodson, West Lafayette, IN, on "Protein Chemistry Renascence". It is interesting to read this chapter today. Hermodson stated that the sensitivity of current analyses was approaching a million-fold improvement with a 100-fold greater speed, and that investigations yielded information simply not obtainable by older methods. Amounts in the range of 1 nanomole of pure protein could be applied to proteolytic cleavage and the resulting fragments purified by HPLC for further amino acid and sequence analysis. Many more automatic methods came onto the market, avoiding transfer of the costly peptides from vessel to vessel, which was a hard problem at that time. Again, he discussed the limiting technology of the amino acid analyzers. Even separation by HPLC met its drawbacks. Mass spectrometry started to "become the winner", although its application for studies of the "normal protein chemist seemed still to be difficult". The new tools of nucleic acid chemistry that deduced protein sequences also received appropriate attention. Big problems that also were addressed included "the folding problem", the "crystallisation ability of proteins", and the "assembly and disassembly of multimeric complexes", all of which were unsolved.

An Excursion to Lake Tegel on an old Paddle-Steamer (sponsored by Herbert Knauer GmbH, Berlin) took place on an exceptionally wonderful warm and windless day. Since the conference was held in the downtown area of West-Berlin, a rented subway brought all attendants and guests safely to the lake site.

MPSA1990, Kiruna, Sweden, July 1-6, 1990, 8th MPSA Conference.
Organizer: Hans Jörnvall, Karolinska Institutet, Stockholm, Sweden.
Proceedings Book: Methods in Protein Sequence Analysis, H. Jörnvall, J.-O. Höög, and A. M. Gustavsson (Eds.), Birkhäuser Verlag, Basel, Switzerland, 1991, 396 pp.
Pehr Edman Award: Geoffrey Begg, Ludwig Institute for Cancer Research and Brigitte Wittmann-Liebold, Max-Planck-Institute of Molecular Genetics, for pioneering contributions to the methodology of protein sequencing analysis. Sponsor: Milligen/Biosearch Division of the Millipore Corporation.

Unfortunately Hans Jörnvall, the main organizer, could not attend, but the conference was well sustained by his collaborators, who really made the arrangements perfect. We experienced not only the Northern Swedish scenery at the midsummer with the long days light, but also a sound and well adapted program. Parties on a mountain peak near Kiruna were flourished with reindeer bacon, good wine, clouds of flying insects and rain suddenly appearing at the scene. But a bag-pipe band and good beer made all of us happy.

We mourned the loss of Gerhard Braunitzer, Heinz-Günter Wittmann and Takashi Murachi all of whom died much too early after a long time engaged in research. Shortly after the congress, Staffan Magnussson, Aarhus, who was still lively while attending the conference, suddenly passed away. Hans Jörnvall gave a summary on the scientific carriers of these scientists in the Proceedings Book.

Central topics at the conference were "Sequencer Methodology and Instrumentation", "Sample Preparation and Analysis", "Elucidation of Modified Residues and Examples of

Structural-Functional Relationships", and "LC/MS and LC/MS/MS as Applied to Screening for Sites of Post-translational Modifications".

In the first part, mainly C-terminal sequence technologies, primarily the chemistry of Schlack and Kumpf (1926), was applied for modern sequencing by Adam Inglis, Victoria, Australia. Inglis had spent a sabbatical in our group at Berlin and had participated in construction of a new C-terminal sequencer. An automated C-terminal degradation method, similar to the Edman sequencer, was requested and would be advantageous. An interesting new degradation scheme was presented by David Hawke and Victoria Boyd (Boyd et al., 1992), both at Applied Biosystems, Foster City, CA; this technique finally made the C-terminal chemistry more suitable for automation.

Richard Laursen, Boston, developed a capillary column packed with DITC-glass beads for directly attaching proteins from gels for solid-phase sequencing. Further, a cross-flow reactor was constructed for automated sequencing by Harald Tschesch's group, Bielefeld, which was applied to micro-sequence analysis in the Knauer sequencer. Another presentation dealt with the introduction of capillary electrophoresis for separation sciences (T. Large, Warrington, UK).

John Shively, Duarte, CA, addressed the structural analysis of membrane proteins, such as microsomal cytochrome P-450 proteins and highly glycosylated plasma membrane-anchored glycoproteins. He applied the proteins to a PVDF strip and incorporated this into a continuous flow reactor. Blotting procedures from gels to PVDF were investigated (Paul Matsudaira, Cambridge, MA), and, for the first time, a "proteomics study" (the name "proteomics" was not yet invented) was described by Joel Vandekerckhove and his group, Ghent, Belgium. Vandekerckhove established an integrated human protein database of [^{35}S]-methionine-labeled proteins from a human amnion cell line that had been separated by IEF and NEPHGE gel electrophoresis.

Critical comparisons were made on an analytical competition of various amino acid analysis systems in different laboratories; forty-three core facilities provided an evaluation of individual laboratory performance. The question was raised as to possible realistic expectations (Lowell H. Ericsson et al., Seattle, WA). The best performing laboratories (33%) reached an accuracy of about 87-93% with a 2.5-5.5% standard deviation, depending on the amounts employed. It is interesting to note that in 1991, state-of-the-art amino acid analysis and sequencing as evaluated in 112 protein chemistry laboratories (R. Niece et al., Madison, WA) requested about 100 picomoles of protein for determining the first 15 or more residues but wanted 4 micrograms for a triplicate hydrolysis and amino acid analysis.

John Findlay, Leeds, UK, gave suggestions about modelling integral membrane proteins. Several papers considered structural-functional implications: Henry Weiner et al., Lafayette, IN, on acetylated mitochondrial aldehyde dehydrogenase; Herwig Ponstingl et al., Heidelberg, on ligand binding sites in polypeptides by photoaffinity-labeling with aryl azides; Tsezi Egorov, Moscow, on thiol proteins; Ettore Appella et al., Bethesda, MD, on zinc finger structures in MHC class I proteins; Jan Johansson et al., Stockholm, Sweden, on hydrophobic surfactant proteins; Robert S. Fuller et al., Stanford, CA, on subtilisin-related serine proteases; Alexander Wlodawer et al., Frederick, MD, on inhibitor complexes of HIV-1 protease; Richard Perham et al., Cambridge, UK, on lipoyl domains in multienzyme complexes; László Polgár, Budapest, Hungary, on the protease specifity of other than the commonly used proteases for protein structure analysis; Gunnar von Heijne, Huddinge, Sweden, on cleavage sites in protein targeting signals; Béatrice S.Vallee and Douglas S. Auld, Boston, MA, on zinc chemistry in the function and structure of zinc proteins; M.

Mattras et al., Montpellier, France, studied a dimeric aspartic acid protease from a single domain of pepsin.

