M. MUTTER

STRUCTURAL ASPECTS OF PEPTIDES AND PROTEINS

FROM PEPTIDES TO PROTEIN DESIGN

IASOC IV, 1990

CONTENTS

" Die Peptidbindung im Sinne Fischers dürfte nicht ausreichen, um alle physikalischen und chemischen Eigenschaften der Proteine zu erklären. Deshalb hat man einiger theoretischer Begriffe und Anschauungen über das allgemeine Bauprinzip der Proteine zu gedenken."

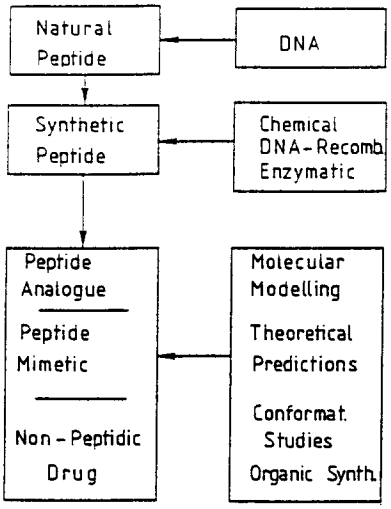
Max Bergmann, 1924

1. PEPTIDE AND PROTEIN CHEMISTRY: PAST AND PRESENT

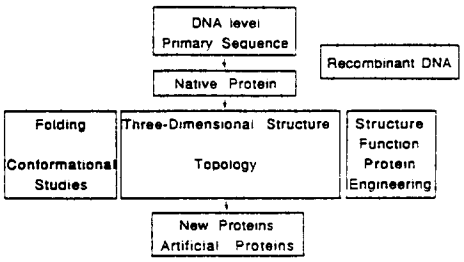
HISTORICAL SURVEY OF SYNTHESIS AND CONFORMATIONAL ANALYSIS OF PEPTIDES AND PROTEINS

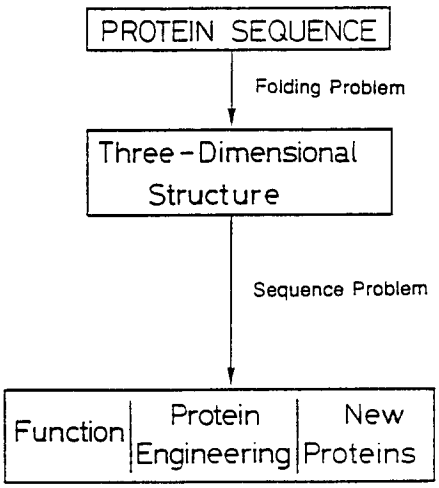
1750 - 1800	:	Chemistry becomes a "Science" (Cavendish, Priestley, Lavoisier, Berzelius)
1828	:	Woehler's Synthesis of urea
1838	:	Berzelius introduces the term "protein"
1848 - 1860	:	Pasteur discovers the optical activity and the first -aminoacid as component of proteins
1899 - 1920	:	Fundamental contributions of Emil Fischer
1902	:	Peptide bond in Proteins is postulated
1906	:	Term "Polypeptide" established
1907	:	Synthesis of a 18-peptide (E. Fischer)
1920 - 1925	:	Staudinger's hypothesis of "macromolecule"
1930	:	Bermann's hypothesis of the relationship between conformation and properties of peptides
1931 - 1936	:	Denaturation of proteins as consequence of conformational transitions (Pauling); X-ray of peptides (Corey and Pauling)
1945 - 1955	:	Sanger's contributions to the primary sequence of insulin
1953	:	Du Vigneaud's synthesis of Oxytocin
1960	:	Three-dimensional structure of myoglobin by Perutz and Kendrew
1962	:	Anfinsen's denaturation / renaturation experiments of ribonuclease
1963	:	Merrifield's solid-phase peptide synthesis; Hypothesis, that the native conformation of proteins corresponds to the minimum of the free energy (Scheraga, Anfinsen)
since 1963	:	Conformational energy calculations, folding studies, investigation of model peptides
1958 - 1970	:	Primary sequence of several hundreds of proteins determined
1970 - today	:	DNA recombinant techniques Three-dimensional structure of more than 200 proteins

