Structure-Activity Studies - New Peptides
Topic C16: Pharmacology of Peptides

P444 A synthetic peptide creates a new strain of prion disease in transgenic mice
Ball Haydn L*, Kaneko Kiyotoshi, Baskakov Ilia, Wille Holger, Prusiner Stanley B, DeArmond Stephen J, Baldwin Michael A, Cohen Fred E.

P445 New cholecystokinin CCK_2 agonists displaying highly favourable "CCK_{2B}" properties in vivo
Bellier Bruno*, Daugé Valérie, Million Marie-Emmanuelle, Crété Dominique, Beslot Françoise, Pommier Blandine, Roques Bernard P, Garbay Christiane.

P446 Single amino-acid substitutions in a mimetic tetradecapeptide agonist of the human $P2Y_2$ receptor prevent second messenger activation
Brown Julia*, Howl John, Martin Ashley, Langel Ulo, Soomets Ursel, Brown Colin.

P447 Characterization of potent new vasopressin analogs to study vasopressin V_{1b} receptors
Derick Sylvain*, Andres Miriam, Guillon Gilles, Cheng Lingling, Stoev Stoytcho, Manning Maurice.

P448 A discontinuous HIV-gp 120 C3/c4 domain derived, branched, synthetic peptide that binds to CD4 and inhibits MP-1 binding
Howie S E M, Cotton G J, Heslop I, Martin N J, Harrison D J, Ramage R*.

P449 Differential signalling pathways upon activation of rat and mouse cholecystokinin A receptor
Ibarz Géraldine*, Poosti Roya, Gagne Didier, Martinez Jean.

P450 The role of opiod receptors and NMDA receptors in analgesic action of TRH
Kharitonov Alesey V, Guseva A A, Romanovskis P Ya.

P451 Isolation of genes differentially over-expressed on gastrin treated astrocytoma cells and their implication on cell motility
Kucharczak Jérôme*, Pannequin Julie, Martinez Jean.

P452 Molecular and biological studies of the middle portion of endothelin-1
Langlois Chantal*, Tessier Sophie, Brkovic Alexandre, Fournier Alain.

P453 Ion channel activation by SPC3, a synthetic peptide inhibitor of HIV infection
Mabrouk Kamel*, Carlier Edmond, Moulard Maxime, Rochat Hervé, Van Rietschoten Jurphaas, De Waard Michel, Sabatier Jean-Marc.

P454 The effect of divalent ions on binding and signal transduction in cells having stably expressed oxytocin receptor
Maletinska Lenka*, Kuncarova Pavla, Prochazka Zdenko, Gimpl Gerald, Slaninova Jirina.

P455 Vasoactive intestinal peptide receptor (VPAC1) is desensitized and internalized independently of its phosphorylation at consensus sites for PKC and PKA
Marie Jean-Claude, Rouyer-Fessard Christiane, Maoret Jean José, Lorinet Anne-Marie, Couvineau Alain, Laburthe Marc.

P456 Inhibition of vasopressin binding by C-terminal phosphorylated peptide segment of $G\alpha q/\alpha 11$ GTP-binding protein
Muller Dany, Schmit Pierre-Olivier, Pascal Robert*, Guillou Laurent, Guillon Gilles, Dufour Marie-Noëlle, Mendre Christiane.

P457 Lipophilic modifications of bradykinin agonists
Nardi Elena*, Chelli Mario, Ginanneschi Mauro, Meini Stefania, Quartara Laura, Altamura Maria R, Maggi Carlo A, Formaggio Fernando, Toniolo Claudio, Broxterman Quirinus B, Rovero Paolo, Papini Anna M.

Structure-Activity Studies - New Peptides
Topic C16: Pharmacology of Peptides

P458 An analogue of the glycine-extended bombesin induces its biological activities by interacting with the GRP/bombesin receptor.
Oiry Catherine, Pannequin Julie, Bernad Nicole, Artis Anne-Marie, Galleyrand Jean-Claude* , Devin Chantal, Cristau Michèle, Fehrentz Jean-Alain, Martinez Jean.

P459 Characterization of non CCK-A, non CCK-B binding sites using the C-terminal heptapeptide of Gastrin (G-7) on human astrocytic tumoral cell lines
Pannequin Julie*, Oiry Catherine, Kucharczak Jérôme, Camby Isabelle, Kiss Robert, Galleyrand Jean-Claude, Martinez Jean.

P460 Synthesis and biological evaluation of new h/rCRH peptide analogs
Papazacharias Spyridon, Pairas Georges*, Kouimtzoglou Elena, Dermitzaki Eirini, Manessi-Zoupa Evy, Gravanis Achilleas, Cordopatis Paul.

P461 The third intracellular loop of the rat and mouse cholecystokinin-A receptors is responsible for different patterns of gene activation
Poosti Roya*, di Malta Laure*, Gagne Didier, Bernad Nicole, Galleyrand Jean-Claude, Escrieut Chantal, Silvente-Poirot Sandrine, Fourmy Daniel, Martinez Jean.

P462 Biosensor analysis for mapping the discontinuous interleukin-10 / interleukin-10 receptor α binding site
Portwich Michael*, Reineke Ulrich, Sabat Robert, Schneider-Mergener Jens, Volkmer-Engert Rudolf.