The new area of analysis by mass spectrometry was discussed by Akira Tsugita and M. Kamo, Noda, Japan, who increased the sensitivity to femtomole sequencing by derivatizing the anilino-5-thiazolinone (ATZ)-amino acids from Edman degradations by reaction with 4-aminofluorescein. The released phenylthiocarbamyl-aminofluorescein amino acids were identified by HPLC and FAB mass spectrometry. Tandem mass spectrometry in combination with micro-capillary HPLC was used to establish modified amino acid sites (Thomas Covey et al., Thornhill, Canada; Werner Schröder and Ferdinand Hucho, Berlin, Germany). Partial peptide sequence determinations were investigated in combination with capillary electrophoresis (CE) or micro-capillary HPLC (Donald Hunt et al., Charlottesville, VA). Two papers on plasma desorption mass spectrometry for modified forms of recombinant proteins (M. Hartmanis et al., Stockholm, Sweden) and for monitoring phosphorylation reactions (A.G. Craig et al., Uppsala, Sweden) were given (see also Jensen et al., 1991).

Secondary structure predictions and databank strategies were reported by Gerald D. Fasman, Waltham, MA (Chou & Fasman, 1978), and Lois T. Hunt, Washington, DC. The mechanism of protein folding was addressed by Rainer Jaenicke, Regensburg. Pattern sequence variations in homologous proteins were described by Tom Blundell et al., London, UK.

MPSA1992, Otsu, Japan, September 20-24, 1992, 9th MPSA Conference.
Organizer: Kazutomo Imahori, Mitsubishi Kasei Institute of Life Sciences Tokyo and Fumio Sakiyama, Osaka University.
Abstracts and Short Communications: J. Protein Chem. 11 (1992) 347-432; Special Issue.
Proceedings Book: Methods in Protein Sequence Analysis, (Proceedings of the 9th International Conference on Methods in Protein Sequence Analysis), K. Imahori and F. Sakiyama (Eds.), Plenum Press, New York, 1993, p. 310.
Pehr Edman Award: Donald Hunt, University of Virginia, and Klaus Biemann, Department of Chemistry, MIT, MA, for developments in mass spectrometry. Sponsor: Millipore Corporation.

For the first time, a location in the beautiful Lake District of Otsu near Kyoto, Japan, was selected for the MPSA conference, where we enjoyed a nice conference and a big boat party on the lake.

Micro-preparation and micro-sequencing by the use of liquid chromatography (Moritz & Simpson, 1992; Bergman, 1992) and sequence analysis of protein spots from 2-dimensional gels collected on a concentrating gel (Joel Vandekerckhove et al., Ghent) now became almost routine. Gas-phase protein sequencing by fluorescein isothiocyanate (Koji Muramoto et al., Iwate Prefecture, Japan) and by thiobenzoylation (Mark Stolowith et al., Pasadena, CA) were proposed. Practical deblocking procedures of proteins suitable for microsequence analysis were designed by Susumu Tsunasawa, Osaka, and Hisashi Hirano, Tsukuba City, Ibaraki, Japan.

Again, C-terminal sequencing was discussed by Jerome Bailey et al., Duarte, CA, and Adam Inglis and De Luca, Padua, Italy). This time a novel method using partial degradation of the peptide by acid vapours (HFBA, and PFPA) and analysis of the released fragments by FAB-MS were reported by Akira Tsugita et al., Noda, Japan (Tsugita et al., 1992).

A method of *in situ* cleavage of blotted proteins with cyanogen bromide and subsequent electro-elution directly on a new separation gel, described by Gerhard Frank et al., Zürich, resulted in an easy manual separation procedure.

Disulfide-bond determinations, a very tedious process in the past, now became much easier in combination with mass analysis (Johann Schaller et al., Bern, Switzerland). Several papers dealt with the isolation of novel enzymes applied to structure and functional analyses and use in peptide generation (e.g. Shin-ichi Ishii et al., Shiga, Japan; Shigemi Norioka and Fumio Sakiyama, Osaka, Japan; and Sadaaki Iwanaga & Hiroyuki Takeya, Fukuoka, Japan).

The two Edman Awardees, Klaus Biemann and Donald Hunt, gave impressive data on the state-of-the-art structure analysis of peptides and proteins by mass spectrometry employing FAB fragmentation and CID (collision-induces dissociation) in combination with microcapillary HPLC attached directly to the mass spectrometer. The first example of high precision mass spectrometry by use of an ion trap was shown (Graham Cooks et al., Purdue, West Lafayette, IN).

An impressive list of observed translational modifications in proteins was compiled by Radha Krishna and Finn Wold, Houston, TX, with appropriate protein references included (Wold, 1981; Krishna & Wold, 1993). Since this first list, the number of known modifications increased greatly year by year and only by mass spectrometric identification is it feasible to determine the precise location where these modifications specifically occur in the polypeptide chain. Several papers dealt with such modifications and their functional implications (Toshifumi Takao & Yasutsugu Shimonishi, Osaka, Japan (Takao et al., 1990; Fukada et al, 1990); Peter Roepstorff and Peter Hoejrup, Odense, Denmark (Jensen et al., 1991); Fred Sherman et al., Rochester, NY; Virginie Redeker et al., Gif-sur-Yvette, France; Hiroh Ikezawa, Nagoya, Japan; Sumihiro Hase, Osaka, Japan; and Tsuneko Ushida et al., Tokyo, Japan). The papers showed high quality measurements important for identification of modified polypeptides and the assignment of their functional role in biological systems.

Jui-Yoa Chang, Basel, discussed how well-populated intermediates represent the pathway of protein folding, and he investigated methods for obtaining reliable folded intermediates. Prediction of protein structure and fold from multiple sequence alignments became increasingly important with the great increase in protein sequences now available; approaches to characterize protein families with a common fold were undertaken (Geoffrey Barton et al., Oxford, UK; Mark Johnson & Thomas Blundell, London, UK). Domain structure and the modular structure of proteins (Mitiko Go et al., Fukuoka, Japan) and homology searches for prediction of biological functions (Hiroyuki Toh, Furuedai, Japan) began to open up new chapters in protein structure and evolution. Russell Doolittle, San Diego, CA, stressed the importance of sequence comparison for evolutionary studies. Other papers addressed structure-function relationships within individual protein families, the nature of the natriuretic peptide family (Hisayuki Matsuo, Osaka, Japan), the role of activin-binding proteins and activin receptors (Hiromu Sugino et al., Toyoake, Japan), the pattern of protein evolution of alcohol dehydrogenases (Hans Jörnvall et al., Stockholm), the notification of a new family of protein domains (Richard Perham et al., Cambridge, UK), and the topography and molecular organization of protein-RNA complexes in ribosomes (Brigitte Wittmann-Liebold, Berlin).

