Biophysical Chemistry

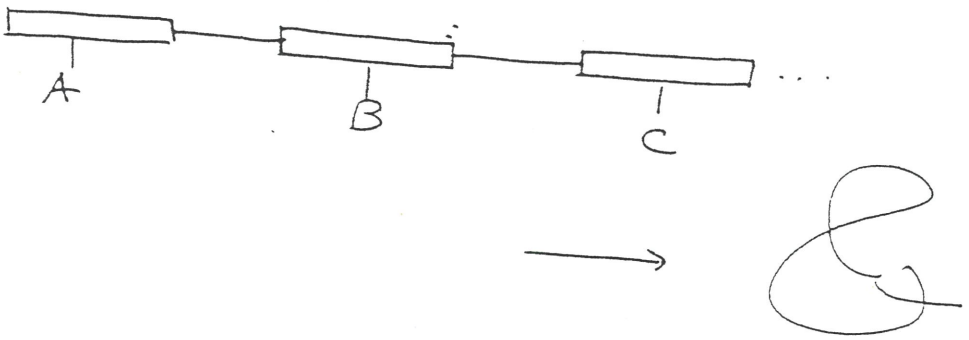
NTHU

May 5, 2001

Sunney I. Chan

Protein Folding and Unfolding

I. Three-dimensional Folding of a Heteropolymer



A, B, C monomers of varying hydrophilicity and hydrophobicity

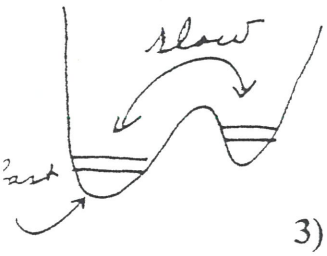
Examples:

1) Proteins
2) RNA
3) DNA
4) Others

II. Three Aspects to the Problem

1) Equilibrium structure (native structure or Three-dimensional fold)

2) Dynamics



Breathing motions (small amplitude fluctuations About native structure)
Conformational transitions (density of states $\rho(E)$)

3) Pathways and kinetics of folding of macromolecule

Complicated
Often spontaneous in solution (about ms – seconds for small proteins)
In cells, larger proteins fold with the help of Chaperons or chaperonins

(1) and (3) is usually referred to as the PROTEIN FOLDING PROBLEM in proteins.

Bottom of the Well

Density of states near the global minimum is still high.

Proteins are characterized by:

a) high frequency small amplitude fluctuations (10^{-12} - 10^{-9} sec) of individual side chains (NOISE!)

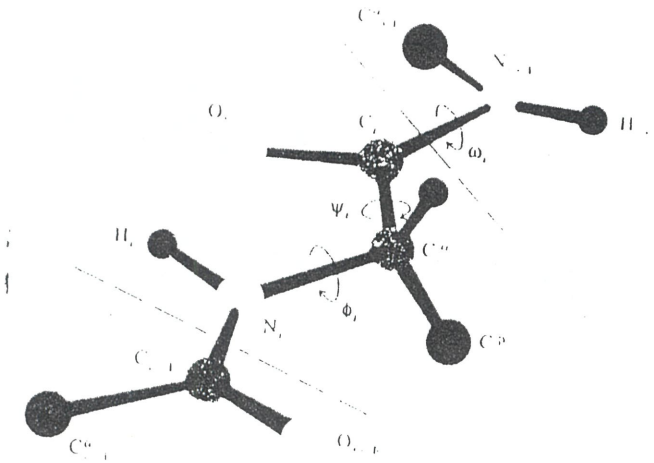
b) larger amplitude collective fluctuations of protein domains (10^{-6} sec)

c) slower conformational transitions

Proteins are not rigid but "soft" structures!

The Protein Folding Problem

FLHARDHCVA HKLFNSLK
MAPNIRKSHP LLKMINNSLI DLPAPSNISA WWNFGSLLAV CLMTQILTGL
LLAMHYTADT SLAFSSVAHT CRNVQYGWLI RNLHANGASF FFICIFLHIG
RGLYYGSYLY KETWNTGVIL LLTLMATAFV GYVLPWGQMS FWGATVITNL
FSAIPYIGHT LVEWAWGGFS VDNPTLTRFF ALHFLLPFAI AGITIHILTF
LHESGSNNPL GISSDSDKIP FHPYYSFKDI LGLTLMLTPF LTLALFSPNL
LGDPENFTPA NPLVTPPHIK PEWYFLFAYA ILRSIPNKLG GVLALAASVL
ILFLIPFLHK SKQRTMTFRP LSQTLFWLLV ANLLILTWIG SQPVEHPFII
IGQMASLSYF TILILFPTI GTLENKMLNY
SDLELHPPSY PWSHRGPLSS LDITSIRRGF QVYKQVCSSC HSMDYVAYRH
LVGVCYTEDE AKALAEEVEV QDGPNEDGEM FMRPGKLSDY FPEPYPNPEA
ARAANNGALP PDLSYIVRAR HGGEDYVFSL LTGYCEPPTG VSVREGLYFN
PYFPGQAIGM APPLYNDVLE FDDGTPATMS QVAKDVCTFL RWAAEPEHDH
RKRMGLKMLL MMGLLVPLVY YMKRHKWSVL KSRKLAYRPP K
SHTDIKVPNF SDYRRPPDDY STKSSRESDP SRKGFSYLVT AVTFLGVAYA
AKNVVTQFVS SMSASADVLA MSKIEIKLSD IPEGKNMAFK WRGKPLFVRH
RTKKEIDQEA AVEVSQLRDP QHDLERVKKP EWVILIGVCT HLGCVPIANA
GDFGGYYCPC HGSHYDASGR IRKGPAPLNL EVPSYEFTSD DMVIVG
AQRPAVSASS RWLEGIRKWY YNAAGFNKYG LMRDDTIYEN DDVKEAIRRL
PLNLYDDRMF RIKRALDLNM RQQILPKEQW TKYEEDVPYL EPYLKEVIKE
RKEREEWDK
GRQFGHLTRV RHLITYSLSP EQRPFPHYF SKGVPNVWRR LRACLRVAP
PFLAFYLLYT WGTQEFEKSK RKNPAAYVND R
GDPKEEEEEE EELVDPLTTV REQCEQLEKC VKARERLELC DERVSSRSQT
EEDCTEELFD FLHARDHCVA HKLFNSLK
VAPTLTARLY SLLFRRTSTF ALTIVVGALL FERAFDQGAD AIYEHINEGK
LWKHIKHKYE NK



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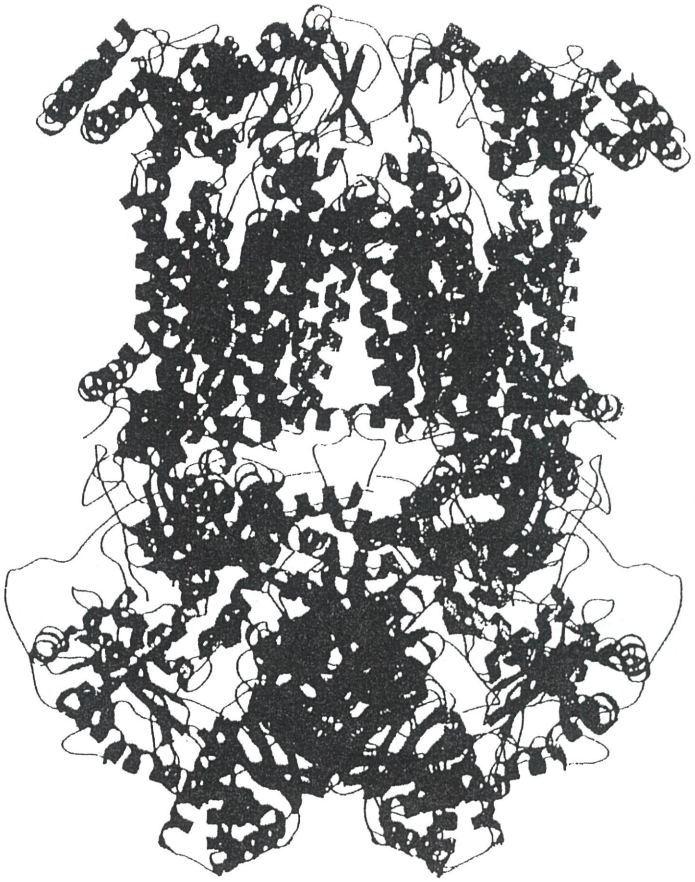


