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## Welcome to APIPS-JPS 2004

It is my very great pleasure and honor to welcome you to the joint meeting of the First Asia-Pacific International Peptide Symposium and the 41st Japanese Peptide Symposium, APIPS-JPS 2004, to be held in Fukuoka, Japan from October 31 to November 3, 2004. The Japanese Peptide Society has decided to introduce a plan to organize future International Symposia on a triennial basis, and it is my pleasant duty that this new step has been scheduled to start in 2004 in Fukuoka. Meanwhile, we, the committee members, have a strong consensual appreciation that the Post-Genome Era serves to highlight the rapidly growing importance of peptides and proteins, underscoring the central contribution to be made by peptide research towards securing a fundamental understanding of all aspects of the molecular basis of peptide substances. Acknowledging the dawning of this new era, we have decided to make plans for International Symposia and to convene APIPS meetings in the Asian and Pacific regions also on a triennial basis. Thus, the first joint meeting of JPS and APIPS have been arranged to coincide in 2004 in Fukuoka, emphasizing the most recent developments and challenges in peptide science and related areas of research in this new era.

I participated in four different Peptide Symposia held in the Asia and Pacific area the past year. These were the 5th Australian Peptide Conference in Day Dream Island (October 5-10, 2003), the 40th Japanese Peptide Symposium in Kisarazu (October 28-30, 2003), the 7th Korean Peptide Symposium in Suwon (November 28, 2003), and the 8th Chinese International Peptide Symposium in Kunming (July 3-6, 2004). All these meetings already had a post-genomic flavor and I could find many friends in these different peptide communities. By drawing together this mood of optimism and many workers in the field, APIPS-JPS 2004 will, we hope, confirm for all to see that this new period has indeed come. For instance, the symposium includes three core programs: "Genome-



Yasuyuki  
Shimohigashi

based peptides and peptidomes", "The machinery of bioactive peptides and proteins", and "The molecular basis for bioactive conformations in peptides".

The symposium program covers recent topics in peptide syntheses, analyses, functions, conformation, structure-activity relationships, immunology, pharmaceuticals, nanosciences, peptidomics, and proteomics. The conference participants who will present these scientific topics will gather from all over the world, from more than 15 countries. Enthusiastic discussion is eagerly anticipated for each of these presentations.

On the first day of the meeting, a Citizen's Forum entitled "Amino Acids, Peptides, and Proteins Essential for Human Life and Welfare" will be held to inform the general public, including high-school students, about the importance of amino acids, peptides, and proteins in our everyday human life. The Forum will include six lectures delivered in Japanese as well as experimental demonstrations aimed at a general audience.

Fukuoka, located in Kyushu, the southernmost island in Japan, has a rich mixture of both modern and classical cultures, steeped in history and with some of Japan's most distinctive culture and cuisine. Fukuoka has flourished since ancient times as a city oriented towards international commerce, for which it has been aptly designated a Portal for the Asian and Pacific region. Standing in contrast to its long history, the striking line-up of modern culture along the seashore of Hakata Bay includes a city waterfront complex that houses the Fukuoka International Congress Center, the meeting place of the Symposium.

As members of Program and Executive Committees, a number of my colleagues in the University Laboratories and Research Institutes in Kyushu are actively working towards the organization of this Symposium, and also the students of my laboratory and other laboratories will be working with me to support the activities of the Symposium. We all sincerely welcome all investigators world-wide who work on peptides and related fields to join us at APIPS-JPS 2004.

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## Recent Development of Peptide Research in China

On behalf of my colleagues and Chinese Peptide Society, I am very happy to congratulate the glorious opening of The First Asian-Pacific International Peptide Symposium with good wishes on its great success. Since the world-first synthesis of insulin crystals by Chinese scientists on September 7, 1965, peptide research has advanced greatly in this country. The Chinese Peptide Symposium (CPS), started in Shanghai in 1990, is a biennial forum to share and discuss such progresses among leading international experts in peptide and protein researches. Following the success of 7th CPS in Dalian, the 8th Symposium took place on July 3-7, 2004 in Kunming. The scientific program was organized into nine sessions covering key areas of current peptide research, ranging from the design of peptidomimetics, peptide binding and signaling, synthetic methodologies, bioactive peptides, structure researches, anticancer and antiviral peptides, peptide microarray, to peptide pharmacology and therapeutics etc. There were 54 presentations and 128 articles at this Symposium. Here are highlights representing the main achievements and current trends of peptide research in China.

**Discovery of novel peptides.** The ultimate goal of peptide research is to serve the people by using peptidyl compounds. One of our top tasks is to discover new peptide drugs, whether it comes from native peptide or synthetic peptidomimetics. Although numerous peptide hormones and brain peptides have already been discovered, novel bioactive peptide is still of great interest. Drs. R.W. Huang and X.Z. Liu discovered recently two novel antifungal peptides, EAFP1 and EAFP2, from the bark of *E. ulmoides* Oliv. (Garrylaes order, Eucommiaceae family), commonly known as Du-Zhong, a hardy rubber tree. The EAFP molecules consist of 41 residues. Its N-terminus is blocked by pyroglutamic acid. An outstanding feature is the pairing of its ten cysteines into a unique disulfide motif. Bioassays showed that EAFP exhibited evident antifungal effects on a relatively broad spectrum of fungi, including a series of pathogens for cotton, wheat, potato, tomato and tobacco.

Dr. Qi and his colleagues of Shanghai Institute of Biochemistry found a novel scorpion toxin, BmSKTx1 (31 residues). It is active on both small conductance calcium-activated potassium channel and SKCa channels. More importantly, its selectivity to SKCa subfamilies was higher than other toxins and it employs a novel binding mechanism.



Yu-Cang Du

It is well known that a linear tripeptide Hemiasterlin from marine sponge is a potent cell growth inhibitor, which showed extremely high inhibitory activity to many kinds of advanced human cancer cell lines. Dr. J.K. Qie et al. investigated the relationships between the N- or C-terminal residue characteristics and the antitumor effects. A series of tripeptide analogs were synthesized with unnatural amino acids, 3-(4-N-morpholinemethylphenyl)-alanine and N-[(3-Azabicyclooctane)-1-carbonyl]-leucine in its N-terminus. Antitumor activities of the analogs were screened by MTT against human leukemia tumor cell K562, human ovarian tumor cell OVCA2780, human breast tumor cell MCF7, human gastric tumor cell Kato-III, and murine leukemia tumor cell P388. Some of the synthetic tripeptide analogs exhibited promising antitumor activities.

**Studies on structure and function.** Small toxins usually have tight structure by forming of inner disulfide bonds. For instance, VxII (21 residues) and VxVI (27 residues) are two novel conotoxins isolated from the worm-hunting cone snails by Dr. Fan from Beijing Institute of Pharmaceutical Chemistry. Both of them have three inner disulfide bonds. Nevertheless, recent studies have shown that  $\alpha$ -synuclein ( $\alpha$ -Syn), as well as amyloid  $\beta$ -protein (A $\beta$ ) and prion protein (PrP), is prone to aggregation depending upon conformation transition from  $\alpha$  to  $\beta$ . Based on CD study, Dr. H.Y. Hu and his colleagues of Shanghai Institute of Biochemistry indicated that a short 9-residue peptide segment in human  $\alpha$ -Syn (66-74) and GAV motif might play crucial roles in  $\alpha$ -Syn aggregation or fibrillization. The novel sequence may be exploited as targets for designing inhibitors that retard amyloidogenesis of GAV-motif related proteins and would likely mitigate progression of neurodegenerative diseases. The general research revealed that the  $\beta$ -sheet peptide system could undergo self-assembly into nanofibril structure following a cross-model. Dr. Y.L. Sha of Peking University synthesized an undecapeptide (T1) and found that it had  $\beta$ -hairpin forming potency and self-assembled into nanofibrils in solution. The mature fibrillar structure has a diameter of near 4 nm and lengths of several micrometers. The fibril is left-handed growing and it has helical pitch near 30 nm along the fibril axis.

