AD-A253 030

ORT DOCUMENTATION PAGE

	(U)	**1b RESTRICTIVE MARKINGS** NA
	NA	**3 DISTRIBUTION/AVAILABILITY OF REPORT**
2b DECLASSIFICATION/DOWNGRADING SCHEDULE	NA	Distribution Unlimited
4 PERFORMING ORGANIZATION REPORT NUMBER(S)	NA	**5 MONITORING ORGANIZATION REPORT NUMBER(S)** NA

6a NAME OF PERFORMING ORGANIZATION University of Washington	**6b OFFICE SYMBOL** (If applicable) NA	**7a NAME OF MONITORING ORGANIZATION** Office of Naval Research
6c. ADDRESS (City, State, and ZIP Code) Department of Chemistry, BG-10 Seattle, WA 98195		**7b ADDRESS (City, State, and ZIP Code)** 800 N. Quincy Street Arlington, VA 22217-5000

8a NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	**8b OFFICE SYMBOL** (If applicable) ONR	**9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER** N00014-91-J-1393 (and 88-K-0202)

8c ADDRESS (City, State, and ZIP Code)
800 N. Quincy Street
Arlington, VA 22217-5000

10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO
61153N	RR04106	441n002	

11 TITLE (Include Security Classification)

(U) Magnetic Resonance Elucidation of Molecule/Macromolecule Interaction Stereochemistry

12 PERSONAL AUTHOR(S)
Andersen, Niels H.

13a TYPE OF REPORT Final Technical	**13b TIME COVERED** FROM 2/91 TO 3/92	**14 DATE OF REPORT (Year, Month, Day)** 92 JUL 09	**15 PAGE COUNT** 7

16 SUPPLEMENTARY NOTATION

None.

17 COSATI CODES			**18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)**
FIELD	GROUP	SUB-GROUP	Nuclear Magnetic Resonance, Molecular Recognition, NOESY Spectral Simulation, Structure Refinement, Software Development
06	03		

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Our primary accomplishments were the development of software for the computer–aided structure elucidation of structural features of biological systems based on NOESY data: a) simulating accurate theoretical NOESY spectra based on structural models, motional assumptions and NMR experiment parameters; and b) the automated extraction of modeling constraints directly from raw NOESY data. The most recent software developments are the incorporation of model–free order parameters into NOESY simulations and the addition of routines for comparing computer–extracted distances (or cross–rates) with the expectation ranges based on the full spectrum of torsional freedom so as to generate precision estimates for NOE–distances from a single NOESY spectrum. These methods have been used to define the conformational states of a variety of polypeptide and drug molecule systems including: prostaglandin and steroid analogs, peptide hormones such as dynorphin–related peptides and GnRH analogs, rigid and flexible endothelin analogs, and a small protein allergen. The methods have also been applied to receptor–bound peptides.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	**21 ABSTRACT SECURITY CLASSIFICATION** (U)
22a NAME OF RESPONSIBLE INDIVIDUAL M. Marron	**22b TELEPHONE (Include Area Code)** (202)-696-4760 **22c OFFICE SYMBOL** ONR

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE (U)

S/N 0102-LF-014-6603

GRANT #: N00014-91-J-1393   R&T Code: 441n002

PRINCIPAL INVESTIGATOR: Niels H. Andersen

INSTITUTION: University of Washington

GRANT TITLE: NMR Elucidation of Molecular/Macromolecular Complex Stereochemistry

REPORTING PERIOD = AWARD PERIOD: 01 February 1991 → 31 March 1992

OBJECTIVE: To develop methods and software for quantitative analysis of NOESY data and the extraction of experimental constraints for the refinement of structures of biomacromolecules and their complexes. as a means for studying molecular recognition phenomena.

ACCOMPLISHMENTS (since the 6/91 progress report):

1) The DISCON algorithm for the model-free extraction of accurate cross-rates (and estimates of their experimental precision) from a single NOESY data set has been fully implemented in user-friendly software. The theoretical basis of the method and validation tests are the subject of two papers that are now in press. These papers include demonstrations of: a) the use of DISCON-derived rates (and distances) to ascertain that a multi-conformer equilibrium must be assumed and to define major conformer structures, and b) the superiority of DISCON (versus nOe growth rate estimates followed by BKCALC distance adjustment) for deriving constraints for DNA structure refinement.