P463 Functional responses to kinins in a novel bovine hepatic artery cell line
Reading Sarah J, Jones Sarah, Howl John, Martin Ashley, Alexander Barry, Benjamin Irving S, Brown Colin A.

P464 Effect of a daunomycin-polypeptide conjugate in multidrug resistant tumor cell lines
Reményi Judit, Hegedüs Tamas, Sarkadi Balazs, Hudecz Ferenc.

P465 Analogs of [L-(pEt)PHE^2] or [D-(pEt)PHE^2] Oxytocin having an α-helix inducing amino acid Aib or β-Ala in position 3 or 7 or 9
Slaninova Jirina*, Nazarov Elsan S, Kuncarova Pavla, Zertova Miroslava, Koumentakos Stamatis, Magafa Vassiliki, Pairas George, Theodoropoulos Dimitrios, Cordopatis Paul.

P466 Structural modifications of highly potent bradykinin antagonists and their pharmacological consequences
Stewart John M*, Gera Lajos, York Eunice J, Chan Daniel C, Bunn, Jr Paul A.

P467 Studies of the interaction between TAT arginine rich domain peptides and TAR RNA HIV-1 by capillary electrophoresis
Szyk Agnieszka, Mucha Piotr, Barciszewski Ja, Rekowski Piotr*.

P468 Pharmacological studies of photolabile ligands derived from TTA-386, a selective ET_A receptor antagonist
Tessier Sophie*, Langlois Chantal, Brkovic Alexandre, Coupal Martin, DeLéan André, Fournier Alain.

P469 The "PYY-prefering" receptor in rat jejunal crypt cells: a peripheral Y2 receptor ?
Voisin Thierry*, Goumain Mathieu, Lorinet Anne-Marie, Laburthe Marc.

OC41 Design of vasopressin and oxytocin agonists, antagonists, radioiodinatable and fluorescent ligands with good affinities for human receptors
Manning Maurice*, Cheng Lingling, Stoev Stoytcho, Durroux Thierry, Mouillac Bernard, Guillon Gilles, Morin Denis, Derick Sylvain, Barberis Claude.

Structure-Activity Studies - New Peptides
Topic C16: Pharmacology of Peptides

OC45 New amino-acidic antagonists of the excitatory amino acid receptor
Paruszewski Ryszard*, Strupinska Marzanna, Stables James P.

OC59 Inhibition of the Ubiquitin-proteasome pathway in Alzheimer disease
Lam Y Amy, Pickart Cecile, Alban Andrew, Landon Michael, Jamieson Craig, Ramage Robert*, Mayer R John, Layfield Robert.

OC81 RXP 407, a selective inhibitor of the N-domain of angiotensin I-converting enzyme, blocks the in vivo degradation of the hemoregulatory peptide Acetyl-Ser-Asp-Lys-Pro with no effect on angiotensin I hydrolysis
Junot Christophe*, Cotton Joël, Gonzales Marie-Françoise, Michaud Annie, Vazeux Gilles, Vassiliou Stamatia, Yiotakis Athanasios, Azizi Michel, Ezan Eric, Corvol Pierre, Dive Vincent.

OC82 Glucagon receptor interactions with two $G\alpha_s$ splice variants
Unson Cecilia G*, Wu Cui-Rong, Merrifield R B.

Topic D: Combinatorial Chemistry

P470 Combinatorial synthesis of cyclic RGD derivatives by solid phase Heck coupling
Akaji Kenichi*, Aimoto Saburo.

P471 Hydroxyproline-containing 2,5-diketopiperazines : synthesis on different solid supports and characterization by HR-MAS NMR
Bianco Alberto*, Furrer Julien, Limal David, Guichard Gilles, Elbayed Karim, Raya Jésus, Piotto Martial, Briand Jean-Paul.

P472 Identification of new antagonists of HIV-1 infectivity from synthetic combinatorial libraries
Blondelle Sylvie E*, Reixach Natalia, Boggiano César.

P473 Backbone cyclic CD4 mimetic peptides: Novel anti HIV-1 drug leads
Cohen Shirra*, Briant-Longuet Laurence, Denisova Galina, Devaux Christian, Gershoni Jonathan, Gilon Chaim.

P474 Optimization of potency, stability and cell permeation of pseudoproline leads from soluble peptide libraries as inhibitors of S-farnesyl transferase
Fauchère J L*, Henlin J M, Kucharczyk N, Desmet-Beaufort C, Loynel A, Bertrand M, Ginot Y M, Gordon B, Genton A, Tucker G C, Boutin J A.

P475 Substituted and non-substituted guanidines introducing diversity for combinatorial chemistry
del Fresno M, Royo M*, El-Faham A, Carpino L A, Albericio F.

P476 Solid phase synthesis of restricted alanine surrogates
Garcia Monica*, Rubiralta Mario, Diez Anna, Segarra Victor, Lozoya Estrella, Ryder Hamish, Palacios José M.

P477 Sequencing of individual peptides from combinatorial libraries via specific generation of chain-terminated sequences
Hoffmann Christian*, Blechschmidt Dierk, Karas Michael, Griesinger Christian.

P478 Preparation of indexed library of amides and oligopeptides by means of triazine condensing reagent immobilized on cellulose
Kaminski Zbigniew J*, Kolesinska Beata, Cierpucha Maciej.