PEPTIDES

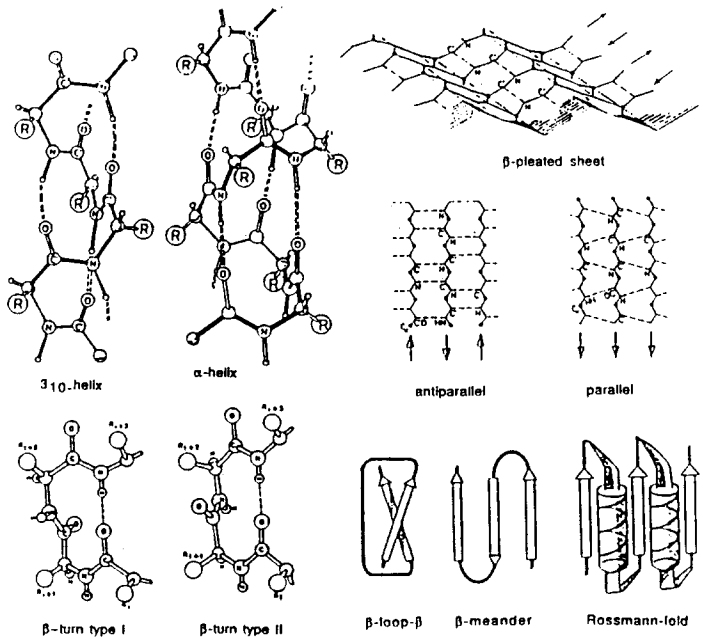


PROTEINS

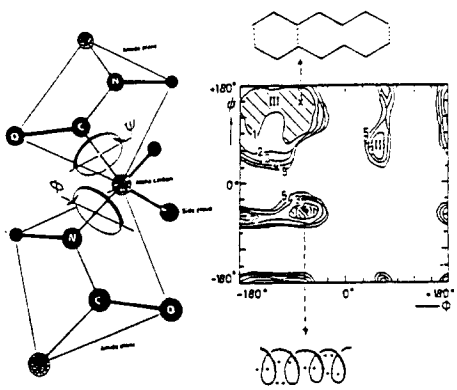




2. SECONDARY STRUCTURE: PREDICTIONS, MODEL PEPTIDES, SYNTHETIC ASPECTS



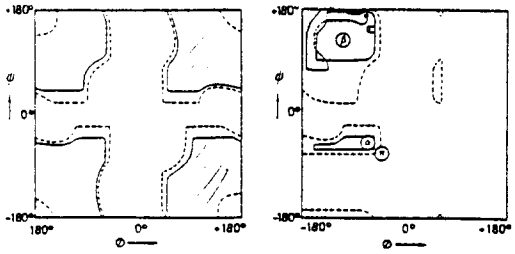
Conformational Energy Map



CONFORMATIONAL RESTRICTIONS IN PEPTIDES

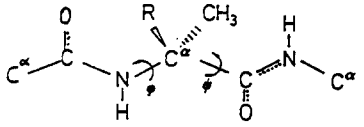
$$E_{total} = \sum_{bonds} K_r (r - r_{eq})^2 + \sum_{angles} K_\theta (\theta - \theta_{eq})^2 + \sum_{dihedrals} \frac{V_n}{2}[1 + \cos(n\phi - \gamma)] +$$

$$\sum_{i<j} \left[\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} + \frac{q_i q_j}{\varepsilon R_{ij}}\right] + \sum_{H\text{-bonds}} \left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right]$$

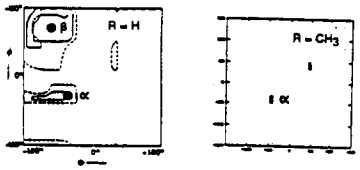


Comparison of the conformational space of glycine and alanine (Ramachandran map)

Conformational energy diagram of the dialkyl amino acid α-amino isobutyric acid (Alb)



$$E(\varphi,\psi) = E_{VW}(\varphi,\psi) + E_{ROT}(\varphi,\psi) + E_{C}(\varphi,\psi)$$



Comparison of the conformational space of Alb (R = methyl) and Ala (R = H)

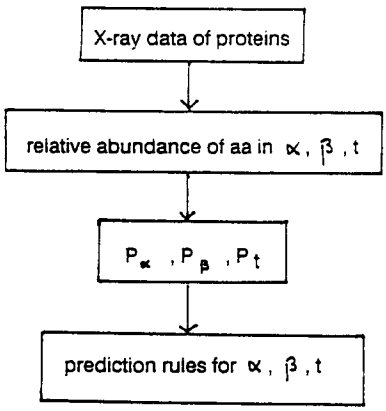
"Structural characteristics of α-helical peptide molecules containing Alb residues"

I.L. Karle, P. Balaram, Biochemistry 29, 6747 (1990)

From this rule man should realize that he is far off the reality.

Demokrit

CONFORMATIONAL PREDICTIONS



G. Fasman, TIBS 14, 295 (1989)

Types of β-turns

Turn	i + 1 φ	i + 1 ψ	i + 2 φ	i + 2 ψ
β turns*				
Type I	−60	−30	−90	0
Type I'	60	30	90	0
Type II	−60	120	80	0
Type II'	60	−120	−80	0
Type III	−60	−30	−60	−30
Type III'	60	30	60	30
Type VIa (cis)*	−60	120	−90	0
Type VIb (cis)*	−120	120	−60	0
γ turns*				
Turn	70 to 85	−60 to −70		
Inverse turn	−70 to −85	60 to 70		

* As originally defined by Venkatachalam (1968), except as noted.
* Angles are taken from data presented in Richardson (1981).
* As originally defined by Némethy and Printz (1972).

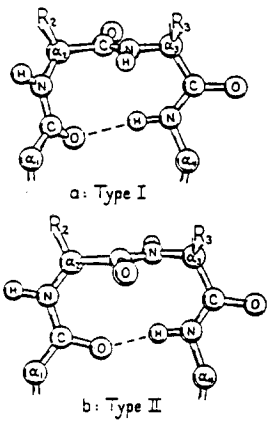
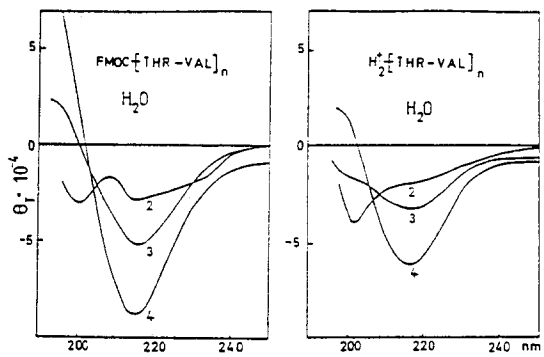


Fig. The two major types of eight turn (I and II). In type II (bottom), R_3 is generally glycine.