2) The incorporation of model-free order parameters in NOESYSIM, our program for calculating the expectation NOESY spectrum for a structural and dynamic model and the experimental conditions, including the non-ideal effects of incomplete return to equilibrium magnetization values during truncated preparatory delays.

3) The structure elucidations of endothelin-1, an endogenous vasoconstrictor = 1/15,3/11–C S C S S L M D K E C V Y F C H L D I I W, and a number of both more rigid and more flexible analogs that retain biological activity have been completed and reported. All permissable bioactive conformers are α-helical from K9→C15.

Figure 1

In the case of the more rigid analogs in which the $Cys^{3,15}$ residues were replaced by $\beta\beta$-Me$_2$-Cys (=Pen), the helical region extends to Leu^{17} and the backbone conformation is well defined from residue 1→18. The backbone conformation over that region is shown to the right. In the case of the native form of endothelin-1, four additional contributing conformers were located.
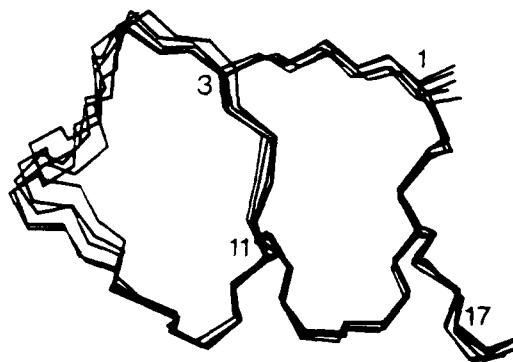
In this conformational mixture, NH $\Delta\delta/\Delta T$-values near zero do not correspond to persistent H-bonds. A novel analytical paradigm for using $\Delta\delta/\Delta T$-values in conjunction with exchange rate and solvent induced shift data to locate and characterize loci of conformational motion has been developed.
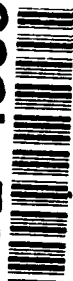
4) Studies of a series of endothelin analogs and hevein (below) revealed that the chemical shift index (CSI) method of Wishart *et al.* (*Biochemistry* **31**, 1647) can be extended to peptides and less rigid portions of proteins. Our modified Hα-CSI also detects the rarer α_L conformation and can thus locate left-handed reverse turns. **Figure 2** illustrates the CSI plots for hevein.

Figure 2. A CSI plot for hevein — the categories of deviation from 'random coil' chemical shift values are 0.11–0.20, 0.21–0.30, 0.31–0.44, 0.45–0.99 and ≥ 1ppm. Solid, upward directed bars indicate downfield shifts.
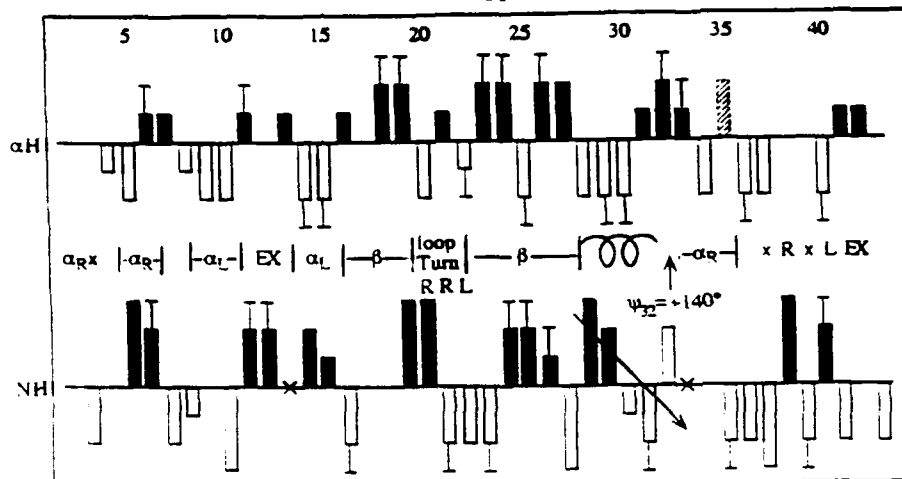
5) A series of analogs of the C-peptide from RNAse have been examined by both CD and NMR methods. These studies have provided insights into the relationship between the classic '%-helicity' measure available from CD measurements and the extent of a helical region and its population in a conformational equilibrium. The latter can be extracted from quantitative NOESY analysis. As it turns out, CD amplitude is surprisingly insensitive to end-fraying; however, we have provided, in these studies, the first experimental verification of a theoretically predicted change in the CD signature of α-helices with persistence length -- the 207nm band shows diminished relative intensity *versus* the 221nm band, and the latter is red-shifted, in helices shorter than 10 residues in length.