MPSA1994, Snowbird, Utah, USA, September 8-13, 1994, 10th MPSA Conference.
Organizer: M. Zouhair Atassi, Houston, Texas USA.
Abstracts: J. Protein Chem. 13 (1994) 433-545; Special Issue: Abstracts and Short Communications.
Proceedings Book: Methods in Protein Structure Analysis (Proceedings of the 10th

International MPSA Conference, Snowbird, Utah), M. Z. Atassi and E. Appella (eds.), Plenum Press, New York, 1995, 534 pp.
Pehr Edman Award: Ruedi H. Aebersold, University of Washington, Seattle, WA, USA and Joël Vandekerckhove, University of Ghent, Belgium; for contributions to the sequencing of samples after electrophoretic separations. Sponsor: Millipore Corporation.

Snowbird is a sport-ski resort in the high mountains (ca. 1800 m) outside of Salt Lake City, UT; with beautiful views all around, my collaborators and I used our early arrival to go to the peak. My doctoral students, for the first time in America, immediately went up by cable car and then rushed down hill on a steep and slippery pathway on mountain bikes at a high rate of speed. This they repeated several times. I almost lost my nerves expecting broken bones. Fortunately, they came to the conference without much damage. We also had a nice walk through Salt Lake City and found it a well kept and nicely preserved town for living. The Town Hall, one of the finest examples of "Art Nouveau", was especially marvellous.

The first part of the program dealt with urgently needed peptide and protein micro-preparation. These talks were followed by N-terminal and C-terminal sequencing techniques. Ruedi Aebersold addressed the analysis of proteins from regulated biological systems in differentiation, development, and signal transduction pathways. He stated that the global approach to tackling these problems must include temporal and spatial interactions of all elements involved and should be accompanied by annotation of all available data in the various databases (indeed an excellent preview of "Proteomics"!). He described a protein chemistry workstation consisting of a 2-dimensional gel apparatus, micro-HPLC, and LC/CE-ESI-MS with simultaneous split for UV-detection and fraction collection followed by computer searching to gain fast and reliable sets of data. He proposed his famous 311 Edman reagent, which was considered the best of several novel Edman-type reagents (see the article by Edward Bures and Darryl Pappin, Herts, UK). Analysis might be done in subpicomole quantities by ESI-MS. However, use of this reagent had limitations due to the expense of the LC-ESI-MS technique and to solubility problems during the degradation. Much later, Michael Karas and Christian Wurzel tried the 311 reagent for use in the so-called "Chip-Sequencer" by combining step-wise degradation with MALDI-MS identification. This more recent approach was hampered by a loss in sensitivity when the degraded 311-PTH-amino acid fractions were spotted onto the target, and by problems with yield quantification and with solubility of the reagent in the machine. At the Snowbird conference, the application of "mass fingerprinting" (Pappin et al., 1993; Mann et al., 1993) was spread for every researcher's use.

Joel Vandekerckhove, Gent, Belgium, described a protein-in-gel-concentration device and a procedure starting with about 50 picomoles protein to enable low-abundant protein analysis. He used an agarose gel as a holding matrix for *in situ* protein cleavage. His group and that of Robert Moritz, Melbourne, Australia, used narrow-bore and high speed RP-HPLC for high sensitivity, micro-sequence analysis. A comparison of non-porous stationary HPLC-column packings, e.g. pores of 300 Å material (Unger et al.,1986) versus macro-porous packings with very large pore diameters of >8000 Å made from divinylbenzene cross-linked polystyrene (PS-DVB) or soft agarose gel encased in a PS-DVB spherical lattice (Boschetti, 1994), was investigated, and the stability, binding capacities and mass transfer kinetics were analyzed.

High sensitivity analysis of PTH-amino acids employing a miniaturized automated protein sequencer and a capillary electrophoresis device with laser-based photothermal detection for minute sample amounts was proposed as a new innovation for fast and simple sequence analysis by Karen Waldron and Norman Dovichi, Edmonton, Alberta (Waldron & Dovichi, 1992). The authors claimed that this PTH-aa identification would be simpler than HPLC-gradient elution, would not require re-equilibration after the run and would allow sub-femtomole detection of the released PTH-amino acids. Unfortunately, the machine was never

commercialized. Anthony Pisano, who was on sabbatical leave in Sydney, Australia, described a nice on-line HPLC identification of glycosylated PTH-amino acids by applying 5 mM TEAF-buffer at pH 4.0. This buffer was compatible with the common PTH-amino acid separation since the PTH-glycosylated amino acids eluted prior to the common ones and the volatile buffer was suitable for ion-spray MS-analysis. A paper by Alyan Rao Anumula, SmithKline Beecham, Pennsylvania, discussed a novel fluorescent method for quantification of monosaccharides and sialic acids in glycoproteins.

The group of Akira Tsugita presented peptide C-terminal analysis by MS; another approach achieved C-terminal sequence data by MS detection after proteolytic treatment with carboxypeptidases, such as Case Y, and monitored at various stages of the reaction (Scott Buckel et al., Ann Arbor, MI). The group of Victoria Boyd, Foster City, had enhanced C-terminal sequencing by introducing a “begin cycle” (Boyd et al., 1992). Now they were testing several thiocyanates for automatic C-terminal sequencing, e.g. ammonium thiocyanate and piperidine thiocyanate, and the introduction of an initial activation step with acetic anhydride, tetramethylchlorouronium chloride, or diphenyl chlorophosphate under basic conditions. They evaluated difficulties encoutered with e.g. Glu, Asp, Ser, Thr, and Tyr. They showed that a few steps of C-terminal degradation were possible starting with one nanomole of protein. The group of Chad Miller et al., Hewlett Pachard, Palo Alto, CA, showed several steps by degradation with diphenyl phophoro-isothiocyanatidate (DPPITC) in a Hewlett-Packard C-terminal sequencer.

Keith Ashman, Heidelberg, demonstrated a practical method for pre-electrophoretic labeling of proteins with S-DABITC (4-N,N-dimethylaminobenzene-4´-isothiocyanate-2´-sulphonic acid), a water soluble reagent that omits fixing and staining of the gel and also allows rapid elution of protein from the gel. Of course, as with all later attempts to prelabel proteins prior to the gel electrophoretic separation, he noticed slight minor shifts to higher molecular masses during gel migration.

A great part of the conference dealt with new and diverse strategies for protein and peptide characterization: disulfide bond determination in the human complement C9 (Stephan Lengweiler et al., Bern); in vivo phosphorylation sites in multiphosphorylated proteins (Esben Sorensen et al., Odense, Denmark); characterization of functional amino acid residues (José Bubis et al., Caracas, Venezuela); X-ray photoelectron spectroscopy of polypeptides and carbohydrates (Kenneth Dombrowski et al., Amarillo, TX.); Asp/Asn damage in aging proteins (Jonathan Lindquist & Philip McFadden, Corvallis, Oregon); identification of ribosomal peptides cross-linked to rRNA (Henning Urlaub et al., Berlin); protein-protein interaction studies in mitochondrial steroid hydroxylase systems (Rita Bernhardt et al., Berlin); and modifications in chromatin structure (Subhendra Mattagajasingh and Hara Misra, Blackburg, VA).