P479 Cellulose-bound peptide libraries as tools for kinase drug discovery programs
Müller Carola, Reineke U, Schtkowski Mike*, Germeroth Lothar.

P480 Arrays of peptides & carbohydrate molecules on self-assembled monolayers and their application in blood serology
Ortigao Flavio Ramalho*, Mecklenburg Michael, Galanina Oxana, Klingel Sven, Cieplik Michael, Pfeiffer Matthias, Videnov Georg, Bovin Nikolay, Nifant'ev Nikolay.

P481 4-amino-3-(aminomethyl)benzoic acid : a novel amino acid for pseudopeptide synthesis and combinatorial chemistry
Pascal Robert, Sola Régine, Labéguère Frédéric, Jouin Patrick*.

P482 Novel high loaded spacer polymers in peptide synthesis and combinatorial chemistry
Rapp Wolfgang*, Wegmann Jürgen, Albert Klaus, Kallus Christoph.

P483 Microwave assisted parallel synthesis of aminoacids using poly(ethylene glycol) as solvent and polymeric support
Sauvagnat Bérengère, Varray Stéphane, Lamaty Frédéric*, Lazaro René , Martinez Jean.

P484 Solid supported parallel synthesis of dimers libraries
Subra Gilles*, Amblard Muriel, Durand Philippe, Komesli Sylvianne, Renaut Patrice, Martinez Jean.

Topic D: Combinatorial Chemistry

P485 Proteinchip arrays : a rapid method for screening combinatorial peptide mixtures
Warder Scott E*, Schenone Monica M, Martin J. Andrew, Prorok Mary, Castellino Francis J.

OC42 Structure-based design of the first high potent inhibitors of botulinum B-toxin
Anne C*, Turcaud S, Cornille F, Fournié-Zaluski M-C, Roques B P.

OC68 Combinatorial split synthesis libraries of glycopeptides for the identification of high affinity ligands for carbohydrate receptors
Meldal Morten*, Halkes Koen, St Hilaire Phaedria.

OC69 The question of mixtures in combinatorial libraries : the balance between completeness and efficiency - single compound arrays, small mixtures or large mixtures ?
Houghten Richard A*.

OC80 Combinatorial libraries for sensor arrays
Jung Günther*, Tünnemann Rolf, Leipert Dietmar, Mack Jürgen, Wiesmüller Karl-Heinz.

Topic E: Miscellaneous

P486 Evolutionary principles for generating protein mimetics: directed assembly of peptide loops on topological templates
Banfi Damiano, Mutter Manfred, Patiny Luc*.

P487 Influence of backbone modification on the hybridization potency of peptide nucleic acids (PNAs)
Ehrlich Angelika, Heyne Hans-Ulrich, Beyermann Michael, Dathe Margitta, Bienert Michael*.

P488 Tritium labeling of an Oxytocin antagonist
Flouret G*, Chaloin O.

P489 Novel concept of resin polymerization : preparation of bifunctionalized polyethylene glycol (PEG) based resins by reductive amination
Groth Thomas*, Grotli Morten, Lubell William, Miranda Les, Meldal Morten.

P490 New insight into the stereospecificity of the intestinal H^+/peptide symporter
Hartrodt Bianka*, Theis Stephan, Börner Volker, Knütter Ilka, Born Ilona, Brandsch Matthias, Daniel Hannelore, Neubert Klaus.

P491 Investigation of non-specific cleavage of a recombinant human monoclonal antibody during papain digest
Kalbag Suresh*, Tan Jason, Truong Long, Harris Reed, Gong Christopher, Karunatilake Chulani.

P492 Solid phase synthesis of thymosin beta-15 and investigation of its biological activity in the chorioallantoic membrane angiogenesis model
Koutrafouri V, Leondiadis L, Ferderigos N, Avgoustakis K, Livaniou E, Evangelatos G P, Ithakissios D S.

P493 Molecular dynamics of the gelsolin 150-169 binding to phosphoinositide lipids
Liepina Inta*, Czaplewski Cezary, Janmey Paul, Liwo Adam.

P494 A new fluorescent probe to mimic lipophilic moieties for interaction studies of bioactive lipopeptides with membrane models
Peroni Elisa, Caminati Gabriella, Baglioni Piero, Chelli Mario, Papini Anna Maria*.

P495 New data on generation of hemoglobin derived peptides by erythrocytes
Philippova Marina M, Blishchenko Elena Yu, Karelin Andrei A, Ivanov Vadim T.

P496 Synthesis of glutathione amide, the main low molecular weight thiol of Chromatium species
Vergauwen Bjorn, Jacquemotte Françoise*, Van Beeumen Jozef.

P497 Cellular delivery of oligonucleotide-peptide chimeras and preliminary pharmacological studies by MALDI-TOF spectrometry
Vivès Eric*, Sauveplane Manuel, Vasseur Jean-Jacques, Lebleu Bernard.

P498 Pseudoprolines in drug design: cyclosporin C derivatives as a novel class of active site inhibitors of cyclophilin A
Wöhr Torsten, Keller Mike, Patiny Luc, Guichou Jean-François*, Mutter Manfred.

P499 Bioactive peptides obtained from natural library
Yoshikawa Masaaki*, Yunden Jinsmaa, Yan Shuzhang, Tsuruki Takahiro, Lipkowski A W.