6) Our primary emphasis during the last year of study was to establish the potential advantages (and risks) associated with a less conservative use of NOESY derived constraints in structure refinement. The main element of our approach is the use of 'tight distance bounds' or their equivalent, high precision cross-rate estimates from a DISCON analysis of the NOESY data. A paper describing this work is in preparation. **Figure 4**, *vide infra*, is taken from that paper.

7) A high resolution NMR structure has been obtained for hevein
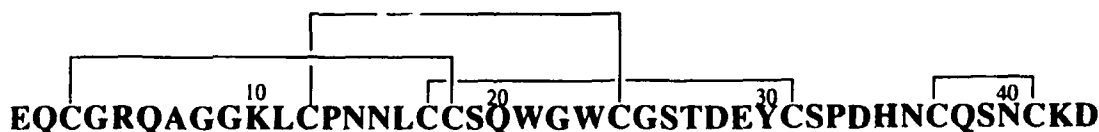
Our NMR work provides the first solution structure for a member of the agglutinin-toxin fold family of proteins which retains the diagnostic disulfide linkage pattern. The conformational features shown in the Hα-CSI correlation are taken from the structure ensemble obtained using tight NOE distance bounds for an XPLOR/CHARMm refinement.
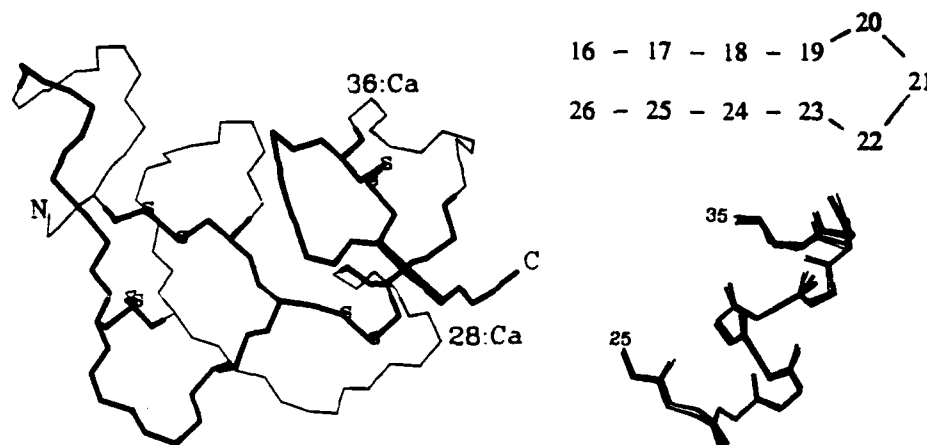
The structural features of the major conformer cluster found in the NMR structure ensemble are illustrated below (**Fig. 3**).