Prediction of Protein Structures

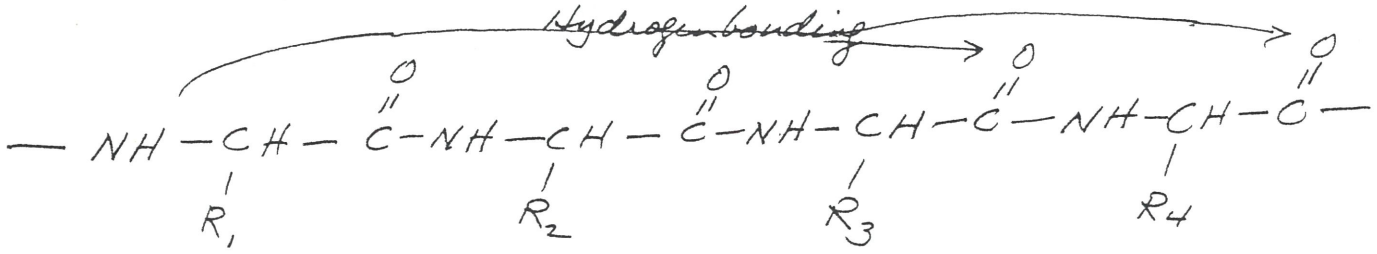
a) Need reliable force fields

b) A way to deal with the role of solvation and water structure

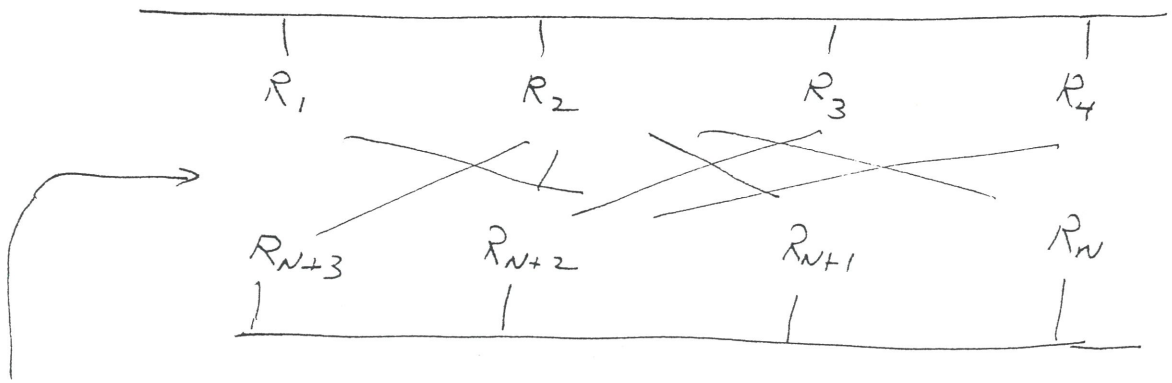
c) Mathematics to get to global minimum without getting trapped in local minima.

Protein

A "string" of L-amino acids:



R_1, R_2, R_3, R_4 amino acids



Hydrogen Bonds
Salt bridges
Hydrophobic Clustering

Also

Specific Hydration
Vander Waal Interactions
Electrostatic Coulombic
Charge - charge interactions
Steric effects

Solvation parameters (typically used)

Atom type	R, A°	σ, kcal/mol
C	1.9	0.024
N	1.7	−0.029
O	1.4	−0.029
N+	1.7	−0.372
O−	1.4	−0.350
S	1.8	−0.018

Other "Force Fields"

- CFF ≡ Consistent Force Field

  Developed by Molecular Simulations, Inc.

  Ref e.g. Maple, J. R., Hwang, M.-J.; Jalkanen, K.J.; Stockfisch, T.P.; & A.T. Hagler, J. Comp. Chem. 19, 430–458 (1998)

- CHARMm ≡ Chemistry at Harvard Macromolecular Mechanics

  Ref. Brooks et al J. Comp. Chem. 4, 187–217 (1983)

- Coverage of macromolecules including proteins, nucleic acids, carbohydrates, and lipids.
- Broad coverage of small organic compounds.
- Range of computed properties: molecular structures, energies, forces, and vibrational frequencies.
- Transferability between small and large molecules and between systems.
- Consistency between types of molecules, allowing simulation of complex systems (such as protein/DNA, enzyme/substrate).
- Scientific validation.
- Automated atom type and partial charge assignment software.
- Complete integration with Discover for structure optimization or molecular dynamics simulations.
- Full commercial support and maintenance.

Range of CFF Functional Groups

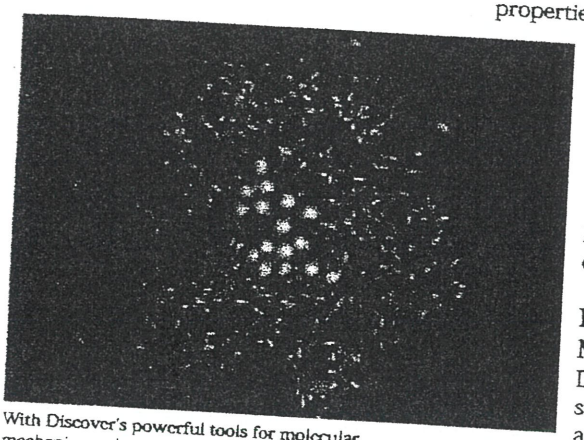
Principal Functional Group Types

alcohols	guanidinium	phenyl amines
aldehydes	hydrazines	phenyl imines
alkanes	imines	sulfides
alkenes	ketones	sulfonamides, N-alkylsulfonamides
amides and alkylamides	monatomic halide anions	sulfones
amine and alkylamine cations	monatomic metal cations	sulfoxides
amines and alkylamines	nitrobenzenes & nitroheterocycles	thiazoles
azo compounds	nitrogen heterocycles	thioamides
biphenyls & bisphenyls	N-heterocycle amides	thiols
carbohydrates	N-heterocycle imines	ureas and aromatic ureas
carbonates	oligazoles, 2-6 ring N's per ring	water
carbamates	oxazoles	1-4 bi-functional compounds
carboxylates	phenyl, N-heterocycle acids/esters	570 aromatic rings
carboxylic acids	phenyl alcohols and ethers	
dialkylphosphates	phenyl amides	
disulfides		
esters		
ethers		

Discover

Molecular Mechanics and Dynamics Simulation Engine

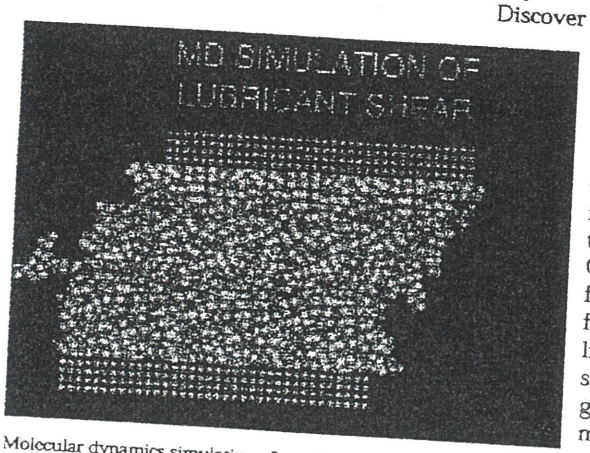
Discover, is a molecular simulation program for applications in computer assisted molecular design in both life and materials sciences. Discover applies a range of well validated forcefields for dynamics simulations, minimization, and conformational searches, allowing you to predict the structure, energetics and properties of organic, inorganic, organometallic, and biological systems. Discover provides you with the ability to study a wide range of molecular systems and materials types. The insight gained through Discover can help you develop and refine working hypotheses as well as guide your experimental directions.



With Discover's powerful tools for molecular mechanics and molecular dynamics simulations, you can study biomolecular structure and interactions. The example shown here is a peptide inhibitor bound to alpha-thrombin (Brookhaven PDB file 1ad8).

Discover Methodologies for Molecular Design

Discover is designed for rigorous simulations and incorporates assorted molecular mechanics and dynamics methodologies that have proven value for molecular design. Using a range of state-of-the-art force fields as the foundation, you can confidently compute minimum energy conformations, as well as families of structures and dynamics trajectories. Supported forcefields for Discover include the default CVFF[1-4], the Amber forcefield[5], CFF[6-11], and COMPASS[12-15]. Through the efforts of the Potential Energy Functions Consortium (PEFC), the CFF forcefield undergoes continuous development to incorporate the latest innovations and parameter values. Currently, CFF, a Class II forcefield, includes parameters for proteins, carbohydrates, lipids, nucleic acids, mixed species (such as common glycoproteins), small organic molecules, many common mono- and divalent cations, and several common anions. Similarly, the COMPASS forcefield has been developed through the efforts of the highly successful Polymer Consortium. With such an array of forcefields at your disposal you are free to explore virtually any type of molecular system.



Molecular dynamics simulation of a model lubricant fluid (5-butyl nonane) undergoing shear between metal plates.

Discover provides flexible control over simulation strategies, ranging from the ability to set atomic level parameters right through to controlling molecular-level behavior through template forcing and geometric restraints. You can constrain structures by setting interatomic distances, bond angles or torsions to desired values. NOE restraints allow you to fit structures to distance and dihedral information derived from NMR experiments. You can investigate binding strengths and thermodynamic stabilities. Full support for symmetry and periodic boundary conditions allow you to simulate infinite crystalline lattices, bulk liquids and mixtures, amorphous solids, interfaces and solvated systems. In addition, comprehensive analysis features allow you to extract the most pertinent results from the simulation.

