Fourth Annual Report
Interactive Graphics for Molecular Graphics System

TR78-02

February 1978

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NATIONAL INSTITUTES OF HEALTH
DIVISION OF RESEARCH RESOURCES
BIOTECHNOLOGY RESOURCES BRANCH

SECTION I - RESOURCE IDENTIFICATION

Report Period		Grant No.
From: January 1, 1977 To: December 31, 1977		RR00898 03 and 04
		Date of Report Preparation: March 1978
Name of Resource	Resource Address	Resource Telephone No.
Interactive Graphics for Molecular Graphics System	273 Phillips Hall, UNC Chapel Hill, N.C. 27514	919 - 933-1074
Principal Investigator	Title	Academic Department
Dr. F: P. Brooks, Jr.	Kenan Professor and Chairman	Computer Science
Grantee Institution	Type of Institution	Investigator's Telephone No.
University of North Carolina at Chapel Hill	State University	919 - 933-2148

Name of Institution's Biotechnology Resource Advisory Committee:

Scientific Advisory Committee

Membership of Biotechnology Resource Advisory Committee: (* Committee Chairman)

Name	Title	Department	Institution
Frederick P. Brooks, Jr.*	Kenan Professor & Chairman	Computer Science	UNC
Ernest L. Eliel	Professor	Chemistry	UNC
Jan Hermans	Professor	Biochemistry	UNC
Sung-Hou Kim	Associate Professor	Biochemistry	Duke
Edward Perl	Professor & Chairman	Physiology	UNC
David Richardson	Associate Professor	Biochemistry	Duke

Typed Name & Title of Principal Investigator	Signature	Date
Frederick P. Brooks, Jr., Kenan Professor & Chairman	F P Brooks Jr	14 March 1978

Typed Name & Title of Grantee Institution Official	Signature	Date
George R. Holcomb, Dean Research Administration-UNC at Chapel Hill	George R. Holcomb	March 14, 1978

II. RESOURCE OPERATIONS AND PROGRESS

A. DESCRIPTION OF RESOURCE OPERATIONS AND PROGRESS

General

The past year has been marked by an expansion both of our community of clients and of the amount of time they spent using the GRIP systm. We served a total of twelve groups of chemists during 1977, eight of which were new users. Only three of our past users did not return during 1977, and we expect two of these to return in the coming year.

Objectives and Operating Policies

We are building a comprehensive and effective interactive computer resource for seeing, manually manipulating, and computationally modifying mathematical models of complex molecules. We believe that our present resource has been shown to be as complete and useful as any in existence; we are aware of many inadequacies and needs.

Fundamental to our approach are the following objectives:

-- The GRIP system is designed to help chemists get results from their research, and its success is measured only by theirs.

-- GRIP is designed to help the chemist visualize his molecules, his density maps, etc., so that he can use his knowledge to guide computation processes. That is, it is an aid to, not a surrogate for, human thinking and manipulation. Hence a strong emphasis on human factors research and on human engineering of the system.

-- GRIP is designed to serve many users, not one or two, so it must include an armory of alternative tools and techniques.

-- GRIP is designed to interface smoothly with any batch computations its users must do, and to incorporate on-line facilities for all computations that can reasonably be done "while you wait."

-- We as computer scientists are interested in GRIP as a test-bed for research in man-machine systems design, in man-machine interaction, and in the design of distributed computing systems.

A corollary of these objectives is that we are heavily dependent on observation of and feedbck from real users attempting to solve real problems. Hence when cuts were necessary, we have heretofore cut development in order to keep on getting user experience.

We currently offer the facility, and such help as we can give, free of charge to any chemist:

-- who has a scientifically interesting problem, as assessed by our Resource Advisory Committee,

-- whose work is at a stage where our facility might be useful,

-- who is willing to commit his time, travel money, and effort to a serious use of the facility, and

-- who is willing to give us written and oral feedback from his experience.

We have extended this offer publicly at the Columbia Workshop on Molecular Graphics (June, 1976), at the South Atlantic Protein Crystallography Workshop (April, 1977), and by word-of-mouth at other occasions. So far, we have had as many users as we have been able to handle.

Our users are almost exclusively working on the structures of molecules of considerable biochemical interest: proteins and nucleic acids. We aim to advance health-oriented biochemical research by enhancing the productivity of individual reseachers through better tools.

Administrative and Scientific Organization

Figure 1 shows the current organization of the GRIP project. At present, Mr. Leonarz is not only managing the Graphics Laboratory but also serving part time as the Acting Resource Director. In this capacity, he arranges all client service and supervises the graduate assistant team that furnishes it. The project continues to need a full-time person skilled in computers and biochemistry for that role.

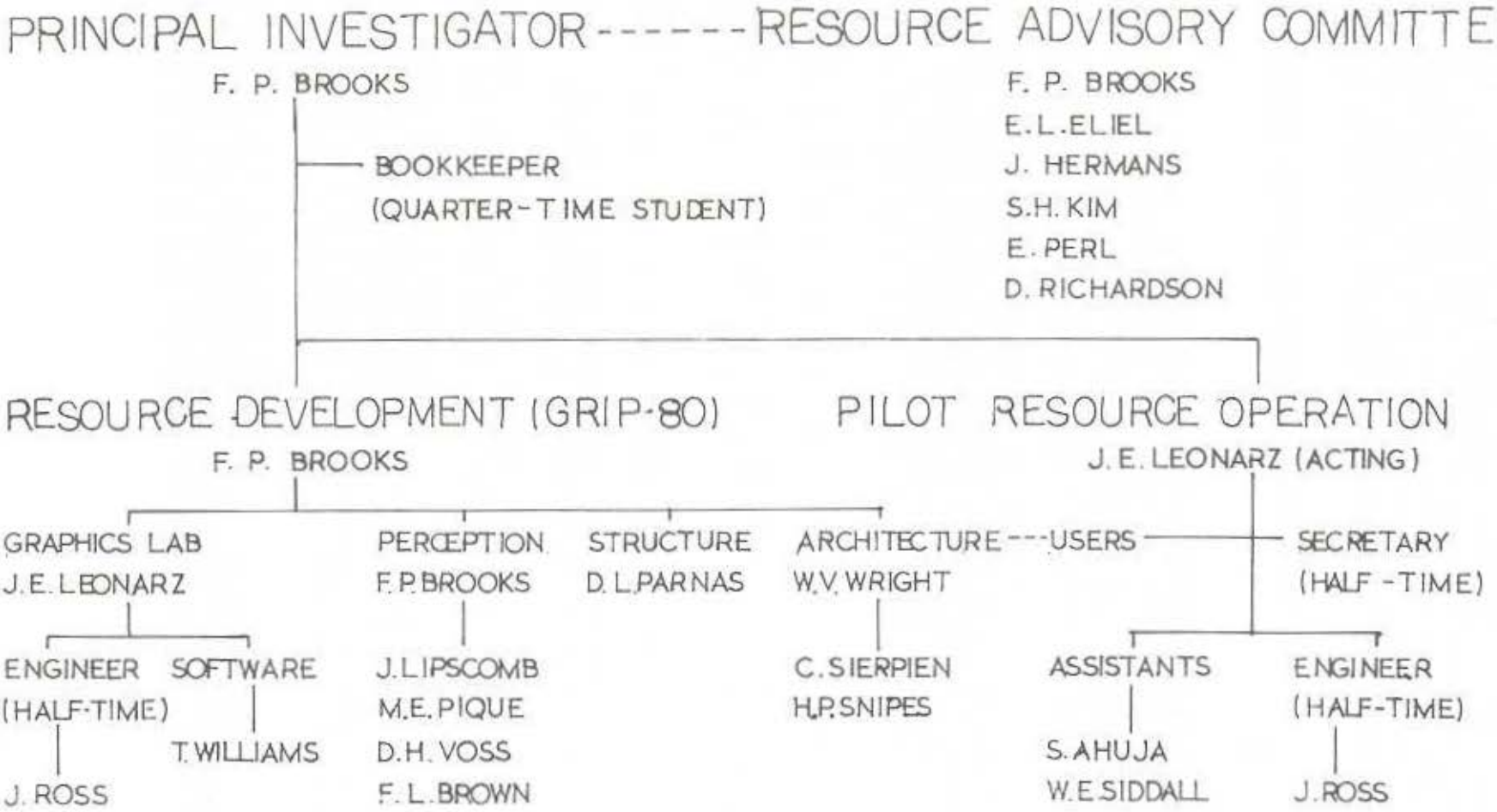

FIGURE 1: ORGANIZATION of UNC MOLECULAR GRAPHICS PROJECT

Core Research and Development

A substantial part of our development effort during 1977 and all project tasks which were brought to completion during the past year were concerned with making our pilot version of GRIP more useful to our clients and tightening our control over the system implementation.