Figure 3.


```
                                       20
           16 – 17 – 18 – 19              
                                          21
           26 – 25 – 24 – 23           
                                    22
```

Parallel refinements were carried out with tight bounds obtained using the DISCON algorithm and with these converted to 'conservative' bounds as commonly used in protein structure elucidation. Only when tight bounds were used was it possible to define the few remaining loci of conformational isomerism in this structure. The two major conformational forms of the N-terminal loop are shown here --

note the high degree of similarity outside of the loop region and the convergence within the short helical segment of the hevein structure. The helical portion which can best be characterized as an 8 residue α-helix with a single kink at ψ_{32}, is shared by all conformers even though His[35] is another locus of conformational change.
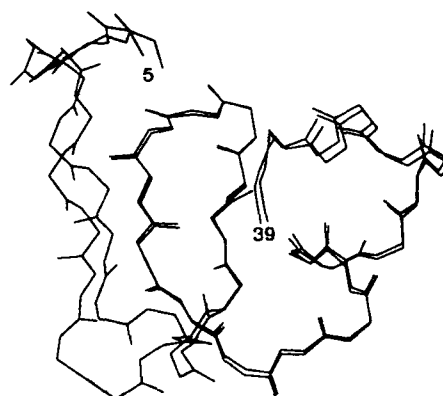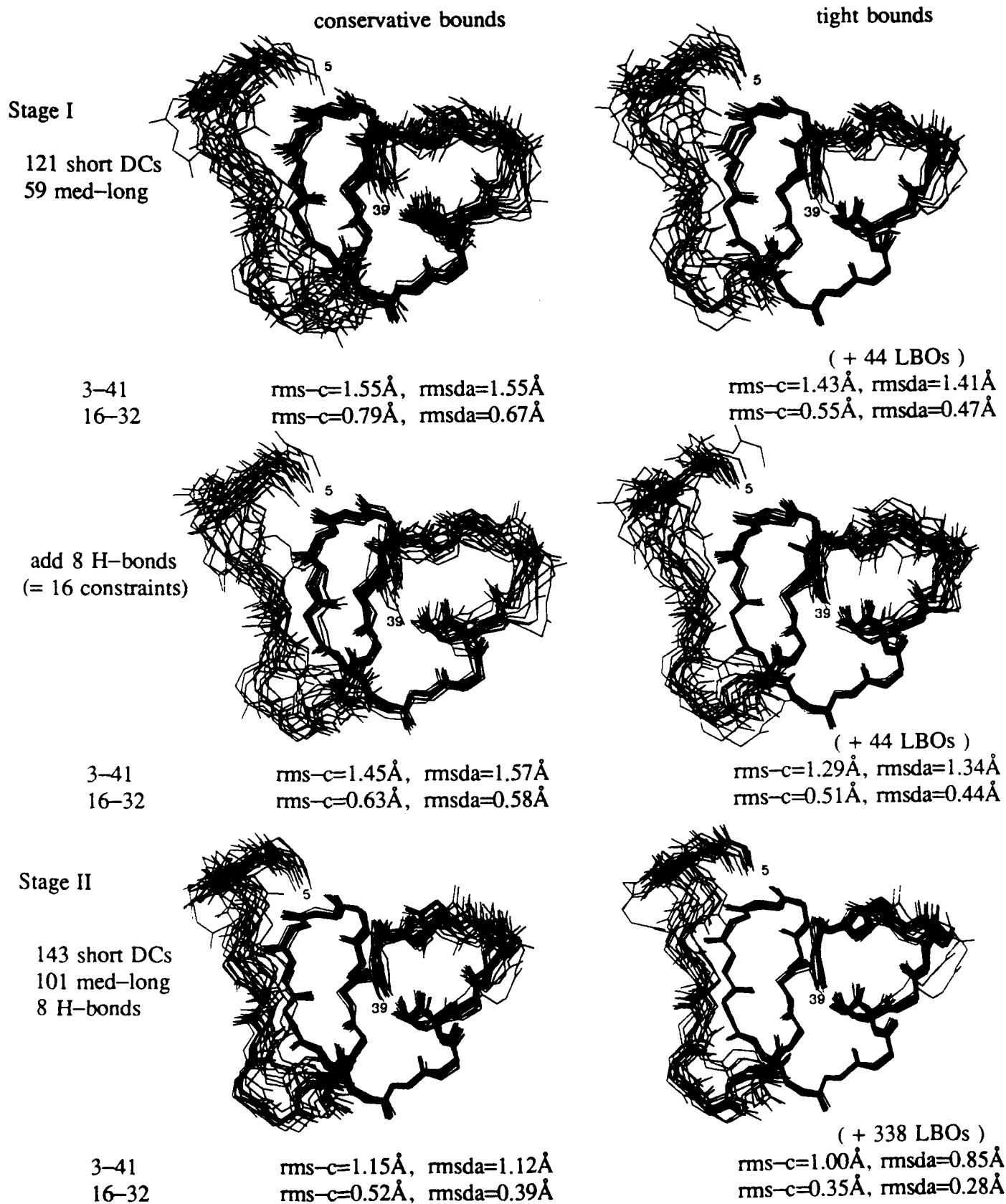

