Michael Bienert

Peptide Chemistry

Peptides and proteins are the most abundant biological macromolecules, ranging in size from relatively small peptides to huge polymers. They are constructed from a set of 20 amino acids, covalently linked in characteristic sequences, exhibiting strikingly different biological properties. Even the smallest peptide can exert biological effects at very low concentrations.

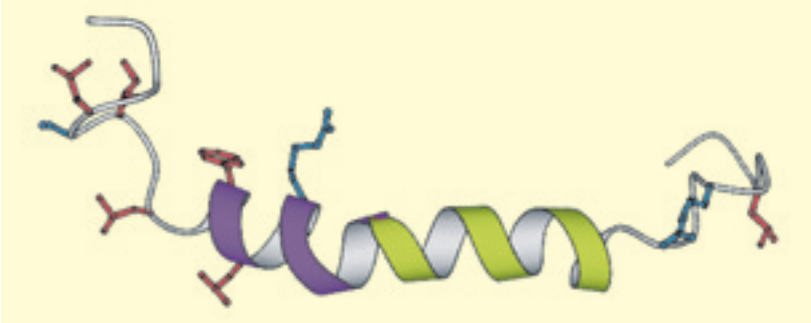
Research efforts of the Department of Peptide Chemistry concentrate on the study of the molecular mechanisms of peptide-protein and peptide-lipid interactions, leading to a better understanding of the action of physiologically active peptides and drugs on their respective targets.

Our present studies focus on the neuropeptide corticotropin-releasing factor (CRF) and the analysis of the molecular basis of its interaction with its membrane-spanning heptahelical (7TM) receptor subtypes. CRF is an important modulator of the stress-response and has various, as yet unclarified, functions in peripheral organs. The development of subtype-specific peptides and small ligands of CRF receptors is, therefore, of pharmacological and clinical importance.

The synthesis of CRF-derived peptide libraries and testing the individual analogues in a novel high-throughput screening assay unravelled the influence of structural moieties on the process of subreceptor-selective recognition of ligands. In order to localize the binding regions of CRF receptors for their peptidic ligands, the extracellular domains have been produced chemically or by expression in *Escherichia coli*, and their structural and binding characteristics are now under investigation.

The lipid matrix of cell membranes may also serve as a target for peptides. The group Peptide-Lipid Interaction is using physico-chemical approaches to understand the lytic actions and to improve the selectivity of naturally occurring peptides towards microbial cells.

Fig. 1
Solution structure of the CRF-derived peptide DDPPLSIDLTFHLLRTLDEIEKEEKEKKRKEQNRKLLDEV, determined by two-dimensional NMR spectroscopy.



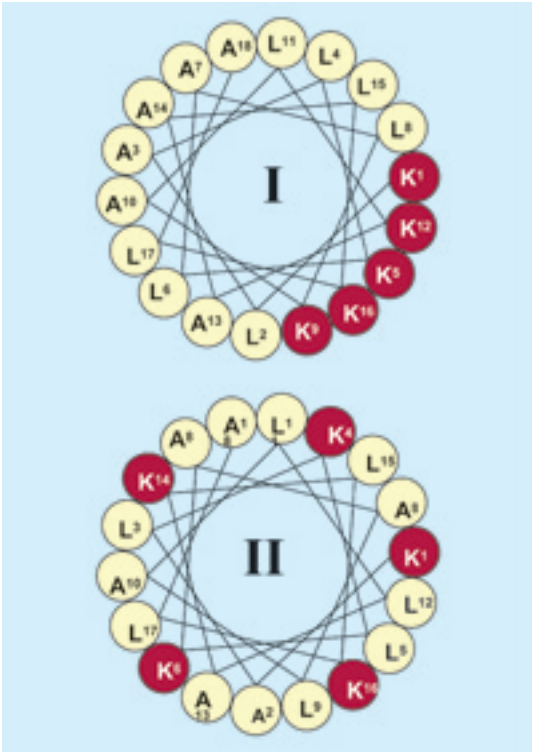


Fig. 2
Helical wheel projections of KLA-derived peptides. The synthesis of peptides containing exclusively the hydrophobic amino acids leucine (L), alanine (A) and the hydrophilic, positively charged amino acid lysine (K) permit the construction of peptide models with distinct biophysical features (I = amphipathic, II = non-amphipathic). We used sets of these peptides in order to gain insights into mechanisms of peptide-lipid interaction.