G.D. Rose, L.M. Gierasch, J.A. Smith "Turns in Peptides and Proteins" Adv. in Protein Chemistry 37, 1 (1985)



Critical chain-length for β-structure formation showing the influence of the Fmoc- protecting group

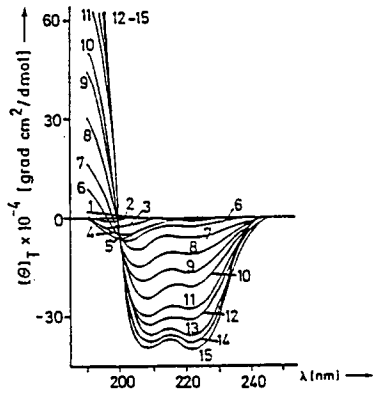
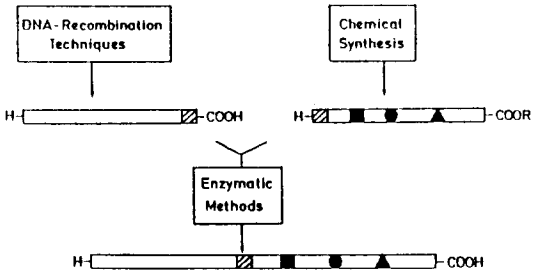


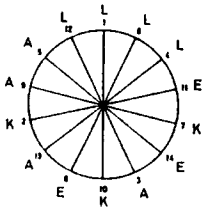
Abb. . Kritische Kettenlänge c_r für die α-Helixbildung: Bei Kettenverlängerung geht das Homooligopeptid Boc-(Met)$_c$-PEG in Trifluorethanol von der ungeordneten Konformation (n = 6) stufenweise in die α-Helixstruktur über, die bei ca. 15 Aminosäureresten vollständig ausgebildet ist (charakteristische Cotton-Effekte im CD-Spektrum bei λ = 207 nm und 222 nm)

Perspectives of Peptide Synthesis

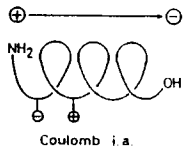


DESIGN OF AMPHIPHILIC HELICES

"Amphipathic Helix Motif: Classes and Properties"
J.P. Segrest et al. PROTEINS 8, 103 (1990)

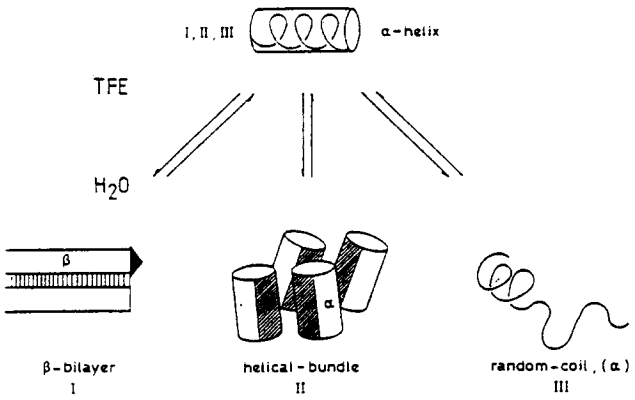


Helical Wheel



Coulomb i.a.

"Peptides as Conformational Switch: Medium-Induced Conformational Transitions of Designed Peptides"
M. Mutter, R. Hersperger, Angew. Chem. Int. Ed. Engl. 29, 185 (1990)

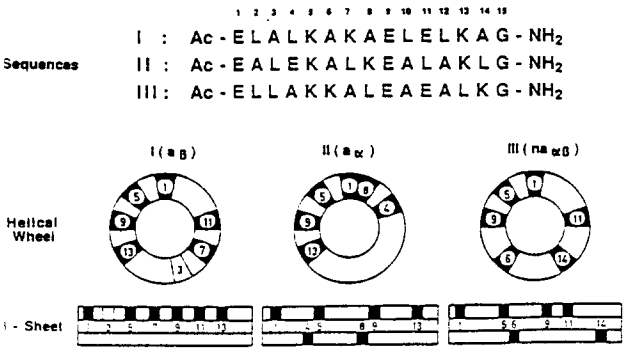


Conformational predictions : Pα > 1.0

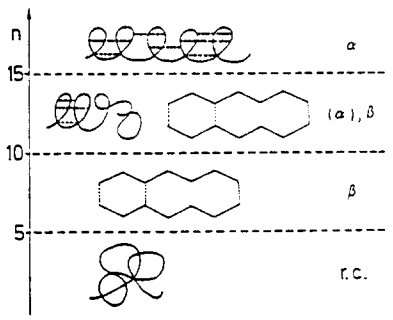
H_2N-E'-A-L-E-K-A-L-K-E-A-L-A-K-L''-OH

Structural stabilization : C^{α}-Me-AA

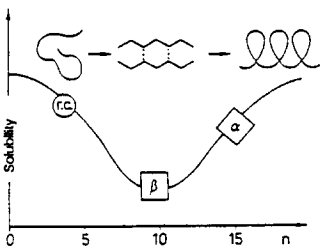
14 - mer : 4A , 4L , 3E , 3K



CRITICAL CHAIN LENGTH FOR SECONDARY STRUCTURE FORMATION



CONFORMATION-DEPENDANT PROPERTIES OF PEPTIDES



M. Mutter et al. in PEPTIDES, 1985

CHARACTERISATION OF PEPTIDES

Amino Acid Analysis

Sequencing

Peptide Maps

Rev. Phas-HPLC

Thin Layer Chromatography

Ion Exchange Chromatography

Mass Spectrometry

Capillary Zone Electrophoresis

NEW ASPECTS IN PEPTIDE SYNTHESIS

(21st EPS, 1990)

I. BOP Family

II. New Solvent Systems

III. New Resins

IV. Enzymes, Catalytic Antibodies

V. Templates, Handles

VI. Protecting Groups, Coupling Reagents

VII. Recombinant Methods

VIII. Design of:
- Immunogens, hormones etc.
- Specific conformations

IX. Cheaper Synthesis

Synthetic peptides can be more versatile than natural peptides, and represent a way by which chemists will be capable of reaping the fruits of biotechnology"
in : Trends in Biotechnology (1986)

3. DRUG DESIGN, PEPTIDE MIMETICS

State of Art: Generation of graphical models consistent with experimental data → suggests new experiments

Present limitations: -experimental data are scarce
-software

Trends: Better understanding of drug-receptor interactions :

- rapid growth of X-ray (NMR) data of pharmacologically important compounds
- advances in molecular biology and protein engineering techniques