Another symposium was concerned with “Immunological Recognition, Phage and Synthetic Libraries”. The following presentations were given: regions of toxin poisoning (Zouhair Atassi and Behzod Dolimbek, Houston, TX); studies on the structure of oxidized low density lipoproteins (Chao-Yuh Yang et al., Houston, TX); synthetic combinatorial libraries (Michal Lebl et al., Tucson, AR); DNA-protein and protein-protein interactions in filamentous bacteriophage assembly (Richard Perham et al., Cambridge, UK). A third session, which cannot be reviewed here in detail (see Proceedings Book), was dedicated to the analysis of protein structures of special interest.

The last part of the conference was dedicated to database analysis, theoretical studies of protein folding and three-dimensional protein structures (Bowie et al., 1991). This topic was discussed by Harold Scheraga et al., Ithaca, NY, and by J.B.C. Findlay et al., Leeds, UK.

Winona Barker et al., Washington, DC and Friedhelm Pfeiffer, Martinsried, discussed superfamilies and domain structures, and the organization of data for molecular evolution studies. David Goldenberg et al., Salt Lake City, Utah, investigated the bovine pancreatic inhibitor (BPTI) folding pathway by mutational analysis. Marius Clore & Angela Gronenborn, Bethesda, MA, employed three- and four-dimensional heteronuclear NMR to investigate proteins in the range of 15-30 kDa and explained this technology. I.A. Vakser and Nikiforovich, St. Louis, MI, presented an instrument for practical low-resolution docking studies as a first, preliminary stage to evaluate ligand binding. Hugh Nicholas et al., Pittsburgh, PA, developed an algorithm for determining distances between sequence alignments. This allowed investigation of different alignments simultaneously as a useful tool without the need to define a standard sequence.

Since it is not possible to deal with all the highly interesting papers and projects in detail, the reader is advised to read the Proceedings Book carefully, since it documents the enormous output of novel approaches, structures and biological knowledge that had advanced so much since the beginning of the MPSA conference series.

MPSA1996, Annecy, France, September 1-5, 1996, 11th MPSA Conference.
Organizers: Michel van der Rest, Director, Institute de Biologie Structurale, Grenoble, France, and Joël Vandekerckhove, University of Ghent, Belgium.
Short communications and abstracts: Vandekerckhove and J. and M. van der Rest (Eds.), J. Protein Chem. 16 (5): 321-564, 1997; Special issue.
Pehr Edman Award: Winona Barker, National Biomedical Research Foundation, Washington, DC, for the development of a comprehensive protein classification database, and Jerker Porath, University of Uppsala, for the development of affinity chromatographic methods. Sponsor: Millipore Corporation.

The Edman Award to Winona Barker demonstrated the importance and thorough effort of the work by her and her team throughout the years developing the Protein Information Resource (PIR) Protein Sequence Database. W. Baker continued the work initiated by Margaret O. Dayhoff who, under many difficulties and especially financial debates, began collecting in the 1960s as many as possible of the known protein and nucleic acid sequences. Dayhoff initiated the “Atlas of Protein Sequence and Structure” in 1965, the collection of all known protein and nucleic acid sequences, under the sponsorship of the National Biomedical Research Foundation at Georgetown University Medical Center, Washington, DC. Collected were not only the known sequences and their modifications, but also comparisons of the various sequenced structures and their evolutionary relationships. For us, the young protein chemists, these Collections, Tables and Supplements were like the Bible. I kept the different volumes, mainly the Presentation Copy of 1969 and 1972, with great enthusiasm. The 1972 Atlas, Vol. 5, was dedicated “To those who would know the biochemical structure, function and origin of man and would strive to improve his lot”. Unfortunately Margaret Dayhoff died much too early, and we mourned the tragic loss. Winona Baker, who was among the scientific staff at the PIR, took over the challenging and difficult task of data collection. Often, it was not clear whether it would be possible for these valuable data collections to be continued. Without Winona Baker’s tremendous efforts and her steady input and that of her co-workers over the years, the collection of all these data in a computerized manner accessible to everyone in the world would not have been possible (for more details see Baker & Hunt, 1997).

Also honoured by receipt of the Edman prize was Jerker Porath, well known in the protein world since the early 1950s for his outstanding discoveries of the starch and cross-linked

dextran materials for protein adsorption and purification (see this Proceedings, pp. 463-468). Through development of cellulose-based ion exchangers, early precursors of Mono Q- and Mono S-type materials were introduced, and the enormous variety in media for column chromatography - gel filtration, size exclusion, ion exchange and hydrophobic interaction - was initiated. With much engagement, Jerker Porath persuaded Pharmacia to commercialize the cross-linked dextran and agarose materials, which was finally done and supplied as "Sephadex" and "Sephasose", respectively. The thorough study of the physico-chemical parameters of the various materials and their variations using ligands and elutions under different salt concentrations, the incremental improvements led to the final bio-affinity chromatography media used today.

Mass spectrometric characterization of peptides and proteins covered large sections of the conference. Technical developments were demonstrated by the combination of capillary liquid chromatography and micro-electrospray ion trap mass spectrometry (Axel Ducret et al.. Seattle, WA). Andrej Shevchenco, Matthias Wilm and Matthias Mann, Heidelberg, discussed the possibility of obtaining sequence information from gel-separated proteins by mass spectrometry at levels too low for conventional approaches (Mann & Wilm, 1995). With these sequence data, it became possible to clone eight proteins based on this method. The results clearly showed mass spectrometry to be an excellent tool for database identification and de-novo sequencing of gel-separated proteins in the low picomole range.

Howard Morris et al., London, UK, designed a novel geometry mass spectrometer, the Q-TOF, for the use in the femtomole/attomole range. This instrument drew wide attention in the protein chemist community, and soon many laboratories obtained this type of mass spectrometer, first commercialized by Fisons/Micromass in Manchester, England. This instrument gained sequence information of the peptides though collision activated decomposition electrospray mass spectrometry (CAD ES-MS/MS) using as a detector the time-of-flight (Q-TOF) principle instead of triple-quadrupole MS/MS. The benefits of this novel instrument were:

- good signal to noise ratios in the MS/MS spectra corresponding to low femtomole/attomole sample consumption.
- Daughter-ion mass (FWHM).
- Easy charge state differentiation.
- Simple low-energy MS/MS fragmentation.
- Definitive unambiguous sequence assignment.

