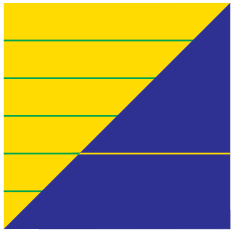
RECOVERY OF BIOLOGICAL PRODUCTS



Conference Series

The Recovery Conference Series is the premier forum for the presentation and discussion of the status, direction and trends in the recovery of biological products of therapeutic, diagnostic and nutritional interest to society. The biennial Conferences are an international crossroads where academia meets industry in the pursuit of cutting edge and trans-disciplinary science and technology. The meetings are characterized by active participation, dynamic exchange of ideas and views on nascent and emerging technologies and discussion of the future impact of peripheral sciences on the industrial application of recovery technologies.

Oral and poster sessions, workshops and panel discussions are organized to focus on a systematic and integrated approach to process design, and not simply on individual unit operations. Further, the sessions seek to strengthen the bridge between academic discovery and industrial implementation, resolution of industrial issues and decision-making in product development. Leading regulatory presence assures a dialog essential to product safety and the timely introduction of novel, ground breaking treatment of unmet medical needs. To maintain the scientific standards and quality of the Conference, participation is limited and by invitation. Leaders in the field of biological product recovery and related sciences are able to exchange innovative ideas in an atmosphere free from commercial, corporate or publishing pressures, in locations which are conducive to networking, building fruitful professional relationships, making new friends and renewing old friendships.

Established in 1981, the Conference Series brings an inimitable array of disciplines together to provide a special platform to develop guidelines for biorecovery processes, promote the image and global agenda of downstream processing and foster the relationship with governmental and international agencies. With a clear focus on the future, the Conference Series will play a proactive role in catalysing the flow of new ideas and their progress from theoretical function to beneficial use. The Series will draw on its intellectual capital, maintain continuity of theme and quality and ensure an influx of ideas from new generations who will take Recovery of Biological Products far into the 21st Century and the second Millennium.

John Curling
Inger Mollerup
Kenneth Taksen

The Recovery of Biological Products Conference Series was initially sponsored by the Engineering Foundation and three meetings were later co-sponsored with the American Chemical Society. Today, the Conference Series is associated with the American Chemical Society's Division of Biochemical Technology that is the organizational sponsor. This relationship maintains the Not-for-Profit and tax-exempt status of the Conference Series and provides certain fiscal services.

The Conference Series is dependent on financial sponsorship from industry. Contributions are used to offset meeting expenses for academic participants and invited academicians and are vital to keeping a balance between theory and practice, exploration and development, and to provide a continuous, innovative flow of ideas that will bring long term benefit. Each Conference has a balanced budget of expense versus income and a balance between sponsor donations and academic support.

Since the R8 Conference in 1996, the Conference Series has been the responsibility of a non-remunerated Board. The Board was formed to guarantee not only the scientific standards, but also the financial stability and continuity of the Conference Series. Members of the Board are active past and current Conference co-chairs with the Chair chosen from one of the co-chairs of the previous meeting. The Board meets during the Conference and may meet in the interim as required and when convenient, often in association with ACS conventions.

On the nomination by current co-chairs, the Board appoints the co-chairs for the next Conference, acts in a consultative manner to the new chairs, approves the Conference budget and controls and administers reserve funds. Members of the Board have developed the administrative infrastructure to meet the needs of organizing the Recovery Conference in the future. This support function will allow co-chairs to focus on the meeting program and scientific content. The prime function of the Board is to preserve and develop the premier position of the Conference Series platform in recovery and downstream processing of biological products.

The home of the Conference Series from June 2001 is www.RecoveryConferences.org. This web site contains the history of previous Conferences and the Conference Series, is the hosting site for the current Conference, provides information for sponsors, feedback from participants, contacts and links, and is the portal for the administrative structure.

Stuart Builder
Strategic Biodevelopment
San Francisco, USA
(formerly Director, Strategic Development, Genentech, Inc.)

Charles Cooney
Executive Officer and Professor of Chemical and Biochemical Engineering
Department of Chemical Engineering
Massachusetts Institute of Technology
Cambridge, USA

Professor Steven Cramer
Isermann Department of Chemical Engineering
Rensselaer Polytechnic Institute
Troy, USA

John Curling
John Curling Consulting AB
Uppsala, Sweden
(formerly President, BioProcess Division, Pharmacia Biotech AB)

Professor Stephen Drew
Department of Chemical Engineering
Princeton University
Princeton, USA

Professor Dr. Maria-Regina Kula
Institute of Enzyme Technology
Heinrich-Heine University Düsseldorf
Jülich, Germany

Michael Ladisch
Distinguished Professor and Director
Laboratory of Renewable Resources Engineering
Purdue University
West Lafayette, USA

Ann Lee
Senior Director, Vaccine Bioprocess R & D
Merck & Co., Inc.
West Point, USA

Inger Mollerup
Vice President, Protein Process Development
Novo Nordisk A/S
Copenhagen, Denmark

Helmut Sassenfeld
Senior Investigator, Process Science
Immunex Corporation
Seattle, USA

Kenneth Taksen
Vice President, Bio and Chemical Process R & D
Pfizer Global Research and Development
Pfizer, Inc., USA

Chairs:
1996-1999 *Kenneth Taksen*
1999-2001 *Inger Mollerup*
2001-2003 *John Curling*

Ad hoc member, ACS Division of Biochemical Technology Executive Committee representative:
Arin Bose, Director, Biologics, Bioprocess R & D, Pfizer Global Research and Development, Pfizer, Inc., USA

Recovery Series representative to the Executive Committee, ACS Division of Biochemical Technology:
Inger Mollerup, Novo Nordisk A/S, Denmark