There is strong evidence indicating that  $\alpha$ -MSH besides being a pigmentary hormone also plays a crucial role in the regulation of immune and inflammatory reactions. Dr. Y.P. Tian et al. recently reported a protective effect of  $\alpha$ -MSH on the mice with experimental allergic encephalomyelitis, a mouse model for human multiple sclerosis and found that  $\alpha$ -MSH has the suppressive effects on the development and the function of dendritic cell (DC) from the bone marrow of C57BL/6 mouse. They provided new evidences to immunomodulation and anti-inflammation

mechanisms of  $\alpha$ -MSH.

Based upon structure-activity relationship studies on endothelins antagonists, Dr. Liu had previously designed and synthesized linear hexapeptides and octapeptides containing unusual aromatic amino acids. Tripeptides were also designed and synthesized based on BQ-485. Some of them exhibited high antagonistic effect.

**Pharmacology of peptides.** Aglycin, a novel bioactive polypeptide, was extracted and purified from porcine intestine recently. It is chemically characterized as having an N-terminal Alanine and a C-terminal Glycine, thus coined Aglycin. The chain length of aglycin is 37 residues containing three intrachain disulfide bonds located at Cys3 to Cys20, Cys7 to Cys22 and Cys15 to Cys32, respectively. Aglycin is capable of lowering postprandial hyperglycemia of KK mice, the diabetes type II model animal. Dr. Z.W. Chen in Wuhan has found that aglycin can intensely resist hydrolyses by trypsin, pepsin and Glu-C proteases *in vitro* and observed that this peptide can prolong the life span of pancreatic beta-cells for three days comparing with the condition of routine incubation for this cell line. It would be very interesting to see if aglycin could be developed into novel drug for treatment of diabetes.

At present, no existing drug for the type 2 diabetes can slow, delay or reverse the progressive failure of the  $\beta$ -cells. Dr. Y.K. Sun has found that glucagon-like peptide 1 (GLP-1) and Exendin-4 not only stimulated insulin synthesis and secretion to reduce the blood glucose without hypoglycemic risk, but also increased the  $\beta$ -cell mass, the sensitivity of insulin receptor and reduced the HbA1c. Dr. Sun and his coworkers have recently developed a biotechnological process including construction of engineered bacteria, fermentation and downstream processing technology to produce the analogs of GLP-1 and Exendin-4 in pilot scale in Shanghai.

Looking ahead, more peptide drugs will be synthesized and provided for Chinese market to meet the social requirement in the near future. Since virus diseases, AIDS and SARS attacked this country in last year, it reminds us that we had better to pay more attention to exploit those peptides capable to interact with nucleotide, RNA or DNA. Fortunately, Dr. M. Yang and her colleagues of Peking University found that short peptides containing the basic region of the HIV-1 Tat protein bind specifically to a bulged region in TAR RNA and their results suggested that this kind of peptides as inhibitors of Tat binding to RNA may provide an important advantage for drug design. On the other hand, researches on the so-call peptide nucleic acid (PNA) are undergoing smoothly in a few laboratories.

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## Structure-Activity/Mechanism Relationships of Antimicrobial Peptides -Peptide Engineering Laboratory, RCPM-

As a member of the international organizing committee, it is my honor to welcome everybody participating the First Asia-Pacific International Peptide Symposium. I hope we can all enjoy the meeting and the city of Fukuoka. Like any other scientific meetings, I think one of the most important things to achieve is to strengthen our existing relationships among us and to establish new network, which is becoming more and more important for competitive scientific environment. It is also a great pleasure for me to introduce our laboratory and some of the research works presently going on in my group.



Kyung-Soo Hahm

Antimicrobial peptides have been recognized as an important component of the non-specific host defense system and innate immunity of insects, amphibians and mammals. Among the more potent antibiotics reported so far are small bioactive peptides like cecropin A, magainin 2, melittin, and plant defensin. These antimicrobial peptides possess a potent antibiotic activity against bacteria, yeast and even certain enveloped viruses. At present, quite a few number of antimicrobial peptides are reported and their antibiotic activities are related to joining of phosphatide membrane of target cell, and forming of ion channel or pore in cell membrane which leads to cell lysis by destroying cell membrane.

The peptide engineering laboratory focuses on the structure-activity/mechanism relationship studies in order to develop novel antimicrobial peptides which can be applied as therapeutics, agrochemicals, cosmetics, food additives, etc. These peptides were designed and synthesized and their structure-activity/mechanism relationships and synergism with known antibiotics were investigated by analyzing antibacterial (especially, oral- and food-borne pathogens), antifungal, and/or antitumor activities, secondary and tertiary structures utilizing CD and NMR spectra.

Antibacterial and antifungal activities were measured and expressed as MIC values using various gram positive or negative bacteria and fungi. And, for anti-viral activity, vPE 16, incorporated gene that encodes gp 120 and 41, which is an envelope protein that is crucial for HIV infection and HeLa/CD4+cell

for CD4 receptor to virus surface were used, and after cell fusion, syncytia forming is measured. Anti-tumor activity of the peptides was measured against tumor cells including AML-2/WT, AML-2/D100 and AML-2/DX100 for acute marrow leukemia and SNU-638 and SNU-668 for gastric cancer. In order to test the cytotoxicity of peptides, we measure hemolysis and select peptides that do not show any hemolytic effect.

For structural determination and mechanism study, CD spectroscopy was used in solvent, including phosphate buffer solution, TFE or SDS for secondary structure, and for the tertiary structure, NMR was used mainly. For the action of the peptides against membranes, liposomes such as large unilamellar vesicles (LUVs) with phosphatidyl choline (PC) and (PC)/phosphatidylserine (PS) (3:1) are used to treat with peptides and the phospholipid membrane disruption activity was measured as the amount of released fluorescent material such as carboxyfluorescein (CF). 1, 6-diphenyl-1, 3, 5-hexatriene (DPH) is also used to measure membrane dynamics. FACScan (Fluorescence Activated Cell Scanning and Sorting), SEM (Scanning electron microscopy), and Confocal laser scanning microscopy are also used to study the mechanism of action of the peptides.

In addition, we have been isolating novel antimicrobial peptides from various sources including potatoes, bacteria and fungi. These novel antimicrobial peptides possess a potent antibiotic activity against bacteria and fungi. Potide-J is isolated from the potato, *Solanum tuberosum* L cv. Jopung and consists of 10 amino acid residues and is not cytotoxic. Bacil-

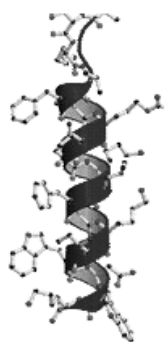
lin, a 10-mer peptide obtained from *Bacillus subtilis*, shows powerful antibacterial properties. And, ubidin (18-mer) from *Aspergillus niger* possesses potent antimicrobial activity and has ubiquitin-like *N*-terminal sequence.