John Yates, III, et al., Seattle, WA, developed searching algorithms for identifying proteins derived from mixtures of peptides in mass spectra after micro-column liquid chromatography and automated tandem mass spectrometry in conjunction with protein and nucleotide database searches; this assisted the rapid characterization of proteins by mass spectrometry (Yates et al., 1995; 1998).

Improvements in peptide micro-preparation and the generation of peptides, an important bottleneck in protein characterization, was overcome by the development of a new digestion robot (Houthaeve et al., 1995), commercially available through ABIMED GmbH, Heidelberg.

The determination of phosphorylation sites in peptides and proteins again was widely discussed. For identification of the phosphorylation sites in ^{32}P-labeled HPLC-purified peptides, Wolfgang Fischer et al., La Jolla, CA employed a volatile Edman reagent, trifluoroethyl isothiocyanate, in combination with volatile buffers to perform manual Edman degradation in subpicomole quantities. The successively shortened peptide was applied to a thin-layer plate and subjected to electrophoresis at pH 1.9 until the phosporyl-amino acid was

detected by migration of the negatively charged phosphate released from the unstable azathiazoline to the anode. Sites of phosphorylation in homo- and heterodimeric proteins were further tested in adaptor proteins that modulate interactions between components of the signal transduction pathways (Thierry Dubois et al., London, UK).

Kris Gevaert et al., Ghent, Belgium, reported the identification and characterization of proteins from 1-dimensional and 2-dimensional gels in femtomole amounts by analysis with MALDI-TOF-MS. Since analysis of low protein amounts yield only a low number of informative peptides, correct assignment is often difficult; therefore, they used additional information from PSD (post source decay) spectra and read out of $^{18}O/^{16}O$-labelled fragmentation ions in the PSD-spectra. Those peptides not clearly identified from the complex PSD spectra were characterized by partial ^{18}O-labelling of the C-terminal carboxyl group during enzymatic hydrolysis in D_2O/water utilizing the computer algorithm MassFrag.

Christoph Eckerskorn et al., Martinsried, presented scanning IR-MALDI mass spectrometric data on peptides obtained by micro-LC with on-line membrane blotting (Strupat et al., 1994). Accurate mass measurements of a few parts per million (ppm), using MALDI-TOF with delayed extraction (DE) and a reflecting analyzer, were reported for peptides in the mass range of 900 to 3700 Da (Edward Takach et al., PerSeptive Biosystems, Framingham, MA). Due to the accurate measurements, the authors could distinguish Lys from Gln in peptides and determine the elementary composition of small molecules in the mass range of 100-500.

Another method to discriminate between these two amino acids during C-terminal sequence analysis by MALDI-MS was accomplished by converting Lys to homoarginine by guanidination (Valentina Bonetto et al., Stockholm, Sweden). They performed C-terminal sequence determination of modified peptides by treatment with carboxypeptidases Y and P in combination with MALDI-MS. Henning Urlaub et al., Berlin, proved amino acid-nucleotide-contact sites on the molecular level in ribosomes of cross-linked peptide-oligonucleotide complexes by MALDI-MS after appropriate fragmentation of the complex (Urlaub et al., 1995).

Several papers covered assignments of post-translational modification by LC-ES-MS/MS or MALDI-MS, respectively, e.g. in carboxy-terminal mammalian sperm tubulin variants (Uwe Plessmann and Klaus Weber, Göttingen), on protein glycosylation and phosphorylation sites (Roland Annan and Steven Carr, SmithKline Beecham Pharmaceuticals, King of Prussia, PE), and on the heterogeneous axonemal tubulin C-terminal tail in sea urchin spermatozoa (Jean Mary et al., Paris, France). Pulsed-FAB mass spectrometry was applied by Toshifumi Takao et al., Osaka, Japan, to improve the sensitivity of tandem MS/MS data by a factor of ten-fold in a four sector instrument upon high-energy collision induced dissociation for investigation of histone fractions obtained from starfish testes.

The combination of micro-preparative capillary electrophoresis and MALDI-MS was examined by Ann-Charlotte Bergman and Tomas Bergman, Stockholm, Sweden, to overcome difficulties concerning an efficient electrophoresis electrolyte in combination with laser desorption /ionization. Superior over phosphate buffer, which suppresses the MS-signal, was citrate, TFA or hydrochloric buffer as electrolyte. Stable, capillary, reversed-phase polyimide fused-silica columns (< 320 µm ID) were produced for low-picomole amounts of peptides for on-line CC-ES-MS by D. Tong from Richard Simpson's group (Parkville, Australia) performing rapid chromatography with flow rates of 0.4 to 40 µl/min and loading capacities of up to 500 µl. A detailed description of the construction of the capillary columns was given. Information on complex protein structures and protein interactions was also determined by mass spectrometry to address the non-static mode of interactions in time course experiments, e.g. by analyzing the deuterium exchange in the mass spectra (Robert Anderegg et al.,

GlaxoWellcome, North Carolina). Similarly, the influence of mutations on the stability of ferridoxin by hydrogen/deuterium exchange in mass spectrometry was examined by Hervé Rémigy et al., Grenoble, France.

Usually difficulties are encountered when proteins in scarce quantities must be recovered from gels or proteins must be renatured in gels. Both questions were addressed by Lila Castellanos-Serra et al., Habana, Cuba, who made use of imidazole-SDS-Zinc detection (reverse staining) and recovery of the proteins from the gel slurry in a solution of Zinc-complexing agent, a method worth noticing. Rice gene screening from a cDNA library was enabled by a compilation of sequence data of proteins derived from a complex 2-dimensional gel pattern (Hisashi Hirano, Yokohama, Japan). Ian Humphery-Smith, Eveleigh, Australia, and Walter Blackstock, GlaxoWellcome, Stevenage, UK, summarized the large input of proteomics data when predicted gene products are translated, and they opened to view the difference between the “static genome” versus the “dynamic proteome” of cells. They noted that direct proteome analysis can detect the relative levels of translated gene-products, the degree of post-translational modifications, and their nature. Years later, this view became common to all researchers in life sciences. Hedvig Hegyi and Peer Bork, Heidelberg, summarized their efforts to classify the functional units (modules) of many proteins. To trace the evolution of modules, they developed a sequence and module alerting system to check new sequence data.

The last part of the conference shifted to 3D-structural problems. In vivo folding studies with detection of 2-4 msec transient intermediates were investigated by Alain Chaffotte et al., Paris, France, and the thermodynamic properties of the pre-molten globule characterized on the F2-fragment of tryptophan synthase was described. The environment of protein residues within protein structures was classified by investigating the solvent-accessible molecular surface area (David Deerfield II. et al., Pittsburgh, PE). Protein homology modeling with a dead-end elimination algorithm was investigated to assign the global minimum-energy conformation and to identify sequences compatible with a given scaffold structure (Ignace Lasters et al., Leuven, Belgium). Bengt Persson, Stockholm, and Patrick Argos, Heidelberg, developed an algorithm to predict membrane topology (intra- and extra-cellular sides). The ratio was calculated for the various amino acids that were extracellular or intracellular in 42 protein families. Residues mostly found to be intracellular were Ala, Arg, Cys and Lys, while those on the extracellular side were Asn, Asp, Gly, Phe, Pro, Trp, Tyr and Val. Good results with this new algorithm were achieved with 12 membrane protein test families that differed from the original test set. An electronic prediction service was offered.