Advances in Fermentation
Recovery Process Technology
Banff, Alberta, Canada, 7-12 June, 1981
Co-chairs:
Harvey Blanch, University of California, Berkeley, USA
Arnold Kaufman, Merck, Sharp & Dohme, USA
Alan Michaels, Stanford University, USA

Recovery of Fermentation Products
Sea Island, Georgia, USA, 29 Jan.-3 Feb., 1984
Co-chairs:
Charles Cooney, Massachusetts Institute of Technology, USA
Kenneth Taksen, Pfizer, Inc., USA

Recovery of Bioproducts
Uppsala, Sweden, 11-16 May, 1986
Co-chairs:
Charles Cooney, Massachusetts Institute of Technology, USA
Nils-Ingvar Olsson, Pharmacia Biotechnology AB, Sweden
Günther Schmidt-Kastner, Bayer AG, Germany

Fourth Conference on Recovery of Bioproducts
Keahou-Kona, Hawaii, USA, 17-22 April, 1988
Co-chairs:
Michael Ladisch, Purdue University, USA
W. Courtney McGregor, Xoma Corp., USA
Irving Holzberg, Syntex Research, USA
Saburo Fukui, Bioindustry Development Center (MITI), Japan

Recovery of Biological Products V
St. Petersburg, Florida, USA, 13 - 18 May, 1990
Co-chairs:
Clark Colton, Massachusetts Institute of Technology, USA
Louis Fries, Collagen Corp., USA
Arnold Hershman, Monsanto Company, USA

Recovery of Biological Products VI
Interlaken, Switzerland, 20-25 September, 1992
Co-chairs:
Stuart Builder, Genentech, Inc., USA
John Curling, John Curling Consulting AB, Uppsala, Sweden
Maria-Regina Kula, Heinrich-Heine University Düsseldorf, Germany

Recovery of Biological Products VII
San Diego, California, USA, 25-30 September, 1994
Co-chairs:
Stephen Drew, Merck & Co., Inc., USA
Charles Goochee, Chiron Corporation, USA
Dennis Lanfear, Amgen, Inc. USA

Recovery of Biological Products VIII
Tucson, Arizona, USA, 20-25 October, 1996
Co-chairs:
Michael Ladisch, Purdue University, USA
Helmut Sassenfeld, Immunex Corporation, USA
Kenneth Taksen, Pfizer, Inc., USA

Recovery of Biological Products IX
Whistler, British Columbia, Canada, 23-28 May, 1999
Co-chairs:
Stuart Builder, Strategic Biodevelopment, USA
Charles Cooney, Massachusetts Institute of Technology, USA
Inger Mollerup, Novo Nordisk A/S, Denmark

Recovery of Biological Products X
Cancún, Quintana Roo, Mexico, 3-8 June, 2001
Co-chairs:
Steven Cramer, Rensselaer Polytechnic Institute, USA
John Curling, John Curling Consulting AB, Uppsala, Sweden
Ann Lee, Merck & Co., Inc., USA

ALAN MICHAELS recognized very early the important role that chemical engineering, and interfacial phenomena and transport in particular, would play in translating the revolutionary changes in life sciences into commercial products and processes. The first "Advances in Fermentation Recovery Process Technology" conference held at Banff, June 7-12, 1981, was a result of his vision and ability to bring together engineers, biologists and chemists to tackle the problems of the newly emerging biotechnology industry.

Trained as a Chemical Engineer at Massachusetts Institute of Technology (MIT) (S.B. in 1944, M.S. in 1947 and Sc.D. in 1948), Alan joined the MIT faculty teaching surface, colloid and polymer chemistry. The research and teaching program he developed became one of the nation's most influential centers for engineering applications of surface and colloidal phenomena. The mechanism of gas transport through polymers drew his attention in 1958, and in a groundbreaking series of papers he described the relationship between permeability of polymers and polymer structure. This lead to his development of "perm-selective" polymer membranes for separating gaseous and liquid mixtures. Desalting sea water was an early application that subsequently resulted in membrane-based separation processes becoming an important unit operation in the chemical industry.

In 1962, Alan founded Amicon Corporation, whose research and development activities were applied to colloid, surface and polymer chemistries and to new separation technologies. Amicon developed low-pressure, high-flow semi-permeable ultrafiltration membrane systems that have become the workhorses of today's biotechnology industry, a new artificial kidney and plastics for prostheses. Alan moved to the Bay Area in 1970, to found Pharmetrics, which was subsequently acquired by Alza. He served as President of Alza Research and as Director of Alza until 1977. At Alza, he developed systems for drug

delivery, including ocular administration of drugs for treatment of glaucoma, transdermal delivery devices, and an osmotically-driven oral system for therapeutic delivery to the gastro-intestinal tract.

Alan joined Stanford University's Chemical Engineering department in 1977. At that time there was tremendous excitement in molecular biology with the advent of recombinant DNA, and, in a number of publications and National Academy reports, Alan was instrumental in bringing a strong engineering perspective to the "new biology". He emphasized the important role that separations would play in manufacture of therapeutic proteins and specialty chemicals. The first Recovery Conference thus brought together academic and industrial leaders in molecular and cell biology, biochemistry and biochemical engineering.

After joining the Chemical Engineering department at North Carolina State University in 1986, Alan retired in 1989 to continue his consulting practice. He played an important role throughout his professional career as mentor to young faculty and to colleagues in industry. His creative abilities at the interface of science and technology were astonishing, and inspired generations of young engineers. Biotechnology, and bioseparations in particular, owe much to his scholarship, innovation and ability to bring disciples together to solve important issues.