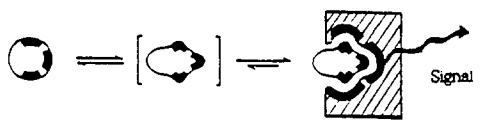
Increasing availability of modelling tools to experimentalists :

- faster experimental feedback for computer-assisted design

Substrate-Receptor Interaction

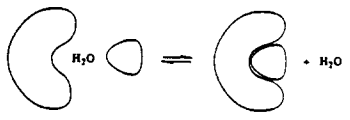
Hormone
Pharmaceutical

Receptor



Inactive Conformation Active Conformation Substrate-Receptor Complex

On the importance of peptide conformation



van der Waals
H-Bond
Electrostatic
Water
Conformational Change
$\Delta G = \Delta H - T\Delta S$

Molecular Recognition (S.M. Roberts, Ed) 1990

CHEMICAL SYNTHESIS IN PEPTIDE RESEARCH

Peptides in research
- synthetic analogues (n < 20 residues)
- screening of structure-activity relationships

Synthesis of peptide mimetics
- main chain modifications
- unusual side chains

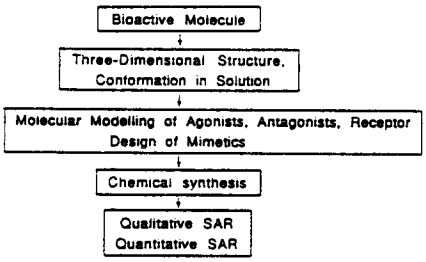
DRUG DESIGN

Classical Approach :

Synthesis of a set of peptide analogs — Structure-Activity Relationships (SAR)

- Shortening of the natural peptide sequence: finding the minimal chain-length for full biological activity
- Side and main chain modifications
- Correlation of structure with activity

Rational Drug Design



"Emerging approaches in the molecular design of receptor-selective peptide ligands: conformational, topographical and dynamic considerations" V. Hruby et al., Biochem. J. 268, 249-262 (1990)

"The molecule knows how to fold, so we may learn how it does it"

J. T. Edsall, 1968

4. PROTEIN FOLDING

"The capability of adopting a dense globular conformation which is at once

space filling, nonoverlapping, free of residue conformations of high energy, and so arranged as to place polar side chains on the exterior

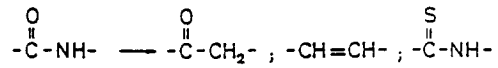
are characteristics peculiar to the chain molecules of globular proteins alone."

P.J. Flory, in "Statistical Mechanics of Chain Molecules" 1969.

PEPTIDE ANALOGS AND MIMETICS

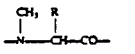
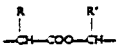
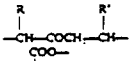
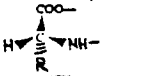
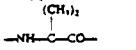
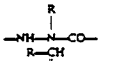
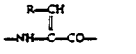
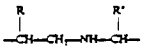
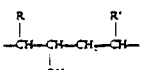
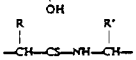
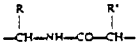
Unusual α-amino acids
- (D) α-amino acids
- dehydro α-amino acids
- non proteinogenic α-amino acids

Amide bond mimetics

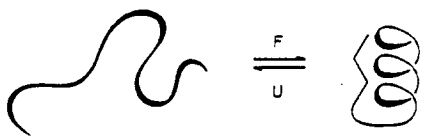


Introduction of conformational constraints
- cyclization
- dialkyl amino acids (Φ, Ψ - restrictions)
- β-turn mimetics

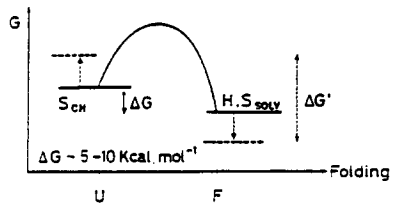
Backbone modifications

Modification	Structure	Effects of the modification
N-methylation		Loss of one hydrogen bond, reduction of conformational freedom, tendency to racemization, tendency to cis-amide configuration, increase of resistance to proteases
Ester (depsipeptide)		Loss of hydrogen bond, almost unchanged proteolysis
Ketomethylene		Loss of peptide bond rigidity, retention of hydrogen bond capacity, increase of resistance to proteases
D-configuration at C_α		Increase of resistance to proteases, local change of peptide backbone conformation, or of side chain orientation
Aminoisobutyric acid		Loss of chirality, strong restriction of conformational freedom, tendency to helix formation
α-Aza		Loss of chirality, intermediate configuration between D and L
α,β-Dehydroamino acids		Z (stable) and E isomers possible, loss of chiral center, rigidity of side chain orientation, increased resistance to proteolysis
Carba replacement of carbonyl		Loss of planarity, introduction of new ionizable group, increased water solubility, increased enzyme resistance
Hydroxyethylene		Loss of planarity and basicity, partial retention of hydrophilicity
Thioamide		Nearly isosteric amide substitution
Olefinic double bond		High analogy with planar, rigid and trans-substituted peptide bonds, protection against proteolysis
Retro-amide		No topological equivalence with peptide bond, can be combined with inversion of configuration of adjacent residues, increased enzyme resistance

THE FOLDING PROBLEM



$$\Delta G = \Delta H - T\Delta S_{SOLV} - T\Delta S_{CH}$$



MODELS FOR THE INITIATION OF PROTEIN FOLDING

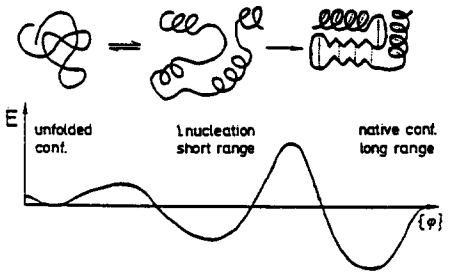
1) Hydrophobic Collapse ("molten globule")
2) Formation of Secondary Structure
3) Specific Interactions