We also predict the peptide structure by using computer, and design highly value-added peptides from this structure. Lately, we are introducing the peptidomimetics in order to develop more effective and applicable peptides. Moreover, our laboratory also maintains peptide library in the internet for our designed antibiotic peptides to provide information to people interested in this field (<http://www.chosun.ac.kr/~rcpm/>).

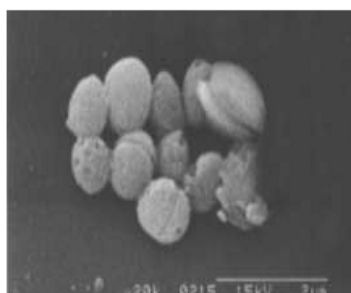
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NMR structure of a perfect amphipathic  $\alpha$ -helical antimicrobial peptide, Hp (2-20) analogue 3 (Anal 3).



Pore forming action of antimicrobial peptide (Anal 3) on *Staphylococcus aureus* as visualized by scanning electron microscopy (SEM).



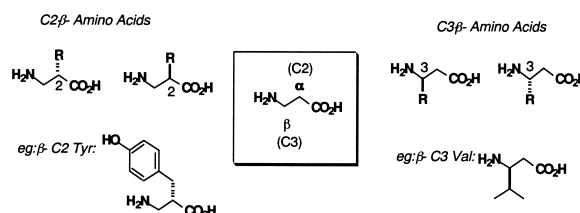
## Novel Approaches to the Design of Peptide Based Enzyme Inhibitors

Advances in peptide chemistry and biology continue to occur at an ever-increasing rate. This acceleration has been further enhanced following the decoding of the human genome together with access to the ever-increasing protein and peptide structural data being generated through sophisticated high throughput structural technologies. These developments shedding even more light on the role specific peptides and peptide motifs play in regulating biological function. Thus the discovery of new peptide entities, the development of new methods for functional and structural analysis together with new synthetic strategies, necessitates the need for peptide scientists from around the globe to meet, exchange ideas and further accelerate this rapidly moving field. The **1st Asian-Pacific International Peptide Symposium** provides an excellent opportunity for scientists from the Asia Pacific region as well as those from Europe and the US working in the peptides sciences to get together for formal and informal scientific interactions. The Australian peptide community is delighted to be involved and to have the opportunity to contribute toward what promises to be an outstanding scientific meeting, covering the many exciting developments in all areas of peptide science. I am particularly honoured to have the opportunity to present our work on the development of Novel Approaches to the Design of Peptide Based Enzyme Inhibitors.

It is well established that proteases and peptidases play a crucial role in many physiological and pathological events including apoptosis, blood clotting and hormonal regulation. Indeed, aberrant proteolytic events are known to be involved in many disease states such as hypertension, thrombosis, autoimmunity and viral invasion and neurodegenerative disease. Given the importance of proteases and peptidases in these various inter- and intracellular processes, inhibitors of these enzymes continue to serve as both molecular probes of major cellular networks as well as potential therapeutic agents for human disease. Although there have been many advances and successes in enzyme inhibitor design, new approaches to the development of inhibitors of different classes of enzymes are clearly required, particularly for the design of molecules which can differentially inhibit members of closely-related enzymes families (a major problem with the metalloproteases, for example). Indeed, both genomic and proteomic analysis is identifying a rapidly increasing number of



A. Ian Smith



target peptidases and proteases thus creating an urgent need for new approaches to accelerating the inhibitor design process. In this article we describe the application of beta amino acids substituted into peptide substrates as stable specific peptidase inhibitors.

-Amino acids have the potential to provide a new approach to the design of both peptide- and protein-mimetics and we believe that -amino acids could play an important role in the design of stable enzyme inhibitors and in generating molecules with selectivity between closely related enzymes, with a particular focus on the metalloproteases. The effect of incorporation of one additional carbon atom in the target molecule results in a powerful combination of peptide bond stabilisation and conformational manipulation not readily available with other classes of peptidomimetics. Given the similarity in the structure of -amino acids to -amino acids and the ability of -peptides to subtly change the binding affinity of peptides to their target, it is clear that their use in the design of potent enzyme inhibitors has enormous potential in the design of novel therapeutics to treat a variety of diseases.

We have recently developed a novel peptidomimetic strategy, which involves the strategic incorporation of -amino acids into peptides<sup>1,2,3</sup>. -Amino acids are similar to -amino acids in that they contain an amino terminus and a carboxyl terminus. However, in -amino acids two carbon atoms separate these functional termini as shown in Fig. 1. As such, -amino acids with a specific side chain can exist as the *R* or *S* isomers at either the (C2) carbon or the (C3) carbon, resulting in a total of 4 possible isomers for any given side chain (Fig. 1). The flexibility to generate a vast range of stereo- and regioisomers, together with the possibility of di- and poly-substitution, significantly expands the structural diversity of -amino acids while at the same time conserving the functional group, and provides enormous scope for molecular design. Furthermore, it is also evident that many more isomers are available in -amino acids than is possible for the corresponding -amino acids. -Amino acid-containing compounds now have significant potential in a range of peptide-related therapeutics, largely reflecting their resistance to proteolytic degradation. However, in two examples, as outlined below, we have demonstrated in a range of applications that incorporation of -amino

acids has been successful in creating peptidomimetics that are not only stable to enzyme hydrolysis, but also have potent biological activity in their own right<sup>1-4,6,7</sup>.

We have shown for example that substitution of  $\alpha$ -amino acids at the scissile bond of peptides can stabilize the peptide against hydrolysis with only a moderate decrease in enzyme affinity<sup>1,5</sup>. We have also demonstrated the potential of  $\alpha$ -amino acids as peptidomimetics in the design of selective enzyme inhibitors. In particular, the selectivity between closely related endopeptidases has been manipulated whereby affinity has been maintained for EP 24.15 and/or EP 24.16, while binding to EP 24.11 has been completely eliminated. Further, this has been achieved via changes in stereo- and regiochemistry of the  $\alpha$ -amino acids<sup>1,5</sup>. Together with their ease of incorporation into peptide synthesis strategies, this sets  $\alpha$ -amino acids apart from many other classes of peptidomimetics in terms of flexibility in molecular design.

Finally, the recent identification of nanomolar inhibitors for the *E. coli* aminopeptidase P (APP) by our group clearly demonstrates the utility of  $\alpha$ -amino acids inhibitor design. APP is a very specific metalloenzyme capable of cleaving peptides with an X-Pro amino acid sequence at their N-terminus. We thus synthesised a library of  $\alpha$ -substituted sets of Pro-Leu and Pro-Pro peptides. A number of compounds, specifically where the  $\alpha$ -substitution was at position 1, exhibited nanomolar inhibition against APP, indicating enzyme affinities orders of magnitude greater than the corresponding  $\alpha$ -dipeptides. Nevertheless, the different effects of some of these inhibitors also demonstrated the subtle effects of introducing extra carbon atoms on binding and inhibition and the potential for exploring differences in active site topography of closely-related enzymes.