Harvey Blanch

Much has changed from the first conference on "Advances in Fermentation Recovery Process Technology" organized in 1981 by the late Alan Michaels together with Harvey Blanch and Arnold Kaufman at Banff. It is hard to imagine today, that in the oral presentations there was a single contribution on recombinant proteins by M. Ross, (Genentech) reporting on the recovery of pharmaceuticals from *E. coli*. At that time proteins of about 20 kD, or smaller, were mainly produced as fusion proteins requiring chemical cleavage by e.g. cyanogen bromide, and extensive purification.

Science and industry have come a long way to the present, diversified array of expression and production systems and from no approved recombinant product in 1981, 15 products in 1991 to circa 100 approved products on the market in 2001.

At the conference, production of vaccines was an important topic and lessons learned to design equipment and facilities for safe processing were intensively discussed. At that time vaccines were attenuated and inactivated viruses, bacterial pili or specific cell envelope antigens shed into the culture medium. Biotechnology owes much, and not only regulatory procedures for "biologicals", to the pioneering work in vaccine production.

In a similar way blood plasma fractionation provided much impetus in protein separation technology, which was apparent in a paper on design of precipitation systems for the recovery of proteins by centrifugal separation by D. J. Bell, M. Hoare and P. Dunnill (University College London) and in the single paper (G. Mitra, Cutter Laboratories) addressing a chromatographic process with human antithrombin III as an example isolated by affinity chromatography on Heparin-Sepharose® from plasma fractions.

Some separation problems, such as the intrinsic difficulties of solid/liquid separation in biotechnological processes, addressed in Banff are still with us, although our arsenal of methods has been enlarged and become much more sophisticated.

Ultrafiltration and other pressure driven membrane processes have developed steadily over the years since 1981, but scale up of electrophoretic protein separation advocated in Banff remains elusive as our perception of what is "large scale" is changing. The recovery of many biological products logical products of low molecular weight from antibiotics to organic acids and ethanol received much attention in Banff, as did questions of waste treatment, focusing on metal

ion recovery and water management in large scale fermentation processes. These topics have disappeared from the Recovery Series due to the exciting developments in the New Biotechnology Industry. Nevertheless, small molecules are important products of the traditional biotechnology industry. With the new tools of metabolic design and the emphasis on sustainable chemical processing a new junction is presently formed and proteins as well as other biotechnology products will profit from progress in biochemical engineering in the recovery field. After all, besides molecular biology, separation science and technology is the basis of successful development.

Maria-Regina Kula

Following on from the initial Recovery Conference at Banff represented a challenge to the organizers of the second conference – because we wanted to convert what had been an extremely successful, but a nonetheless one-off, conference into a "series". To do that, we felt that we had to sustain the quality of the science, the ambience of the venue, and the collegial atmosphere and small conference feel that allowed us to attract the true leaders of the field. And we knew that by attracting the experts, we would in turn be able to garner high interest and hopefully generous sponsorship from the industrial sector.

The Cloister Hotel, a small, intimate resort located on Sea Island, Georgia, and the then favorite retreat of Jimmy Carter was chosen as the venue for R2. The tranquil south Georgian sea coast offered an interesting contrast to the Canadian Rockies, and a locale that would be more accessible to prospective participants from Europe.

We decided to continue the policy of not publishing proceedings – in the hope that that would encourage more open contributions from the private sector. For the same reason and to encourage inclusion of the absolutely latest results, this policy continues today. The "afternoons off, evenings work" format was continued, as it has into the 10th conference, so that participants could talk science more informally while indulging in the amenities and building professional friendships.

Professor Elmer Gaden was invited to give the Conference's opening plenary lecture: Elmer is considered by many to be the father of biochemical engineering, and perhaps more importantly, came with the reputation of being a *raconteur par excellence*! Elmer did not disappoint and his lecture on "Separations Technology: Achilles Heel of the Bioprocess Industry" was peppered with the more personal asides and anecdotes that are his trademark.

The scientific program grouped the downstream papers into more or less their chronological processing order, primary separations, concentration/extraction, chromatography/electrophoretic processes, a practice that has been continued at subsequent Recovery meetings. Three other program items were particularly notable. Firstly, we introduced a session entitled "Integrated Fermentation Recovery Systems" in order to emphasize the interdependency of those downstream sciences on what was happening

in the preceding upstream stages and what better way to do that than to have those upstream and downstream sciences converge. Second, to further stimulate "out of the box" interdisciplinary thinking, we sought participation by scientists who do not generally attend the bioprocess and bioengineering oriented meetings, and were particularly pleased in that regard by the presentation of Peter O'Farrell from University of California San Francisco, who talked about "New Electrophoretic Methods that give High Capacity Equilibrium Separations". Lastly, in addition to formal oral and poster presentations, we initiated workshops on "Economics and Scale up ", which were extremely well received and have also become a standard feature at subsequent Recovery sessions.

As we convene this 10th anniversary of the Recovery Series, the organizers of R2 and each successive conference can look back proudly at the role they have played in establishing what has become the premier downstream conference, a conference that now has the momentum to carry it well into this new Millennium.

Kenneth Taksen

The third Conference on Recovery of Bioproducts was held in Uppsala, Sweden from May 11-16, 1986. The co-chairs of the conference, Charles Cooney, Nils Ingvar Olsson and Günther Schmidt-Kastner sought to create a program that balanced a focus on classical unit operations with newer applications. After 15 years we can see how well this snap shot of downstream bioprocess technology represented the state of the art and how well we were able to look forward and anticipate where the field was moving.