Other GRIP development work currently under way is directed toward the addition of functions which will make our system useful over a wider range of the molecular biology application and toward a better understanding of the perception and manipulation of three-dimensional objects by means of computer graphics. This development work will be tested in GRIP-75, and it will become part of our next generation system, GRIP-80. We are currently identifying the system requirements for GRIP-80.

This work is described in Section IV.

Collaborative Research

The increased number of GRIP users this year yielded a wide variety of experiences. Their research represents a range of macromolecule crystallography from crude maps with no trial structure to refinement and study of advanced models. A list of the users is given later in this section, and sketches of their work are given in Section IV. During 1977 we noted almost as many distinct strategies for fitting molecules to maps as we had user groups. This demonstrates that a molecular graphics resource system must offer a wide choice of facilities for display, perception, and manipulation of models and electron density data if it is to service many users effectively.

Most of our users were quite successful, achieving some or all of their goals. Some were dramatically successful such as Mrs. Liu who constructed a model of 324 residues in just 13 days. The most serious failure was experienced by Dr. Delbaere and Mr. Brayer, colleagues of Prof. M. James of the University of Alberta. So we learned the most from their visit. We tried a new model-builder on them; it was clearly not ready for use, producing an initial configuration too far from the real one to be manually repaired in the time available.

The new technical issues raised by the Delbaere and Brayer experience has to do with when, how, and under what control, departures from ideal geometry are to be allowed into the molecular model being fit to a density map. Because the valence bonds of a real molecule are under stress and because of experimental error, the geometry of the molecular model which is the result of a fitting process is expected to depart slightly from ideal. Different users want to inject these discrepancies at different stages in the fitting process and by different means. The geometry of the trial model we built as the starting point for this group was far from ideal. Even more importantly this geometry differed from ideal in ways to which the users were not accustomed. This experience supports our thesis that a widely-used system must accommodate the several preferred methodologies of all potential users.

We append letter reports written by our several visiting users since our last report. They indicate a great deal about the lessons to be learned from such collaborative research.

Service

The Department of Computer Science owns a block of core memory which is installed in the S/360 model 75 at the UNC Computation Center. This memory is shared by GRIP, OCCAM (a character string editor), and a number of other application programs. In July 1977 this block of memory was enlarged from 374 K to 630 K bytes, making possible simultaneous service to a GRIP user and up to four OCCAM users. As a result we no longer limit GRIP users to a few arbitrary time slots of each day but now take their reservations for sessions of any length beginning at times convenient to them. This has greatly simplified the scheduling of GRIP usage and has enabled our users to work more efficiently.

We have also made a number of additions and refinements to the GRIP system and its supporting utility programs to improve system reliability and convenience to the user. These are described in Section IV.

Training

Our chief training activity is teaching our clients to use GRIP. This is an integral part of the service we offer all visiting users, and it is a major consumer of our human resources. We believe it is essential to the effective use of our system.

The GRIP project has played an important part in the educational program of 17 graduate students in the Department of Computer Science. Of these nine are members of the system operation and development teams at present.

During 1977, GRIP was also used by the Department of Biochemistry in two graduate-level courses and by the Department of Computer Science as an example of an interactive computer graphics system for several classes.

Support

Most of the hardware used to implement GRIP-75 was owned by the Department at the beginning of the project. The salaries of our student research assistants, charges for our use of the host computer, and maintenance of our hardware were paid from this grant. Dr. Brooks received half his salary during June and July 1977, Dr. Parnas received one quarter of his 1977 summer salary, and Mr. Leonarz received half of his salary during 1977 from the same NIH grant. The services of Dr. W. V. Wright were made available under a joint study agreement with the International Business Machines Corporation, which was renewed in December, 1977, for the 1978 calendar year.

Plans and Implications for Future NIH Support

NIH's BRB originally stressed to us the importance of getting a usable, useful, and used system as quickly as possible and demonstrating chemically significant results. This has been done. The natural next steps are the construction of a product-quality, second-generation system and the establishment of a service-oriented multi-user facility. We have the experience, the team talents, and the user guidance to build such a major molecular graphics

research resource. To accomplish this we must have committed NIH support over the next three years at a level adequate to hold our team together, to continue our collaboration with GRIP users and to purchase new hardware which is more typical of the resources that NIH is currently placing in biochemistry laboratories. The real, tangible and usable results to biochemistry may well exceed, however, those from all of the millions heretofore spent on molecular graphics. The planned systems are not flashy or esoteric; they will, however, be carefully designed, user-oriented, and constructed to professional standards of quality. The probability of success is very high.

The plans are set forth in some detail in our renewal application of May, 1976, in the two supplements to it, in our supplementary application of April, 1977, and in our request for supplementary funds on Januay 3, 1978. We will not repeat them here. Basically they provide for an economical and exportable system providing facilities like those of GRIP-75.

B. SUMMARY OF RESOURCE USAGE

Table I identifies the twelve service projects which made use of the GRIP system during 1977. The first line of each project identification gives the last name of the principal investigator and the chemical substances(s) investigated. This project title is used to correlate the information in this table with the details of computer usage given in Table II and the narrative description of these service projects in Section IV.

Tables II and III summarize the computer usage for all projects carried out under this grant. Computer usage by each service project for data preparation, data storage, and interactive sessions is given in Table II. The computer resources required for the entire project are given in Table III. The batch computing time in these tables is reported as the number of minutes that were or would have been required on a S/370 model 165, the fastest of four computers available to us at the UNC Computation Center. Because our PDP 11 is always connected to the S/360 host throughout an interactive session, dedicated computer hours and time-sharing connect hours are the same. An analysis of 19 sessions showed that about three minutes of host CPU time are required for each session hour. This ratio was used to calculate the host CPU time for each service project. Host

core memory utilization was also calculated from the total connect time for each project because 374 K bytes of core are allocated to GRIP throughtout the session.

Table I: Identification of Service Projects

1. Amma -- Deer Hemoglobin
   Investigator: Elmer L. Amma
   Department: Chemistry
   University of South Carolina
   Grant: HL-15158

2. Davies -- Acid protease
   Investigator: David R. Davies
   Department: Molecular Biology, NIH
   Grant: NIH Internal Project

3. Hendrickson -- Myohemerythrin
   Investigator: Wayne A. Hendrickson
   Department: Lab for Structure of Matter
   Naval Resesearch Lab
   Grant: NRL Internal Project

4. Hermans -- Neurotoxin/Rubredoxin
   Investigator: Jan Hermans
   Department: Biochemistry
   University of North Carolina
   Grant: PCM 74-21633

5. James -- Alpha-lytic Protese
   Investigator: Michael James
   Department: Biochemistry, University of Alberta
   Grant: Medical Research Council
   of Canada 55-42289

6. Jensen -- Methemerythrin
   Investigator: Lyle H. Jensen
   Department: Biological Structure
   School of Medicine
   University of Washington
   Grant: NIH AM-03288

7. Kim -- Phenylalanine tRNA
   Investigator: Sung-Hou Kim
   Department: Biochemistry, Duke University
   Grants: NIH CA-15802
   NSF GB-40814

8. Low -- Erabutoxin b
   Investigator: Barbara W. Low
   Department: Biochemistry, Columbia University
   Grants: NIH NS-07747
   NSF BMS-73-01430

9. Richardson -- Superoxide Dismutase
   Investigator: David C. Richardson
   Department: Biochemistry, Duke University
   Grant: NIH GM-15000

10. Schiffer -- Mcg Bence-Jones
   Investigator: Marianne Schiffer
   Department: Biological & Medical Research
   Argonne National Laboratory
   Grant: Argonne Internal Project