Based on these results, we have shown that  $\alpha$ -amino acids represent a new and very viable peptidomimetic technology for the design of specific and potent substrate- and product-based peptidase inhibitors. We propose to further develop this approach to inhibitor design using focussed libraries of inhibitors based on substrate or product-based mimics incorporating  $\alpha$ -amino acids which will be screened for potent and selective inhibitors of other closely related metallopeptidases. This will not only further validate the utility of  $\alpha$ -amino acids in peptidomimetic inhibitor design but may also generate lead candidates for both functional probes and perhaps therapeutic applications. This new technology has we believe, the potential to significantly impact on the field of drug design by providing a new approach to accelerating the identification of lead compounds and their subsequent development into highly potent and stable drug candidates.

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## Synthetic Insulin Superfamily Research in Melbourne

For more than 20 years, I have worked as a synthetic peptide chemist at the Howard Florey Institute at the University of Melbourne, Australia, principally in the field of relaxin. This is a two-chain peptide member of the insulin superfamily and is characterized



John D. Wade

by the presence of the three insulin disulfide crosslinks. In most mammals, relaxin (also known as relaxin-1) has a key role in reproduction and causes a relaxation of the connective tissues in the birth canal during parturition thus enabling easier passage of the young. Relaxin-1 has since been demonstrated both in cell culture experiments and *in vivo* models to regulate proliferation, angiogenesis and connective tissue remodelling through activation of molecular targets such as vascular endothelial growth factor and matrix metallo-proteinases. Remarkably, the receptor for relaxin-1 is a G-protein coupled receptor known as LGR7 and which contains a large extracellular domain with multiple leucine-rich repeats. This is in contrast to other members of the insulin superfamily, namely insulin and insulin-like growth factors 1 and 2 which have protein tyrosine kinase receptors. More recently, with the availability of the human genome sequence, our laboratory identified a novel human relaxin (relaxin-3) in the Celera Genomic database. The predicted peptide sequence retains all the key structural features of a relaxin peptide and detailed mRNA screening studies have subsequently shown to be a brain peptide. Recently, colleagues at Johnson & Johnson Pharmaceutical Research laboratories (USA) have made the surprising finding that relaxin-3 has its own G-protein coupled receptor known as GPCR135 which differs structurally and functionally from LGR7. Our laboratory has been actively engaged in chemically preparing each of the human relaxins with the aim of undertaking structure-function studies to determine the molecular basis of their receptor specificity and biological activity. Curiously, whereas relaxin-2 can be readily prepared by simple combination in solution of each of the two synthetic S-reduced A- and B-chains, such methods do not work for relaxin-3. Consequently, we have had to devise novel regioselective disulfide bond formation methods in which each of the three disulfide bonds are formed sequentially. Such methods work well in our hands and multi-milligrams of synthetic relaxin are acquired. Together with our collaborators, we have also recently completed a tertiary structural study of relaxin-3 which shows key differences between the peptides and which will allow us better elucidate the determinants of receptor specificity.

Our experience in this area of expertise has extended to another member of the insulin superfamily known as insulin-like peptide 3 (INSL3). This was originally identified in the testes of the boar and later shown by others in gene knockout studies to be essential for the maturation of a ligament that controls the descent of the testes during male fetal development. Female homozygous mice show impaired fertility associated with abnormal oestrus cycle length. Overexpression of the INSL3 protein in female mice causes the ovaries to descend into the inguinal region due to an over-developed gubernacu-

lum. Like relaxin, the receptor for INSL3 is also a leucine-rich G-protein coupled receptor known as LGR8. Together with our collaborator, Aaron Hseuh at Stanford University, we have used synthetic INSL3 and its biotinylated form to show that LGR8 is expressed in oocytes in ovary and in male germ cells in the testis. Further, we have shown that LH stimulates INSL3 transcripts in those cells. Thus INSL3, in turn, binds LGR8 expressed in germ cells to protect follicles and corpora luteal cells from entering the apoptosis pathway and may play a role in follicle selection. Other researchers have shown that both INSL3 and its receptor are present in tumour cells including in the mammary gland, thyroid and testes. Additionally, LGR8 has been found to be expressed in various other tissues, including brain, thyroid, testis, ovary, kidney and bone marrow. However, the roles of the INSL3 in these tissues remain unknown.

The future of synthetic peptide science is very bright. The recent deciphering of the human genome and the corresponding discovery of many new peptides and proteins together with their post-translationally modified forms leaves no doubt that much remains to be achieved in our understanding of the mechanism of action of these biomolecules. There is also no doubt that synthetic peptide chemistry will continue to have a key role to play in such studies. One of the great advantages of the technology is allowing the preparation of peptide analogues that contain functional modifications that cannot otherwise be obtained by recombinant DNA methods. However, it is important that the next generation of peptide researchers appreciates that the field is vibrant, exciting and rewarding and one that represents a fulfilling career option. Towards this aim, conferences such as the 1st Asian Pacific International Peptide Symposium are vitally important forums for fostering such interest and eventual adoption of peptide chemistry as a life long passion.

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## Fibrillar Assemblies of the Peptide Fragments Derived from Amyloidogenic Proteins

### Abstract

Fibrillization of amyloidogenic proteins is implicated in association with some neurodegenerative diseases. Our recent research is focusing on the mechanism of fibrillar assemblies of proteins by applying peptide fragments derived from



Hong-Yu Hu

$\beta$ -synuclein as well as other amyloidogenic proteins. The observations show that both the core sequence and the unstructured region are required for  $\beta$ -synuclein fibrillization. Structural transformation often concurs with the fibrillar assembly, and  $\beta$ -sheet formation from random coil structure is predominant in fibril formation. A circular dichroic method has been developed to measure the secondary structures of peptides and proteins in solid state that are structurally analogous to the amyloid deposits.

## Introduction

Alzheimer's disease, Parkinson's disease and Prion disease are common neurodegenerative disorders characterized by the deposits of insoluble protein inclusions in human brains<sup>1</sup>. Studies on the molecular mechanism have revealed that aggregation or fibrillization of  $\beta$ -amyloid peptide (A $\beta$ ),  $\beta$ -synuclein ( $\beta$ -Syn) and prion protein (PrP) are strongly associated with the fatal neurodegeneration<sup>2,3</sup>. The fibrillar deposits of these amyloidogenic proteins are extremely toxic to neural cells that then cause the brain loss of function<sup>4</sup>. Thus, exploring fibrillization of these amyloidogenic proteins in molecular level will help us understand the pathogenesis of these diseases and develop the strategies for clinical treatment. Homologous peptide fragments of the disease-related proteins have been commonly used in identifying the fibrillization core<sup>5</sup>, mimicking the structural transformation<sup>6</sup> and elucidating the mechanism of amyloidogenic aggregation<sup>7</sup>. This review will describe our recent studies on protein fibrillization by use of homologous peptide fragments.

## Structural transformation of the peptide fragments from the reactive center loops of serpins

Serpin (serine proteinase inhibitor) family has a unique ability to regulate its activity by dramatic structural transformation in the reactive center. The crystal structures of serpins revealed that a long flexible loop or loop-helix-loop constitutes the reactive center and its conformation varies strikingly, which implicates that the flexible reactive center loop is in relation to the aggregation of serpins and the pathogenesis of diverse diseases<sup>8</sup>. To further understand the structural transformation of the novel reactive center<sup>9</sup>, we studied two peptide fragments homologous to the reactive centers of ovalbumin (OVARC) and plasminogen activator inhibitor-1 (PAIRC)<sup>10</sup>. OVARC shows a nascent helical structure in aqueous solution, and its helical content increases under acidic conditions, while PAIRC is in a random-coil form. Hexafluoroisopropanol (HFIP) prompts the helicities of the peptides in solution. The novel structural transformation from random coil or nascent helical structure in aqueous solution to the  $\beta$ -helix in HFIP may reflect the conformational polymorphism

of the serpin reactive centers and is implicated in the structural features of the amyloid aggregates.