Addressing membrane processes, there was the continuing debate of centrifugation vs. filtration and where in the process do membranes fit best. This debate still exists. It was interesting to hear of innovative applications such as the use of selective aggregation and affinity adsorption as complimentary chemistries to enhance the selectivity in membrane filtration. There was a common complaint that may still be present, that the quality of predictive models was poor.

Considerable attention was given to extractive technologies with biphasic aqueous partitioning - not surprising given the origins of this technology in Sweden through the work of Albertsson. Despite the enthusiasm, applications have been few as evidenced by the paucity of examples in future meetings.

Chromatography, as in all the Recovery meetings was in center stage. The focus was on scale-up, affinity adsorption, and application of HPLC at a process scale. As with membranes, there was considerable lamenting about the poor quality of the models as predictive tools. In reflection, the expectations laid out at the meeting have proven true; one may still complain about the predictive ability of models in chromatographic separation of complex materials.

The session on process technologies for the future was intended to provide an opportunity to look forward with speculation and provide some vision of things to come. Topics included genetic solutions to separation problems, continuous sorption processes, the use of reverse micelles for affinity capture and an increased focus on affinity chromatography. We still have not learned to use reverse micelles in bioseparations, but there is frequent incorporation of affinity steps in many commercial processes.

Genetic solutions to create affinity tags, promote secretion and remove problem amino acids have been successful. In reflection, the vision was not bad.

Process monitoring and control was also addressed. It was clear that a major barrier was the sampling and analysis of proteins. The time scale of the analysis was on the same order as the process and control was difficult. On the other hand, sequence control for batch process like chromatography showed good promise.

There was considerable interest in the production and recovery of proteins from mammalian cell culture; affinity capture with antibodies was presented as an exciting approach. The need for efficient technologies was apparent as the secreted proteins were large molecular weight, glycosylated, and contained multiple disulfide bonds. In addition, they were in low concentration relative to other proteins in the medium. One of the major barriers was the analytical technology available to support the process development and manufacturing quality control. Mammalian cell culture has become a critical technology and many of the ideas discussed have become standard practice.

In retrospect, we see that the paradigm for recovery of biological products was already set in 1986 and in many ways has changed little in the last 15 years. Many refinements in the unit operations used in 1986 have taken place in the intervening years but the strategy for recovery has been invariant. The fact that the speculation of the future has become reality speaks well for the role that this series of meetings has played in the development modern recovery processes for biological products.

Charles Cooney

Hawaii was the location for the fourth Recovery Conference, held under the auspices of the Engineering Foundation. The poster session was developed by Maria-Regina Kula and Harry Bungay. Then, as now, the posters were a huge success and well attended event, on a patio overlooking the beach. Chromatography formed a central theme of the meeting and was chaired by Juan Hong and Bob Sitrin. Bob Sitrin addressed issues of what the industry really needed. Leaders in the field presented advances in chromatographic separations development and scale-up using commercially important stationary phases, followed by several discussions on strategies for industrial scale chromatography.

This conference introduced the concepts of the recovery-fermentation interface organized by Jim Schwartz of Genentech. In an early version of industrial case studies, Schwartz's session addressed recovery-fermentation interactions and the production of human growth hormone, the recovery of biopharmaceuticals from perfusion cell culture, and the production of antibiotics. This theme was complimented by a discussion of bioproducts at interfaces, led by John Brash and Joseph Andrade and addressing protein interactions with stationary phases at the molecular level.

Membranes, filtration, and centrifugation continued to be an important theme of the fourth conference and evolved from previous conferences in this series. This session, led by Alan Michaels, gave particularly valuable insights into challenges of membrane filtration and microfiltration of cell suspensions and bioproducts. Industrial case studies helped to illustrate the impact of research advances on industrial practice.

Biosensing was of re-emerging interest in 1988 and was covered in workshops. The lively discussions anticipated some of the important developments that would occur in the coming years as nanoscale and microscale technology for separations and monitoring became feasible. Future developments in membrane technology and discussions on analytical methods for biological products were other areas of concentration. The influence of government agencies on recovery of bioproducts was a continuing theme and was discussed in the context of the purification of epidermal growth factor, purification of tissue plasminogen activator, and a review of criteria for purity of biological products. Kathryn Zoon (FDA - Office of Biologicals) gave valuable insights into regulatory issues. Purification of epidermal growth factor was presented by R. Johnson of Chiron

Corporation while the purification of tPA was presented by Stuart Builder, then at Genentech. Both fostered discussions of how purification processes might evolve as the types of therapeutic proteins increased in complexity.

Aqueous two-phase partitioning was another elegant method discussed here by Maria Kula. The session on product stability addressed case studies on stability of human growth hormone produced by Genentech as well as interferon-ß-Ser 17 (Betaseron™) used at that time by Cetus Corporation.

The keynote address by Bill Young, who was Vice President of Manufacturing Process Science at Genentech, on "Biotherapeutics from rDNA Technology: Challenges, Past, Present, and Future." This presentation anticipated many of the challenges that would be encountered by the industry, and subsequently resolved, over the coming decade. In retrospect, this address, together with the sessions that followed it , addressed purification issues for various types of recombinant products and gave an uncanny view to the future.

As we look back at this meeting, we find many of the issues still remain. These include regulatory issues, and approaches for improving the processes for purification of recombinant products. Purification of proteins was done in an environment where the costs of processing were small relative to the overall cost of the product, and being first to market was the most significant factor in fielding a new product. The costs of purification alone were not a driving factor in scale-up in 1988. Times have changed and many issues of biopharmaceutical product purification are being revisited in this context. Many of the new products are not proteins, but rather molecules that act on proteins.