R.L. Baldwin, TIBS 14, 291, (1989)

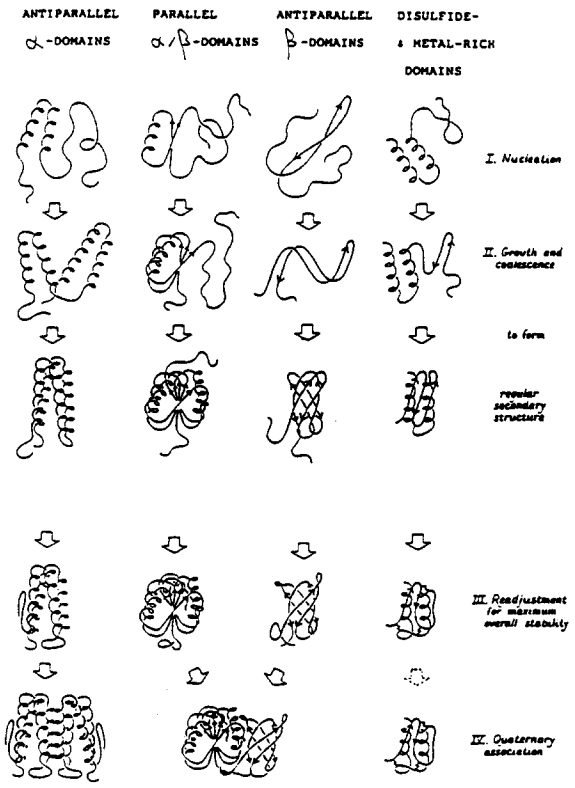
"Dominant Forces in Protein Folding" K.A. Dill, Biochemistry 29, 7133 (1990)

"Pieces of the Folding Puzzle" R.L. Baldwin, Nature 346, 405 (1990)

Model of the Folding Mechanism of Proteins



REFINED CONCEPT OF PROTEIN FOLDING



STABILITY OF PROTEINS

$$\Delta G_{F-U} = \Delta H - T\Delta S_{SOL} - T\Delta S_{CH}$$

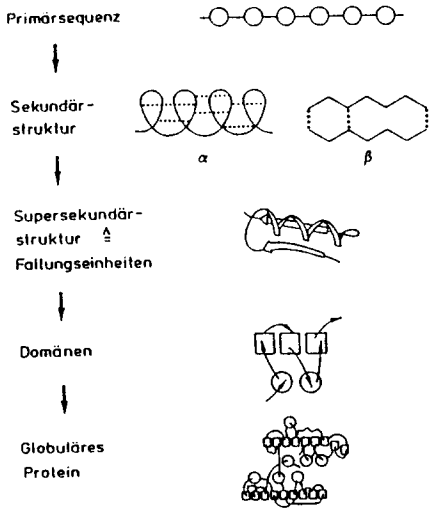
Factor (n = 100)	ΔG_{F-U} (Kcal mol^{-1})
Conformational Entropy	+ 330 / + 1000
Unfavorable i.a. in F	+ 200
Hydropnobic i.a.	- 264
Van der Waals i.a.	- 227
H-Bonds, other	- 49 / - 719
Observed ΔG_{F-U}	- 5 / - 10

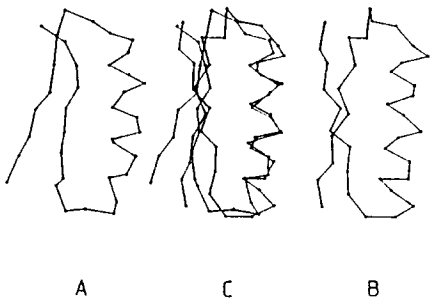
PROTEINS, 1984; T.E. Creighton

"To be astonished is the first step for a discovery".
L. Pasteur

5. PROTEIN TOPOLOGY

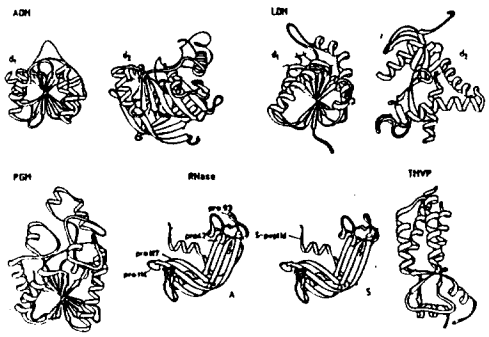
Hierarchie der Proteintopologie





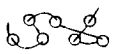
Vergleich der räumlichen Architektur von βαβ-Faltungseinheiten aus 2 unterschiedlichen Proteinen :
A : (S)-Lactat Dehydrogenase (D47-D84)
B : (L)-Arabinose-Binding Protein (L3-P41)
C : Ueberlappung von A und B.

TOPOLOGY OF PROTEINS



Structural Patterns in Globular PROTEINS (Levitt, 1976)

Class I : All α

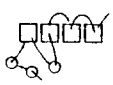


MBN (MHN, MGN)

Class II : All β

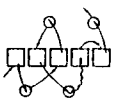
RUB (IGC, IGV, PBN, SDM, CON, CHT)

Class III : α + β



RNS (INS, CB5, PTI, LZM, SNS, LZ4, PAP, TLS)

Class IV : α / β



TRX (FLN, ADH, AKN, PGM, TIM, SUB, CPA, LDH, PGK, GPD, HKN)

"Wer immer nur nach dem Zweck de Dinge fragt, wird ihre Schönheit nic entdecken"