For the first time in this conference series, the phage display techniques for fast, sensitive and systematic protein-protein interaction studies was presented (Stefaan Rossenu et al., Ghent, Belgium), a system originally used to create large libraries of antibodies for selection purposes (Smith, 1985). Now it was used in combination with PCR saturation mutagenesis to present peptides at the surface of phages in order to identify high-affinity interactions with ligands. A screening of diverse libraries of small molecules created by combinatorial synthetic methods was employed by Surinder Kaur et al., Emeryville, CA, to identify lead compounds involving affinity selection and mass spectrometry.

At Annecy, we went through the old town, admired the narrow streets and houses from the middle-ages in this beautiful city and had a nice boat party on the lake. I still remember the great dancing on the upper deck!

MPSA1998, Halkidiki, Greece, September 5-10, 1998, 12th MPSA Conference.
Organizers: D. A. Kyriakidis and Theadora Choli-Papadopoulou, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Short communications and abstracts: Choli-Papadopoulou, Y. and D. A. Kyriakidis (Eds.) J. Protein Chem. 17: 507-577, 1998; Special issue.
Proceedings Book: Proteome and Protein Analysis (Selected papers from MPSA 1998), Kamp, R.M., Kyriakidis, D., Choli-Papadopoulou, T. (Eds.), Springer-Verlag, Heidelberg, 2000, pp. 1-372.
Pehr Edman Award: Mathias Wilm, European Molecular Biology Laboratory, Mathias Mann, University of Southern Denmark, and John Yates, The Scripps Research Institute, for contributions to the identification of proteins and protein complexes by mass spectrometry.
Sponsor: Millipore Corporation.

The conference mourned the tragic loss of Diane and Don Sheer, whose plane crashed off the coast of Nova Scotia on their way to the conference. This came as a shock to us; many attendees knew both very well from the former MPSA exhibitions of the Analytical Division of Millipore Corp.

Advances in the methodologies of protein structural analysis were discussed mainly in the light of the determinations of modifications and in respect to structural and functional evaluations. Among the topics discussed, of course, were mass spectrometric applications to biological systems in proteomic studies.

The term “Proteome” had been invented two years previously by Wilkins et al. (1996) to describe the specific protein complement expressed from distinct states of an organism, in selected tissues or cells, and the word rapidly spread among protein chemists in the world. The technology and the new concepts involved were summarized (see Anderson & Anderson, 1998). Comprehensive proteome projects, such as from the cells of *Saccharomyces cerevisiae, Salmonella enterica, Spiroplasma melliferum, Mycobacterium tuberculosis, Ochrobactrum anthropi, Haemophilus influenca, Synechocystis spp., Escherichia coli, Rhizobium leguminosarum* and from various tissues such as human and mouse bladder, fibroblasts, kidney, liver, plasma and serum (see e.g. Celis, 1999; Garrels et al., 1997; Dunn 1997; Otto et al., 1996; Wilm et al., 1996; Dunn, 1992) were reported. Ruedi Aebersold and David Goodlett, Seattle, WA, foresaw the need to develop integrated analytical tools for generating multidimensional protein expression maps. Since it now became possible to shift from single protein analysis to a comprehensive investigation of biological systems and pathways, a suite of new technologies for the description of proteomes were presented. Included was a sensitive capillary extraction method in a micro-electrophoresis tool by directing the electric current towards different outlets for separation and appropriate mass analysis by MS and MS/MS. Efforts and difficulties encountered in these global approaches with protein modifications and isoforms visible in 2-dimensional gels as well as the choice of separation methods and sample preparation for mass analysis of individual proteins were discussed. Ian Humphery-Smith, Eveleigh, Australia, brought up new technical ideas for high throughput robotics to handle sample preparations in an automatic manner for proteome analyses of biological systems.

Peter Roepstorff, Odense, Denmark, questioned alternative ways of protein and genome sequencing and discussed where mass spectrometry fits into the picture. He gave a general scheme for proteome analysis, starting with proteins separated from 2-dimensional gels for identification by MALDI and nano-ESI-mass spectrometry and on the design of oligonucleotide probes from sequence tags for cloning and sequencing of a protein’s gene. He showed several examples starting from NEPHGE 2-dimensional gels (Klose & Kobalz, 1995; Klose 1975; O`Farrell, 1975) obtained from rat Langerhans’ islets and micro-purification data of proteins from an immune precipitated human protein complex. He also gave examples of glycopeptides isolated by HPLC and characterized by MALDI-MS. He stated that there is no

doubt that mass spectrometry will play an increasingly important role as a tool in protein analysis in the post genome area.

Matthias Wilm et al., Heidelberg, discussed the problem that *de novo* sequencing is far more difficult to achieve than the identification of known proteins in a proteome project. His group used C-terminal labeling of peptides and their identification by tandem triple quadrupol mass spectrometry. Labels employed were made by methylation or by treatment of in-gel spots with trypsin in 33% ^{18}O-water. The peptide mixture was eluted directly into a nano electrospray pulled glass capillary of the MS-system. Two tandem MS spectra were acquired, one by transmitting the complete ^{16}O/^{18}O isotopic distribution into the collision zone and the second by selecting exclusively the ^{18}O containing ions, and the spectra were then subtracted. However this technique was not applicable to peptide mixtures. When working with 50% isotope labeling in a quadrupole time of flight machine, the proposed scanning technique generated isotopically resolved fragment spectra and nearly noise free y-ion spectra thereof, which allowed good interpretation of the MS data in an automatic manner.

Structural and functional analysis of protein modifications by MALDI-MS were reported by several investigators. Philipe Bulet et al., Strasbourg, France, analyzed antimicrobial peptides in the hemolymph from an immune-challenged Drosophila. The samples were obtained from a single fly using a glass capillary, and they acquired time course data from the induction and degradation process of the peptides. Wade Hines et al., Framingham, MA, investigated post-source decay analysis (PSD MALDI-MS) of yeast proteins in 2-dimensional gels. Jeffrey Gorman et al., Melbourne, Australia, employed PSD MALDI-MS to study modified peptides, e.g. a superactive by-product of peptide synthesis, Nß-butyl asparagine modified peptides, in the attempt to obtain cytotoxic T lymphocytes. Furthermore, the authors characterized the proteolytic activation of fusion protein precursors of Newcastle Disease Virus isolates by PSD MALDI-MS. They determined S-S-bridge patterns in the ectodomain of the Respiratory Syncytial Virus attachment protein; they showed examples of oligonucleotide structure analysis of PMP- (phenyl-methyl-pyrazolone) derivatized pentaglucose. Carl W. Anderson, Upton, NY, described the detection of posttranslational modifications produced as a consequence of DNA damage using affinity-purified immunological tools. Sviatlana Astrautsova, Grodno, Belarus, studied chemical modifications essential for heart 2-oxoglutarate dehydrogenase activity.

