Fifth Annual Report
Interactive Graphics for Molecular Graphics System

TR79-03

March 1979

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21 March 1979

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DIVISION OF RESEARCH RESOURCES

BIOTECHNOLOGY RESOURCES BRANCH

SECTION I - RESOURCE IDENTIFICATION

Report Period:		Grant No.
From: Jan. 1, 1978 (mo/day/year) TO: Dec. 31, 1978 (mo/day/year)		RR00898 04, 05 & 05S1 Date of Report (mo/day/year) March 20, 1979
Name of Resource Interactive Graphics for Molecular Graphics System	Resource Address 273 Phillips Hall, UNC Chapel Hill, NC	Telephone (Area Code) 919 933 1074
Principal Investigator Dr. F. P. Brooks, Jr.	Title Kenan Professor & Chairman	Address (Department) Computer Science
Grantee Institution University of North Carolina at Chapel Hill	Type of Institution (State, Private, Univ., Hosp., etc.) State University	Telephone (Area Code) 919 933 2148

Name of Institution's Biomedical, Laboratory Animal Committee or Equivalent

Scientific Advisory Committee

Membership of Institutional Committee (Indicate Chairman and those who have reviewed this report)

Name	Title	Department	Institution
F.P. Brooks, Jr. (Chair)	Kenan Prof. & Chairman	Computer Science	UNC
J. Hermans	Professor	Biochemistry	UNC
S.H. Kim	Assoc. Professor	Biochemistry	Duke
E. Perl	Professor & Chairman	Physiology	UNC
D. Richardson	Associate Professor	Biochemistry	Duke
J. Richardson	Res. Assoc.	Anatomy	Duke
W. V. Wright	Adjunct Assoc. Prof.	Computer Science	UNC

Typed Name & Title of Principal Investigator	Signature	Date
Dr. F. P. Brooks, Jr. Kenan Professor & Chairman	F P Brooks Jr.	March 20, 1979
Typed Name & Title of Grantee Official Dr. George R. Holcomb, Dean, Research Administration	George R Holcomb	March 21, 1979

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Research Highlight -- GRIP Resource

RR-00898-05

University of North Carolina

1. Head-Motion Parallax Added to Computer Graphic System

An important means human beings use to perceive depth is called head-motion parallax. As one moves his head, objects nearer to him change angular position more rapidly than those farther away. For example, as one drives down a straight road, the telephone poles fly by, distant billboards appear to move more slowly, and the moon stays at a constant angle. This effect is separate from stereopsis; even one-eyed people perceive this depth cue perfectly.

The UNC GRIP Molecular Graphics Resource added a head-position sensor to our system on an experimental basis. As one moves his head, the computer-generated image on the screen rotates as it would if one were looking at a real 3-D object.

To our surprise, this cue alone is not an especially effective aid to depth perception. It does, however, very powerfully enhance the synthetic stereopsis cue available on the system. The two together given an effect much more realistic than either alone.

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Research Highlight -- GRIP Resource

RR-00898-05

University of North Carolina

2. Display of Molecular Surface Shows Up a Previously Unsuspected Reactive Site in Erabutoxin

Dr. Martha Kimball and Professor Barbara Low, of Columbia, working with the UNC GRIP Research Resource, have discovered and proposed a probable reactive site region for a powerful nerve toxin, erabutoxin.

An experimental system capability, built by Michael E. Pique, Lee R. Nackman, and William E. Siddall, allows the using chemists to plot an opaque-surface view of the molecule they are studying, to supplement the usual stick-figure view. Professor Low says, "Van der Waal's interactions are most elegantly characterized by the visual examination of the shaded-surface representation ... The information about intermolecular interactions, so easily established by display, has been totally unexpected."

II. RESOURCE OPERATIONS AND PROGRESS

A. DESCRIPTION OF RESOURCE OPERATIONS AND PROGRESS

General

The past year has been marked by an substantial expansion in the amount of productive system use by clients. Indeed, we have reached a saturation point beyond which client use cannot increase without additional equipment. Production usage increased from 781 hours in 1977 to 1034 hours in 1978. We served a total of thirteen groups of chemists during 1978, three of which were new users. Only four of our past users did not return during 1978.