The differences in the convergence and structural precision obtained at different constraint density levels and with a tight *versus* conservative treatment of bounds at each stage are illustrated in **Figure 4**, the following page of this report.

OTHER RESULTS: The other results that came from the studies undertaken as a result of O.N.R. grant and contract support during the 1988-1992 period are adequately described in the papers and meeting abstracts that have appeared. These are listed in a later section.

SIGNIFICANCE: Until our most recent studies, regions of proteins which were ill-defined in the ensemble of NMR-refinement structures were dismissed as "disordered" and the standard NOESY methodology did not work for smaller peptides that display conformational isomerism. The methods that we have developed should extend the range of systems that can be structurally characterized by NMR to include peptides displaying multi-conformer equilibria and the loop dynamics in proteins.

Figure 4. A comparison of the convergence (rms-c) and accuracy (rmsda) of the hevein structure ensembles obtained at stages I and II using the same nOe distances as tight *versus* conservative bounds in XPLOR simulated annealing. Residues 5→39 are displayed, rms fitted over 16–32.

conservative bounds   tight bounds

Stage I

121 short DCs
59 med–long

(+ 44 LBOs)

	conservative bounds	tight bounds
3–41	rms–c=1.55Å, rmsda=1.55Å	rms–c=1.43Å, rmsda=1.41Å
16–32	rms–c=0.79Å, rmsda=0.67Å	rms–c=0.55Å, rmsda=0.47Å

add 8 H–bonds
(= 16 constraints)

(+ 44 LBOs)

3–41	rms–c=1.45Å, rmsda=1.57Å	rms–c=1.29Å, rmsda=1.34Å
16–32	rms–c=0.63Å, rmsda=0.58Å	rms–c=0.51Å, rmsda=0.44Å

Stage II

143 short DCs
101 med–long
8 H–bonds

(+ 338 LBOs)

3–41	rms–c=1.15Å, rmsda=1.12Å	rms–c=1.00Å, rmsda=0.85Å
16–32	rms–c=0.52Å, rmsda=0.39Å	rms–c=0.35Å, rmsda=0.28Å

The endothelin structure elucidation was presented in posters or oral presentations at the Spr.'91 ACS Mtg. and at the Keystone Symposium on "Frontiers of NMR in Molecular Biology" (4/91). This structure elucidation has also been featured in numerous guest lectures in which our NOESYSIM/DISCON software was presented. FOUR (4) posters based on ONR-supported research were presented by my group at the Apr.'92 E.N.C. Meeting, the titles appear below as published abstracts.

PUBLICATIONS (cummulative for N00014-91-J-1393 & -88-K-0202):

Throughout this section the following symbols are used:
† acknowledges ONR support, previously submitted to the Scientific Program Officer, *Will be submitted when reprints become available, § Utilize methods developed under ONR support but acknowledge primary support from other sources.

previously reported

† 1) Eaton, H.L., Andersen, N.H., and Lai, X. (1988), Recent Extensions of NOESYSIM, A Program for Rapid Computation of NOESY Intensity Matrices from Atomic Cooridinates and Experimental Conditions, Abstract #112, 29th E.N.C. (Rochester, N.Y., 4/88).