11. Tsernoglou -- Sea-snake Neurotoxin
   Investigator: Demetrius Tsernoglou
   Department: Biochemistry, Wayne State University
   Grant: NIH HL-15958

12. Wright -- Glycine tRNA
   Investigator: H. Tonie Wright
   Department: Chemistry, Princeton
   Grant: GM-23598

TABLE II: COMPUTER USAGE FOR SERVICE PROJECTS

Project Identifier	Batch Minutes on S/370 Model 165	Dedicated Hours on PDP 11/45 & Connect Hours to S/360 Model 75 Host CPU	Host CPU Minutes to Service Interactive Commands	Host Core in Megabyte -Hours	Host Disk Space in Track-Months of IBM 3330 Tracks or Equivalent
Jensen - Stenkamp	0.02	62	186	23.2	15
James - Delbaere	2.40	19	57	7.1	15
Davies - Liu	14.56	85	255	31.8	90
Petsko - Tsernoglou	2.64	11	33	4.1	15
Low - Sato	0.82	55	165	20.6	--
Hermans	2.57	42	126	15.7	684
Schiffer	13.70	35	105	13.1	60
H. T. Wright	15.35	41	123	15.3	70
Amma - Girling	44.19	163	489	61.0	140
Hendrickson	7.94	72	216	26.9	84
Richardson	0.53	91	273	34.0	--
Kuntz	8.75	--	--	--	15
Zucker	1.40	--	--	--	15
Kim	--	105	315	39.3	--
Totals	114.87	781	2343	292.1	1203

TABLE III: COMPUTER USAGE SUMMARY

Project Class	Batch Minutes on S/370 Model 165	Dedicated Hours on PDP 11/45 & Connect Hours to S/360 Model 75 Host CPU	Host CPU Minutes to Service Interactive Commands	Host Core in Megabyte -Hours	Host Disk Space in Track-Months of IBM 3330 Tracks or Equivalent
System Development	172.3	198	594	74.1	8261
Service Projects	114.9	781	2343	292.1	1203
Demonstrations	--	161	483	60.2	--
Totals	287.2	1140	3420	426.4	9464

28 February 1978

On March 17 and 18 we demonstrated GRIP to the attendees and speakers of the Symposium on Structure and Dynamics of Macromolecules sponsored by the UNC Department of Chemistry.

During 1977 we also demonstrated the system to the following individuals:

Dr. Chris Bedell	Burroughs Wellcome, England
Prof. G. A. Blaauw	T.H.S. Enschede, Netherlands
Dr. Robert Clark	Argonne National Laboratory
Mr. John Fairclough	IBM Corp., Hursley, England
Prof. Herbert Freeman	Rensselaer Polytechnic Inst.
Dr. Karl Ganzhorn	IBM Corp., Stuttgart, Germany
Mr. Don Gavis	IBM Corp., Harrison, N.Y.
Dr. Kris L. Gimmy	E. I. Dupont de Nemours
Dr. Carl Hammer	Sperry-Univac, Washington, D.C.
Dr. William Heller	IBM Corp., East Fishkill, N.Y.
Prof. Richard Helwig	UNC-Chapel Hill, Psychology
Prof. Derek Hodgson	UNC-Chapel Hill, Chemistry
Dr. Wim Hol	U. of Groningen, Netherlands
Mr. David Huemer	VISA, Washington, D. C.
Prof. Thomas Insenhour	UNC-Chapel Hill, Chemistry
Dr. Carroll Johnson	Oak Ridge National Lab
Prof. Daniel Jones	UNC-Charlotte, Chemistry
Prof. Martin Karplus	Harvard, Chemistry
Dr. Brian Kernighan	Bell Telephone Labs
Prof. Michael Klapper	Ohio State U., Biochemistry
Dr. Martha Miller	U. of Penn., Pharmacology
Dr. Craig Mudge	Digital Equipment Corp.
Dr. V. Adrian Parsegian	National Institutes of Health
Mr. Gregory Riccardi	SUNY, Buffalo, N. Y.
Dr. Milton Rose	ICASE, Langley Field
Mr. Steve Schwarz	IBM Corp., Kingston, N.Y.
Prof. A. M. Starfield	U. of Witwatersrand, South Africa
Prof. Thomas Wallsten	UNC-Chapel Hill, Psychology
Prof. Maurice V. Wilkes	Cambridge U., England
Dean Samuel Williamson	UNC-Chapel Hill, College of Arts & Sciences
Prof. Niklaus Wirth	ETH Zurich, Switzerland
Mr. Frank Zucker	Yale, Chemistry

Our research assistants spent the full-time equivalent of about 40 man-months on all work carried out under this grant. This time was used for preparation of the users' data, shepherding them through interactive sessions, and system development. Much of the system development carried out was done in response to the users' immediate needs and in this sense was done in collaboration with them.

A major organizational change during 1977 split off the service to clients from system development and placed this service function under Mr. John Leonarz, associate chairman of the department. The tables below list the personnel associated with the two GRIP teams at the end of 1977 and gives the developmental assignment of each person for the next generation system. Two other research assistants, Gary Kennedy and Rose Motley were members of the GRIP team during the first four months of 1977.

Development team

Dr. F. P. Brooks Dr. D. L. Parnas Dr. W. V. Wright	System structure
M. E. Pique J. S. Lipscomb	Documenting GRIP-75 and proposing structural changes for GRIP-80
H. P. Snipes	Advanced technology -- display of solid models of molecules
D. H. Voss	Perception effects of depth cues
C. E. Sierpien	Storing and contouring structure factors rather than density maps
F. L. Brown	Incorporation of Feldman database

Service team

Mostly working on supporting GRIP-75 but responsible for some new investigations.

	New Investigation
J. E. Leonarz	Equipment
W. E. Siddall	Fitting strategies and tools

T. V. Williams	PLCD optimizing compiler
S. Ahuja	Attachment of Bausch and Lomb stereo shutter and substitution of data tablet for some joysticks

All service projects were health-related, and we charged none of our clients for their use of the system. All local user groups (Duke and UNC) and Prof. Jensen, however, paid the UNC Computation Center directly for some batch processing of their data and its storage in our host computer. These direct payments to the Computation Center are not included in the financial data in the next section of this report.

Because we use our department computer graphic system for projects unrelated to this grant and because the system is sometimes down for maintenance, repair and system development, it is not always available to our clients. We have not determined the total number of hours available to our users.

C. EQUIPMENT

The resource continues to operate as part of the UNC Department of Computer Science, and it uses the Department's Graphic Facility. This is a multi-purpose facility used for several other research projects and learning activities in the Department. Funds from this grant were used to purchase a Bausch and Lomb Stereo Viewer and a Digital Equipment Type DD11DF Interface for our PDP11/45. We needed this interface in order to attach a new data tablet and printer/plotter to our system. In addition, this grant supplemented funds from other sources in the purchase of a Hewlett-Packard Display Station and a Summagraphics Data Tablet. The details of these purchases are given in Section III of this report. We also purchased a Versatek Printer/Plotter and a Vector General Three-axis Joystick for our graphics facility during 1977, but no funds from this grant were used for these.

A block of 256 K bytes of core memory was added to the UNC S/360 model 75 with NIH grant funds. Its use is described above in the subsection "Service."

D. PUBLICATIONS BASED ON WORK DONE ON GRIP-75

1. K. M. Beem, D. C. Richardson, and K. V. Rajagopalon, "Metal Site of Cu, Zc Superoxide Dismutase," Biochemistry, 16, 9 (3 May 1977), pp. 1930-1936.

2. C. W. Carter, "X-ray Analysis of High-poential Iron-sulfur Proteins and Ferredoxin, Chapter 6 in Iron-sulfur Proteins, ed. by W. Lovenberg (Academic Press, 1977).

3. S. H. Kim and J. L. Sussman, "Turn in a Conformational Pattern in RNA Loops and Bends," Nature, 260, 5552 (1976), p. 645.

4. D. C. Richardson, "Three-dimensional Structure of CU, Zn Superoxide Dismutse," in Superoxide and Superoxide Dismutase: Proc. EMBO Workshop, ed. by J. W. McCord and A. M. Michaelson (Academic Press), in press.