Using either the Insight II or Cerius2 graphical user interface and visualization module, you can immediately begin complex molecular dynamics simulations. Both the Insight II and Cerius2 environments have a straightforward user interface that eliminates the need to know the intricacies of Discover commands. In addition, the powerful 3D graphics features in Insight II and Cerius2 allow you to display your results visually providing a clearer and more immediate understanding of your system.

Built into Discover are useful tools for performing analysis of structures and dynamics trajectories. You can analyze pair correlation functions, mean square displacement, elastic properties, arbitrary distributions of lengths and angles, as well as other interesting properties. For life science applications, in-depth analysis of trajectories can be enhanced with MSI's flexible analysis module, DeCipher. DeCipher acts as a dynamic molecular information system, allowing interactive definition of molecular properties and dynamic processing of simulation data to obtain the desired molecular information.

With Discover and DeCipher, you can address serious projects in computer aided molecular design.

msi
molecular simulations

Discover

discovery, protein design, genomic therapeutics, NMR spectroscopy, and X-ray crystallography.

COMPASS is a class II force field designed for modeling materials systems comprising organic and polymeric systems and selected inorganics and metals. COMPASS has been specifically parameterized with a view to performing accurate predictions of the properties of condensed phase materials - such as PVT behavior and cohesive properties.

References

1. Lifson, S., Hagler, A. T., Dauber, P., Consistent Force Field Studies of Intermolecular Forces in Hydrogen-Bonded Crystals. 1. Carboxylic Acids, Amides and the C=O...H - Hydrogen Bonds. *J. Am. Chem. Soc.* **101**, 5111, **1979**
2. Hagler, A. T., Lifson, S., Dauber, P., Consistent Force Field Studies of Intermolecular Forces in Hydrogen-Bonded Crystals. 2. A Benchmark for the Objective Comparison of Alternative Force Fields. *J. Am. Chem. Soc.* **101**, 5122, **1979**
3. Hagler, A. T., Dauber, P., Lifson, S., Consistent Force Field Studies of Intermolecular Forces in Hydrogen-Bonded Crystals. 3. The C=O...H-O Hydrogen Bond and the Analysis of the Energetics and Packing of Carboxylic Acids. *J. Am. Chem. Soc.* **101**, 5131, **1979**
4. Dauber-Osguthorpe, P., Roberts, V. A., Osguthorpe, D. J., Wolff, J., Genest, M., et al., Structure and Energetics of Ligand Binding to Proteins: *Escherichia coli* Dihydrofolate Reductase-Trimethoprim, A Drug-Receptor System. *Proteins: Struc. Func. Gen*, **4**, 31-47, **1988**
5. Weiner, S. J., Kollman, P. A., Nguyen, D. T., Case, D. A. An All Atom Force Field for Simulations of Proteins and Nucleic Acids. *J. Comp. Chem.* **7**, 230-52, **1986**
6. Maple, J. R., Hwang, M. J., Stockfisch, T. P., Dinur, U., Waldman, M., et al., Derivation of Class II Force Fields. 1. Methodology and Quantum Force Field for the Alkyl Functional Group and Alkane Molecules. *J. Comp. Chem.* **15**, 162-82, **1994**
7. Hwang, M. J., Stockfisch, T. P., Hagler, A. T., Derivation of Class II Force Fields. 2. Derivation and Characterization of a Class II Force Field, CFF93, for the Alkyl Functional Group and Alkane Molecules. *J. Am. Chem. Soc.* **116**, 2515-25, **1994**
8. Maple, J. R., Hwang, M. J., Stockfisch, T. P., Hagler, A. T., Derivation of Class II Force Fields. III. Characterization of a Quantum Force Field for Alkanes. *Israel J. Chem.* **34**, 195-231, **1994**
9. Peng, Z., Ewig, C. S., Hwang, M. J., Waldman, M., Hagler, A. T., Derivation of Class II Force-Fields. 4. van der Waals Parameters of Alkali Metal Cations and Halide Anions. *J. Phys. Chem. A* **101**, 7243-52, **1997**
10. Maple, J. R., Hwang, M. J., Jalkanen, K. J., Stockfisch, T. P., Hagler, A. T., Derivation of Class II Force Fields. V. Quantum Force Field for Amides, Peptides, and Related Compounds. *J. Comp. Chem.* **19**, 430-58, **1998**
11. Hwang, M. J., Ni, X., Waldman, M., Ewig, C. S., Hagler, A. T., Derivation of Class II Force Fields. VI. Carbohydrate Compounds and Anomeric Effects. *Biopolymers* **45**, 435-68, **1998**
12. Sun, H., Rigby, D., Polysiloxanes: ab initio force field and Structural, Conformational and Thermophysical Properties, *Spectrochimica Acta* A53, 1301-23, **1997**.
13. Rigby, D., Sun, H., Eichinger, B.E., Computer Simulation of Poly(ethylene oxide): Force Field, PVT Diagram and Cyclization Behavior, *Polymer International* 44, 311-330, **1997**
14. Sun, H., Ren, P., Fried, J. R., The COMPASS forcefield: Parameterization and Validation for Polyphosphazenes, *Computational and Theoretical Polymer Science*, In press.
15. Sun, H., COMPASS, An ab initio Force Field Optimized for Condensed-Phase Applications - Overview with Details on Alkane and Benzene Compounds, *J. Phys. Chem.* In press.

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molecular simulations

CHARMm

Analysis Options

Structural

- Powerful commands to manipulate coordinates including rigid body RMS comparisons, reorientation, mass-weighting, calculate radius of gyration, and scaling
- Powerful commands to analyze internal coordinates
- Calculation of molecular volumes and surfaces
- Calculation of solvent averaged properties
- Correlation function analysis from dynamics trajectories
- Identification of reaction coordinates for a given trajectory such as conformational transition

Energetics

- Interaction energies between any two sets of atoms
- Hydrogen bond energy

Vibrational/Normal Mode

- Analyze fluctuations of atoms or internal coordinates
- Explore the energy surface along one or two modes
- Analysis of crystals with any space group symmetry

Hardware Platforms

Versions available for Silicon Graphics workstations, Cray servers (T3D, T3E, J90, C90, and T90), IBM RS/6000 workstations.

Software Requirements

Insight II or QUANTA 3D graphics program

Complementary Software

MBO(N)D is a multibody dynamics program which allows you to produce simulations of molecular movements and properties up to 30 times faster than conventional methods.

DeCipher is a powerful and flexible program for high-level analysis of molecular structure and the results of molecular dynamics simulations.

MCSS searches potential active sites in proteins for potential binding points using a unique, computationally efficient approach.

References

1. Brooks et al., CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. *J. Comp. Chem.*, **4**, 187-217 (1983)
2. MacKerell, Jr. et al., All-atom empirical potential for molecular modeling and dynamics studies of proteins. *J. Phys. Chem B*, **102**, 3586-3617, (1998)
3. MacKerell, Jr. et al., An all-atom empirical energy function for the simulation of nucleic acids. *J. Amer. Chem. Soc.*117, 11946-11975, (1995)
4. Pavelites et al., A Molecular Mechanics Force Field for NAD+, NADH, and the Pyrophosphate Groups of Nucleotides. *J. Comp. Chem.*, **18**, 221-239, (1997)
5. Feng et al., Structural Characterization of the Phosphotyrosine Binding Region of a High-Affinity aSH2 Domain-Phosphopeptide Complex by Molecular Dynamics Simulation and Chemical Shift Calculations. *J. Amer. Chem. Soc.*, **118**, 11265-11277, (1996)
6. Schlenkrich et al., Empirical Potential Energy Function for Phospholipids: Criteria for Parameter Optimization and Applications in "Biological Membranes: A Molecular Perspective from Computation and Experiment," K.M. Merz and B. Roux, Eds. Birkhauser, Boston, pp 31-81, (1996)
7. Feller et al., Molecular Dynamics Simulation of Unsaturated Lipids at Low Hydration: Parameterization and Comparison with Diffraction Studies.*Biophys. J.* **73**, 2269-2279, (1997)
8. Neria et al., Simulation of Activation Free Energies in Molecular Systems. *J. Chem. Phys.*, **105**, 1902-21, (1996)
9. Momany, FA, and Rone, R., Validation of the General Purpose QUANTA 3.2/ CHARMm Force Field, *J. Comp. Chem.*, **13**, 888-900, (1992)