## The core role of the GAV motif in $\alpha$ -Syn fibrillization

$\beta$ -Synuclein ( $\beta$ -Syn) is a presynaptic protein possibly involved in synaptic plasticity and neurodegeneration<sup>11</sup>. Amyloid-like fibrillization of  $\beta$ -Syn and deposit in Lewy bodies are associated with the pathogenesis of Parkinson's disease<sup>12</sup>. We studied the secondary structural transformation and fibrillization of human  $\beta$ -Syn and its N- or C-terminally truncated fragments in TFE<sup>13</sup>. The NAC (non-amyloid component) containing proteins (intact  $\beta$ -Syn,  $\beta$ -Syn<sub>100</sub>) undergo a three-state transition: from native random coil to  $\beta$ -sheet and then to  $\beta$ -helical structure, while the NAC deficient fragments ( $\beta$ -Syn<sub>60</sub>,  $\beta$ -Syn) undergo a typical two-state coil-to- transition. The  $\beta$ -sheet form is highly hydrophobic that strongly binds to ANS and ThT (thioflavin T), and is prone to self-aggregation. The observations suggest that the NAC sequence ( $\beta$ -Syn<sub>61-95</sub>) is essential to  $\beta$ -sheet formation and the fibrillization originates from the  $\beta$ -sheet intermediates.

$\beta$ -Syn contains a novel short sequence (namely GAV motif) that is implicated in initiating amyloidogenic fibrillization. We obtained several fragments derived from  $\beta$ -Syn (Fig. 1A). The experimental results show that, like full-length  $\beta$ -Syn, the synthetic GAV motif-containing fragments ( $\beta$ -Syn<sub>1-74</sub> and  $\beta$ -Syn<sub>61-140</sub>) and the GAV peptide are prone to aggregating into fibrils and the fibrillar deposits are toxic to PC12 cells, whereas deletion of the GAV sequence ( $\beta$ -Syn<sub>1-65</sub> and  $\beta$ -Syn<sub>61-74</sub>) completely eliminates fibrillization and neural toxicity<sup>14</sup>. There is a structural transformation from random coil to  $\beta$ -sheet-dominant structure for the GAV motif-containing fragments. This strongly demonstrates that the GAV motif is essential to  $\beta$ -Syn fibrillization and cytotoxicity.

## $\alpha$ -Syn, A $\beta$ , and PrP share a consensus core sequence for initiating fibrillization

Although the three amyloid-prone proteins,  $\beta$ -Syn, A $\beta$ , and PrP, show little homology in full-length sequence, sequence alignment suggests that they share a short consensus sequence<sup>15,16</sup>, normally VGGAVVAGV (Fig. 1). We named this consensus sequence as GAV motif<sup>16</sup>, because it contains mainly three kinds of amino acid residues, glycine (G), alanine (A) and valine (V). The homologous sequences are from the central segment of  $\beta$ -Syn (66-74), the C-terminus of A $\beta$  peptide (36-44) and the conversion region of PrP (117-125). Since the GAV motif sequences are resided in the unstructured regions of these proteins, it is speculated that the GAV segments are conducive to structural transformation from random coil to  $\beta$ -sheet, which is likely accom-



panied by amyloid fibrillization. Our recent studies indicate that not only the core sequences but also the unstructured regions where the core sequences are embedded is required for amyloidogenic fibrillization.

When the three sequences are attached to the C-terminus of  $\alpha$ -Syn<sub>1-65</sub> (Fig. 1B), the resultant hybrid proteins ( $\alpha$ -CT, PrP-CT and GAV-CT) become amyloidogenic. They readily aggregate into fibrils and partly transform into  $\beta$ -sheet structures<sup>17</sup>. This means that the nine-residue peptide segments originating from other amyloidogenic proteins can also induce the non-amyloidogenic  $\alpha$ -Syn<sub>1-65</sub> to form amyloid fibrils, which further demonstrates that the GAV motif acts as a critical core for some amyloidogenic proteins.

### $\beta$ -Sheet formation of peptide fragments in solid state

Elucidating the structures of the amyloid aggregates is the prerequisite for understanding the mechanism of disease pathogenesis. We developed an applicable circular dichroic methodology to investigate the solid-state secondary structures of some peptides and proteins associated in thin films<sup>18</sup>. Among the peptides we tested, insulin, lysozyme, DsbA protein, luciferase and OVARC peptide fall into one group; they show no or slight structural rearrangement from solution to solid state. Another group, including BSA, ovalbumin,  $\alpha$ -Syn, PAIRC pep-

tide and A  $\beta$  peptides undergo structural transformation with an increase of  $\beta$ -sheet structure in solid state. The  $\beta$ -sheet formation of PAIRC peptide may reflect the structural transformation of the serpin reactive center that is relevant to the inhibitor activity; and the  $\beta$ -sheet structure of  $\alpha$ -Syn in solid state may correspond to the amyloid-like aggregates found in amyloid deposits (Lewy bodies, amyloid plaques) in patients' brains. Interestingly, salmon calcitonin (sCT) presents a typical  $\alpha$ -helix in solid state while human calcitonin (hCT) forms a  $\beta$ -sheet structure, although both of them adopt random coil structures in aqueous solution<sup>19</sup>. This may explain why hCT aggregates more readily than sCT, and provide information for developing potent calcitonin analogues by reducing aggregation and prompting biological activity for clinical usage.

### Conclusion

Amyloid-like aggregation or fibrillization of peptides and proteins is strongly associated with cellular dysfunctions, which are relevant to the devastating neurodegenerative diseases. Our finding shows that both the core sequence and the unstructured region are required for  $\alpha$ -synuclein fibrillization. Further work will gain insights into the morphology of these peptide fibrils in nanoscale<sup>20</sup>, which may provide information for designing anti-amyloid drugs.

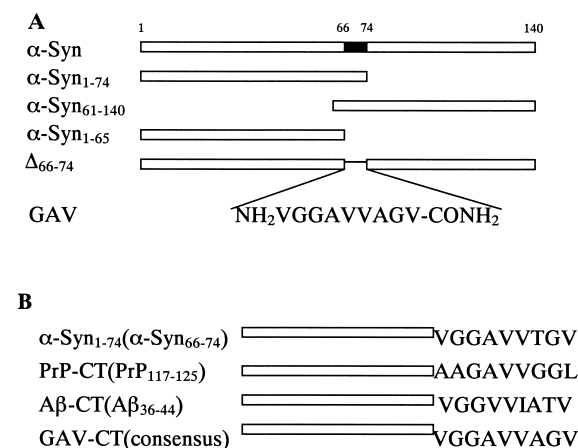


Fig. 1 Amino-acid sequences of the peptide fragments used in this work.