Gene therapy has brought a new class of molecules to the attention of bioseparations scientists and engineers. The technical challenges continue to evolve, as does the value of the biorecovery conferences.

This meeting was remarkable in that it began to address limitations and barriers to scale-up relevant to new products that were yet to be developed as the New Biotechnology Industry grew five-fold in the ensuing 10 years.

Michael Ladisch

Recovery of Biological Products V took place on the white sand shores of the Gulf of Mexico in the splendid Don Cesar Resort, which hosted beach games to facilitate renewal of old friendships and establishment of new ones. The keynote speaker was Dr. George Rathmann, Chairman of Amgen Corporation, who spoke on the Biotech Revolution, in particular the strategy employed by Amgen in development of recombinant protein therapeutics. This presentation set the tone for the meeting which featured increased participation by industrial representatives in discussing bioprocess recovery applications. More than two-thirds of all oral presentations came from the biotechnology industry.

A highlight of the meeting consisted of a series of five presentations from Monsanto Company, all of which related, at least in part, to the company's large-scale process for commercial production of recombinant bovine and porcine somatotropins (rBST) from *E. coli*. This series was the first public disclosure of its type in a Recovery Conference, and it still remains the most detailed and extensive description of a commercial process. One presentation by G. L. Backman traced the process development effort for somatotropins, showed how improvements early in the process substantially improved overall product quality and yield, and discussed the role of automated analytical methods in leading to effective on-line process control. Four other papers focused on recovery issues. In one, S. M. Balaban described rBST inclusion body formation (resulting from high expression), composition, and stability. In another, S. B. Storrs described a method to solubilize inclusion bodies and subsequently oxidize and refold rBST, a difficult challenge because the protein contains disulfide bonding. B. L. Haymore described metal-affinity purification of rBST and other recombinant proteins by use of site-specific mutagenesis to produce strongly binding metal recognition sites on exposed protein surfaces. Lastly, T. A. Kewer discussed problems and considerations in the design and operation of industrial-scale ion exchange chromatography (e.g., for production of a ton or more per year of purified protein) based on actual experiences.

There were several prominent themes throughout the conference. Protein refolding was discussed from the standpoint of achieving high yields by appropriate control of key solution variables, by use of gene fusion (i.e., fusing the coding DNA sequence of the desired protein to that of a polypeptide

domain with high affinity to a ligand), and through use of quasi-elastic light scattering to observe aggregation. Solid/liquid separations described included biospecific, continuous, and fractional precipitation.

The growing use of membrane processes was highlighted by presentations by T. W. Strickland (Amgen) on the use of ultrafiltration in production of recombinant erythropoietin and by R. van Reis (Genentech) on fluid dynamic optimization of tangential flow filtration of mammalian cell suspensions.

Other work on membrane separations included new ultrafiltration membranes capable of high resolution removal of viruses from protein solutions, functionalized hollow fiber microfiltration membranes for affinity and ion exchange chromatography, and development of a fundamental understanding of protein deposition and membrane fouling.

Adsorption and chromatography filled a significant fraction of the program. Fluidized bed adsorption, which was in its infancy and has grown to be an important technique, was the subject of two presentations: F. P. Gailliot (Merck) described his experience with whole broth extraction in the very early stages of fermentation, and H. A. Chase described the principles of operation and analysis of performance. Other studies reported dealt with perfusion chromatography, HPLC on a production scale, validation of chromatographic processes, visualization of concentration profiles inside resins, and liquid-liquid partitioning and extraction.

The meeting was notable for the extent of interaction between participants and for the high quality of presentations that provided a blend of fundamentals and applications.

Clark Colton

The site of the sixth meeting of the Recovery of Biological Products in 1992, the elegant Victoria-Jungfrau in Interlaken, Switzerland, was chosen to acknowledge that the biotechnology industry had turned a corner to profitability. It had been ten years since recombinant insulin had been approved and five years since tPA's launch. With the acceptance of both bacteria and transformed mammalian cells as approved hosts, the floodgates were opening. Recombinant monoclonal antibodies were helping to fill the pipeline.

As with any evolving technology, each success brought with it new opportunities, challenges, and excesses. As we look back it is interesting to note both the new paths taken and the attempts that withered. By 1992, it was accepted that most protein sequences could be expressed in either bacteria or mammalian cells. "Six Central Questions" of the day were addressed at the meeting.

First. When more complex human proteins were expressed in bacteria, could they be correctly folded, giving both efficacy and safety from immunological reactions? Much progress has been made in both performing and monitoring protein refolding. However, there still seems to be an economic cross-over between 20-30 kDaltons as yields from bacteria drop with increasing peptide size and complexity. Secondly, would mammalian cell expression in cell lines such as CHO and BHK lead to "proper" glycosylation and hence desired solubility, stability and pharmacokinetics, in addition to lack of immunogenicity? Analogous questions accompanied expression in yeast and insect cells. To a significant extent the question of "proper" has since been supplanted by the concept of "acceptable".

As analytical tools became more powerful, we asked the third question - how can we demonstrate safety and control of the many observed post-translational modifications, such as: deamidation, desialylation or under-sialylation, peptide bond cleavage, oxidation of methionine residues? A few years later, this same analytical power gave us the ability to simplify the work needed to make process changes (no clinical trials) through the "Well-Characterized Products" initiative.

Question number four "Is the product safe from viral contamination?" has always been

critical for biologics licensure. The CJD incident was the basis for the withdrawal of human cadaver pituitary hGH from the market and the licensure of *E. coli*-derived Protropin®. It was one of the most important issues of concern in the acceptability of CHO-derived products. The AIDS epidemic and the decimation of the hemophilia community by contaminated blood-derived Factor VIII led to the transition to a recombinant method of production of this product as well.