5. J. S. Richardson, D. C. Richardson, K. A. Thomas, E. W. Silverton, and D. R. Davies, "Similarity of Three-Dimensional Structure Between the Immuo-Globulin Domain and the Cu, Zn Superoxide Dismutase Subunit," Journal of Molecular Biology, 102, (1976), pp. 221-235.

6. J. L. Sussman and S. H. Kim, "Idealized Atomic Coordinates of Yeast Phenylalanine Transfer RNA," Biochemical and Biophysical Research Communications, 68, 1, (1976), pp. 89-96.

7. J. L. Sussman and S. H. Kim, "Three-Dimnsional Structure of a Transfer RNA Common in Two Crystal Forms," Science, 192, 4242 (1976), pp. 853-858.

8. D. Tsernoglou and G. A. Petsko, "Three-Dimensional Structure of Neurotoxin a from Venom of the Philippines Sea Snake," Proc. National Academy of Science USA, 74, 3 (March 1977), pp. 971-974.

9. D. Tsernoglou, G. A. Petsko, J. E. McQueen, and J. Hermans, "Molecular Graphics: Application to the Structure Determination of a Snake Venom Neurotoxin," Science, 197, (30 September 1977), pp. 1378-1381.

10. D. Tsernoglou, G. A. Petsko, and A. T. Tu, "Protein Sequencing by Compuer Graphics," Biochemistry and Biophysics ACTA, 491, (1977), pp. 605-608.

11. J. Sussman, S. Holbrook, G. Church, and S. H. Kim, "A Structure-factor Least-squares Refinement Procedure for Macromolecular Structures using Constrained and Restrained Parameters," ACTA Crystallographica, A33, (1977), pp. 800-804.

12. R. W. Warrant and S. H. Kim, "α-Helix-Double Helix Interaction Shown in the Structure of a Protamine-Transfer RNA Complex and a Nucleoprotamine Model," Nature, 271, (12 January 1978), pp. 130-135.

SUMMARY OF RESOURCE EXPENDITURES
Calendar Year 1977
(Parts of budget years RR00898-03 and RR00898-04) *

1.	Personnel	
	a. Salaries & Wages	$ 44,307.00
	b. Fringe Benefits	1,341.00
	Subtotal	$ 45,648.00
2.	Consultant Services	$ 290.00
3.	Equipment	
	a. Main Resource - Purchased	$ 30,147.68
	b. Supporting Equipment	4,582.49
	c. Maintenance	8,008.93
	Subtotal	$ 42,739.10
4.	Supplies	$ 3,566.26
5.	Travel	$ 1,323.09
6.	Alterations & Renovations	$ 550.36
7.	Publication Costs	$ 948.50
8.	Other	
	a. Computer Services	$ 8,610.92
	b. Other	574.42
	Subtotal	$ 9,185.34
9.	Subtotal - Direct Costs	$104,250.65

* The figures presented in this report represent the expenditures from Grant RR-00898 supporting the UNC Molecular Graphics Facility during calendar 1977 and are drawn from the official accounting reports of the University of North Carolina at Chapel Hill. Since neither the -03 nor -04 budget periods coincided with calendar 1977, these figures will not be the same as the official reports of expenditures for the final figures for those budget periods.

EXPENDITURE DETAILS
Direct Costs Only

		Effort	Amount
1.	Personnel		
	1. Brooks, F. P., Principal Investigator; Kenan Professor and Chairman	9%	$ 4,300.00
	2. Leonarz, J. E., Associate Chairman	30%	6,735.00
	3. Parnas, D. L., Professor	5%	1,456.00
	4. Graduate Research Assistants		
	Ahuja, S.	19%	2,000.00
	Babich, W.	11%	930.00
	Bellovin, S.	15%	1,240.00
	Bittinger, D.	13%	996.00
	Brown, L.	19%	2,000.00
	George, J.	4%	310.00
	Jin, G.	19%	1,517.00
	Kennedy, G.	19%	1,920.00
	Lipscomb, J.	19%	2,000.00
	Motley, R.	19%	1,920.00
	Pique, M.	10%	1,111.00
	Siddall, W.	46%	2,515.00
	Sierpien, C.	19%	2,000.00
	Snipes, H.	19%	2,000.00
	Voss, D.	42%	3,860.00
	Williams, T.	46%	4,170.00
	5. Clerical Support: Uhlig, J.	5%	327.00
	Subtotal: Direct Salaries		$43,307.00
	Fringe Benefits		1,341.00
	Total		$45,648.00

EXPENDITURE DETAILS (continued)

2.	Consultant Services:		$ 290.00
	a. Dr. J. Foley		
	b. Dr. V. Wallace		
3.	Permanent Equipment:		
	a. Main Resource - Purchased		
	IBM 2365 (256K Memory)	$29,406.00	
	DEC DD11-DF Backplane	741.68	
	Subtotal	$30,147.68	
	b. Supporting Equipment		
	Part of Summagraphics Data Tablet	$ 422.60	
	Stereo Image Alternator	897.86	
	Part of Hewlett-Packard Terminal	3,262.03	
	Subtotal	$ 4,582.49	
	c. Equipment Maintenance		
	Contract	$ 5,836.80	
	Other	2,172.13	
	Subtotal	$ 8,008.93	
	Total Equipment		$42,739.10
4.	Consumable Supplies		
	a. Electronic	$ 1,798.41	
	b. General	1,767.85	
	Total Consumable Supplies		$ 3,566.26
5.	Travel		
	a. Brooks: CA	$ 224.53	
	b. Brooks: DC	166.73	
	c. Brooks: TX	153.89	
	d. Brooks: NY	106.59	
	e. Brooks: TX	266.20	
	f. Parnas: NY	115.95	
	g. Leonarz: NY	211.89	
	h. Miscellaneous local	77.31	
			$ 1,323.09
6.	Alterations and Renovations		$ 550.36

EXPENDITURE DETAILS (continued)

7.	Publication Costs a. Printing and copying		$ 948.50
8.	Computer Services		$ 8,610.92
9.	Other		
	a. Postage	$ 263.50	
	b. Telephone	310.92	
	Total Other		$ 574.42
Grand Total - Direct Costs			$104,250.65

IV. CORE RESEARCH AND DEVELOPMENT

A. DEVELOPMENT WORK COMPLETED DURING 1977

Reliability

The scope of the automatic checkpoint feature of GRIP was extended to include the result of a molecular idealization operation and a temporary copy of each molecular structure deleted from a user's library. Dates were added to the checkpoint files to eliminate an occasional ambiguity in deciding whether a file is relevant to the current working session.

The session-logging feature was modified to write directly on disk without buffering, thus avoiding the loss of usage data as a result of a failure of the host computer or its operating system.

When any output file becomes full the user is now notified so he can take corrective action. These incidents no longer cause system crashes.

Convenience

In the early stages of the interpretation of an electron density map, the chemist does not know the positions of individual atoms but knows only an approximately position for each residue. We have developed a program for building an initial trial model of the molecule from this data and have used this program successfully. We have also taken over maintenance and control of our own copy of Dr. Hermans's model-building program that allows the user to specify initial positions for all atoms.

We made substantial improvements to our programs and procedures for bringing electron density data into the GRIP system. Also, names were added to the header records in the electron density data sets and the contour maps. Primitve verbs were added to display these to the user on command.

The interface to the molecular idealization program was modified to:

a) Display the greatest geometric deviations in the idealized part of the molecule.

b) Permit the user to invoke a display of these deviations without any change of the structure.

c) Make considerations of interactions between all pairs of atoms in the structure while idealizing a specified substructure the default case. (Previously the default was to consider no atom pairs, which is meaningless.)

d) Permit the user to examine and modify independently the weights applied to the various classes of geometry parameters during an idealization operation.

We modified the system to allow the user to direct output data for the off-line generation of plots to any of several data sets. This removed from our staff the burden of coordinating use of a single data set for output to the plotter.