Halldor Laxness

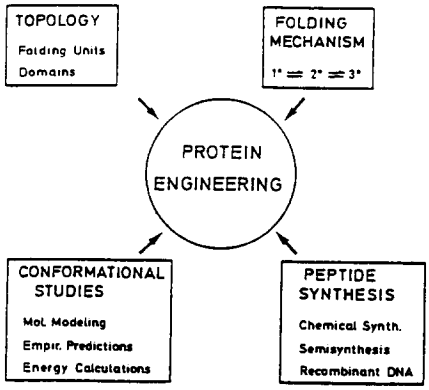
6. PROTEIN ENGINEERING, DE NOVO DESIGN OF PROTEINS

THE PHILOSOPHY OF
ARTIFICIAL PROTEIN DESIGN

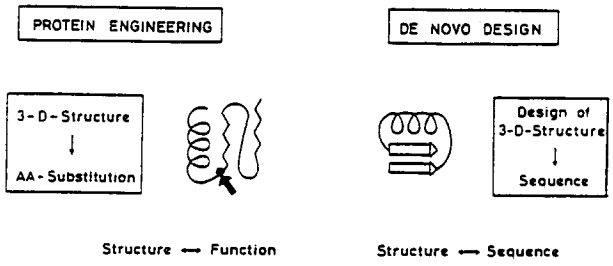
- Synthesis of new and interesting macromolecules
- Introduction of function in tertiary structures
- Test of our knowledge of proteins
- Learn about folding, structure, function

"The de novo design of protein structures"

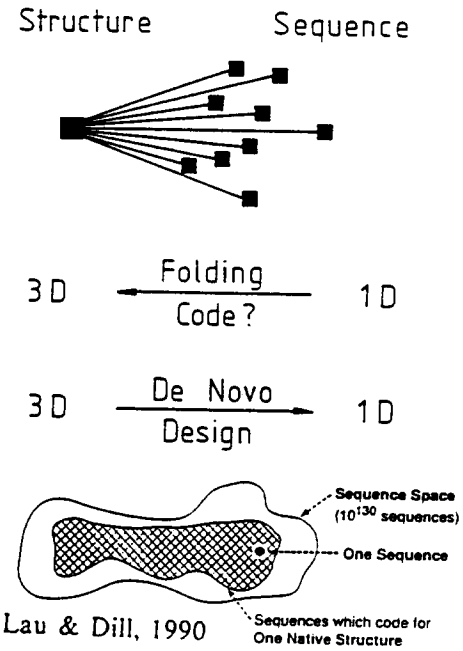
J.S. Richardson, D.C. Richardson TIBS 14, 304 (1989)



NEW PROTEINS



FOLDING CODE AND PROTEIN DESIGN



RULES OF PROTEIN STRUCTURE

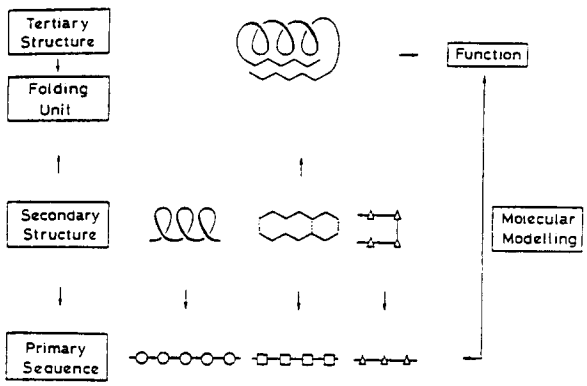
Hydrophobic interior

Hydrophilic residues on surface

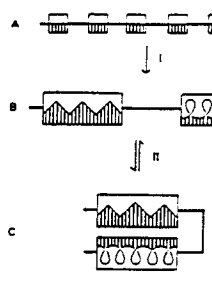
φ / ψ -angles in favourable area of map

Conformational preferences, e.g. E, L, A, in α

Interior closely packed



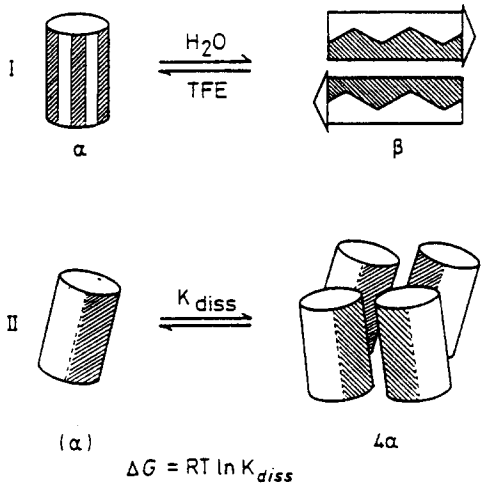
THE CONSTRUCTION OF NEW PROTEINS



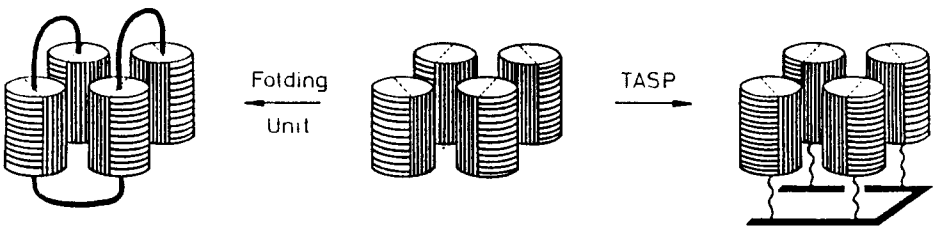
Faltungsschema künstlicher Faltungseinheiten (A = Polypeptidkette in der r.c.-Konformation) : Im ersten (Nukleations-) Schritt (I) kommt es zur Ausbildung stabiler amphiphiler Sekundärstrukturen als Nukleationszentren (B). Die Haupttriebkraft zur intramolekularen Faltung (II) erwächst aus der Amphiphilie der Sekundärstrukturblöcke, die unter Erhöhung der Wasserentropie zur Ausbildung eines hydrophoben Kerns der künstlichen Supersekundärstruktur (C) führt.