A) Truncations of  $\alpha$ -synuclein.  $\alpha$ -Syn: full-length  $\alpha$ -Syn, 140 residues;  $\alpha$ -Syn<sub>1-74</sub>: N-terminal 74 residues;  $\alpha$ -Syn<sub>61-140</sub>: C-terminal 80 residues;  $\alpha$ -Syn<sub>1-65</sub>: N-terminal 65 residues;  $\Delta$ <sub>66-74</sub>:  $\alpha$ -Syn with deletion of 66-74 residues; GAV: chemically synthetic peptide corresponding to residues 66-74 of  $\alpha$ -Syn.

B) Amino-acid sequences of the core region from  $\alpha$ -Syn (66-74), PrP (117-125), A  $\beta$  (36-44) and their consensus GAV sequence. These sequences were engineered attaching to the C-terminus of the  $\alpha$ -Syn<sub>1-65</sub> host.

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## Peptide Synthesis in the Era of Protein Chemical Synthesis

Peptides are fascinating biologically active molecules. In peptide science, the development of methods for the chemical synthesis, structural engineering and functional discovery of peptides are of high value. Moreover, the advancement of such synthetic methods in terms of reliability, efficiently, and scope is still required, and will underpin peptide-based research & discovery efforts. Although chemoselective ligation methodologies for reproducible chemical protein synthesis have been firmly established<sup>1</sup>, it must not be overlooked that stepwise-solid-phase peptide synthesis (SPPS)<sup>2</sup> lays the foundation for such endeavors, and in part, still imposes limitations on its scope. In this short letter, one of our published contributions aimed at the refinement or expansion of the art of peptide chemistry are described.

A major challenge with Merrifield's SPPS<sup>2</sup> approach has historically been the establishment of synthetic routes to homogeneous products of defined covalent structure. A main advantage of Merrifield's solid-phase approach is the exclusion of intermediate isolation or purification steps during peptide assembly. At the same time, one of the main disadvantages is the gradual accumulation of impurities from incomplete transformations or side-products at each step. Arguably, the most complicated step in SPPS is the final global side-chain deprotection and resin-cleavage step. In contrast to the repetitive coupling and deprotection steps, which usually occur exclusively at the *N*<sup>α</sup>-terminus, final global side-chain de-

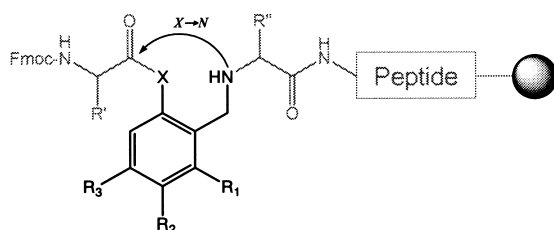


Les P. Miranda

protection and resin-cleavage occurs collectively at many more sites of reactivity thorough the peptide. Furthermore, this overall cleavage process exploits several cleavage mechanisms to remove common side-chain protecting groups, and also generates several types of reactive species that can in turn modify the target peptide. Elucidation of the nature of deleterious side-reactions in peptide synthesis, such as those derived from acylium ions, is an important key to the improvement of contemporary SPPS. In *tert*-butyloxycarbonyl (Boc)-based solid-phase synthesis, final global side-chain deprotection and resin-cleavage is performed in anhydrous hydrogen fluoride (HF). Under these conditions glutamyl acylium ions (among other species) are efficiently formed from protected or unprotected glutamyl or long-chain carboxylic acids and will form side-products if not scavenged<sup>3</sup>. Until relatively recently, comprehensive analysis of complex crude synthetic mixtures represented a significant challenge and was quite labor intensive. Today, with routine accessibility of powerful analytical techniques in many laboratories, such as LC/MS and MS/MS, data on peptides and peptide derivatives can be acquired thoroughly and quickly. In our glutamyl acylium ion work<sup>3</sup>, LC/MS and electrospray Fourier-transform ion cyclotron resonance mass spectrometry (FT-MS)<sup>4</sup> analyses, in combination with more traditional techniques (e.g. NMR), were used as tool to gain insight into this chemical system; and allowed for the correct identification of a key side-product leading to a new scavenging condition that, on the one hand, rapidly traps acylium ions, and on the other hand, enables the reconversion of these trapped products into the target peptide via a mild chemical procedure. Going forward, chemists armed with increasingly sophisticated and powerful analytical tools amendable to the analysis of synthetic peptide, such as FTMS, will be able to elucidate the nature of unexplored side-products/reactions that were previously too difficult or numerous to study. Ultimately, effort in these areas will lead to more appropriate synthetic peptide procedures, cleaner chemistry, higher quality crude peptides, and the extension of the scope of chemical protein synthesis. Furthermore, this task provides an excellent basis and opportunity for the in-depth education and training of young peptide chemists on the art of peptide chemistry that has evolved to make the chemical synthesis of small proteins a reality.

Complementing deleterious side-reactions, the phenomenon of "difficult" sequences has plagued the history of SPPS, often resulting in low yields and poor homogeneity of peptides >15 residues. Even though considerable advances in coupling methods, resin properties, and the choice of coupling solvents have been made, the firm establishment of a universal method for the robust and clean synthesis of pep-

tides still remains a considerable challenge. Nevertheless, one of the most powerful strategies used to combat “difficult” sequences is reversible backbone substitution introduced by Sheppard and co-workers in the form of the 2-hydroxy-4-methoxybenzyl (Hmb)  $N^\alpha$ -auxiliary<sup>5</sup>.



<b>Hmb</b>	1. X = OH,	R <sub>1</sub> = H,	R <sub>2</sub> = H,	R <sub>3</sub> = OCH <sub>3</sub>
	2. X = OH,	R <sub>1</sub> = H,	R <sub>2</sub> = NO <sub>2</sub> ,	R <sub>3</sub> = H
<b>Hnb</b>	3. X = OH,	R <sub>1</sub> = NO <sub>2</sub> ,	R <sub>2</sub> = H,	R <sub>3</sub> = H
	4. X = SH,	R <sub>1</sub> = H,	R <sub>2</sub> = H,	R <sub>3</sub> = H
	5. X = SH,	R <sub>1</sub> = H,	R <sub>2</sub> = NO <sub>2</sub> ,	R <sub>3</sub> = H
	6. X = Cl,	R <sub>1</sub> = H,	R <sub>2</sub> = NO <sub>2</sub> ,	R <sub>3</sub> = H

Reversible backbone substitution involves  $N^\alpha$ -substitution of one or more amide bonds several residues before the identified or expected “difficult” sequence in a peptide is encountered. Amide-backbone substitution appears to prevent or disrupt troublesome intermolecular hydrogen bonding networks by the removal of the native amide hydrogen bond after N-substitution, and also by alteration of the backbone conformation caused by the introduction of tertiary amide bonds. Unfortunately the scope of the strategy with the Hmb backbone chemistry group is somewhat limited. It has been observed that the acyl capture and acyl transfer reaction between adjacent small residues such as glycine or alanine proceeds well with the Hmb group and has been performed successfully many times, but when more sterically demanding residues such as phenylalanine, leucine, and valine are encountered, the efficiency of N-acylation can decrease to unacceptable levels<sup>6</sup>. Working on the premise that the low acylation problem was not entirely due to incomplete *O*-acylation of Hmb auxiliary (acyl capture) but also poor *O*  $\rightarrow$  *N* acyl transfer reaction kinetics, we explored the utility of mildly activated N-benzyl auxiliaries to improve acyl transfer rates and yields in hindered difficult peptide synthesis. We examined mercapto- and nitro- substituted benzyl-based auxiliaries, in particular the 2-hydroxy-6-nitrobenzyl (Hnb) auxiliary<sup>6</sup>. After performing a series of comparative acyl transfer experiments, we found significant improvements in  $N^\alpha$ -acylation yield in comparison to the Hmb group in hindered acyl transfer reactions with nitro-substituted auxiliaries which could also be used to overcome difficult sequences containing  $\beta$ -branched amino acids. The applicability of these auxiliaries to peptide cyclization has also been examined with constrained peptides, such as pentapeptides<sup>7</sup>, where initially a more favored large ring structure is first formed by