OC33 A novel group of peptidic biological response modifiers
Blishchenko Elena Yu*, Karelin Andrei A, Ivanov Vadim T.

OC58 Calcitonnin derived peptide carriers are novel tools for selective drug delivery
Beck-Sickinger Annette G*, Krauss Ulrike, Meinecke Martina, Merkle Hans Peter.

OC77 Reductive samariation as a means for the selective C-alkylation of glycine residues: application to the synthesis of unnatural peptides
Ricci Marina, Blaksjaer Peter, Skrydstrup Troels*.

Lectures and Oral Communications

L 01 Monday Morning: Berlioz Auditorium L 02

The proteasome and ATP-dependent proteolysis
Robert Huber
Max-Planck Institut für Biochemie, D-82152 Martinsried

Structural [1,2] and mutational [3-7] studies of the 20S proteasome from *T. acidophilum* and *S. cerevisiae* defined architecture, subunit maturation, substrate binding, enzymatic mechanism, and regulation in atomic detail. They are also a basis for structure based design and development of specific inhibitors [8]. Structural studies of HslV [9] and HslVU [10] provide a first view at basic mechanisms underlying ATP dependent regulation of proteolytic activity.

1. Löwe, J., Stock, D., Jap, B., Zwickl, P., Baumeister, W. and Huber, R. Crystal structure of the 20S proteasome from the archaeon *T. acidophilum* at 3.4 Å resolution. (1995) Science **268**: 533-539.
2. Groll, M., Ditzel, L., Löwe, J., Stock, D., Bochtler, M., Bartunik, H.D. and Huber, R. Structure of the 20S proteasome from yeast at 2.4 Å resolution. (1997) Nature **386**: 463-471.
3. Seemüller, E., Lupas, A., Stock, D., Löwe, J., Huber, R. and Baumeister, W. Proteasome frrom *Thermoplasma acidophilum*: a threonine protease. (1995) Science **268**: 579-582.
4. Ditzel, L., Huber, R., Mann, K.-H., Heinemeyer, W., Wolf, D.H. and Groll, M. Conformational constraints for protein self-cleavage in the proteasome. (1998) J. Mol. Biol. **279**: 1187-1191.
5. Nussbaum, A., Dick, T., Keilholz, W., Schirle, M., Stefanovic, S., Dietz, K., Heinemeyer, W., Groll, M.,, Wolf, D., Huber, R., Rammensee, H.G. and Schild, H. Cleavage motifs of the yeast 20S proteasome β subunits deduced from digests of enolase 1. (1998) Proc. Natl. Acad. Sci. USA **95**(21): 12504-12509.
6. Jäger, S., Groll, M., Huber, R., Wolf, D. and Heinemeyer, W. Proteasome β-type subunits: unequal roles of propeptides in core particle maturation and a hierarchy of active site function. (1999) J. Mol. Biol. **291**: 997-1013.
7. Groll, M., Heinemeyer, W., Jäger, S., Ullrich, T., Bochtler, M., Wolf, D.H. and Huber, R. The catalytic sites of 20S proteasomes and their role in subunit maturation: A mutational and crystallographic study. (1999) Proc. Natl. Acad. Sci. USA **96**: 10976-10983.
8. Loidl, G., Groll, M., Musiol, H.J., Huber, R. and Moroder, L. Bivalency as a principle for proteasome inhibition. (1999) Proc. Natl. Acad. Sci. USA **96**: 5418-5422.
9. Bochtler, M., Ditzel, L., Groll, M. and Huber, R. Crystal structure of heat shock locus V (HslV) from *Escherichia coli*. (1977) Proc. Natl. Acad. Sci. USA **94**: 16070-6074.
10. Bochtler, M., Hartmann, C., Bourenkov, G.P., Bartunik, H.D. and Huber, R. The structure of HslVU and the mechanism of ATP-dependent proteolysis (submitted).

MULTIVALENT INHIBITION OF EUKARYOTIC PROTEASOMES AND HUMAN β-TRYPTASE
Luis Moroder, Norbert Schaschke, Günther Loidl, Michael Groll, Gabriele Matschiner,[a] Christian P. Sommerhoff,[a] Wolfram Bode, Robert Huber, Max-Planck-Institut für Biochemie, 82152 Martinsried; [a]Abt. für Klin. Chemie und Klin. Biochemie, LMU, 80336 München, Germany.

In nature, multivalency is a general principle that is used to enhance via the entropy effect both selectivity and avidity in bimolecular recognition processes.[1] With the discovery of the multicatalytic proteases proteasome and tryptase and with their structure elucidation by X-ray crystallography[2,3] it became obvious to attempt an application of this principle to the design and synthesis of inhibitors that address the characteristic geometry of the spatial arrays of the catalytic sites in the digestion chambers of these protease complexes. From the X-ray structures the distances between the geometrically arranged active-sites were determined and molecular modeling served for the design of suitable spacers of two identical or different binding heads. Taking into account the free access to the digestion chamber of the tryptase and the impeded entrance in the case of the protesome that recruits from outside only fully unfolded linear proteins for digestion, PEG of defined length as mimic of an unfolded polypeptide chain was selected as spacer for peptide aldehydes to construct double-headed inhibitors of the proteasome, whereas β-cyclodextrin served as rigid core to present to the recognition by tryptase two 3-(aminomethyl)-benzenesulfonyl-glycine moieties. Whilst with the monovalent inhibitor constructs both enzyme complexes were poorly inhibited by the non-selective binding heads, the principle of bivalency was realized with the double-headed constructs with remarkably enhanced selectivity and avidity which in the case of the bivalent cyclodextrin-based inhibitor reached almost the theoretical exponential cooperativity value of 1.9 *vs* β = 2 as expected from theory.[1]