Whereas ten years before there had been a psychological preference for the "natural" material from animals, now there was a preference for the "virus-free" recombinant source material. This feeling of relative safety was short lived, as production cultures came down with a number of viral and mycoplasmal infections. It spurred the drive for removal of all animal-sourced raw materials from all pharmaceutical production in order to eliminate the potential of human exposure to BSE. This was in addition to comprehensive viral kill/removal in the recovery process as well as in-process and final product viral testing.

Five. As more products were being worked on simultaneously, we asked: how can we design and operate multi-product pilot and production facilities? This was central to conservation of precious capital and it is now common practice.

Lastly, how can products be brought to market faster? All the information and skill in a company was brought to bear to answer this most pressing business question. It remains an elusive goal today. The major positive impact on human health of the biotechnology industry is a measure of how successfully these questions have been answered.

Stuart Builder

Co-sponsored by the ACS, the seventh Conference on Recovery of Biological Products convened in San Diego, California on September 25, 1994, drawing 135 participants from 11 countries. A session on "Regulatory Issues in Pharmaceutical Manufacturing", organized by Wolfgang Berthold and John Poulos, highlighted change control and the evolving trend toward more rigorous process validation. The paper by Annette Baeckman, "Variations: Process Changes and their Regulation in the EU" presented a vision of global harmonization in methodology for process validation and change control. This theme was further advanced by two excellent workshops on Regulatory Issues lead by Susan Vargo and Kenneth Seamon, then both of the US FDA and continued the long-established dialog between key regulatory agencies and the pharmaceutical and biotechnology industries. Discussions and debate on these issues were so fruitful and so well subscribed by the attendees that this topic has now become a routine component of the conferences – a forum for learning, sharing and evolving best practices.

Stuart Builder and Thomas Boone organized one of the most popular sessions of the conference, "Case Studies of Industrial Separation". The topics ranged from historical insights from the chymosin process (Kenneth Taksen, Pfizer) to troubleshooting methodology (Amgen) and detailed evaluation of the process for IGF-1 (Genentech). The paper by Orella, Hagen, Sitrin and colleagues at Merck & Co., Inc. presented one of the first process views of a highly purified vaccine for Hepatitis A. That work went on the win the American Chemical Society Industrial BioProcess Award in 1998 for industrial innovation. The hepatitis A inactivated vaccine paper typifies the hallmark of the Recovery Conferences, early sharing of important insights in process design and development.

The evolution of new materials for chromatographic and adsorptive separations played an important role in R7. Frances Arnold (Cal Tech) presented her important work on the new materials for selective separation of biological molecules based of affinity for specific ligands. Her paper introduced advances in metal ion affinity for specific peptide sequences and the use of template polymerization for *de novo* synthesis of affinity ligands. Richard Willson (University of Houston) introduced his exciting use of phage

surface display libraries of Protein A for searching out novel affinity ligands in high throughput assays. And Michael Flickinger described surface-modified zirconia supports for fluidized-bed protein recovery. Howard Chase (University of Cambridge) and Jörg Thömmes (Heinrich-Heine University) demonstrated how high-density, product-specific chromatographic materials could be used in expanded bed and fluidized bed applications. Scale up of chromatographic separations was also discussed by Shuichi Yamamoto (Yamaguchi University) who described design calculations for gradient elution and by Susan Behrens (Merck & Co., Inc.) who described a commercial scale HPLC process for recovery of lovastatin.

New methodology for monitoring and understanding purification was described. Michael Fountoulakis (Hoffmann-La Roche) described how to use soluble interferon receptors to follow recombinant protein heterogeneity; Inger Mollerup (Novo Nordisk) used reverse phase HPLC to monitor degradation of coagulation Factor VII and Rainer Rudolph (Boehringer Mannheim) presented a new method for *in vitro* refolding of inclusion body recombinant proteins.

The conference was augmented by 64 posters from laboratories around the world on topics such as direct extraction of glucose-6-phosphate dehydrogenase by affinity chromatography in expanded beds, protease activity during purification of recombinant proteins and cleaning validation in recovery systems. The papers were great, the skies were sunny and the company and conversation were grand!

Stephen Drew

In October of 1996 the Recovery of Biological Products VIII was held in Tucson, Arizona. The Conference featured two new sessions, one on plasmid recovery processes and the other on process economics. A presentation by D. Clark (BRL/Gibco) illustrated that many of the same issues that make protein separations challenging also apply to plasmid scale-up and separation. If the challenges are similar then the solutions may be as well. This was shown in a paper presented by M. Atkinson (Targeted Genetics Corp.) demonstrating that many of the approaches used for industrial protein separation can also be applied to large scale preparation of gene therapy vectors.

The process economics workshop provided an insight into some of the early models for predicting downstream costs of protein therapeutics. It seems there was a time when the value and potency of protein therapeutics was so great that almost any expense to produce them was a relatively insignificant expense. Clearly that has changed with more proteins being approved for chronic indications and with the steady progress of transgenic production methods where recovery costs should far outweigh raw material expense.

This Conference also featured an expanded session on Industrial Case Studies, organized by John Curling and Stuart Builder, which continues to be one of the strong attractions of this Conference Series. R. Bridenbaugh (Genentech) discussed the issues surrounding process change for a licensed product (Activase®), focusing on the application of extensive analytical chemistry testing to demonstrate product equivalence before and after the change. He highlighted how this approach was used to expedite process change and to avoid additional clinical testing.

These were the days before the "Well-Characterized Products" when one had to avoid process change to ensure safety and a smooth registration. Of course, this approach is now captured in comparability protocols. It has become standard practice and the subject of FDA guidance documents. But at the time, it seemed like a demystification process had begun. Proteins were no longer invariably too complex. The choice of process method had become a little less sacred.