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 feedback from real users attempting to solve real problems. Hence when budget cuts have been necessary, as they were in the first half of 1978, 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 on many 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. Leonard 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.

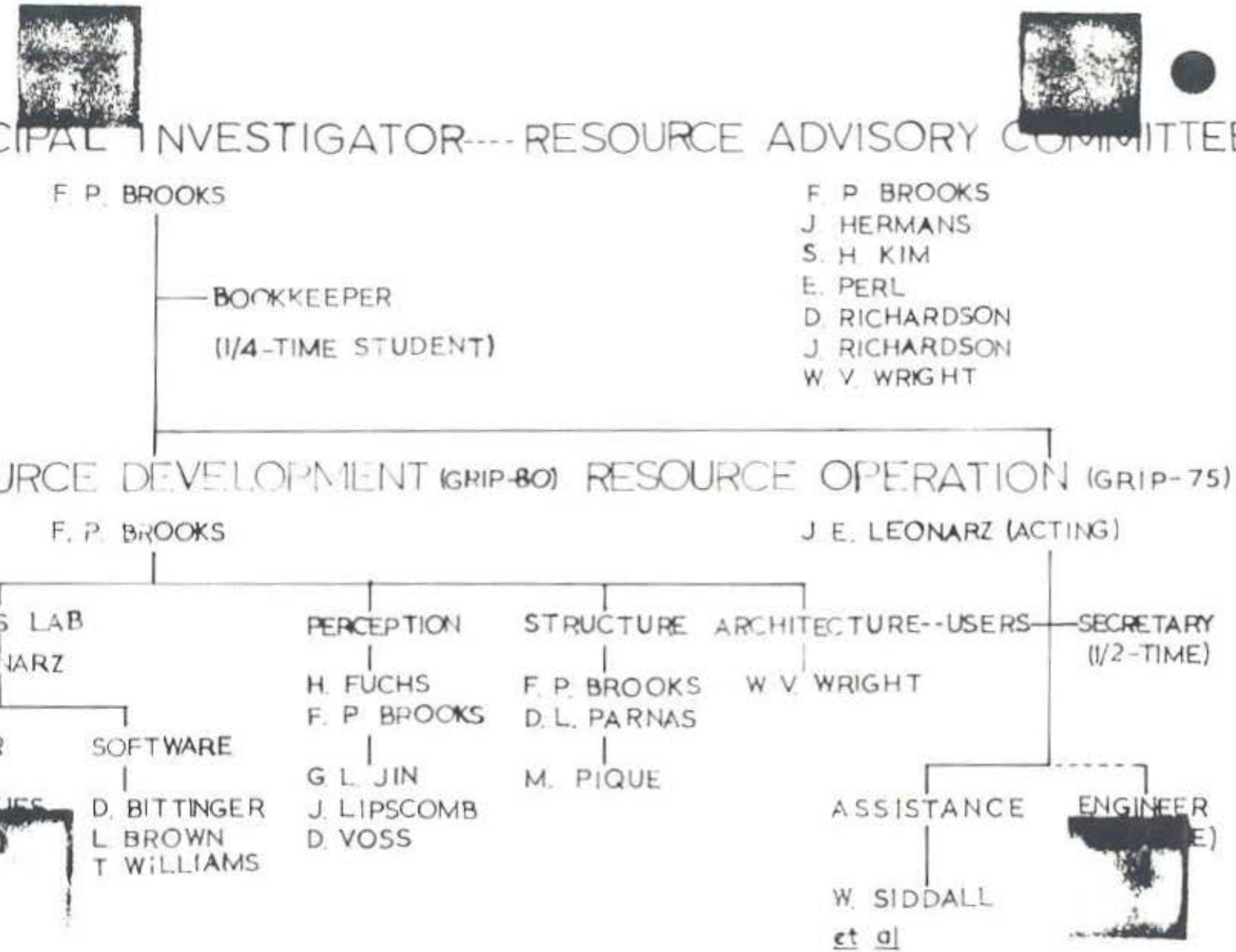


FIGURE 1: ORGANIZATION of UNC MOLECULAR GRAPHICS PROJECT

(AS PLANNED FOR FALL, 1978)

21 March 1979

PROJECT STAFF

Faculty

Prof. F. P. Brooks
Prof. H. Fuchs
Mr. J. E. Leonard
Prof. D. L. Parnas
Prof. W. V. Wright

Research Assistants

T. A. Alspaugh, Jr.
F. L. Brown
J. S. Cohen
G. L. Jin
J. S. Lipscomb
C. S. Orshich
M. E. Pique
P. R. Reintjes
W. E. Siddall
H. P. Snipes
R. J. Traywick
D. Voss
A. Wakabayashi
T. V. Williams

Our research assistants spent the full-time equivalent of about 41 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.