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[2] Groll, M., Ditzel, L., Löwe, J., Stock, D., Bochtler, M., Bartunik, H.D. & Huber, R. (1997) *Nature* **386**, 463-471.
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OC 01 Pasteur Auditorium - Bioactive Peptides OC 02

STRUCTURAL INDUCING PROBES (SIP) - BLOWS NEW HOPE INTO THE GENERAL USE OF PEPTIDES AS DRUGS.
Bjarne D. Larsen[a], Lene H. Jensen[b], Niels E. Mørk[a], Morten J. Bjerrum[c], Axel Meissner[d], Gerd Nielsen[d], Sven Frokjaer[b]; [a]Zealand Pharmaceuticals A/S, DK-2600 Glostrup Denmark, [b]The Royal Danish School of Pharmacy, DK-2100, Copenhagen Denmark,[c]The Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark, [d]Carlsberg Laboratory DK-2500, Valby Denmark.

One of the most obstructive biological barrier hindering a general use of peptides as drugs is the lack of resistance of the peptide bond towards enzymatic degradation.

Proteases and other proteolytical enzymes are ubiquitous, therefore peptides are usually susceptible to degradation in multiple sites. Furthermore, a given peptide is usually susceptible to degradation at more than one linkage within the backbone. Thus, peptide drugs must be, not only suitably protected against degradation, but also in more than one anatomical site for them to reach their target sites intact.

The aim of the present work is to develop a derivatisation capable of protecting the entire peptide backbone against enzymatic degradation. The strategy used to accomplish this goal is based on the use of a new technology "structural inducing probes" (SIP), which are capable of disrupting the enzyme/substrate recognition process by forcing the parent peptide to make intramolecular hydrogen bonds which make the enzymatically labile peptide bonds hindered or inaccessible for the individual proteases.

In vitro data on enzymatic stability as well as data from a structural investigation of SIP-model peptides clearly demonstrating the advantage of the SIP-technology will be presented.

THE ANTITUMOR SOMATOSTATIN ANALOG (TT232) INDUCED SIGNALING CASCADES
György Kéri, Attila Steták, Tibor Vántus, Gyöngyi Bökönyi, Péter Csermely, Jackie Vandenheede[b] Axel Ullrich[c], János Seprodi, Aniko Horváth, István Teplán, Zsolt Szegedi[c], Béla Szende[e] Department of Medical Chemistry and [c]1st Inst. Pathology, Semmelweis Medical University, [d]Natl. Inst of Oncology, Budapest, Hungary, [b]Dept. of Biochem, Catholic University of Leuven, [c]Max Planck Inst. for Biochemsitry, Martinsried, Germany

TT232 is a heptapeptide structural derivative of somatostatin, which has strong in vitro and in vivo antitumor activities and induces apoptosis in tumor cells. Some in vivo tumor models were found to be especially sensitive for TT232 treatment with a strong apoptotic response. Regarding the signaling mechanism we demonstrated that TT232 inhibits tyrosine kinases after a long term incubation, while it activates tyrosine phosphatases in short term incubation. Further elucidating the short term signaling events we found that TT232 activates PI3 kinase on a biphasic fashion. We measured the phosphorylating cascade of various PI3 related proteins and found significant changes in the phosphorylation of various proteins. We also measured the effect of TT232 on the RAS-RAF-ERK pathway and found that it interferes with the Epidermal Growth Factor induced mitogenic pathway causing alterations in the phosphorylation state and in the activation of several MAPK cascade components such as ERK2, Raf-1, p38RK. We investigated the possible role of various caspases (1,3,7) in the TT232 induced apoptosis and found no activation of caspases, and could not inhibit the apoptotic effect with caspase selective peptide inhibitors. On the other hand we have demonstrated the possible role of a chymotrypsin like Ser/Thr protease in mediating the apoptotic effect of TT232. Some other data on fragmentation of certain proteins and effect on some transcription factors also suggest that TT232 induces apoptosis on a caspase independent pathway.

Lectures and Oral Communications

OC 03 Pasteur Auditorium - Bioactive Peptides OC 04

HYDROXAMIC ACID ANALOGS OF NATURALLY-OCCURRING CYCLIC TETRAPEPTIDES, TRAPOXIN, WF-3161, CYL-1, HC-TOXIN AND CHLAMYDOCIN INHIBIT HISTONE DEACETYLASES

Norikazu Nishino[a,b], Kin-ya Tomizaki[a], Makiko Tsukamoto[a], Daisuke Yoshikawa[a], Ryuzo Shinta[a], Hidekazu Nishino[a], Yuji Tanaka[b], Tamaki Kato[a,b], Yasuhiko Komatsu[b,c], Makoto Nishiyama[d], Ryohei Furumai[b,e], Minoru Yoshida[b,e]

[a]Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Japan; [b]CREST, JST; [c]Pharmaceuticals and Biotechnology Laboratory, Japan Energy Corporation, Japan; [d]Biotechnology Center, The University of Tokyo; [e]Department of Biotechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Japan.