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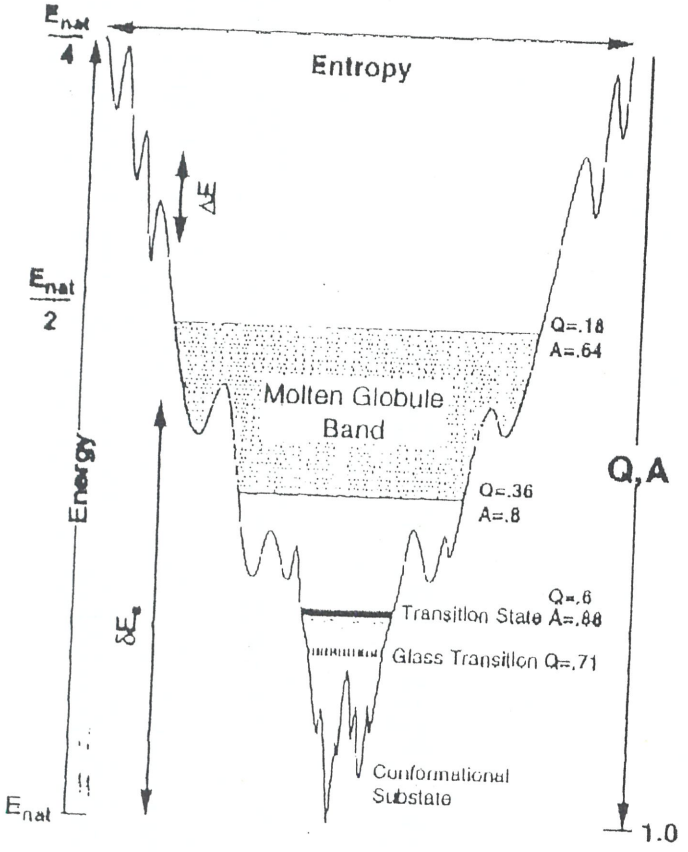
Protein Folding Funnel

Proc. Natl. Acad. Sci. USA 92 (1995)



Peter Leopold and Jose Onuchic introduced the protein folding funnel approach to the problem. *(PNAS 1992)*

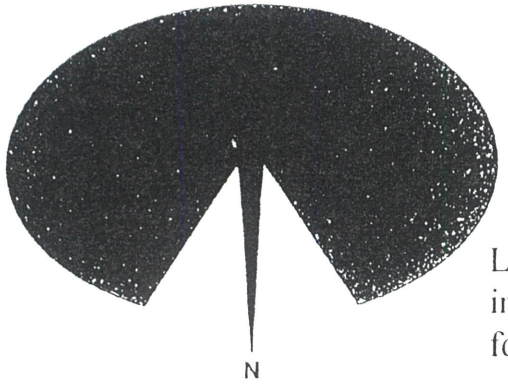
The configurational entropy provides a measure of the density of states.

Roughness of the landscape offers some insight into the kinetic barriers (not directly).

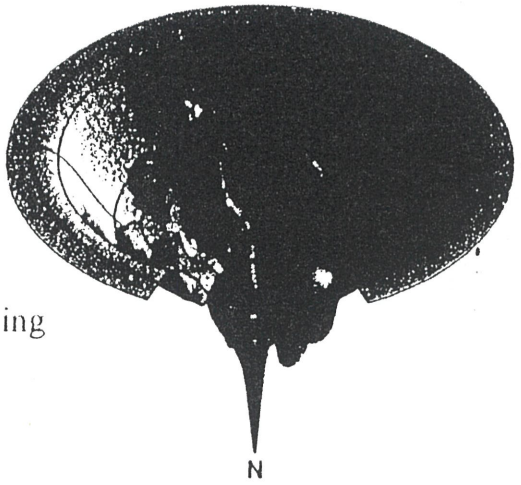
The protein folding funnel offers a way to conceptualize the problem in terms of the pathway(s), and mechanisms, including:

- The nature of transition states: many transition states vs. several entropic barriers.
- Is there an enthalpic collapse?
- Kinetic channeling?
- Annealing intermediates?

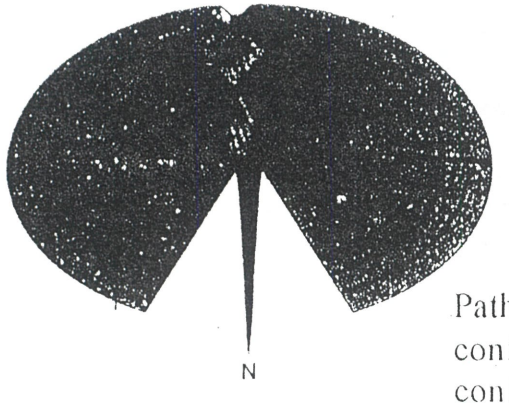
Other Models



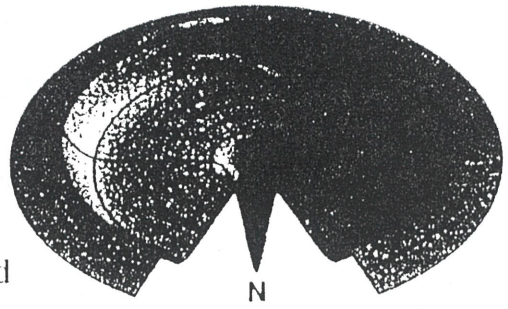
Levinthal landscape involves random searching for the native state.



Rugged energy landscape with kinetic traps, energy barriers, pathways to the native state. Folding can be multi state.



Pathway from the denatured conformation to the native conformation.



Conformational entropy can cause free energy barriers to folding. The rate limiting step is the aimless wandering on the flat plateau.

K. Dill & H. Chan, *NSB* (1997)

Models of Protein Folding

1. Ken Dill -- Heteropolymer collapse

   • The protein is a heteropolymer which collapses

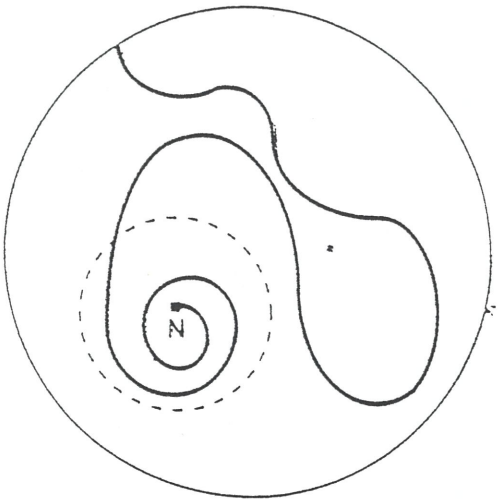
      - to avoid contact with the water solvent
      - to maximize contacts between apolar amino acid side chains

   • Compaction of the polymer

      - creates secondary structure
      - drives the formation of tertiary structure

2. Walter Englander and Robert Baldwin -- Secondary structure drives tertiary structure

   • Contacts form between amino acid side chains
   • Secondary structure forms and molds protein structure



Search for "Transition State"

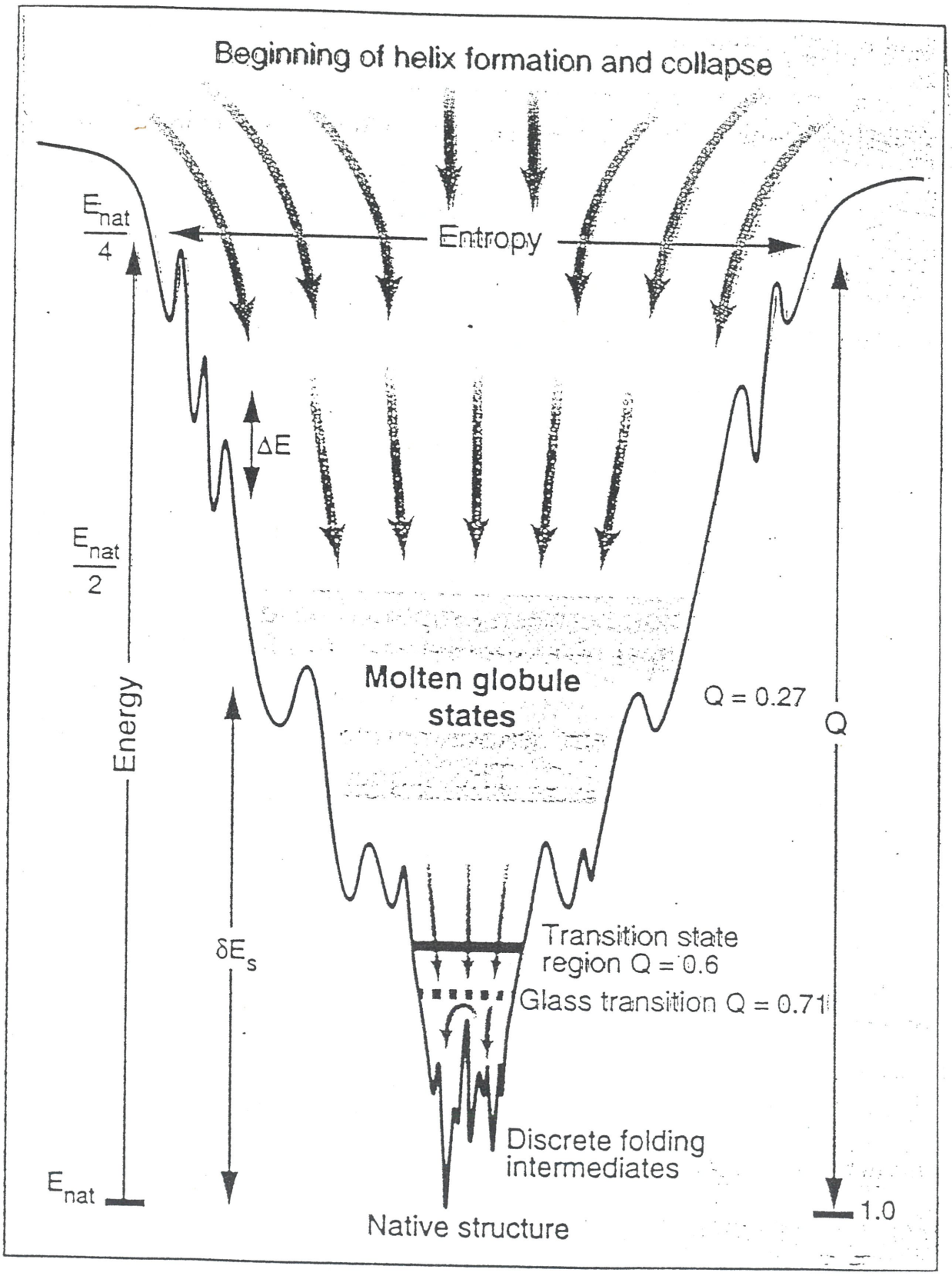
$$\text{Entropic Barrier} \sim \Delta S^{\ddagger} \sim S_{\substack{\text{entropic} \\ \text{barrier}}} - S_{\text{unfolded}}$$