Adjunct verbs were added to:

a) Permit the user to identify atoms by number. (Some users know atoms by number rather than by residue and name.)

b) Turn on or off the display of several contour maps with a single command. (Saves considerable time in the current implementation.)

c) Clear the operand stack with a single command. (Particularly useful after an idealization operation or an aborted contouring operation which leaves diagnostic information in the stack.)

By recoding the PDP 11 routine which samples the analog inputs we were able to coax a little better response from the system to these inputs. We also enlarged the "dead zone" of the velocity input joystick for tumbling a piece of a molecule to elminate a drift problem and rescaled this input for finer control.

A break key is now implemented for aborting any contouring or idealization operation. This is most useful when the user mistakenly specifies a very lengthy idealization operation.

Development Control

There are always many versions of the several modules of GRIP-75. A controlled standard version of the GRIP system was put together in February 1977, distributed to interested parties, such as Dr. Richard J. Feldman of NIH, and archived on tape. We are now routinely maintaining version control.

A standard set of PL/I DECLARE statements for GRIP external variables was prepared and stored on disk for inclusion in each compilation of a program module of the system.

A standard set of LINKAGE EDITOR control statements for the system was prepared and stored.

A convention for naming GRIP data sets was adopted and is being implemented as obsolete data sets are re-allocated.

B. DEVELOPMENT WORK UNDER WAY

We have purchased a Summagraphics Data Tablet and have begun its integration into our computer graphics laboratory and the GRIP system. We plan to experiment with this input device as a replacement for many or all of our current input devices.

We have on order an RK06 disk drive for our PDP 11 satellite computer. This will be used for our clients' data and as a backing store for the next generation of GRIP, giving us more freedom in the distribution of function between the satellite and host pocessors.

We have completed and are testing a program that displays the surface of a molecule as a mesh of triangles. This program also marks the locations of electronic charges and dipoles on this surface. It will be used by our clients to study molecular interactions and packing.

We are designing a program for calculating electron density distributions from x-ray diffraction data. This is the first step toward integrating into GRIP the processing of x-ray data.

Biochemists often publish pictures of molecules as stereo pairs which appear three-dimensional when viewed through a

simple optical device. GRIP facilitates the creation of such stereo pairs by means of either a camera or a plotter. We are currently adding a feature to the system which enables the user to place labels on these stereo pairs which appear to be located at a specified position in three dimensions.

We have acquired copies of the current collections of published biological molecular conformations and x-ray diffraction data maintained by Dr. Richard Feldman at NIH and Dr. Thomas Koetzle at Brookhaven National Laboratory. Conversion of the NIH data to the GRIP format is underway.

GRIP has a facility which enables the user (or a member of our staff who is helping the user) to define a sequence of commands as a new verb. At present this requires preparation of text which specifies the sequence of commands in a high-level programming language and submission of a batch job for translating this text and installing the new verb in GRIP. A student project is underway to provide for the entry, translation, and installation of new verbs from the graphics terminal while a GRIP session is in progress.

Another student project under way is to add an alternative means for displaying and studying a molecular conformation, a Ramachandran Plot.

C. DEVELOPMENT WORK PLANNED

Our clients have adopted a number of different strategies for fitting a molecular model to an electron density map. They also have suggested other strategies which could not be implemented with our current system. We plan to construct a taxonomy of these strategies to guide the design of our next generation system, GRIP-80. We also plan to carry out controlled experiments to compare these strategies quantitatively.

One of the greatest consumers of computer resources in our present system is the display of electron density data. The computation of contour maps is lengthy, the disk storage required for these limits the size of the maps that can be held at any moment, and drawing these maps taxes the performance of our display unit. We plan to explore other algorithms for calculating contour maps, other data structures for storing them and alternative display techniques which require fewer lines.

Probably the greatest advantage of computer graphics over mechanical models for molecular studies lies in the ease with which the user can move back and forth between manual manipulation and algorithmic optimization. This has been most evident in the work of Dr. Sung-Hou Kim, our client and collaborator from Duke. There is still much to be done, however, to take full advantage of this potential. Although we now frequently move data between GRIP and batch optimization programs, this data must be transmitted, reformatted, optimized, reformatted again, and finally transmitted back to the GRIP system. All of this requires the submission of several batch jobs. We plan to couple GRIP more closely to these optimization programs so that an optimization job can be set up and submitted from the graphics terminal. Going farther, we plan to bring the optimization algorithms inside GRIP, so that when an optimization calculation is not too long the user can have it done and view the results immediately. At present, the only interactive optimization function in GRIP idealizes molecular geometry. We plan to add facilities to minimize the internal potential energy of a molecule due to electrostatic forces and to reduce the discrepancy between computer simulated diffraction by a trial molecular structure and the original crystallographic data.

D. COLLABORATIVE PROJECTS

We consider all system development which stems from the use of our pilot system by chemists as collaborative work. Almost all system development during the past year was of this class. What we learned from our interaction with each of our clients is described in the following section on service projects.

E. SERVICE PROJECTS

1. Amma -- Deer Hemoglobin

Professors Elmer L. Amma and Rowland Girling used GRIP on three occasions during 1977 to correct and improve a model of sickling deer hemoglobin and to fit this model to a new electron density map. This is a very large molecule, 5120 atoms including the heme group, and parts of adjacent molecules were included in the model so that

intermolecular interactions could be studied. The resulting model of 8368 atoms was divided into ten overlapping substructures of about 2000 atoms each so they could be handled by our system. Through the combined use of GRIP and a simpler computer graphics system in their own laboratory, Professors Amma and Girling were able to solve and study this molecular structure without constructing a mechanical model or a "Richards Box."

2. Davies -- Acid protease

During June 1977, Mrs. Mamie Liu, who works under Dr. David R. Davies at the NIH, fit a model of the acid protese from Rhizopus chinensis to an electron density map with 2.5 Å resolution. Approximate coordinates for the alpha carbon atoms of this structure were obtained from a mini-map and a complete model was built using R. Diamond's program. Mrs. Liu then fit this rough model of 324 residues to the map in 13 days using the GRIP system. Adjustments of over 1 Å were needed for most residues.

3. Hendrickson -- Myohemerythrin

In September 1977 and again in October, Dr. Wayne Hendrickson used GRIP to fit a model to his maps for myohemerythrin. This oxygen-carrying muscle protein contains 118 residues. Dr. Hendrickson has not built a mechanical model of this molecule, but before coming to our laboratory he had obtained a complete set of coordinates from his electron density maps. Using GRIP he made a number of major changes to his trial model.

4. Hermans -- Neurotoxin/Rubredoxin

During 1977 Professor Jan Hermans used GRIP to study several conformations for Neurotoxin and Rubredoxin which he had obtained using his REFINE2 program to minimize their internal potential energy. He also compared these models with electron density maps supplied by Professors G. Petsko and D. Tsernoglou (Neurotoxin) and Professor L. Jensen (Rubredoxin). While making these comparisons Dr. Hermans found an error in the sequences for Neurotoxin which was corrected subsequently by Professor Petsko.

5. James -- Alpha-lytic Protease

In March 1977, Dr. Louis Delbaere and Mr. Gary Brayer working under Professor Michael James used GRIP-75 to fit the first 46 residues of alpha-lytic protease to their 2.8 Å map starting with a set of alpha-carbon coordinates taken from a minimap. They were not able to complete the interpretation of their map in the time available, and the partial fit obtained was not acceptable in quality. The starting configuration produced by our model builder was not good enough to justify continuing. We have improved our model builder since their experiment (see Low -- Erabutoxin b).

6. Jensen -- Methemerythrin

In January 1977, Drs. Ronald Stenkamp (Molecular Biophysics, Yale) and John McQueen with the support of Lyle H. Jensen used GRIP-75 to fit a 113-residue subunit of methemerythrin to Dr. Stenkamp's 2.8Å map of this molecule. Approximately 70 percent of this structure is in four alpha helices. After unsuccessfully trying the method which had worked for Professor Tsernoglou (see discussion below), this molecule was fit by first forming the four alpha helices with ideal geometry using an interactive command defined in our high-level geometric language. These were then manipulated into place using the manual fitting controls. Once this was done the "random" parts of the structure were successfully fit using the technique that had failed earlier.