† 2) Lai, X., Marschner, T.M., Chen, C., and Andersen, N.H. (1989), DISCON, A New Program for Obtaining Distance Constraints Corrected for Spin Diffusion, Poster #W20, 30th E.N.C. (Asilomar, CA, 4/89).

† 3) Andersen, N.H., Eaton, H.L., and Lai, X. (1989), Quantitative Small Molecule NOESY: A Practical Guide for Derivation of Cross-Relaxation Rates and Internuclear Distances, *Magn. Reson. in Chem.* **27**, 515-528.

4) Andersen, N.H., Lai, X., and Marschner, T.M. (1989), NOESYSIM/ DISCON Documentation, copyrighted by Univ. of Washington. (70 pages)

† 5) Andersen, N.H., Lai, X., Marschner, T.M., Chen, C., Hammen, P.K., and Harris, S. (1990), Programs for Quantitative NOESY Analysis and Applications to Biorecognition Phenomena, Molecular Recognition Res. Mtg., Charleston, SC (1/90).

† 6) Andersen, N.H., Lai, X., Hammen, P.K., and Marschner, T.M. (1990), Computer-aided Conformational Analysis Based on NOESY Signal Intensities in *NMR Applications in Biopolymers* (Finley et al., Eds.). pp 95-134, Plenum Publ. Co., NY.

more recent publications and submitted works

7) Andersen, N.H., Lai, X., and Marschner, T.M. (1991), NOESYSIM/DISCON Documentation, copyrighted by Univ. of Washington. (80 pages, copy available upon request, the original 1989 version has been submitted in conjunction with invention disclosures associated with the reporting for contract # N00014-88-K-0202)

§ 8) Krystek, S.R., Bassolino, D.A., Novotny, J., Chen, C., Marschner, T.M., and Andersen, N.H. (1991), Conformation of Endothelin in Aqueous Glycol Determined by 1H-NMR and Molecular Dynamics Simulations, *FEBS Lett.* **281**, 212-218. (previously submitted to the scientific program officer)

§ 9) Rovnyak, G., Andersen, N., Gougoutas, J., Hedberg, A., Kimball. S.D., Malley, M., Moreland, S., Porubcan, M., & Pudzianowski, A. (1991), Active Conformation of 1,4–Dihydropyridine Calcium Entry Blockers: Effect of Size of 2–Aryl Substituent on Aryl Rotamer Preference, *J. Med. Chem.* **34**, 2521–2524.

§10) Andersen, N. H., & Hammen, P. K. (1991), A Conformation–Preference/ Potency Correlation for GnRH Analogs: NMR Evidence, Biorganic & Med. Chem. Lett. 1, 263–266.

† 11) Andersen, N.H., Chen, C., Marschner, T.M., Krystek, S.R., & Bassolino, D.A. (1992), Endothelin Conformational Dynamics in Acidic Aqueous Media from Quantitative NOESY Analysis, *Biochemistry* **31**, 1280–1295.

§ 12) Krystek, S.R., Jr., Bassolino, D.A., Bruccoleri, R.E., Hunt, J.T., Porubcan, M.A., Wandler, C.F., & Andersen, N.H. (1992), Solution Conformation of a Cyclic Pentapeptide Endothelin Antagonist: Comparison of Structures Obtained from Constrained Dynamics and Conformational Search, *FEBS Lett.* **299**, 255–261.

§ 13) Andersen, N.H., Cao, B., & Chen, C. (1992), Peptide/Protein Structure Analysis using the Chemical Shift Index Method: Upfield α–CH Values Reveal Dynamic Helices and α_L Sites, *Biochem. Biophys. Res. Commun.* **184**, 1008–1014.

14) Cao, B., & Andersen, N.H. (1992), The Solution Structure of Hevein, a Representative of the Allergen–Toxin Motif, 33rd E.N.C., #MP–50, Book of Abstracts, p. 105.

15) Andersen, N.H., Chen, C., Cao, B., & Wandler, C.F. (1992), Peptide Structure Analysis, Amide–NH Temperature Gradients and Exchange Rates Revisted: Extracting Structural and Dynamics Information, 33rd E.N.C., #MP–51, Book of Abstracts, p. 105.