The chromatography sessions featured no less than five talks on the application of expanded bed chromatography. G. Zapata (Genentech) described the implementation

of this technology at the 12,000 litre scale. Eventually, expanded bed absorption (EBA) became the subject of its own conference series and enjoys fairly widespread application today. Something considerably less broadly applied, but nonetheless intriguing, was a paper by E. Beckman (University of Pittsburgh) on carbon dioxide extraction of proteins. It was the first report of an active enzyme being extracted into liquid carbon dioxide with the aid of fluoroether surfactants. Recovery was accomplished by a reduction in pressure. That was an unusual presentation.

The Conference also contained an extensive and very well attended regulatory session organized by A. Bose (Pfizer) that featured Kathryn Zoon (FDA - CBER) commenting on the latest directions of the agency. Regulations affecting the nascent gene therapy area were also discussed.

In the end, our post-conference survey indicated that the right mix of quality science and informal discussion had been achieved. As always, it was the enthusiasm and commitment of the attendees that made the Southwest meeting a success.

Helmut Sassenfeld

Back to Canada. The 9th Conference in the Recovery Series took place in May 1999 in Whistler, British Columbia. It was a fabulous meeting with fantastic weather giving the best conditions both for the interchange of good science and enjoying contacts with new and old friends. A total of 235 scientists participated, and of these 85% had an active role in the meeting as presenters, chairs, sponsors and organizers, resulting in a very active and stimulating meeting.

One of the trends showing clearly in the program was the insight gained at the molecular level. Bioseparations R & D is gradually evolving away from its largely empirical history and increasing attention is paid to molecular level issues in conceiving and designing bioseparation processes. The program covered a range of issues and techniques, a notable common theme being the similarity of the methods used to those that are best known for their use in drug discovery. This kind of convergence is likely to persist as bioinformatics, genomics, proteomics and combinatorial methods continue to grow in importance.

Chromatography played a major part in the program – also starting at the molecular level. Intra-particle phenomena were visualised during protein adsorption, and by taking a detailed look into the stationary phase, experimental evidence may be obtained, which encourages improvements to current approaches to modelling and simulation. Modelling and simulation is not only an intellectually stimulating exercise, it is a powerful tool in process development: Steven Cramer had the special honour of demonstrating the usefulness of models.

The production of proteins in transgenic systems was part of the Industrial Case Studies session for the first time, illustrating that these alternatives are becoming mature. In one example 10,000 litre CHO cell culture is equivalent to 50 goats Also transgenic plant systems were presented yielding 0.5-2g protein/kg seed at $20 M for 300 kg/year. Of course the questions related to contaminants change dramatically going to plants: pesticides and herbicides become an issue instead of viral clearance issues.

The impact of the genomics area was a significant topic for the first time at a Recovery Conference highlighting the challenges in expressing cDNAs as the products of functional genomics programs. The paradigm of drug discovery has changed as a result of the efforts to advance the Human Genome

Project. These efforts have made large numbers of nucleic acid sequences, whose functions are unknown, available in both public and private databases. The primary challenge is to "mine" these databases using the tools of bioinformatics to highlight cDNAs that represent potential targets. A secondary challenge is, for a subset of these targets, to express and purify these as recombinant proteins for functional analysis. There is a strong emphasis on generic methods to make this possible. Helmut Sassenfeld lectured in 1986 on affinity tags, leading to the thought, that this technology would supplant all other downstream processing techniques. Clearly, that did not occur due to structural limitations of the approach. However, significant progress has recently occurred in this area, illustrated both by Georges Belfort's discussion of inteins - self splicing proteins, and Milton Hearn's presentation on ligands that are capable of recognizing secondary structure motifs of proteins, both illustrating the impact of process sciences on the possibilities for utilisation of genomics.

Inger Mollerup

Cancún, Mexico is the venue for the Recovery of Biological Products 10 meeting being held from June 3-8, 2001. This particular site was selected by the co-chairs for both it's natural beauty as well as it's unique connection to the ancient Mayan civilization. This is the 10th , anniversary meeting of this prestigious Conference Series and the first Recovery Conference of the 21st century. Further, it is the first Conference to occur after the completion of the Human Genome Project. We are clearly witnessing a major paradigm shift in the field of biotechnology and this meeting was structured to enable the participants to "learn for the future".

The R10 meeting differs from previous Recovery Conferences in several ways. Instead of having separate sessions on individual separation processes (e.g. chromatography, membranes, etc.) all unit operations were placed into two sequential sessions. In addition, parallel workshops were held to enable those who are more focused in the specific unit operations to delve deeper into the state of the art of these technologies. This opened up space in the program to include two special anniversary sessions with invited speakers. This is a pivotal point in biotechnology, we thought it was an appropriate time to look at lessons from the past as well as to learn for the future. In a session entitled "Lessons from the Past" the speakers will present historical perspectives as well as their visions for the future. Stephen Drew will discuss industrial bioseparations while Georges Belfort and Jan-Christer Janson will give their unique perspectives on membrane and chromatographic technology, respectively. A session on the theme of the conference, Learning for the Future, will expose the conference attendees to new technologies that are expected to have dramatic impacts on the new era of biotechnology. Bernhard Palsson will discuss the phase transition from *in vivo* to *in silico* biology, and Martin Yarmush will look at the future of cell and tissue engineering. Finally, Michael Heller will present his perspective on microelectronic array technology for bioanalysis and diagnostics.