7. Kim -- Phenylalanine tRNA

Drs. Joel Sussman, Stephen Holbrook, and Wade Warrant, and Mr. George Church working under the direction of Professor Sung-Hou Kim continued the crystallographic refinement of the 3-dimensional structure of Yeast Phenylalanine Transfer RNA. A combination of molecular graphics and a new refinement algorithm, the constrained-restrained least squares method, was used.

Professor Kim's group also investigated three RNA-ligand interactions associated with this molecule:

1) Metal binding sites in Yeast Pheylalanine Transfer RNA.
   Drs. Sussman, Holbrook, and Warrant discovered why magnesium ions are essential to the activity of this tRNA molecule. By displaying a F -F difference map on our molecular graphics system, they were able to identify the number and the coordination geometry of

the essential magnesium ions and study their specific stereo-chemical environments.

2) Protamine -- double helix interaction.
Dr. Warrant investigated how Protamine becomes alpha-helical upon interaction with the tRNA and how it stabilizes the packaging of two adjacent double helical segments of tRNA's.

3) Aromatic mutagen -- tRNA interaction.
Dr. Warrant identified tentative binding sites for several aromatic mutagens on the tRNA molecule.

8. Low -- Erabutoxin b

In April 1977, Dr. Atsushi Sato (Chemistry, Tohoku University) and Mrs. Jane Richardson (Anatomy, Duke University) working under Dr. Barbara W. Low interpreted a 2.5 Å map of the sea snake neurotoxin erabutoxin b. This is almost certainly the same molecule that Drs. Tsernoglou and McQueen fit using our system in July and August 1976. Remarkably different mtheodologies were used by these two groups, however. Dr. Low's group started with a molecular model built from the residue sequence and approximate coordinates for the alpha-carbon atoms taken from a mini-map. Our model builder, improved since our experience with Professor James's group, built a sequence of residues with ideal internal geometry and then positioned them in the model space using the given alpha-carbon coordinates. Instead of leaving these residues in the orientation in which they were generated, as our earlier program did, our present model builder makes use of the alpha-carbon coordinates for the adjacent residues to approximate the correct orientation for each residue. Starting with the resulting conformation Dr. Sato and Mrs. Richardson were able to manually fit the model to their electron density map. The resulting conformation will be used as the starting point for further refinement to this structure.

9. Richardson -- Superoxide Dismutase

Dr. David and Jane Richardson obtained a new map with 2 A resolution for their superoxide dismutase molecule and placed it in the GRIP system in June 1976. Fitting was continued from the conformation obtained with their previous map. An interpretation of one of the subunits of this molecule was finished, and the metal sites of all four subunits were compared. Mrs. Richardson also used GRIP to make illustrations for one chapter of

Principles of Biochemistry by White, Handler, Smith, Lehman, and Hill.

10. Schiffer -- Mcg Bence-Jones

Dr. Marianne Schiffer spent a week in July of 1977 using GRIP to study and adjust her model of the Bence-Jones dimer. This model of 432 residues was quite advanced, having been completely fit to its map and refined. Dr. Schiffer's map of 2.3 Å resolution was calculated with phase angles derived from this model. Using GRIP Dr. Schiffer checked the entire structure and adjusted some of the side chains and the main chain at a few points. These adjustments reduced the R-factor from 33% to 32%. Dr. Schiffer is the only GRIP user who has elected to display contour maps on two sets of orthogonal planes. All other users have chosen either one set of planes or complete "cage" contours using three sets of orthogonal planes.

11. Tsernoglou -- Sea Snake Neurotoxin

In July and August 1976, Professor D. Tsernoglou and Dr. J. McQueen fit a 2.2 Å map of a sea snake neurotoxin using GRIP-75. Their starting point was a set of approximate coordinates for the alpha-carbon atoms. The model builder of the Hermans/McQueen refinement program was used to create an initial conformation with approximately ideal molecular geometry. The principal method of fitting was to specify target positions in the density map for selected atoms and then to use the GRIP on-line routine for idealizing molecular geometry to move and twist individual residues until these target locations were achieved. The conformation thus obtained was sufficiently accurate to initiate a crystallographic refinement of the structure.

In November 1976, Dr. G. Petsko (Biochemistry, Wayne State University) used our system and an improved map to check the progress of the refinement and confirm the original fitting.

In June 1977, Dr. Petsko used GRIP again to study a refined structure for neurotoxin and to obtain pictures for publication.

12. Wright -- Glycine tRNA

In August 1977, Professor H. T. Wright brought his electron density map for Glycine tRNA to the GRIP

system. This was a relatively low resolution map, and Professor Wright did not have a trial structure for the molecule. Instead he used GRIP to determine whether the structure of Glycine tRNA is similar to that of Phenylalanine tRNA by trying to manipulate Professor Kim's model for the latter molecule into his map. He concluded that the structures are not similar.

APPENDIX A: CRITIQUES FROM GRIP USERS

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

July 13, 1977

Dr. John Leonarz
Dr. Fred Brooks
Dr. William Wright
Computer Science Department
University of North Carolina
Chapel Hill, North Carolina 27514

Gentlemen:

I wish to say how much we appreciate your hospitality, assistance and encouragement for Mrs. Mamie Liu, during her recent successful attempt to build a model for the acid protease from Rhizopus chinensis. The molecule has a molecular weight of about 35,000 and we reckon that her two-week use of the GRIP-75 Molecular Graphics System saved us approximately half a year compared to mechanical model building and measurement.

As a result of Mamie's success, we are now completely sold on the graphics approach to model building. The advantages of a good model building program are many, and include speed, accuracy and, not least a tremendous saving in space. We hope to incorporate many of the features of the GRIP system into a molecular graphics system now being developed at the NIH by Richard Feldmann.

Finally, we congratulate you on developing such a good system and encourage you to continue to make it available to the scientific community as much as possible.

Sincerely yours,

David Davies

David R. Davies, Chief
Section on Molecular Structure
Laboratory of Molecular Biology
National Institute of Arthritis,
Metabolism and Digestive Diseases

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

July 7, 1977

Dr. John Leonarz
Dr. Fred Brooks
Dr. William Wright
Computer Science Department
University of North Carolina
Chapel Hill, North Carolina 27514

Gentlemen:

I would like to express my appreciation and gratitude toward the Computer Science Department of UNC. By using their GRIP-75 Molecular Graphics System, I was able to build a protein model of 324 amino acid residues in 13 days, which would otherwise take at least 5 months by conventional method. A description of the project and suggestions to the system from a user's point of view are given below.

This is an on-going project at the Arthritis Institute of NIH, under the supervision of Dr. David R. Davies. A 3-dimensional structure of an acid protease from Rhizopus chinensis was solved to 2.5Å resolution by multiple isomorphous replacement method. The electron density map was further improved by Wayne Hendrickson at NRL, using density modification method which takes the solvent into account. The protein is a single polypeptide chain of 324 residues, and sequence information is available for only 45 residues. 324 Cα positions were measured off a minimap at the scale of 3.75mm/Å. A tentative sequence was made up from the combined information of the same minimap, the amino acid composition, and sequence homology between other acid proteases. A starting model was generated from this sequence together with the above Cα coordinates by R. Diamond's model building program. The model building at UNC therefore started with this rough model. Examination showed that the model was pretty far off density, but by using the commands of GRIP-75, one could manipulate each residue and fit them into density. Adjustments of over 1Å in translation and rotations of the peptide planes were necessary for most residues.

I was able to fit all 324 residues in 10 days. Instead of using the on-line refinement program to idealize the geometry of every short stretch of the polypeptide chains fitted, Jan Hermans' refinement program was used to idealize the molecular geometry of the whole molecule in one off-line batch job. The fitting was less interactive this way, but the time saving was substantial. After the refinement, about 60% of the molecule was recontoured on the graphics; this involved mostly minor adjustments. At this moment, no data are available for judging the goodness of fit. Because of the viewing ability on the graphics, the model should have a R-factor at least comparable to those built conventionally. Generally speaking, I was very pleased with the system.

Some suggestions for a future system are given below.

1) The fitting algorithm: The present fitting algorithms are either twisting about a bond only, or breaking the peptide bonds (Ci-Ni+1), together with all the other possible manipulations. Alternatives should be provided to break either the Ni-Cαi or Cαi-Ci bonds. In this case, a command should also be provided to distinguish between a L- and D- amino acid. It would also be helpful if the bond can be twisted in either direction, instead of the positive direction only.