bridging peptide termini through the auxiliary moiety followed by a ring-contraction (acyl transfer) process to enable cyclo-amidation. The extension of the auxiliary concept for peptide ligation is also attractive and has been pursued by several laboratories. Currently, although auxiliary-mediated ligation can be performed at relatively simple sites (e.g. Gly-Gly), considerable more advancement of the chemistry is required to match the robustness and elegance of the native chemical ligation (NCL) process developed in the Kent laboratory<sup>1</sup>.

Peptide synthesis strategies have historically been based on the principle of orthogonal protection and modulated lability<sup>8</sup>. However, the differential protection of several functional groups of similar reactivity in peptide synthesis remains a major challenge with conventional protecting-group strategies, and it is not altogether surprising the number of instances chemists will essentially run out of sufficiently different chemistries when endeavoring to design a complex solid-phase synthesis. This demand for selective deprotection conditions is counteracted by the intrinsic lack of diverse and yet compatible reaction conditions for the clean and efficient removal of one protecting group over another in a given system. To explore a new approach to this problem we devised a strategy termed unichemo protection (UCP)<sup>9</sup>, which in principle only requires a single chemical process for all deprotection reactions. In more detail, UCP protecting groups are derived from a repetitive unit that permits their controlled and efficient step-wise removal. Functional site selectivity is achieved by varying the degree of oligomerization at each site, and, after each deprotection cycle, only the newly liberated functional site is available for derivatization. Oligomeric UCP groups have been used for the protection of amino groups<sup>9</sup> and hydroxyl groups<sup>10</sup>, but advancement in the robustness of chemistry is needed, as well as, the extension of the protection strategy to other groups such as thiols and carboxylates. Although in its relative infancy, we envision the refinement of UCP will open new opportunities in peptide synthesis, the combinatorial synthesis of highly substituted scaffolds, template-assisted synthetic proteins (TASP), oligosaccharide synthesis, and the general goal of automated organic synthesis.

The future of peptide chemistry is certainly bright, and even though the total chemical synthesis of small proteins is now an exciting reality, there are still many advances in the chemistry of peptide synthesis to be made.

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### In memoria m Masahiko Fujino 1931-2004

Dr. Masahiko Fujino, an honorary member of the Japanese Peptide Society died of lung cancer on June 22, 2004 at the age of 72.



He was born on November 7th, 1931 in Yamanashi Prefecture, graduated from Hokkaido University and entered Takeda Chemical Industries Ltd. in 1958. As a graduate student he participated in whale insulin research under Professor Tsuneyuki Saito.

He carried out antibiotics research at Takeda and received his Ph.D. from the Faculty of Science of Hokkaido University in 1963. In 1964 he became interested in bioactive peptides as future pharmaceuticals and joined Dr. Sakakibara's group at the Protein Research Institute of Osaka University to learn peptide synthesis. He later joined Professor Ward's group of The University of Texas MD Anderson Cancer Center where he studied the characterization of proteins and peptides. While working under Professor Ward, he unraveled the structure of glycoprotein Luteinizing Hormone (LH) and discovered that LH consists of two different linear peptides.

In 1966 he returned to Japan and organized a peptide research team in the chemical research laboratories of Takeda and he began synthetic research on Gastrin and ACTH. But after the chemical structures of TRH and LHRH were elucidated by Dr. Gulemin *et al.* and Dr. Schally *et al.*, he changed his research target from ACTH to these hypothalamic peptide hormones.

He considered the methodology of peptide synthesis at that time was not suitable to produce high quality peptides on a manufacturing scale, and this led him to develop protecting groups (isobornyloxycarbonyl for amino group and methoxybenzenesulfonyl and 4-methoxy-2,3,6-trimethylbenzenesulfonyl for Arg), deprotection methods (methanesulfonic acid) and cou-

pling strategies (N-hydroxy-5-norbornene-2,3-dicarboximide (HONB)-DCC, HONB active ester and cyclohexanone oxime method).

Using HONB-DCC method, he developed a very short synthetic route to TRH, in which all the intermediates and the final product, TRH tartrate, were obtained as crystals. TRH is now used as a therapeutic agent for the treatment of spinocerebellar degeneration and traumatic cerebral injury.

Through synthetic research on LHRH derivatives he found a highly potent analog (super agonist), pGlu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHCH<sub>2</sub>CH<sub>3</sub>, which was called Fujino's modification product by Dr. Gulemin. After formulation for drug delivery, the analog was launched as LEUPLIN, which is used as an anti-neoplastic and antiendometriotic agent all over the world.

At the beginning of 1990s progress in gene technology made him pay attention to a new field. He organized research groups to study orphan G-protein coupled receptors (GPCRs) and their ligand fishing, which led to the establishment of a new method to find therapeutic pharmaceuticals. A lot of new peptides, including Prolactin releasing peptide (PrRP), Apelin, Metastatin, Galanin-like peptide (GALP), RFRPs and NPW/NPB and several orphan GPCRs were discovered as candidates for future medicines by the groups.

Through his continued activities in bioactive peptide research he received a Purple Ribbon Medal in 1999 and a Japanese Peptide Society award in 2001, respectively.

Dr. Fujino was an active member of the Peptide Symposium of Japan from its 2nd meeting and he was recommended to be an honorary member of the Japanese Peptide Society in 2001. In 1997 he proposed to found a prize of the Japanese Peptide Society to promote the development of peptide research in Japan.

At the annual meeting of the Japanese Peptide Society and through the Peptide Newsletter of Japan (PNJ), he continued to send out the message to all the members of the Society, that joint research among peptide scientists is the most important strategy to develop peptide research in Japan.

Chieko Kitada  
 Takeda Chemical Industries, LTD.

### In the Memory of Dr. Murray Goodman

In the beginning of last June many e-mails circled the world relating the sad news that Professor Murray Goodman had passed away in Munich, Germany on June 1, 2004. We





were told that he had taken ill with a respiratory infection during his trip with Mrs. Goodman, Zelda. Earlier he had attended, as usual, the Capri Meeting in Italy after visiting Israel. However, when he arrived in Munich he was seriously ill with pneumonia and died with his family by his side.

I was shocked and grief stricken by his sudden death, at the age of 75, and could not believe it to be true for some time. Without hesitation I decided to attend his funeral to personally extend our condolences to the family, and made travel arrangements.