A unique amino acid, (2S,9S)-2-amino-8-oxo-9,10-epoxydecanoic acid (L-Aoe) in variously bioactive cyclic tetrapeptides, Trapoxin, WF-3161, Cyl-1, HC-toxin and Chlamydocin was substituted with ζ-hydroxamido-α-aminosuberic acid (Asu(NHOH)). The cyclic hydroxamic-acid-containing peptides (CHAPs) potently (IC_{50} = 3.3~21 nM) inhibited mammalian histone deacetylases. They are also active in enhancement of MHC class 1 protein production of B16/BL6 mouse melanoma cells. Through the design of more than a hundred of CHAPs, we will discuss the conformation-specificity relationship and the possibility in the use as anticancer drugs.

OPTIMIZATION OF VASOACTIVE INTESTINAL PEPTIDE TOWARDS BETTER TUMOR IMAGING PROPERTIES

Sarah Bhargava[a], Tobias Knaute[a], Andreas Becker[b], Kai Licha[b], Jens Schneider-Mergener[a] and Rudolf Volkmer-Engert[a]

[a] Institut für Medizinische Immunologie, Universitätsklinikum Charité, Humboldt-Universität zu Berlin, Schumannstr. 20/21, D-10098 Berlin; [b]Institut für Diagnostikforschung GmbH an der FU Berlin, Spandauer Damm 130, D-14050 Berlin

The vasoactive intestinal peptide (VIP) is overexpressed on the surface of numerous tumor cells. Therefore VIP labeled with carbocyanine dyes can be used as a contrast agent for the near-infrared optical imaging of tumors accessible to fluorescent endoscopic techniques. Proteolytic degradation *in vivo* limits the diagnostic use and suggests the need for structurally optimized VIP derivatives with improved pharmacokinetics.

For that purpose we have prepared a substitutional peptide library of VIP on cellulose membranes allowing cleavage of the peptides with authentic C-termini. These peptides were directly tested for receptor binding by flow cytometry providing a detailed analysis of amino acid positions essential for receptor binding. The binding studies lead us to the following assertions: (1) key residues are mostly located in the C-terminal region; (2) substitutions with negatively charged amino acids decrease binding affinities substantially and (3) replacement of the majority of residues by proline and glycine is not tolerated.

Molecular modelling on the free form of VIP suggests a largely helical structure of the peptide in aqueous solution. This is in good agreement with the substitutional pattern implying a helix-like conformation in the receptor-bound state. Based on this assumption we propose structural models of modified VIP's which might have increased *in vivo* stability.

OC 05 Einstein Auditorium - Structural studies - Protein Folding OC 06

THE TRP-CAGE: A NOTABLY STABLE MINI-PROTEIN FOLD

Niels H. Andersen, Jonathan W. Neidigh, R. Matthew Fesinmeyer

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Based on the partially formed tertiary structure observed for a naturally occurring 39 residue peptide in aqueous TFE, we have designed a series of 20-mers that are folded and monomeric in strictly aqueous medium at pH 7. The original peptide sequence and that of the most stable miniprotein examined to date are shown below.

Exendin-4 .... AV RLFIEWLKNG GPSSGAPPPS-NH_2
mini-**5b** NLYIQWLKDG GPSSGRPPPS

Mini-**5b** is 98.6% folded at 5°C and displays cooperative unfolding both thermally (T_m = 43°C) and upon GdmCl addition. The fold stability has been confirmed by NH exchange protection. These observations indicate that mini-**5b** is by far the most stable fold observed for any construct of less than 30-residues and lacking cross-links. The key stabilizing feature of the fold is the docking of the $Pro^{18,19}$ unit onto the apolar surfaces of Tyr^3 and Trp^6. The indole ring is also shielded from solvent by one face of Pro^{12} and the methylenes of Gly^{11}/Arg^{16}. The resulting compact, non-fluxional structure produces ring current shifts as large as 3.5 ppm. NMR measures of the fold lifetime (2 – 6 μsec) indicate that the folded/unfolded state equilibrium is relatively rapid; as a result, the extent of folding can be monitored from temperature and pH dependence chemical shift deviations. These studies revealed that the Asp^9/Arg^{16} salt bridge which was introduced provided 1.5 kcal of fold stabilization. Similar studies have been used to probe the effect of the other mutations.

The mutational fold optimization scheme employed and studies of the thermal unfolding of these species will be presented.

STRUCTURAL STUDIES OF YEAST MITHOCHONDRIAL ATP SYNTHASE SUBUNITS.

Céline Aznar, Serge Geoffre, Claude Manigand, Philippe Picard, Gilles Précigoux.

UBS, UMR 5471, CNRS, Université de Bordeaux I, Avenue des facultés, Bât B8, 33405 Talence Cedex, France.

The ATP synthase is a protein complex and reversible enzyme which catalyses the ATP synthesis from ADP and inorganic phosphate by extracting energy from the flow of protons across the membrane. This complex is three-part-formed. The catalytic and hydrophilic sector F1, the hydrophobic FO sector, embedded in the membrane, which permits a proton-conducting pathway, and a stalk connecting both sectors.