16) Harris, S.M., & Andersen, N.H. (1992), Persistent Short Helices *versus* Dynamic Nascent Helices – – The Comparative Information Content of CD and NMR Studies, 33rd E.N.C., #WP–50, Book of Abstracts, p. 197.

17) Chen, C, & Andersen, N.H. (1992), Endothelin Analogs — A Case Study of Helix Stability and Persistence Length, 33rd E.N.C., #WP–51, Book of Abstracts, p. 197.

* 18) Lai, X., Chen, C., and Andersen. N.H. (1992) Extracting Experimental Distances from NOESY Data: The DISCON Algorithm, an Accurate and Robust Alternative to an Eigenvalue Solution. *J. Magn. Reson.*, in press.

* 19) Lai, X., Reid. B., & Andersen, N. H. (1992) A Comparison of BKCALC–Adjusted Constraints and those from DISCON using Simulated Data for a B–DNA Structure, *J. Magn. Reson.*, in press.

* 20) Harris, S.M., Cao, B., Lee, V.G., & Andersen, N.H. (1992) Toward a More Precise Definition of Peptide Structure by CD. I. Generating Secondary Structure Spectra for Medium–sized Peptides. The CD Signature of Very Short α–Helices, *Biopolymers*, submitted.

* 21) Andersen, N.H., Cao, B., Rodriguez, A., & Arreguin, B. (1992) Hevein: the NMR Asignment and an Initial Assessment of Solution–state Folding for the Agglutinin–Toxin Motif, *Biochemistry*, submitted.

* 22) Andersen, N.H., Cao, B., Chen, C., & Wandler, C. (1992) Concerning NOE ^{1}H/^{1}H–Distance Constraints: The Advantages and Risks of Abandoning a Universal 2.0Å Low–bound. *J. Biomolecular NMR*, in preparation.

SALARY SUPPORT FOR P.I. (% of annual salary, 12mo-basis, is indicated):

    N00014-88-K-0202 period   -- 14.3%

    N00014-91-J-1393 period (14 mos) -- 9.0%

STUDENTS AND TRAINEES SUPPORTED (91/02/01 - 92/03/31, on N00014-91-J-1393)

name	status [a]	man-month [b] salary(effort).	requested data [c]
Xiaonian Lai	post-doc	1.3 (2.2)	P.R.C., Han Chinese
Bolong Cao	grad. st.	3.5 (7.0)	P.R.C., Han Chinese
Chinpan Chen	grad. st.	3.7 (7.0)	Taiwan, Chinese
Scott M. Harris	grad. st.	7.8 (9.0)	U.S.A. citizen
Robert Palmer	grad. st.	2.85(4.0)	U.S.A. citizen
Charles F. Wandler	grad. st.	4.2 (6.0)	U.S.A. citizen
TOTALS		23.4 (35.2)	

Note that $39,984.72 in funds from the previous contract were transfered forward to the 1 year extension grant.

STUDENTS AND TRAINEES SUPPORTED (88/03/16 - 91/01/31, on N00014-88-K-0202)

name	status [a]	man-month [b] salary(effort).	requested data [c]
Thomas Marschner	post-doc	12.0 (14.0)	U.S.A. citizen
Xiaonian Lai	grad. st.	15.4 (24)	P.R.C., Han Chinese
Bolong Cao	grad. st.	12.9 (21)	P.R.C., Han Chinese
Phil Hammen	grad. st.	1.5 (6.0)	U.S.A. citizen
Chinpan Chen	grad. st.	11.2 (14)	Taiwan, Chinese
Scott M. Harris	grad. st.	7.0 (10)	U.S.A. citizen
TOTALS		60.0 (89)	

---

a) Status is indicated as post-doctoral research associate (post-doc) or as graduate student research assistant (grad. st.).

b) Many of the research assistants continued to work on this project while supported as teaching assistants. The parenthetic value is the estimated total effort which includes both periods as a TA and as a RA drawing stipend support.

c) Citizenship and ethnicity -- all U.S.A. citizens were caucasian not of hispanic origin.