Masaharu Kamo et al., Tokyo, established chemical methods for specific fragmentation of proteins, e.g. peptide bond cleavage at the N-terminal side of Ser/Thr-peptides in the vapour of S-ethyl trifluorothioacetate at 50°C for 24h for further mass fingerprinting. The authors discussed a novel C-terminal stepwise sequencing method. Ella Cederlund et al., Stockholm, Sweden, presented experiments with a C-terminal sequencer (ABI Procise C) and the problems encountered.

MALDI-MS spectra also served as tools to detect specific zinc finger peptide complexes (E. Lehmann and R. Zenobi, Zürich) as done with 18-residue metal chelated peptides and in complexes with oligonucleotides showing that MALDI-MS can reflect solution-phase chemistry. Overproduction, purification and structural studies on the ribosomal protein S14 from *Thermus thermophilus* also revealed a Zn finger like structure comparable to that of prokaryotic S14 and rat liver ribosomal proteins S27 and S29 (Tsiboli et al., Thessaloniki, Greece). The biomolecular interactions of matrix metallo-proteinases and their inhibitor, TIMPS, were studied by co-crystallization and Biacore binding studies by Harald Tschesche and M. Fah, Bielefeld. These proteinases are involved in the remodelling of the extracellular matrix during embryogenesis and in growth, development and repair. Enzymatic and chemical deblocking techniques of N-terminally modified proteins were reviewed under

applications of a new deblocking aminopeptidase from *Pyrococcus furiosus* for microsequence analysis (Kamp et al., 1998).

John R. Yates et al., Seattle, WA, studied the direct analysis of protein complexes by integration of multidimensional liquid separation methods with mass spectrometry; this was made possible by the recent refinements of ionization methods resulting in improved sensitivities.

The functionalities of glycosylation in host defence were explored using a novel oligosaccharide sequencing technology, as exemplified by human IgG glycoprotein by Paula Rudd et al., Oxford, U.K.; the authors developed a parallel glycan and protein identification scheme including successive cleavage of the side chain bi-, tri- and tetra-antennary complex oligosaccharides, their separation by HPLC and identification with MALDI-MS. Strategies for isolation and determination of proteoglycans, and specifically glycosaminoglycans (GAGs), and determination of their biological roles were addressed by Nikos Karamanos, Patras, Greece. He showed the participation of glycan-containing macromolecules in several cellular events and discussed their biological significance. Juan J. Calvete and Laura Sanz, Valencia, Spain, studied the lectin-carbohydrate binding mechanism of spermadhesins and their ability as primary zona pellucida-binding molecules in mammalian fertilisation by crystal structure analysis and characterization of the ß-galactoside recognition site and the analysis of glycan chains. Gluten structures in wheat seed storage proteins, e.g. the monomeric gliadins and the polymeric glutenins and their disulfide structures, were investigated with the aid of ESI-MS by Tsezi Egorov et al., Moscow, Russia.

Site-directed mutagenesis of the heme-binding domain in flavocytochrom b2 was analysed by Diep Le et al., Gif-sur-Yvette, France, using epitope mapping of the monoclonal antibody, which inhibits flavin to heme electron transfer, by surface plasmon resonance (SPR) analysis in a Biacore instrument (Jäger et al., 1997). Epitope mapping was further employed for the structural analysis of the 20S proteasome by single amino acid replacement studies by Regine Kraft et al., Berlin, Germany. Georg Büldt et al., Jülich, Germany, dealt with the structure, dynamics and function of the proton pump, baceriorhodopsin; they used infrared spectroscopy, electron microscopy, neutron and X-ray diffraction, time-resolved and steady state FITR and site-directed mutagenesis to gain understanding of the proton pumping process and its intermediate states. Molecular dynamic studies were initiated with the design of different thiol-specific cross-linking reagents that are useful for distance determination in proteins, such as for the analysis of the conversion of ATP into mechanical energy in the myosin head (Heinz Faulstich et al., Heidelberg). CD spectra and limited proteolysis data gave experimental support that proSP-B (a saponin-like protein) is composed of three tandem saponin-like domains and shed light on intracellular targeting (Shahparak Zaltash & Jan Johansson, Stockholm, Sweden).

Protein-protein and protein-DNA interactions of the p53 complex were studied by Rodney E. Harrington et al., Tempe, Arizona. This phosphoprotein plays a central role in regulating cellular growth and in tumor suppression. The co-crystal structure of the nucleoprotein complex of the p53 DNA binding domain and wildtype p53 was investigated; structural models were proposed to explain the DNA twisting and bending directionality in the DNA response element upon complex formation. The implications of the molecular model for p53 function were discussed.

The comparison of protein structure and dynamics was investigated using NMR by Beate Bersch et al., Grenoble, France; the authors discussed sample preparation, the different NMR spectral parameters, the introduction of various isotopic labels and their application for different proteins up to 40 kDa in size. The folding pathways during oxidation for disulfide

containing proteins were investigated by characterizing acid and jodoacetate trapped intermediates, as exemplified for hirudin, EGF and TAP, the tick anticoagulant peptide (Jui-Yoa Chang, Houston, Texas). The influence of the prolyl bond on conformational changes of *in vivo* and *in vitro* folding was studied by Sieglinde Menge and Gerd Krauss, Halle, Germany. Hydrophobic clustering as a driving force in protein folding was studied in native and partially unfolded states on alpha-lactalbumin by CD, fluorescence and photolabeling measurements (Geertrui Vanderheeren and Ignace Hanssens, Leuven, Belgium). The effect of hydrophobic interactions in the dimer interphase of citrate synthase of *Thermoplasma acidophilum* on thermostability was discussed by Ipek Erduran and Semra Kocabiyik, Ankara, Turkey. Discrimination of conformational states of mitochondrial cytochrom P-450scc was investigated by modifying several lysine residues (Anna G. Lapko, Minsk, Belarus and Klaus Ruckpaul, Berlin). Structure-function relationships of folded and unfolded amaranth proteins were analysed by Johann Salnikow et al., Berlin.

A new approach for C-terminal polypeptide determination was proposed by combining selective blocking of the proteolytic peptides by anhydrides with isolation by cation exchange in centricon tubes; the retained and non-retained fractions were analyzed by RP-HPLC and mass spectrometry (Laurie J. Gonzáles et al., Habana, Cuba).