Investigation of the basis of the striking selectivity of membrane disturbing peptides may be important for development of a new class of antibiotics and an understanding of other membrane-related effects. Recent experiments have been demonstrated that the

Forschungsinstitut für Molekulare Pharmakologie
Abteilung Peptidchemie

Die Abteilung Peptidchemie untersucht die molekularen und biochemischen Mechanismen der Wirkung von Neuropeptiden sowie von Peptiden mit neurodegenerativer Aktivität (βA4). Ziele sind u.a. die Aufklärung der molekularen Wechselwirkung zwischen Peptid und Rezeptor sowie der Rezeptor-Effekt-Kopplung. Da Peptide auch ohne die Vermittlung spezifischer Rezeptoren biologische Aktivitäten, z.B. antimikrobielle Wirkungen, entfalten können, werden auch die Interaktion mit Lipiden charakterisiert und die Durchlässigkeit der Zellmembran für pharmakologisch interessante Substanzen untersucht. Methoden wie Peptidsynthese, Proteinexpression sowie verschiedene biophysikalische, biochemische und analytische Techniken sind bei der Themenbearbeitung unentbehrliche Grundlagen. Insbesondere die Massenspektrometrie leistet einen wichtigen Beitrag bei der Identifizierung von Peptiden und Proteinen sowie bei der Aufklärung von Proteinmodifizierungen in lebenden Zellen.

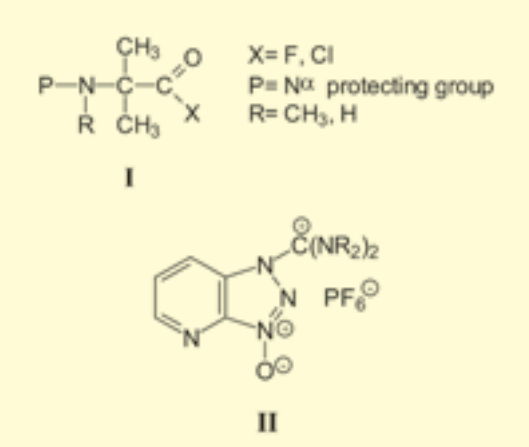


Fig. 3
In order to overcome limitations in peptide synthesis, novel coupling reagents, such as amino acid fluorides and chlorides (I) as well as guanidinium-type compounds (II) have been evaluated in the assembly of various, difficult to synthesize peptides.

optimization of the balance between hydrophobicity and positive charges may increase the selectivity of peptides towards bacterial cells. Moreover, the group contributed to the biophysical and conformational characterization of newly discovered spider toxins.

Unexpectedly, many peptides translocate rapidly across cell membranes, though how this action proceeds is unknown. Elucidation of the mechanisms of translocation, may provide a basis for the development of optimized vectors for the transport of pharmacologically useful molecules (oligonucleotides, peptide nucleic acids, peptide drugs) into cells. Thus, the successful down-regulation of a peptide receptor in distinct cell populations by application of antisense strategy using peptide nucleic acid-peptide conjugates has been demonstrated in collaborative studies with other research groups.

Most of the studies above are based on the preparation and characterization of peptide analogues and protein domains. In order to overcome limitations in peptide synthesis, we have developed improved methods for the coupling of sterically hindered amino acid residues, cyclization of distinct linear penta- and hexapeptides, and the synthesis of protein sequences possessing a pronounced tendency to form aggregates (e.g. transmembrane domains of proteins, fibril-forming Alzheimer βA4 peptide, β-sheet forming model peptides). These chemical studies delivered e.g. the basis for experiments to elucidate the role of biophysical parameters for aggregation and on fibril formation of peptides involved in the pathogenesis of Alzheimer Disease.

The substantial efforts in peptide synthesis are based, at least in part, on the remarkable impact of mass spectrometric methods which allow the rapid identification of side-products. Besides being an invaluable tool in peptide analytics, mass spectrometry in combination with gel electrophoresis has been established in our department as a powerful methodology for protein identification and for the characterization of post-translational protein modifications, which is now of increasing significance in joint projects with other research groups in and outside the institute. In cooperation with other groups, the mass spectrometry group contributed to the analysis of the mouse brain proteom, analyzed the autophosphorylation sites of phosphoinositide 3-kinases, and identified a novel lipid modification motive in $G\alpha_s$ proteins. Further studies are aimed to elucidate the impact of mass spectrometry, in combination with H/D-exchange experiments, for structural analysis of peptide secondary structures and amyloid aggregates.

Scientific Projects
The scientific projects of our department are organized in the following four Research Groups:

- Peptide Synthesis, *Dr. Michael Beyermann,* beyermann@fmp-berlin.de
- Peptide Biochemistry, *Dr. Hartmut Berger,* berger@fmp-berlin.de
- Peptide-Lipid Interaction/Peptide Transport, *Dr. Margitta Dathe, Dr. Johannes Oehlke,* dathe@ fmp-berlin.de, oehlke@fmp-berlin.de)
- Mass Spectrometry, *Dr. Eberhard Krause,* ekrause@fmp-berlin.de

Prof. Dr. Michael Bienert
Born 1943, received his Ph.D. in organic chemistry from the Humboldt-Universität zu Berlin in 1969. 1971 he joined the peptide group at the Institute of Pharmacology and since 1991 he is head of the Department of Peptide Chemistry at the Research Institute of Molecular Pharmacology (FMP) in Berlin-Buch. Since 1998 M. Bienert is Honorary Professor at the Institute of Organic and Bioorganic Chemistry of the Humboldt-Universität zu Berlin.

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