How does a PEPTIDE become a PROTEIN ?

Amphiphilic Structure as Driving Force for Self-Assembly



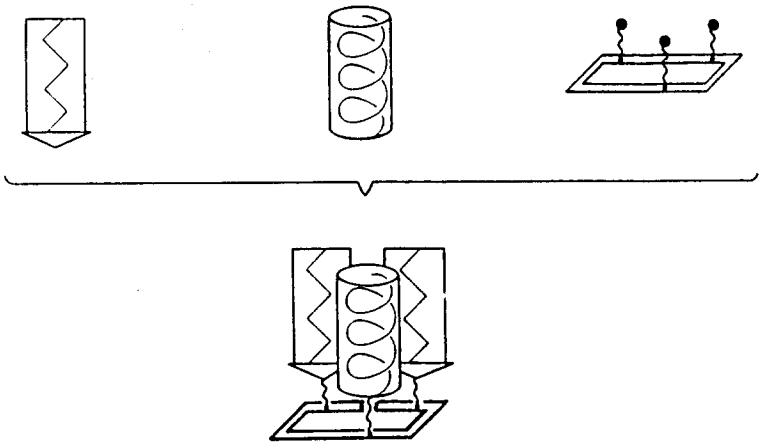
Strategies for the 'De Novo Design' of Proteins



What can PEPTIDES tell us about PROTEINS and vice-versa ?

Protein's Gordon Conference, 1988

TEMPLATE-ASSEMBLED SYNTHETIC PROTEINS (TASP)



A Chemical Approach to Protein Design— Template-Assembled Synthetic Proteins (TASP)

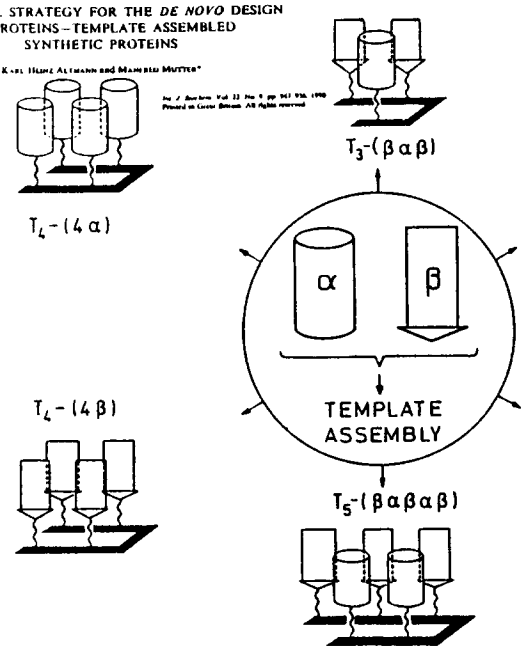
By Manfred Mutter* and Stéphane Vuilleumier

Angew. Chem. Int. Ed. Engl. 28 (1989) 535 554

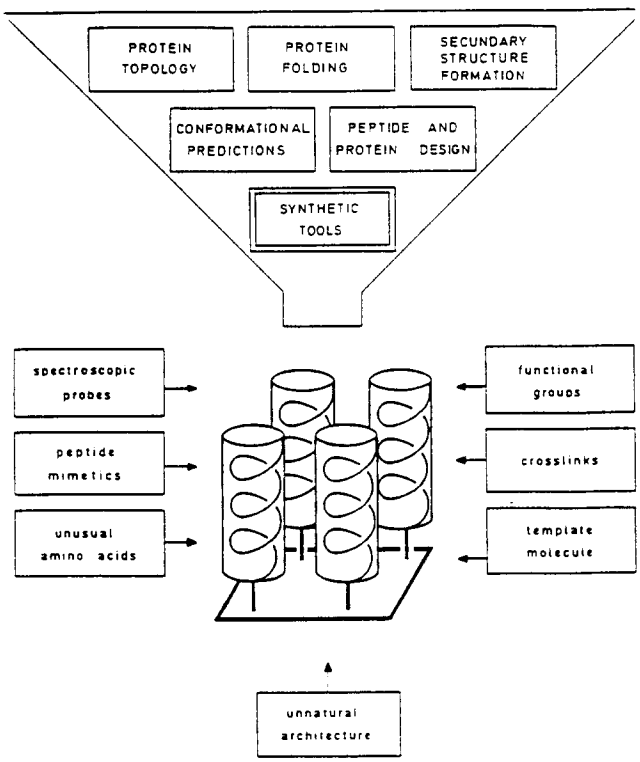
A GENERAL STRATEGY FOR THE *DE NOVO* DESIGN OF PROTEINS – TEMPLATE ASSEMBLED SYNTHETIC PROTEINS

KARL HEINZ ALTMANN and MANFRED MUTTER*

Int. J. Peptide Protein Res. Vol. 32, No. 6, pp. 562–570, 1988
Printed in Great Britain



NATURE'S RULES AND CHEMIST'S TOOLS



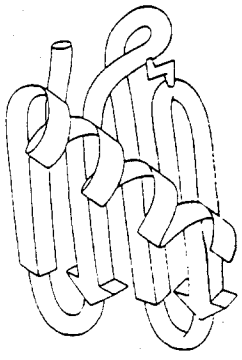
M. Mutter, TIBS 1988

EVIDENCE FOR FOLDED STRUCTURES

spectroscopical evidence
- Circular dichroism : overall conformation, conformational transitions
- Infrared : secondary structure
- NMR : NOE : distances
  ^{2}H-NMR : side chain mobilities
- Fluorescence : charge transfer (D-A) : -> distances
- X-ray : "solid-state" conformation

physicochemical evidence
- Gel permeation chromatography : Stokes radius, molecular weight, aggregation
- Light scattering : <r>, <s>
- Kerr effect: dipole orientation