Lila Castellanos-Serra et al., Havana, Cuba, employed high sensitivity ZnII reversed staining of 2-dimensional gels in connection with mass spectrometry and capillary HPLC (Fernández-Patron et al., 1992). High resolution 2-dimensional gel electrophoresis in combination with nano-ESI-LC-MS and MS/MS was employed to study apoptosis-associated processes in a Burkitt lymphoma cell line (Albrecht Otto et al., Berlin). Anne Bergmann et al., Stockholm, Sweden, outlined an analysis scheme of 1-dimensional and 2-dimensional gels and blotting onto PVDF for high sensitivity Edman degradations of long stretches of sequence. Hydrophobic proteins, the most abundant in bile, were purified by 2-dimensional gel electrophoresis, and both the proteins and the phospholipids were profiled by RP-HPLC techniques (Margareta Stark et al., Stockholm). Hydrophobic peptide sequences and phospholipids present in pulmonary surfactants were characterized by RP-HPLC and their native folds studied (Magnus Gustafsson & Jan Johansson, Stockholm, Sweden).

Comprehensive synthetic strategies were introduced for mapping of subunit-interacting surfaces of oligomeric proteins in solution. A series of uniform-size, consecutive overlapping peptides of an entire subunit of an oligomeric protein were synthesized and used for determining binding to the other subunit(s) (Zouhair Atassi and Naofumi Yoshioka, Houston, Texas).

A “Greek Night” at the beach and the visit to the excavated antiquities of old Thessaloniki were additional highlights of the conference.

Incorporation of the ***International Association for Protein Structure Analysis and Proteomics (IAPSAP)***; in the Commonwealth of Virginia, September 14, 1999.

By the time of MPSA1998 in Halkidiki, Greece, it had become clear to many of us that a more formal organization would be required to perpetuate the MPSA meetings. After much discussion, it was decided that a small committee would explore possible options. Over the summer of 1999, Ettore Appella, Jay Fox, and Carl Anderson drafted by-laws for a formal international association, the International Association for Protein Structure Analysis and Proteomics, which subsequently was incorporated in the state of Virginia, USA, as a non-profit organization. The association consists of an eighteen member Board of Directors and has three officers: President, Secretary and Treasurer. Board Members serve 6 year terms.

The Board is responsible for organizing MPSA meetings and generally meets every two years at the MPSA meetings to conduct necessary business. A website for the IAPSAP has been established at www.iapsap.bnl.gov.

The first meeting organised through the IAPSAP was MPSA2000, which was held in Charlottesville, VA, USA in September, 2000. For this and the subsequent MPSA meetings in 2002 and 2004, meeting reports have been published in *Protein Science* and are available for downloading at the IAPSAP website.

MPSA2000, Charlottesville, Virginia, USA, September 16-20, 2000, 13th MPSA Conference.
Organizer: Jay Fox, University of Virginia, USA.
Meeting Report: Appella, E., J. W. Fox, and C. W. Anderson. Methods in Protein Structural Analysis Conference (MPSA2000), Protein Sci. **10** (2001) 459-461.
Pehr Edman Award: Leroy Hood, Systems Biology Institute, Seattle, WA, and Michael Hunkapiller, Applied Biosystems, for development of the automated, gas-phase protein sequencer. Sponsor: PE Applied Biosystems.
MPSA Distinguished Postdoctoral Fellow Award (first): Kristian Müller, University of California, Berkeley.

MPSA2002, Valencia, Spain, September 8-12, 2002, 14th MPSA Confernce.
Organizer: Juan J. Calvete, Instituto de Biomedicina de Valencia, Valencia, Spain
Meeting Report: Anderson, C. W., J. Calvete, J. W. Fox, E. Appella. Methods in Protein Structural Analysis Conference (MPSA2002). Protein Science **12** (2003) 398-400.
Pehr Edman Award: Prof. Hans Jörnvall, Karolinska Institute, Stockholm, Sweden, for contributions to protein chemistry, structure and function through the development of techniques associated with the use of Edman chemistry and structure-function studies on the alcohol dehydrogenases; and Emeritus Professor Kenneth Walsh, University of Washington, Seattle, WA, USA for contributions to the development of mass spectrometry for the characterization of the chemical structure of proteins and their posttranslational modifications and concepts regarding the evolution of protein structure and function. Sponsor: PE Applied Biosystems.
MPSA Distinguished Young Investigator Award (second): Dorothee Kern, Department of Biochemistry, Brandeis University, Waltham, MA 02454, USA, for studies on the dynamic processes that trigger protein function related to enzyme catalysis, ligand binding and signaling events. Sponsor: PE Applied Biosystems.

MPSA2004, Seattle, WA, USA, August 29 - September 3, 2004, 15th MPSA Conference.
Organizer: Ray Paxton, Amgen, Seattle, WA.
Meeting report: Anderson, C. W., R. Paxton, J. W. Fox, and E. Appella. Meeting Report (MPSA2004). Protein Sci. **14**(1) 272-275 (2005).
Pehr Edman Award: Stephen Altschul, Computational Biology Branch, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, USA, and Amos Bairoch, Swiss Institute for Bioinformatics, Geneva, Switzerland, for their contributions to the development of bioinformatics methods and databases for the analysis of proteins and their structures. Sponsor: PE Applied Biosystems.
IAPSAP Distinguished Young Investigator Award (third): Dr. Niroshan Ramachandran, Harvard Institute of Proteomics, Department of Biological Chemistry and Molecular Pharmacology, Harvard University, Boston, MA 02115, USA, for the development of novel

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Brigitte Wittmann-Liebold is Scientific Advisor and Founder (1992) of WITA GmbH

(Wittmann Institute of Technology and Analysis of Biomolecules, Teltow/Berlin-Brandenburg). She studied chemistry and biology in Giessen, Innsbruck, Tuebingen and Munich and received in 1959 her *Doktor rerum naturalium* at the University of Munich in Chemistry and Biochemistry for studies on the primary structure of human adult haemoglobin, which were performed at the MPI of Biochemistry with Gerhard Braunitzer and Adolf Butenandt. After postdoctoral work (1961-1967) on the elucidation of the Genetic Code using mutants of tobacco mosaic virus (TMV) at the MPI of Biology in Tuebingen, Germany, and on the structure of ribosomal proteins at the MPI of Molecular Genetics in Berlin (1967-1991), together with Heinz-Günter Wittmann, she started a Proteome Research Group at the newly founded Max Delbrück Centrum in Berlin-Buch (1992-2001). Her work involved chemical and technical improvements in methods for sensitive protein structure analysis. The photograph of her and Agnes Henschen (left) was taken in Shanghai, China, in 1980, where they were teaching a course in protein chemistry.

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