2) The present on-line refinement takes a lot of effort to assign all the parameters. A single one line command should be provided with all the parameters pre-defined as default. This will facilitate the interactive model building. The present set up would be useful for later stages of the refinement when different values are needed.

3) Zooming ability would be a very nice feature to have.

4) Joy stick controlled translation of view area. This will have the effect of a bigger viewing area without losing the resolution by scaling the picture down.

5) A better stero viewer, or preferably, a full screen stereo, would be of tremendous help.

6) The present system works with a whole set of starting coordinates. If the graphics system can be used earlier, much of the human effort of measuring Cα coordinates off the minimap could be eliminated. One possible way of doing this would be an on-line assignment of the positions of any subset of atoms of a residue, e.g. Cα, Cβ and O.

The whole set of idealized coordinates could then be generated by any model building program. Several residues could be built at the same time; the polypeptide chain would grow longer as the building goes on. A simple way of changing the side chains could also be provided at the same time. This method would have the merit of conventional model building together with all the extra powers of computer graphics.

Sincerely yours,

Mamie Liu

ARGONNE NATIONAL LABORATORY

September 6, 1977

Dr. William V. Wright
Department of Computer Science
New West Hall
The University of North Carolina at
Chapel Hill
Chapel Hill, NC 27514

Dear Dr. Wright:

Thank you for your hospitality while visiting Chapel Hill. My stay at the University was not only profitable but also enjoyable. I accomplished quite a lot during my stay. I checked all 432 amino acids of the Bence-Jones protein dimer and adjusted the individual residues where it was needed by twisting side-chain bonds. I also made some corrections in the position of the main chain, but could not complete it for the lack of time. The adjustment of the side-chains decreased the R factor from 33 to 32%.

My plan now is to continue the refinement till stagnation incorporating the changes I made, and then hope to come back to use the GRIP system again. My original estimate of completing the next refinement step by September was a little optimistic, some time in November would be more realistic. I wonder how I could fit into your schedule.

I found the GRIP system easy to use especially with the expert help of Bill Siddall and Kurt Voss. I have a couple of comments about the system which I will send to you later.

Please give my regards to Dr. Brooks and Dr. Leonarz. With best wishes,

Sincerely yours,

Marianne Schiffer

Marianne Schiffer
Division of Biological
and Medical Research

MS:gz

9700 South Cass Avenue, Argonne, Illinois 60439 · Telephone 312-739-7711 · TWX 910-258-3285 · WUX LB, Argonne, Illinois

ARGONNE NATIONAL LABORATORY

September 27, 1977

Dr. William V. Wright
Department of Computer Science
New West Hall
The University of North Carolina
at Chapel Hill
Chapel Hill, NC 27514

Dear Dr. Wright:

Here are some comments and suggestions for the GRIP system:

My purpose of using the GRIP system was to check out and adjust the partially refined structure of the Mcg Bence-Jones dimer. This molecule consists of 432 amino acids; the resolution of the data is 2.3 Å. The atomic positions have been previously refined by the method of constrained crystallographic refinement. This consists of alternate cycles of real-space refinement and calculating new electron densities based on the phases of the refined coordinates.

The GRIP 75 system is very easy and convenient to use with each feature carefully thought out. I found the lorgnette stereo mode especially useful and less tiring to work with. Because the use of stereo, to limit the number of vectors displayed, contours were drawn parallel to only two axis. This gave a very good representation of the electron density map.

I spent most of my time at Chapel Hill checking the fit of my model to the $2F_o-F_c$ electron density map with calculated phases, and adjusting the side chains to improve the fit. For this process, the contouring was the rate-limiting step. Only one contour level was used for the whole study. Therefore, for partially refined structures one contour level is adequate, which might simplify the storage of a contour map. A provision for easily going back and forth between a regular and a difference electron density map would be a very useful feature. For a difference map one level of positive and one level of negative contour will again be sufficient.

I spent only limited time adjusting the main chain and therefore did not learn and use fully all the capabilities of the system. All my previous experience was with physical models; I guess I was trying to do the same manipulations with interactive graphics, without learning its way of doing it. I hope my suggestions can still be of some use.

In a number of cases, I wanted to turn a peptide bond. Having used models before, the distortion in the peptide bond introduced by my manipulations was disturbing. I was wondering if a rigid peptide group would be feasible. Also to aid in the visualization could the bonds be broken in the middle leaving the half bonds on the atoms. Since on can align the parts of the broken bond, this feature helps in the estimating of both

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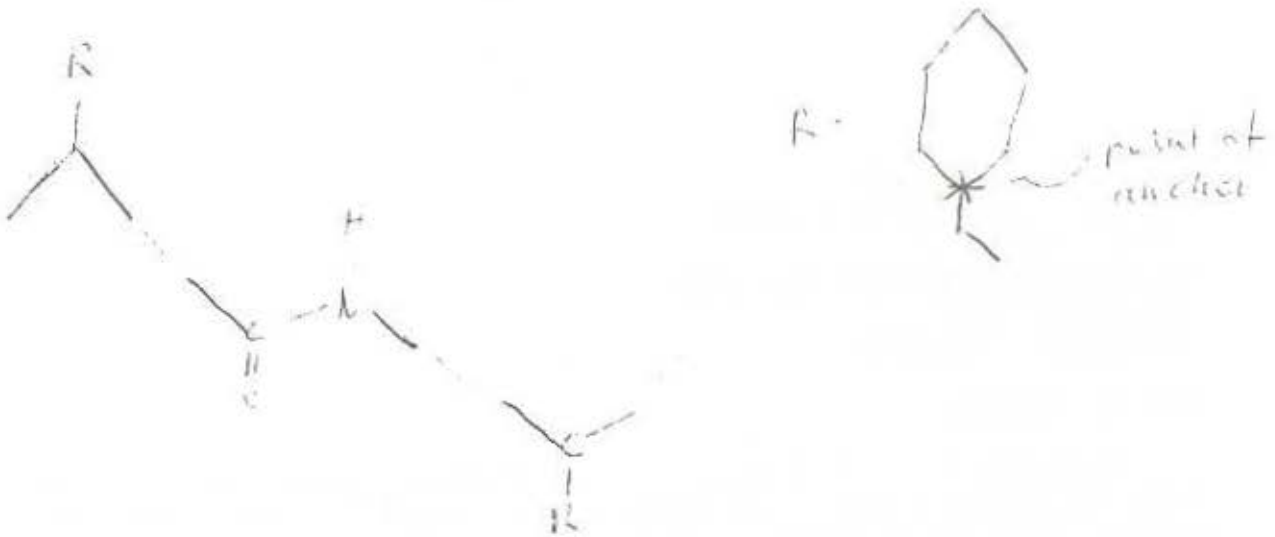

the bond angle and the distance between atoms in the Kendrew type models. I think it would help in the display as well. Another useful feature would be a provision to anchor a side chain group in such a way that it still can not be. In many cases, a density for a side chain is visible but its connection to the main chain is not clear. This way the side chain can be put into the density, then the direction of the connecting bond would be easily determined. The rigid peptide bond, together with the above side chain positioning, will allow independent placing of these parts and their subsequent connecting.

Sincerely yours,

Marianne Schiffer

Marianne Schiffer
Division of Biological
and Medical Research

MS:gz

ARGONNE NATIONAL LABORATORY

October 14, 1977

Dr. William V. Wright
Department of Computer Science
New West Hall
The University of North Carolina
at Chapel Hill
Chapel Hill, NC 27514

Dear Dr. Wright:

Thank you for your letter; I am glad that my suggestions are of some use. Since our conversation, I went through my notes and did some looking at physical models. Your second option, varying the N-CA and CA-C' bonds, treating the CA atom as part of the sidechain, is my choice.