$$k \sim \frac{kT}{h} N_{path} e^{\Delta S^{\ddagger}/R}$$

$$\sim \frac{kT}{h} N_{path} e^{(S_{\substack{\text{entropic} \\ \text{barrier}}} - S_{\text{unfolded}})/R}$$

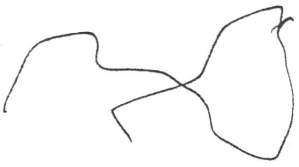
Followed by "Enthalpic Collapse"

(waterfall phenomenon)



Wolynes, Onuchic, & Thirumalai Science 267, 1619, (1995).

T-jump



"Cold Denatured" Protein ~4°C

$\Delta T \sim 20°C$

T-jump (Laser to heat water) $\sim 10^{-9}$ sec.

"Denatured Protein" at 25°C

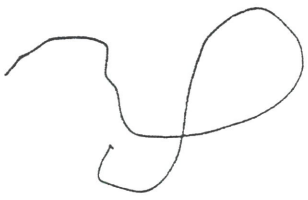
↓

↓

↓

"Folded" Structure

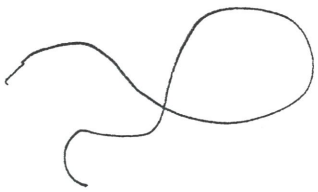
PH-jump



"Denatured" Protein at low or high pH

$\Delta pH \pm 4$

pH-jump $\sim 10^{-6}$ sec



"Denatured Protein" at new pH

↓

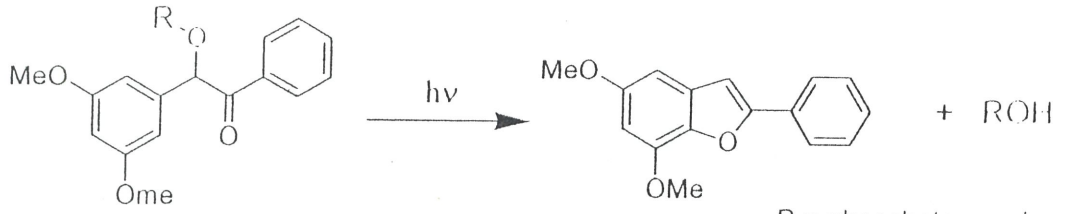
↓

↓

"Folded" Structure

A General Method for Photoinitiating Protein Folding in a Non-Denaturing Environment

Photolabile Benzoins



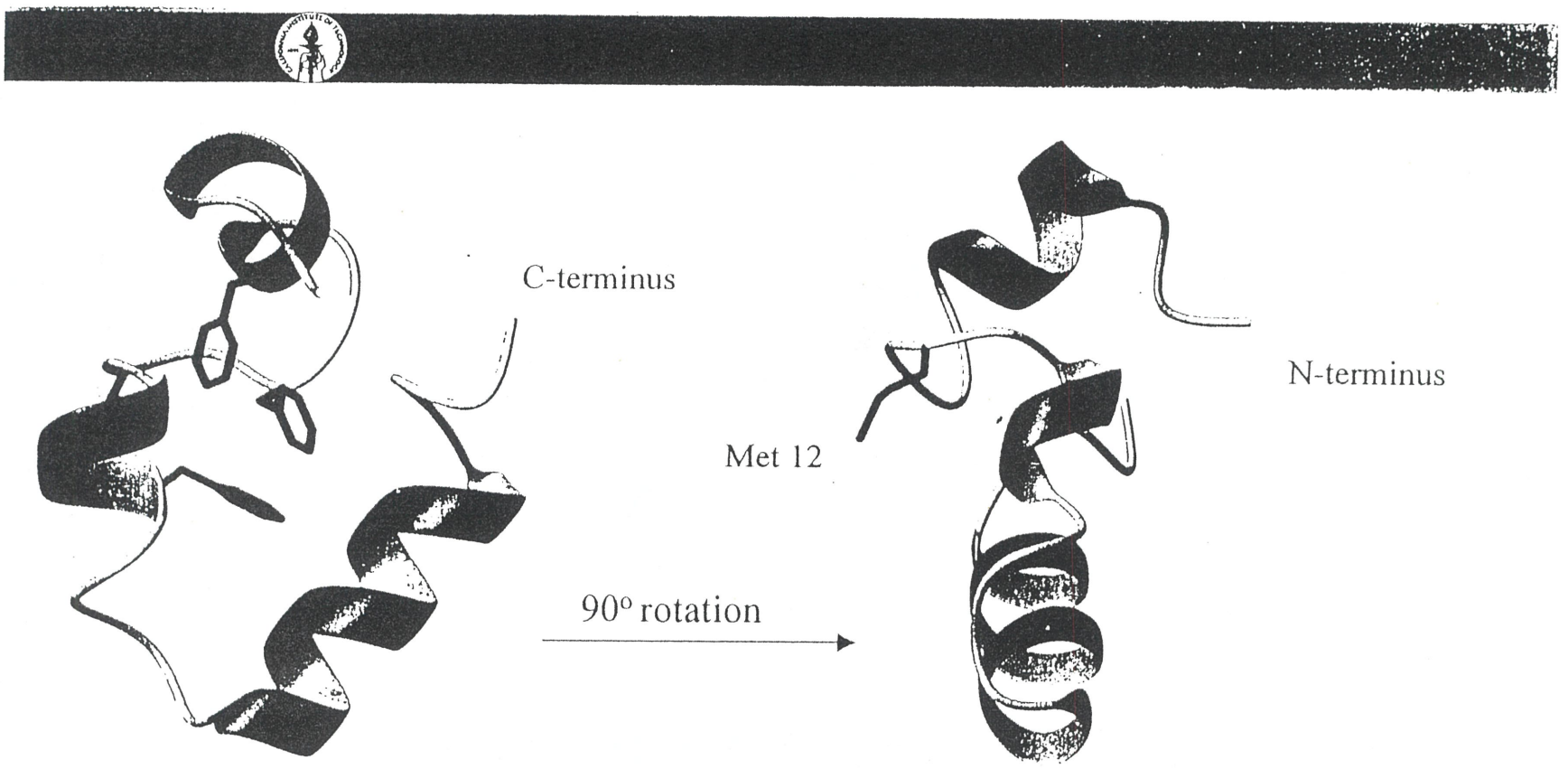
In 1971 Sheehan *et. al.* showed that esters of 3'5'-dimethoxybenzoin undergo efficient and very clean photolysis under near-UV illumination.