While most of the focus of this conference in the past has been on process separations, it is clear that nano-scale separations and analytical biotechnology will play an increasing rôle in the intellectual content of this conference series. Additional topics covered during

this meeting will include combinatorial technology for bioseparations, process integration and optimization, post-approval process changes, gene therapy/plasmid and virus purification, scale-up challenges for recombinant biopharmaceuticals and the Industrial Case Studies session. In addition, a very successful session from R9 will be repeated at R10, namely the molecular science of bioseparations. Clearly, the content of this Conference has changed dramatically over the past 20 years.

The spirit of this Conference is to maintain the unique position of the Recovery Series as the pre-eminent conference on Recovery of Biological Products while expanding the vision of the Conference to include groundbreaking new technologies that will play a pivotal role in the rapidly changing field of biotechnology.

Although this is written before the actual Conference, the co-chairs are confident that this objective will be achieved and that this Conference will set the stage for future Recovery Conferences in the 21st century.

Steven Cramer

The following is an abstract of the Address "Moving downstream processing up to the front" given by Harvey Blanch at a Gala Dinner on 7 June 2001, to mark the 10th Anniversary of the Recovery Conference Series.

Shortly after the advent of recombinant DNA and hybridoma cell fusion technology, the first Recovery Conference in 1981 highlighted the impacts of these astonishing developments in the life sciences on fermentation and bioseparations. Academia and industry were also grappling with a dramatic shift in the rate at which laboratory advances were translated into new products and processes. New bioseparation methods were developed to address the high-purity requirements of recombinant therapeutic proteins, inclusion body isolation and refolding, the integration of fermentation and recovery steps, and an increasing requirement for enantiometrically-pure products.

Advances in bioseparations were driven by the fast-paced discovery of peptides and proteins for medical use, new applications of enzymes in synthesis, production of specialty (bio)chemicals, and to a lesser extent, commodity products such as fuel ethanol from renewable resources. New separations materials, such a chromatographic supports, membranes and extractants containing surfactants or ionic liquids, were developed for these products. Today a second revolution in the biological sciences is occurring. The increasing availability of complete genome sequences of prokaryotes, archaea and eukaryotes as a result of the human genome initiative will accelerate the discovery of new pharmaceuticals and biologics, and substantially impact agriculture and the manufacture of specialty and commodity chemicals. What will distinguish this transformation of genetics and biology is the parallel advance in materials science. The ability to control molecular architecture at various length scales will provide new routes to materials with targeted properties.

Nanoscale materials will clearly have a direct impact on genomics, proteomics and high-throughput processes. But advances in guest-host chemistries, molecular simulation, the ability to design "smart" materials for bioseparations (e.g., pH- or electrochemically-switchable membranes with nanoscale pores), and biomimetic materials that blur the distinction between organic and inorganic, will result in the development of new bioseparation technologies. These technologies can then be used as vital tools in the product discovery process itself. Bioseparation advances have typically relied on the development of new materials. With the molecular-level design of materials now possible, downstream processing will certainly move to the "front" end of product design, discovery, and production.

Harvey Blanch
University of California Berkeley, USA

We are grateful to all the companies and organizations that have sponsored Recovery Conferences and particularly those who have repeatedly supported the Series.

A
Abbott Laboratories
Abgenix, Inc.
American Cyanamid
Amersham Pharmacia Biotech Ltd.
Amgen, Inc.
Arris Pharmaceutical Corp
Aventis Behring L.L.C.
Aventis Pasteur

B
Baxter Healthcare Corporation
Bayer Corporation
Berlex Laboratories, Inc., Bioscience Division
BIA Separations d.o.o
Bioengineering AG
Biogen, Inc.
Bioprocessing Ltd.
Bio-Rad Laboratories, Inc.
BioReliance Corporation
BioSepra SA
Boehringer Ingelheim Pharma KG
Boehringer Mannheim
Bristol-Myers Squibb Company

C
Celltrix Pharm
Centocor
Chiral Technologies Inc.
Chiron Corporation
Cohesive Biotechnologies
Corixa Corporation
Creative Biomolecules

D
DSS Danish Separation Systems
Dyax Corporation

E
Eli Lilly and Company

F
Filtron Tech

G
Genencor International, Inc.
Genentech, Inc.
Genetics Institute
Geneworks L.L.C.
Genzyme Transgenics Corporation
Genzyme Corporation

H
Hoffman-LaRoche

I
Immunex Corporation
Integrated Biosystems, Inc.

K
Kendro Laboratory Products

L
Life Sciences
Life Technologies, Inc.
Lonza Biologics plc

M
Merck & Co., Inc.
Merck KGaA Darmstadt
Millipore Corporation

N
National Science Foundation
Neose Technologies, Inc.
Nestec Research Center
Novasep
Novo Nordisk A/S

O
Oxford Glycosystems

P
Pall BioPharmaceuticals
Pasteur Merieux
PerSeptive Biosystems, Inc.
Pfizer, Inc.
ProMetic BioSciences, Inc.

R
R&S Technology
Repligen Corporation
Rohm and Haas
Roussel Uclaf

S
Sachem, Inc.
Sartorius AG
Schering-Plough Research
Scios Inc.
Searle Division of Monsanto
Sepragen Corporation
SmithKline Beecham
Somatogen Inc.
Sterogene, Inc.
Systemix

T
Takeda Chemical Industries
Targeted Genetics Corporation
TechniKrom
The Separations Group
TosoHaas
Tosoh Biosep

W
Westfalia Separator AG
Whatman International Ltd.
Wyeth Lederle Vaccines

X
Xoma Corporation


RX
RECOVERY OF BIOLOGICAL PRODUCTS 10
CANCÚN MEXICO, 3-8 JUNE 2001