When building physical models, the sequence of events are as follows. One places the sidechain in its density followed by an attempt to connect it up to the main chain. (For large sidechains, the main feature on the map is the electron density for the sidechain.) This process would require: the possibility of translating and rotating of the whole sidechain, fixing it, followed by rotation of the chain so that the CA fits in its proper place. The alternative is to place the CA and then try to reach the electron density for the rest of the sidechain by rotation about each bond in turn. I understand from our conversation that the GRIP system is set up to do the latter. Iterations are required by both methods to get the best fit.

Thank you for the opportunity to use the GRIP system again. I will let you know when my plans are more definite.

Sincerely yours,

/ Marianne

Marianne Schiffer
Division of Biological
and Medical Research

MS:gz

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UNIVERSITY OF SOUTH CAROLINA
COLUMBIA, S. C. 29208

DEPARTMENT OF CHEMISTRY
(803) 777-6263

August 3, 1977

Dr. F. P. Brooks, Jr.
Department of Computer Science
University of North Carolina
Chapel Hill, NC 27514

Dear Sir:

I wish to thank your departmental organization and the individuals who contributed to my profitable stint on the Grip system. Virtually all the day-to-day procedures and problems were handled successfully by Bill Siddel or Dirk Voss.

My objectives in using the Grip were to fit a Deer III Sickling Hemoglobin molecule to electron density maps obtained by a molecular replacement structure solution. The interest in sickling hemoglobin structure vis a vis sickle cells disease is readily apparent and I enclose our initial report of the structure solution. There were at least two contact areas which showed intratomic distances inconsistent with molecular geometry. Side chain and backbone positioning was to be reviewed for consistency with the electron density map.

During the six days I examined the structure by means of Grip, over 110 side chain conformations were altered and one section of 7 residues on the amino end of the β_2 chain was completely rebuilt with the result that two intramolecular contacts were dramatically improved (.6Å → 2.3Å; 0.7A → 3.5A). A more detailed set of results will be published and I will send a preprint of the manuscript.

I found the grip system 1) easy to use for simple fitting operations, 2) to have a good quality picture; in general flicker free and of sufficient resolution, and 3) to contain a convenient idealization refinement package which really works.

The worst feature of the system was the lack of definitive stereo. Although the capability appears to be present, the hardware was not functioning.

There are several features which could be helpful to me and might be appreciated by other users.

(1) The ability to display symmetry related molecular (or parts of them) to define intermolecular contacts.
(2) The ability to display "non-ideal" bonded and non-bonded distances and angles given a threshold value (perhaps on a separate screen).
(3) The ability to create or refine with a preference for helices beta sheet, etc.
(4) The lack of a definitive manual specifying the operation of Grip.
(5) An option on the idealization "refine" which given a residue #, refines that residue and its N nearest neighbors.
(6) An option to reject a "refinement" idealization.

I appreciated the opportunity to use a "state of the art" molecular fitting system and plan to use it again to check the results of refinement procedures based on the energy minimization of Hermann and McQueen and the least squares of KOnnert and Hendrickson.

Sincerely,

Rowland Girling

RG/pk

P.S. Jo Lipscomb and Mike Pique are better than any hand book on the system but it still would be handy to have a quick reference to the system operation.

UNIVERSITY OF SOUTH CAROLINA

COLUMBIA, S.C. 29208

DEPARTMENT OF CHEMISTRY
(803) 777 5263

January 4, 1978

John Leonarz
Dept. of Computer Science
University of North Carolina
Chapel Hill, NC 27614

Dear Sir:

We wish to extend our utmost thanks and appreciation for the scheduling and "real time" assistance during the fitting of Deer III sickled hemoglobin to a much improved set of maps. Extensive progress was made in revising the molecular conformation in two separate sessions of five and two days duration in December 1977. Close examination of the heme groups reveals little electron density (and therefore a low probability of atoms being present) between the distal histidine and the iron of these groups. There is also an unexpected displacement of the distal histidine away from the iron environment. Many amino-acid side chains were repositioned and the interpretation of others questioned. The protein chain termani were carefully checked and three were extensively rebuilt.

The main purpose of the fitting was to improve and change, if necessary, the position of the protein in the intermolecular contacts. Many pictures were taken (thanks to M. Pique) of these critical areas and the repositioning was carried out to our satisfaction using the "standard" procedures in the GRIP molecular graphics package.

With respect to the GRIP system, there are very few improvements which we could mention and most of these have to do with user convenience rather than the function not being available. The improvements we suggest are:

(1) Improvement of the stereo capability either by two screens or the ability to plot more vectors.

(2) The ability to build in constraints (such as α helix, β sheet) to the starting co-ordinates of selected portions of the molecule.

(3) Display, perhaps on a separate unit, the geometrically "unacceptable" parameters such as interatomic distances.

(4) The ability to reject refinements (i.e., refine) in the same way as in the "cancel" command of "fitres."

(5) The ability to display negative contours.

(6) Documentation of all the available functions.

(7) The ability to generate symmetry related molecules.

(8) An alternative camera so that the set up and focal length of the camera do not obscure the picture for editing and slides may be made directly.

The best features of the GRIP systems are:

(1) High resolution graphics with the ability to display large portions of macromolecules superimposed on electron density maps. This display can be viewed from any angle.

(2) Real time interactive fitting made convenient by extensive software.

(3) The absence of major "bugs" in the hardware-software package.

(4) Presence of competent, helpful people to assist the user.

We especially wish to thank J. Lipscomb and M. Pique for their help in solving our problems especially with the electron density maps, Dr. W. V. Wright for his assistance even during the holiday season, D. Vass and W. Siddell for transforming the molecule to the GRIP system and J. Leonarz for his logistical and administrative skill on a day-to-day basis.

Sincerely,

Rowland Girling

RG/pk

Duke University Medical Center

DURHAM, NORTH CAROLINA
27710

DEPARTMENT OF BIOCHEMISTRY

January 4, 1978

To Whom It May Concern:

Re: The GRIP Molecular Graphics System at the University of North Carolina, Chapel Hill

Dear Sir:

This is to illustrate an example where the GRIP system played a critical and essential role in our studies on nucleic acid structure and the interaction between protein and nucleic acids.

We in the Department of Biochemistry, Duke University Medical Center, have been using the GRIP system consistently and very heavily for the last several years, primarily to interpret the electron density maps of yeast phenylalanine transfer RNA at successive stages of refinement. Without this system, our refinement of the structure would have taken at least one or two years longer. The system proved to be extremely useful also in finding the ligands which bind to tRNA. We have successfully located site-specifically bound magnesium ions, spermine molecules and bound water molecules with the help of the GRIP system.

More recently, we have been working on a model system of protein-nucleic acid interaction, namely, the crystal structure of protamine-transfer RNA complex. In this structure, the protamine molecule binds in a space between tRNA molecules, and it would have been almost physically impossible and impractical to build a mechanical model (Richard's Box) to accomodate several tRNA molecules. In this particular study, the GRIP system proved to be extremely valuable in determining the structure, not only because it allowed us to interpret the electron density map without building a mechanical molecular model, but also because it allowed us to examine several electron density maps (F_{obs} map, $F_{obs} - F_{calc}$ map, and $2F_{obs} - F_c$ map) made with different criteria at the same time, thus eliminating the chance of misinterpreting electron density artifacts. The protamine-tRNA complex structure is one of several structure studies we are engaged in, which involves interaction of various molecules with transfer RNA. To make physical models and electron density maps for all of these tRNA-ligand complexes would be impractical and impossible due to the huge physical space one needs. Without a molecular graphics system like the GRIP system, one would hesitate to launch this type of project in any extensive manner.

I cannot overemphasize the useful and sometimes crucial role played by the GRIP molecular graphics system for our structural studies of biological macromolecules. In addition to its usefulness in crystallographic studies, this system is an extremely powerful tool for the model building studies to understand the function of various biological macromolecules. For example, we have been investigating the way two tRNA molecules can line up next to each other on messenger RNA, using the GRIP molecular graphics system. Such model building, which requires a very detailed examination of interatomic contacts, would be impossible with physical models, such as Kendrew models or space-filling models. This type of rigorous model building study can only be done with a molecular graphics system such as the GRIP system. I strongly believe that the GRIP molecular graphics system significantly contributed in facilitating our structural studies of nucleic acid as well as protein-nucleic acid interaction, and I believe it will continue to do so..

Yours sincerely,

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