The benzoin system has the following characteristics which make it the cage of choice for this study:

- Quantum yields in the 0.6-0.7 range
- inert photo-products
- possible derivatization of the benzyl rings
- an estimated rate constant of $>10^{10}$ s^{-1}

Protein System

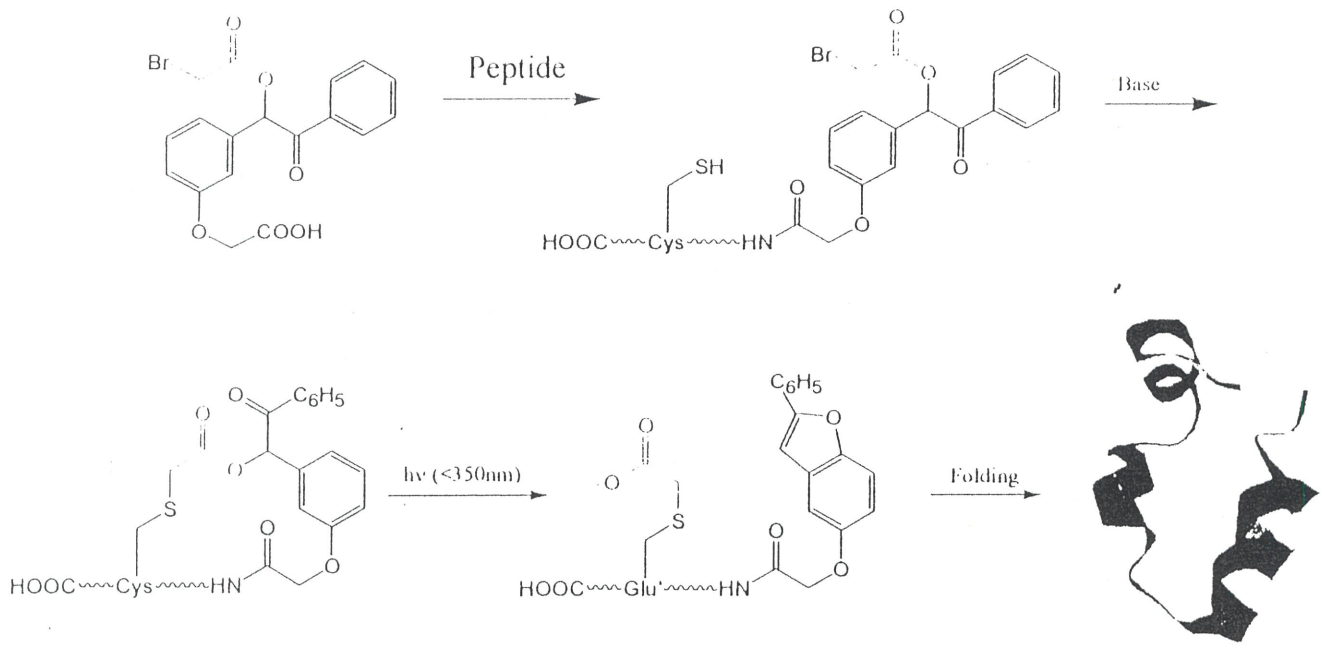
NMR Structure of 35-residue subdomain within the villin headpiece.



- The short length is compatible with standard Fmoc solid phase peptide synthesis
- A high percentage of secondary structure is present
- Monomeric with a high thermostability ($T_m = 70\,^{o}C$ at pH 7.0)[5]
- VHP has a well defined hydrophobic core
- Expected fast folding
- Computational experiments have been performed by Duan and Kollman.

McKnight, Matsudaira, Kim, *NSB* (1997)

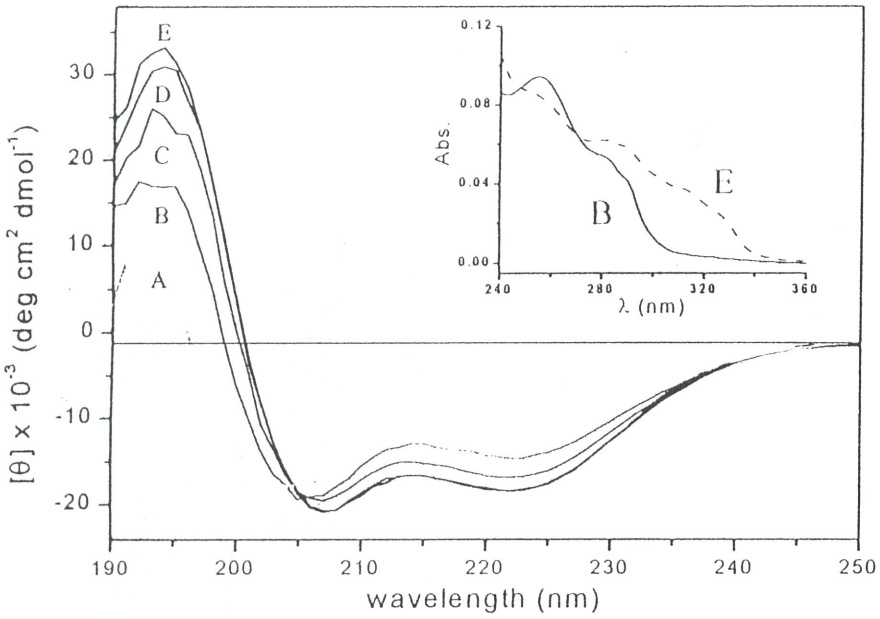
General Synthetic Strategy



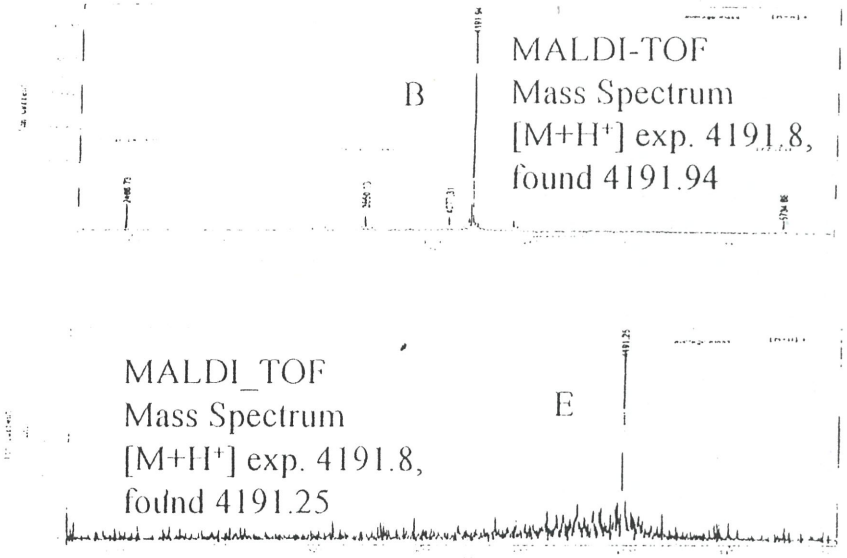
The final product yields a thioether analog of Glu, thus the best residues to target are solvated surface residues.

VHP(34) M12C:
LSDEDFKAVFGCTRSAFANLPLWKQQNLKKEKGL

Steady State Photolysis

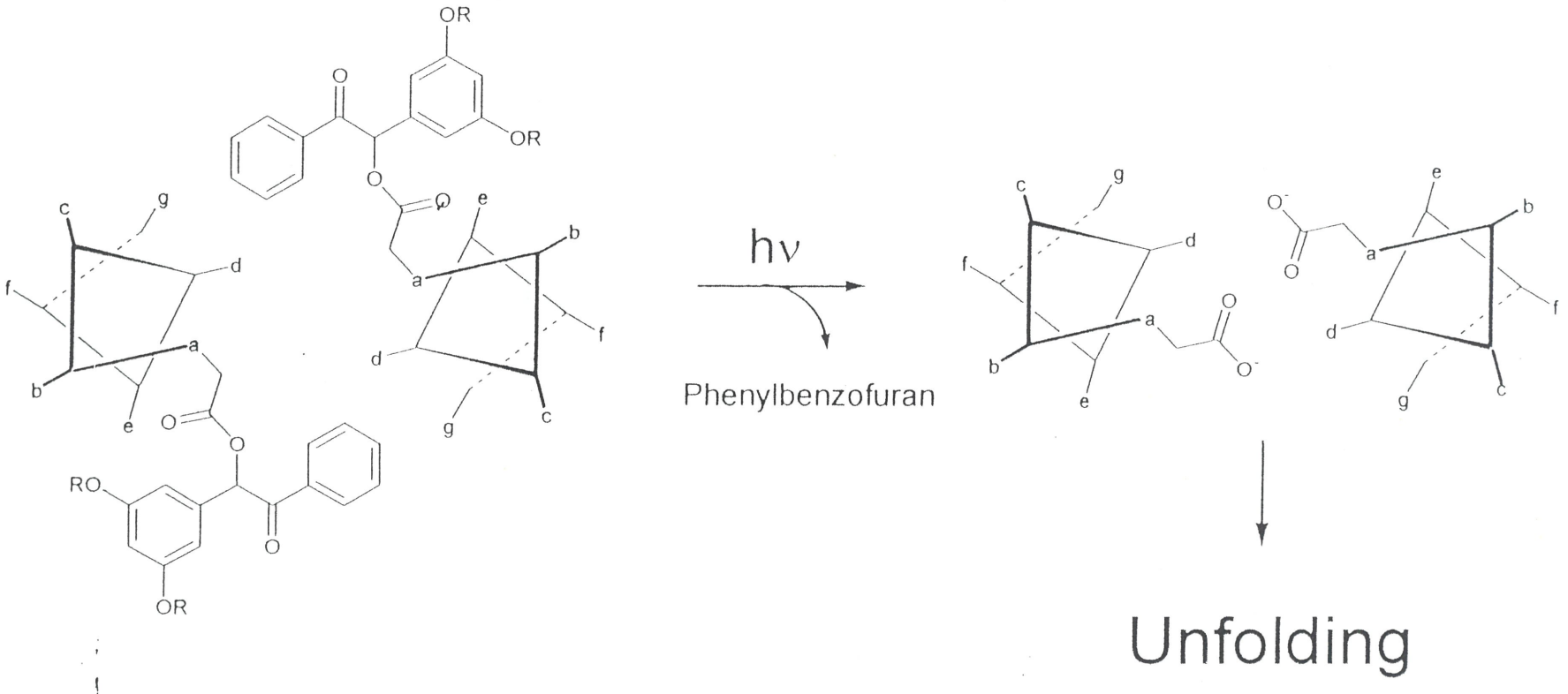


Steady-state photolysis of 28 μM cVHP M12C-CMB in 10 mM $NaPO_4$ buffer, pH 7.4, irradiated from 300 to 400 nm using a filtered high-pressure mercury vapor arc lamp. Linear form at 95 °C (above the melting temperature of VHP-35) (A); cyclized form, irradiation time (s): (B) 0, (C) 10, (D) 30, (E) 90. Inset UV/Vis spectra.