In order to understand how this enzyme works, numerous researches are lead in the world. Our laboratory is interested in yeast mithochondrial *Saccharomyces Cerevisiae* ATPase. The F1 sector contains five different subunits : α, β, γ, δ and ε with a stoichiometry of 3 :3 :1 :1 :1. We are more particularly working on the ε subunit, which is the smallest F1 polypeptide (6612 Da, 61 residues), and on the δ subunit (14555 Da, 143 residues), which works as a complex with ε. The primary structure of these subunits were obtained by direct sequencing, but their functions remains to be established. Therefore, the knowledge of their three dimensional structure will provide very useful informations.

For this purpose, the ε subunit was synthesized by solid phase method (Boc chemistry), purified by RP-HPLC and its purity was checked by Mass Spectroscopy. Circular dichroism studies were undertaken to point out its conformationnal behaviour and were used to set the right solvent conditions. NMR experiments are in progress.

The δ subunit was overexpressed and purified by ion exchange chromatography and RP-HPLC. But its height doesn't permit us to undertake NMR studies. So crystallizations of this subunit and of the complex are in progress.

THERMODYNAMIC CONTROL IN THE FOLDING OF PROUROGUANYLIN BY THE N-TERMINAL REGION

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Uroguanylin, an endogenous ligand for guanylate cyclase C, contains 15 amino acid residues and two intramolecular disulfide bonds. We have previously shown that the propeptide region, which serves as an intramolecular chaperone, in the precursor protein, prouroguanylin, is required for achieving correct disulfide pairing and the native conformation of the mature peptide, uroguanylin [1]. However, details of the folding mechanism of prouroguanylin, mediated by the propeptide, remains unknown.

The N-terminal propeptide in some proteases, such as subtilisin, mediates the folding of the mature protein by serving as an intramolecular chaperone. In this case, the propeptide affects the folding of the mature protein at the kinetic level by diminishing the activation energy in the folding pathway [2]. Recently, we found that the propeptide in prouroguanylin provides thermodynamic assistance for the correct folding of the protein at the final stage in the folding pathway and that the N-terminal region in the propeptide plays an important role in the folding of the mature peptide.

In order to further investigate the role of the N-terminal region in the folding of prouroguanylin, we examined the time course of the in vitro folding of the N-terminal deletion mutants, in which one or two N-terminal amino acids had been deleted, and compared these to the wild type of prouroguanylin. At the initial folding of the deletion mutants, the disulfide isomers with non-native disulfide bonds were kinetically produced, as was also the case for the wild type. However, the deletion mutants were not able to completely fold to the native conformation of prouroguanylin. In addition, circular dichroism measurements of the disulfide isomers revealed that the formation (or stabilization) of the α-helix structure in the propeptide region is important for the construction of the native tertiary structure of prouroguanylin.

In conclusion, the N-terminal amino acid residues of the propeptide provides thermodynamic assistance for the folding of prouroguanylin at the final stage in vitro.

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STRUCTURE AND MEMBRANE PROPERTIES OF TRICHOGIN GB IX FROM *TRICHODERMA LONGIBRACHIATUM*, THE LONGEST SEQUENCE AMONG LIPOPEPTAIBOLS

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Trichogin GB IX is an antimicrobial peptide produced by the *Trichoderma longibrachiatum* fungus, and belonging to the lipopeptaibol group. Only four members of this sub-class of peptaibols are known: they contain either 11 (trichogin GA IV[1]), trikoningins KB[2]) or 7 residues (trichodecenin TD I[3]), among which about 1/4 of α-aminoisobutyric acid (Aib). With 15 residues, trichogin GB IX is the longest lipopeptaibol isolated so far. It was purified by a multi-step chromatography procedure including reversed-phase HPLC. Trichogin GB IX contains the repeating Gly-Leu-Aib-Gly unit; the Aib1 residue is acylated by *n*-octanoic acid, leading to an N-terminal C8 lipidic chain and the C-terminal amino alcohol is leucinol. CD and NMR data (NOEs, $^3J_{NHC\alpha H}$ coupling constants and thermal coefficients of amide protons) obtained in methanol solution indicated that trichogin GB IX has a helical structure. It exerts antimycoplasmic activity directed to several *Mycoplasma*, *Spiroplasma* and *Acholeplasma* species (CMI = 0.5-10 μM). It exhibits remarkable membrane-perturbing properties which are described and compared to those of other related antibiotic peptides.

GB IX	Oc	$U^1G^2L^3U^4G^5G^6L^7U^8G^9G^{10}L^{11}U^{12}G^{13}I^{14}Lol^{15}$
GA IV	Oc	$U^1G^2L^3U^4G^5G^6L^7U^8G^9$ ---------- $I^{10}Lol^{11}$
KB I	Oc	$U^1G^2V^3U^4G^5G^6V^7U^8G^9$ ---------- $I^{10}Lol^{11}$
TD I	Dec	$G^1G^2L^3U^4G^5$ ---------- I^6 Lol^7

(U (Aib): α-aminoisobutyric acid; J (Iva): isovaline; Lol (Leuol): leucinol, Oc: octanoyl; Dec: decenoyl)

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Lectures and Oral Communications

L 03 Monday Afternoon: Pasteur Auditorium L 04

TILTED PEPTIDES: A MOTIF FOR MEMBRANE DESTABILISATION

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Cell life depends on the dynamics of molecular processes: molecule folding, organelle building and transformations involving membrane fusion, protein activation and degradation... To carry out these processes, the hydrophilic/hydrophobic interfaces of amphipathic systems such as membranes and native proteins must be disrupted. In the past decade, protein fragments acting in the disruption of interfaces have been evidenced: they are named the tilted or oblique peptides. Due to a peculiar distribution of hydrophobicity, they can disrupt hydrophobicity interfaces. Tilted peptides should be present in many proteins involved in various stages of the cell life[1,2,3]. We overview their discovery, describe how they are detected and discuss how they could be involved in dynamic biological processes.