The funeral was held on July 7<sup>th</sup> at the Temple Beth El, which is just south of the UCSD campus on Gilman Drive. In the morning before the ceremony, I visited his office in Pacific Hall in the UCSD campus and met with Joseph Taulane; his assistant and laboratory director, and Robyn Swanland; his secretary. We shared our deep sorrow. A great silence had settled over his laboratory. There were some red Dharma dolls (達磨), which are Japanese tumblers for luck, setting by the window where he had left them. On the wall, there were many certificates and awards proclaiming his many remarkable accomplishments such as the Ralph Hirschmann Award in Peptide Chemistry, the Pierce (Merrifield) Award, the Herman F. Mark Polymer Chemistry Award and an award from the Japanese Society of Polymer Science. The last one, dated May 11, 2004, was the Chancellor's Associates Recognition Award for Excellence in Graduate Teaching from UCSD. That award not only reminded me of how proud Murray had been of the more than eighty graduate students whom he had trained, but also reminded me of how much he would be missed as a teacher and mentor.

We drove to the temple which was modern but solemn. The temple, which has a capacity of several hundreds, was filled to capacity with mourners and many more people were standing outside. Among them, I met Prof. and Mrs. Scheraga, Charles Deber, Victor Hruby, Arthur Felix and Stanley Opella.

Following the Rabi, ten people eulogized the deceased. They related that he was a religious man and a true *mensh*. For example, he was the president of the Congregation Beth El and his leadership was essential during the construction of the temple. He also channeled a lot of his energy into social work. Where did he find the spare time and energy to devote to such activities? He was so busy with demanding academic activities, journal editorships, symposia, etc. Throughout the eulogies, the specific word mentor was used many times to celebrate his life. I learned about the scientific family that he mentored. Not only were there more than eighty graduate students in his scientific family, but also he was proud of the more than two hundred postdocs and visiting scientists from all over the world who had

worked in his lab. Many, such as Caludio Toniolo, Luis Moroder, Ettore Benedetti, Andrea Temussi and others, are now leaders themselves in the peptide and polymer fields. Among the Japanese associated with the Goodman lab are the names of Shinsuke Yamashita, Masao Kawai, Ryouichi Katakai, Ken'ichi Ueyama, Yuji Nishiuchi, Shin'ichiro and Kumiko Kumagaye, Toshimasa Yamazaki, and others.

It was rather unusual for me to hear speakers introduce jokes that evoked laughter in a funeral oration. But I quickly understood that it was impossible to describe Prof. Goodman's personality without reflecting on his good humor. As his son, Andy, said Murray always amused his family, friends and collaborators with a good joke and humor. That was just one of many qualities mentioned that made him such an outstanding human being.

After the nearly two-hour ceremony at the church, interment, was at El Camino Memorial Park in San Diego, which is located on the outskirts of La Jolla. Students and postdocs studying in Dr. Goodman lab served as pallbearers. The remains, in a simple unvarnished wooden coffin, were laid to rest in a grave surrounded by a beautiful well-tended lawn. Then each of us shoveled soil on top of the coffin. This ritual goodbye was emotional for me because we have become familiar with memorial services after cremation.

After the interment Mrs. Goodman invited everyone back to her home for refreshments. Family, relatives, colleagues and friends gathered one last time to remember Murray. Zelda talked to everyone and thanked everyone for coming. When she talked to me, I expressed condolences for myself and from my Japanese colleagues. Drs. Shumpei Sakakibara, Tet-suo Shiba, and Terutoshi Kimura had personally asked me to relay their deepest sympathies. I also expressed my gratitude for all of his kindness that Murray had shown me in life. I told her that I feel very fortunate to have known him. She graciously accepted our sympathy and appreciated it. Although there was a traditional shivah (a period of religious mourning) later, I refrained from attending and returned to the hotel with Prof. and Mrs. Scheraga.

I can still remember the occasion when I first met Dr. Goodman. It was at the American-Japanese Peptide Conference that was held in the Institute for Protein Research, Osaka University, in 1980. It was at a round table discussion. At that time he was very interested in high field NMR, which had to be used to determine conformations of bioactive polypeptides. Because I was also fascinated by the capabilities of NMR, we enjoyed discussing conformational analysis. I remember that we continued to talk with each other common interests. One topics that we talked about was the relatively new method of water suppression and the reliability of the signal intensity

after water suppression. Ever since then Murray treated me like a friend and we shared other common interests. We were both interested in application of various physico-chemical techniques to the analysis of structure-function relationships of peptides. His achievements in organic chemistry were many. He developed synthetic methods and applied them in the field of peptidomimetics, which was another of his primary interests. He pioneered the use of peptidomimetics to modify the conformational propensities of peptides for designing drugs and new biomaterials. He had recently completed the five-volume compendium of *Synthesis of Peptides and Peptidomimetics* (Houben-Weyl, 2002-2003) as editor-in-chief working with Arthur Felix, Luis Moroder and Claudio Toniolo. He had often mentioned to me what a difficult job that was and told me how happy he had been to finally finish it. Many books, journals, publications and awards bear the name Murray Goodman. His contributions to peptide and polymer chemistry were numerous.

Murray Goodman was well known as the Editor-in-chief of *Biopolymers* and as a member of the editorial board of the *Journal of Peptide Research* (formerly the *International Journal of Peptide and Protein Research*). He was also known as a founder of *Peptide Sciences*. He kindly invited me to be a member of the editorial board of *Biopolymers* in 1998. I have thoroughly enjoyed working with Murray and he not only taught me many things about editing scientific journals but also interpersonal relationships. He always helped and promoted young scientists. In addition he was very supportive of peptide societies in Asia. For example he helped me by giving an opportunity to present my research, as an invited lecturer, at the APS meeting in La Jolla in 1989. I believe that led to my receiving the John Pisano Memorial Award from NIH. Now as I have to look forward to editing the journal without him, I will try to do my best to follow in his footsteps by thinking of what he would have done.

When I close my eyes and picture Dr. Goodman, the picture that comes to mind is Murray coming into a symposium session and taking a front row seat. After the talk he asks one or more of those penetrating, but instructive, questions. That is followed by a memorable comment. Then I open my eyes and see that we have lost a great friend, mentor and leader. Murray, you will be missed by all. Thank you for everything that you taught me and good bye. 合掌

〔 Yuji Kobayashi 〕  
〔 Osaka University 〕

## 【Symposium】

### 5th Peptide Forum (JPS)

Cooperation between Academia and Industry  
January 29, 2005  
Toyama International Conference Center  
Shin Ono: shinono@eng.toyama-u.ac.jp

### 6th Peptide Forum (JPS)

Explore the Links between Peptide Science and Macromolecular Chemistry  
February 11, 2005, Hokkaido University  
Kazuyasu Sakaguchi: kazuyasu@sci.hokudai.ac.jp  
Motoyoshi Nomizu: nomizu@ps.toyaku.ac.jp

### 42th Japanese Peptide Symposium

October 27-29, 2005, Osaka  
wakamiya@chem.kindai.ac.jp  
www.peptide-soc.jp/42jps (under construction)

### 8th Korea Peptide Symposium

November 5, 2004, Ewha Woman's University  
www.korean-peptide.com  
syhan@mm.ewha.ac.kr

### 9th Chinese Peptide Symposium

Shanghai, July 3-6  
Contact information: bnpc@mail.sioc.ac.cn

### 6th Australian Peptide Conference

October 9-14, 2005, Hamilton Island, Queensland  
www.peptideoz.org

### 19th American Peptide Symposium

June 18-23, 2005, San Diego, California  
www.AmPepSoc.org

### 29th European Peptide Symposium

September 3-8, 2006, Gdansk, Poland  
www.29eps.univ.gda.pl

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