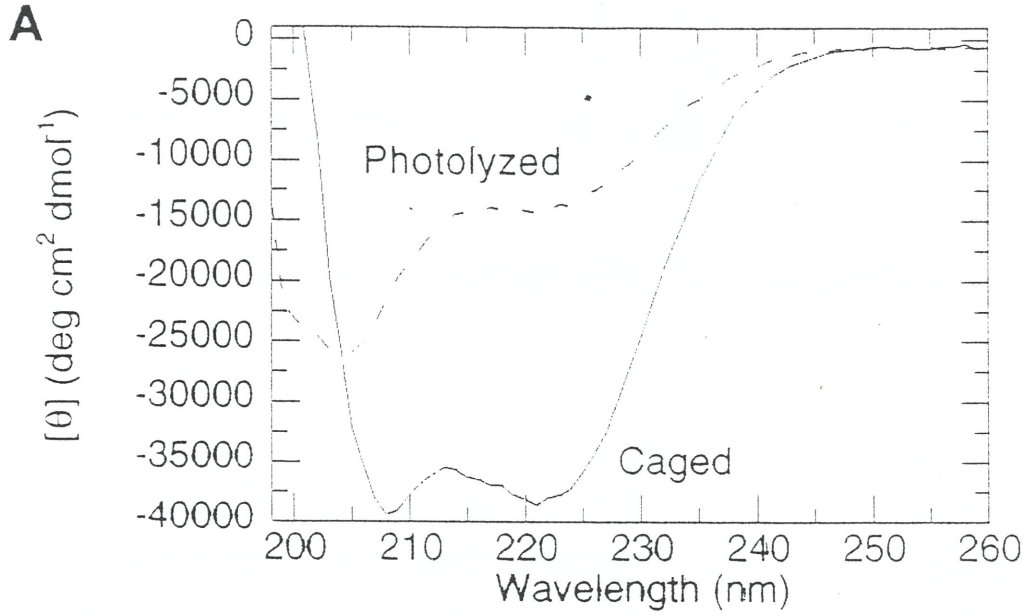


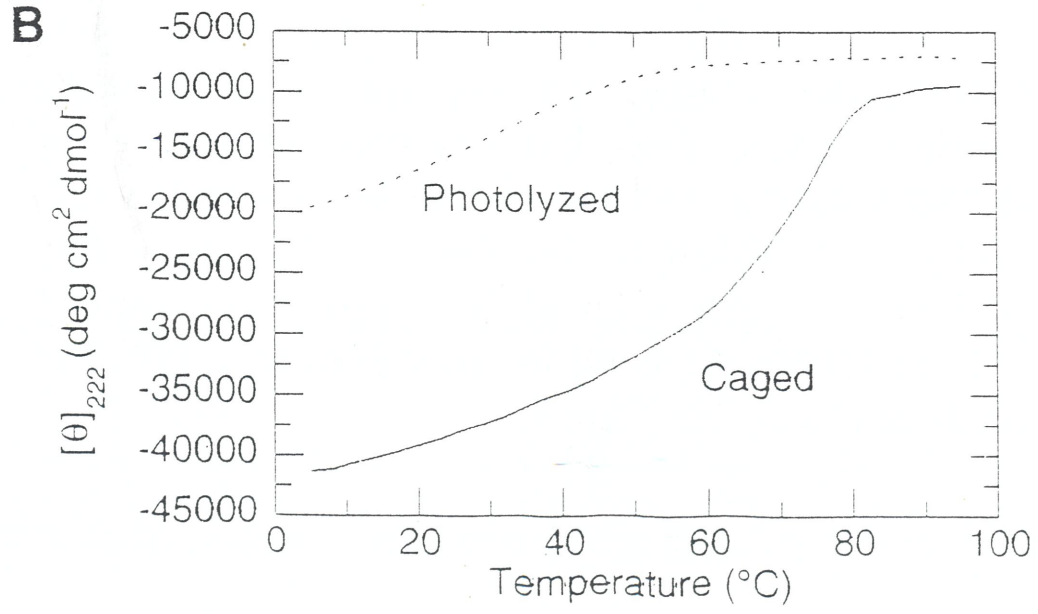
MALDI MS. The molecular mass of the peptide before and after the photolysis, were to be 4191.9 and 4191.2 amu, respectively. This is indicative of cyclized peptide.

Initiation of Unfolding



CD Spectra of GCn4-p1 (N16D(DMB))





In PBD, changes in the refractive index are followed optically by a probe beam.

In PAC, the volume expansion is monitored by detecting the accompanying acoustic wave.

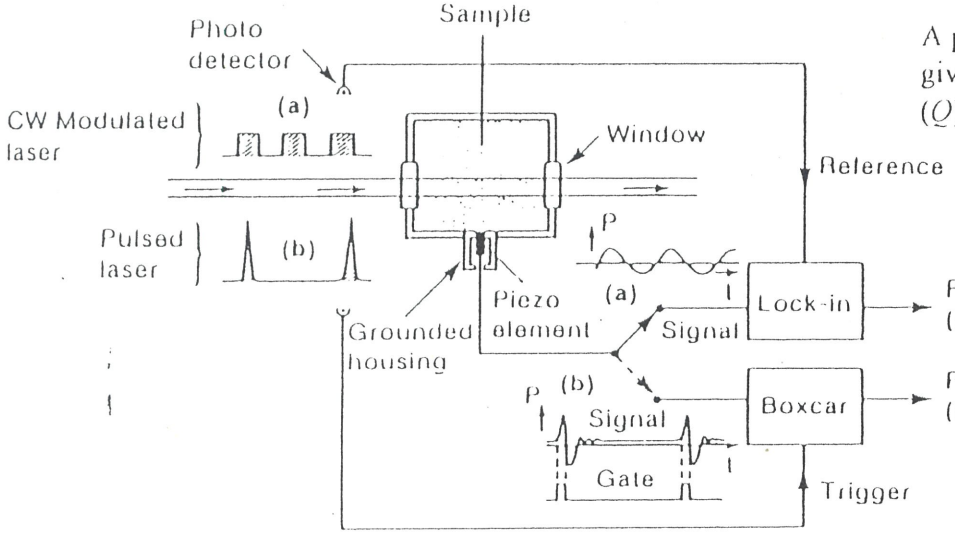
Photoacoustic Calorimetry

➲Pulsed Laser photoacoustics is a technique that measures photoinduced enthalpic and volumetric changes on the nano- and microsecond timescales.

➲Photoacoustic Calorimetric measurements are based on the vibrational relaxation to the ground state of a photo-excited molecule and the accompanied thermal heating of the surrounding solvent.

➲After normalizing the signal acoustic wave with that of an appropriate reference compound (on that is nonfluorescent and does not undergo any photochemistry) the following expression is obtained:

$$\phi E_{h\nu} = (S/S_{ref})E_{h\nu} = Q + [\Delta V_{con}/F(T)]$$



A plot of the normalized photoacoustic amplitude (ϕ) versus 1/F(T) gives a strait line with a slope equal to the heat evolved (Q).Subtracting Q from $E_{h\nu}$ gives ΔH for the reaction.

$F(T) = (\beta/C_p\rho)$
β = thermal expansion coefficient of water in K^{-1}
C_p = heat capacity in cal/g -deg –mol
ρ = density in g/ml
Q is the amount of heat released to the solvent after photolysis
$E_{h\nu}$ is the energy of the excitation photon

In principle, the amount of heat deposited in the solvent could be derived from the deflection angle of the probe beam created by the refractive index gradient within the solution.

In practice, the amount of deflection can be quantified by comparing the signal amplitude of the unknown sample to that of a reference compound. The reference typically absorbs the pulse energy that is deposited, in its entirety, to the solvent as heat without additional photochemistry. Thus, the expression of the PBD signal for the reference compound has no volume change terms, a quantum yield of unity, and a Q_{ref} equal to $E_{h\nu}$, or

$$R = A\, E_{h\nu}(dn/dT)(1/\rho\, C_p)\, E_{h\nu}$$

so that $(S/R)^* E_{h\nu}$

$$= \Phi\, Q + \Phi[\rho(dn/d\rho)\Delta V + B\Delta n_{obs}]/\, dn/dT)(1/\rho\, C_p)$$

PBD Analysis

The deflection signal arising from changes in the refractive index of the sample can be written as:

$$S = AE_{h\nu}\Phi[(dn/dT)(1/\rho C_p)Q + \rho(dn/d\rho)\Delta V + B\Delta n_{abs}]$$

A represents the geometrical parameters of the instrument
$E_{h\nu}$ is the photon energy absorbed
Φ is the quantum yield
ρ is density (g/mL), C_p is the heat capacity (cal/deg K)
Q is the heat released to the solvent by the sample upon excitation
n is the solution refractive index
$B\Delta n_{abs}$ is the change in refractive index due to absorption changes in the sample .