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MINIATURIZED PROTEINS: APPLICATION TO THE DESIGN OF METALLO-PROTEIN MODELS.

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We will report on our efforts in the design, synthesis, and structural and functional characterization of small/medium-size peptides that self assemble to properly incapsulate metal ions with a predetermined coordination geometry. The first objective of these studies is to understand how the polypeptide matrix can modulate and specify the function of metallo-proteins. The final objective is the design of peptide-metal ion complexes that can be used as alternatives to recombinant proteins in bio-catalysis and bio-sensor technology.

We centered our attention on three different classes of proteins: hemoproteins, di-iron proteins, and iron-sulfur proteins. In the design, we first analyzed in detail their 3D-structures as found in the Protein Data Bank. We interestingly observed that the protein backbone folds symmetrically (C2 symmetry) within a sphere of about 10 A around the metal ion(s). C2 symmetrical models were designed for all three classes of molecules with a minimalist approach. The symmetry simplified the modeling, the synthesis, and the structural characterization by X-ray and Nmr techniques.

Mimochromes are representatives of hemoproteins. Four analogues where synthesized and characterized. They adopt the expected structure in solution and elicit important catalytic properties.

We have also developed 48 residue helix-loop-helix (α_2) peptide able to assembly into a four helix dimer. The interior of the four helix bundle was engineered in order to accommodate a dinuclear metal coordination site. Nmr and X-ray structure confirms our expectations.

We also developed a new class of peptide-based miniaturized rubredoxin, named METPs, as models of electron transfer proteins that contain iron-sulfur clusters as redox-active centers. In the early studies we synthesized several analogs that differ for the first co-ordination sphere. Their structural and functional characterization is presently in progress.

OC 09 Pasteur Auditorium - Bioactive Peptides OC 10

AN INTERESTING APPROACH FOR CANCER THERAPY: INHIBITION OF THE ASSOCIATION OF HDM2 WITH THE TUMOR SUPPRESSOR p53

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The p53 tumor suppressor gene is a multifunctional protein that regulates cell proliferation by induction of growth arrest or apoptosis in response to DNA damage and/or stress stimuli. Among the known mechanisms by which the tumor suppressor functions of p53 can be abrogated, we are interested in the regulation of p53 by the human double minute 2 (hdm2) oncoprotein, which targets p53 for degradation by the ubiquitin pathway. The disruption of the p53/hdm2 protein-protein interaction in tumor cells should therefore result in p53 accumulation and induction of cell death by apoptosis.

As part of our drug discovery program to identify antagonists of the association of hdm2 with p53, we have attempted to determine the amino acid specificities of hdm2's binding pockets in order to establish a pharmacophore model for this protein-protein interaction. This work has resulted in the identification of a highly potent peptide antagonist. Combining conformational constrains as selected by molecular modeling with functional groups that are able to establish additional electrostatic and van der Waals interactions with the hdm2 protein, we have been able to increase the hdm2 binding affinity of our initial 8-mer peptide 1,700-fold (IC_{50}= 5 nM *versus* IC_{50}= 8949 nM). The interactions identified and experimentally confirmed in this work could be directly applied to the optimization of non-peptidic leads or incorporated into the "de novo" design of antagonists of the p53/hdm2 protein-protein interaction. Furthermore, the new peptide has been a valuable tool to study the activation of the p53 pathway in tumor cells.

CHEMICAL SYNTHESIS OF MAXADILAN AND ITS RELATED PEPTIDES AS AGONISTS AND ANTAGONISTS OF PACAP TYPE 1 RECEPTORS

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A potent and persistent vasodilator, maxadilan (Maxa), had been isolated from salivary gland lysates of the blood feeding sand fly, *Lutzomyia longipalpis*. Maxa consists of 61 amino acids with two disulfide linkages and acts as an agonist of the PACAP type I receptor (PAC1) [1]. Previously we have synthesized numerous PACAP/VIP and their related peptides in order to study various aspects of their structural biology reviewed in [2] and it is very interesting that Maxa recognizes specifically the PAC1 receptor only, although the primary structure of Maxa is quite different to that of PACAP. To develop agonists and antagonists against PAC1, Maxa with its disulfide isomers and various related peptides such as middle, N- and C-terminal fragments as well as middle-region deleted Maxa have been prepared by highly efficient SPPS with improved synthesis protocols. After purification and characterization the peptides obtained were used in different bioassays that include a PAC1 binding assay using rat brain membranes and the recently developed melanophore technology, reviewed in [3], to elucidate the structural requirements for PAC1 recognition, especially antagonistic actions. The results indicated that the middle section of Maxa and the first disulfide linkage are not essential for recognition. An antagonistic action to PAC1 is observed for the middle-region deleted Maxa fragment.

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