biological evidence
- Binding (ligand, substrate)
- Catalytic activity

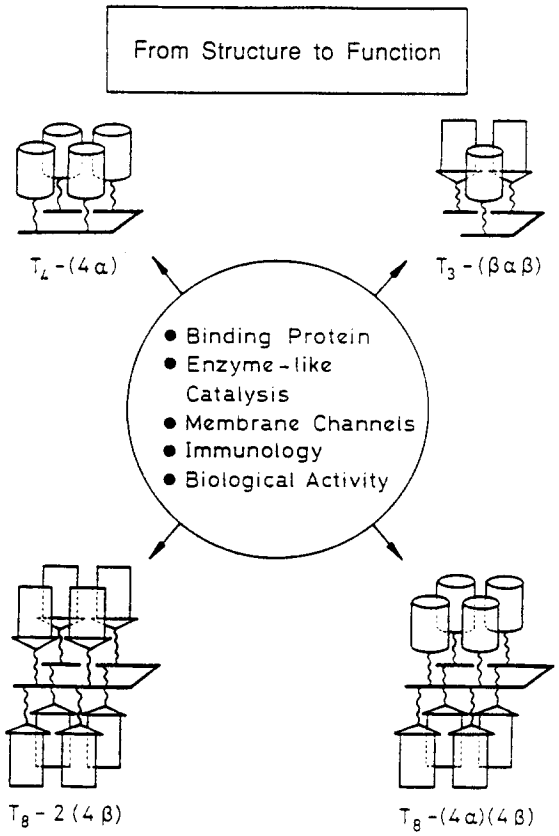


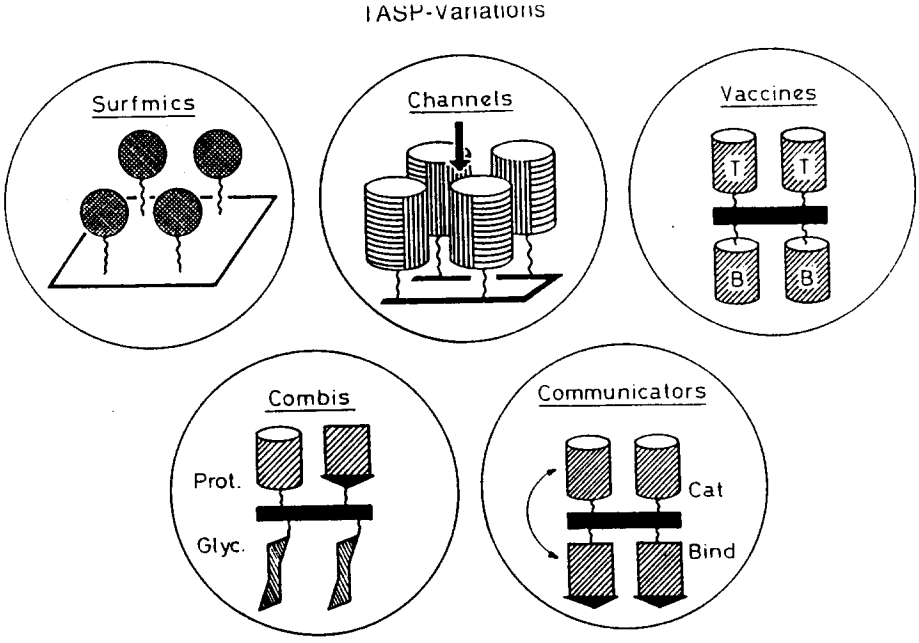
Incredulase

Just a cautionary reminder that not all apparently plausible protein structures will turn out to work.

"As we learn an increasing amount about the redesign of secondary structural regions in molecules where tertiary structure is important, we are moving closer to the time ... that we can consider the construction of whole enzymatic systems with new structural features and catalytic functions".

E.T. Kaiser, Angew. Chem. 1988

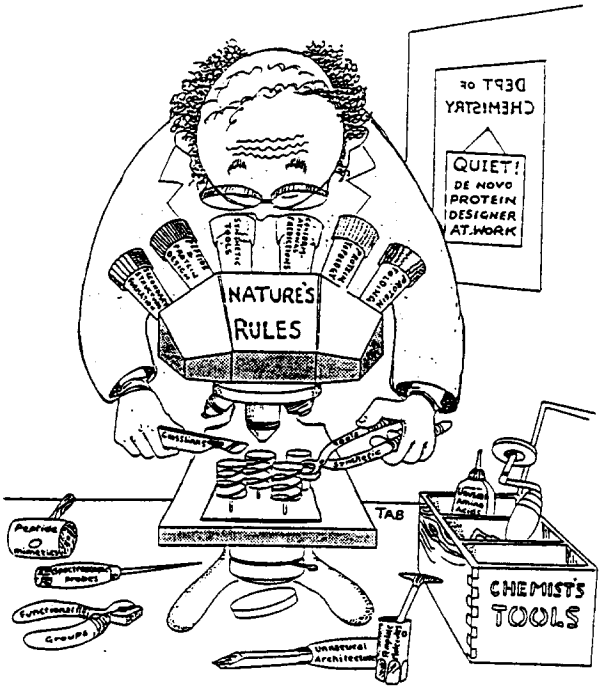




Trends in Biochemical Sciences (TIBS)
June Issue - 1972?
FILE TI006

Nature's rules and chemist's tools: a way for creating novel proteins

Manired Mutter



DESIGNED PEPTIDES AND PROTEINS

J.S. Richardson and D.C. Richardson, Trends, Biochem Sci. 14, 304 (1989)

M. Mutter, S. Vuilleumier, Angew. Chem. Int. Ed. 28, 535 (1989)

D. Eisenberg et al. Proteins 1, 16 (1986)

L. Regan, W.F. DeGrado, Science 241, 967 (1988)

M. Mutter et al. Proteins 5, 13 (1989)

M. Hecht, J.S. Richardson et al., Science 249, 884 (1990)