By using the ratio of the sample signal to that of the reference, the normalized PBD signal provides an expression which eliminates the instrument response factor, A:

$$(S/R)^*E_{h\nu} = \Phi Q + \Phi[\rho(dn/d\rho)\Delta V + B\Delta n_{abs}]/(dn/dT)(1/\rho C_p)$$

Plotting $(S/R)E_{h\nu}$ versus $\rho C_p/(dn/dT)$ gives a slope proportional to the volume change and an intercept equal to the amount of heat released to the solvent. Since Q is the amount of heat released by the photoinitiated reaction, subtracting Q from $E_{h\nu}$ (the amount of energy absorbed by the molecule) gives ΔH for the reaction.

Results

PBD:

ΔV = -2.6 ml/mole on a timescale faster than deadtime of the instrument (initial electrostrictive contraction of the solvent upon generation of the free carboxylate at residue 16)

Fast phase was followed by a single phase of volume expansion of + 1.5 ml/mole, occurring at a rate of 1.8×10^5 sec^{-1} (slower volume expansion upon unfolding due to an exposure of hydrophobic residues in the core of GCN4-p1).

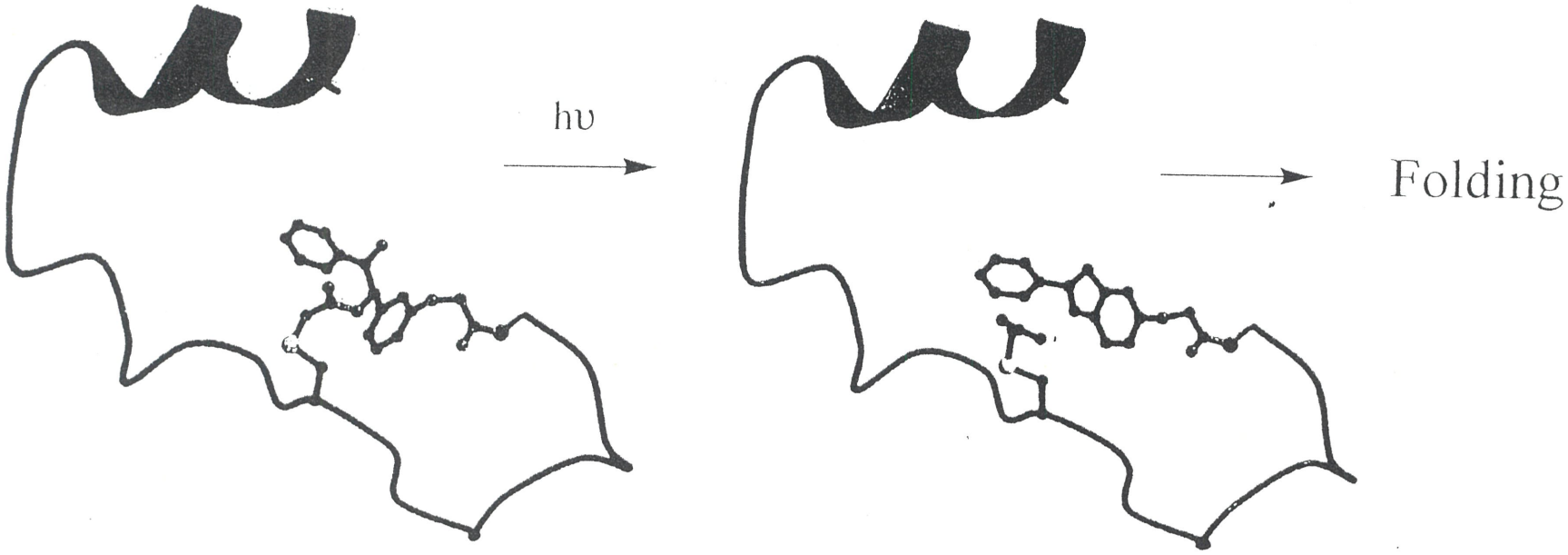
PAC:

Additional kinetic phases of 1.4×10^6 sec^{-1} and 3.3×10^6 sec^{-1}

Control

Photochemical cleavage of a DMB model compound was instantaneous as revealed by PAC and PBD.

cVHP-34 M12E' MODEL



Model was built with BioGraf™
The structure was minimized by running Molecular Dynamics simulation at 1000K and then 300K.

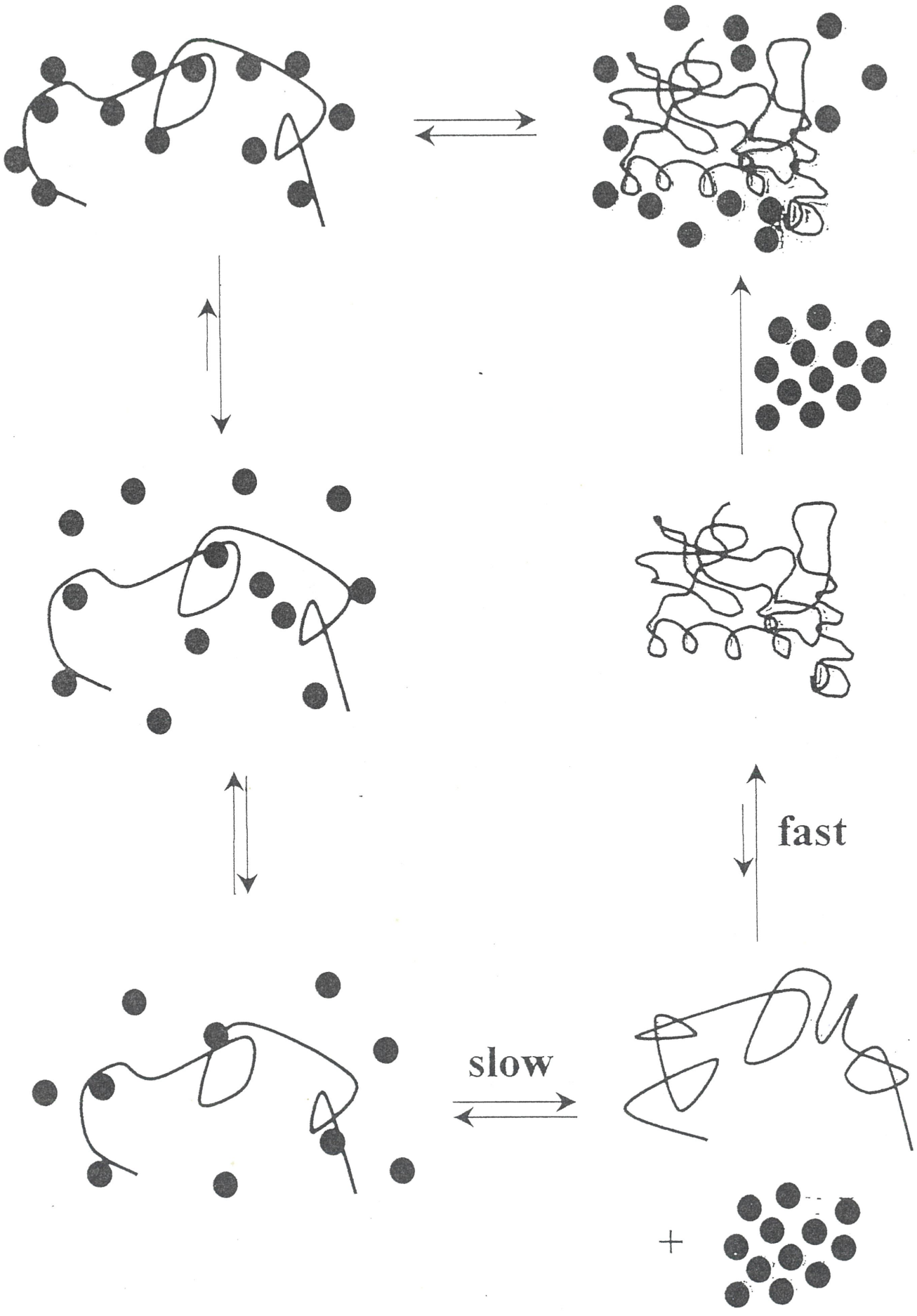
Conclusions

- We have shown that folding can be initiated by releasing a peptide chain from a cyclic-constrained conformation using a caging group that photolyzes on the sub-nanosecond time scale.

- This general strategy can be used to link the N - terminus of a protein to a cysteine side-chain in synthetically accessible proteins.

- This technique can be applied to virtually any protein assuming that the secondary structure will be disrupted by loop formation.

- Kinetics of the refolding event have been studied using PAC and PBD spectroscopy.


fast
slow
+