Core Research and Development

Most development work in 1978 consisted of pilot models of new user facilities, some of which will be incorporated in our production version of GRIP-75, and others of which will be incorporated in GRIP-80. We are currently identifying the system requirements for GRIP-80.

This work is described in Section IV.

Collaborative Research

A list of the users follows in this section, and sketches of their work are given in Section IV. Most of our users were quite successful, achieving some or all of their goals.

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

Service

We have again this year 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 graduate students in the Department of Computer Science.

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 his salary during June and July 1978, Dr. Fuchs received six weeks of 1978 summer salary, and Mr. Leonarz received slightly more than half of his salary during 1978 from the same NIH grant. The services of Dr. W. V. Wright were made available without charge under a joint study agreement with the International Business Machines Corporation.

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 enlargement of a service-oriented multi-user facility.

To accomplish this we now have NIH support committed for the next five years at a level adequate to move forward, to continue our collaboration with GRIP users, and to purchase new hardware to meet our growing needs. We are much gratified at this renewal and excited at the prospect of being able to work at a better rate. The planned systems are not flashy or esoteric; they will, however, be carefully designed, user-oriented, and constructed to professional standards of quality.

The plans are set forth in some detail in our 1979-1984 renewal application of May, 1978. We will not repeat them here.

B. SUMMARY OF RESOURCE USAGE

PRODUCTIVE USE OF THE SYSTEM

The use of GRIP by our clients exceeded 1000 hours during 1978, up about 33 per cent over the previous year. We had only three new user groups during 1978, but the number of returning groups was ten. Only four of our previous clients did not return during 1978.

Table I identifies the thirteen service projects which made use of the GRIP system during 1978. 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 throughout the session.

Table I: Identification of Service Projects

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

2. Carter -- High Potention Iron Sulfur Protein
   Investigator: Charles W. Carter
   Department: Biochemistry
   University of North Carolina - Chapel Hill
   Sponsor: NIH GM-21991

3. Davies -- m603 FAb
   Investigator: David Davies
   Department: Molecular Biology
   National Institutes of Health
   Sponsor: NIH Internal Project

4. Hendrickson -- Myohemerythrin
   Investigator: Wayne A. Hendrickson
   Department: Structure of Matter Laboratory
   Naval Research Laboratory
   Sponsor: NRL Internal Project

5. Hermans -- Neurotoxin/Rubredoxin
   Investigator: Jan Hermans
   Department: Biochemistry
   University of North Carolina - Chapel Hill
   Sponsor: PCM 74-21633

6. Kartha -- Bovine Pancreatic Ribonuclease - A
   Investigator: Gopinath Kartha
   Department: Roswell Park Memorial Institute
   Buffalo, New York
   Sponsor: NIH GM-22490

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

8. Lipscomb -- ATCdse
   Investigator: William Lipscomb

Department: Chemistry
Harvard University
Sponsor: NIH GM-06920

9. Love -- Hemoglobin S
Investigator: Warner Love
Department: Biophysics
Johns Hopkins University
Sponsor: NIH AM-16446

10. Low -- Eraboatoxin
Investigator: Barbara Low
Department: Biochemistry
Columbia University
Sponsor: NIH NS-07747

11. Richardson -- Superoxide Dismutase
Investigator: David C. Richardson
Department: Biochemistry
Duke University
Sponsor: NIH GM-15000

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

13. Sigler -- tRNA f Met.
Investigator: Earl Sigler
Department: Biophysics
University of Chicago
Sponsor: NIH GM-15225

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
Carter	---	4	12	1.5	---
Hermans	---	13	39	4.9	---
Richardson	.62	188	564	70.3	28
Kim	.31	24	72	9.0	14
Amma-Girling	17.02	35	105	13.1	766
Davies-Padlan	20.12	92	276	34.4	906
Sigler-Podjarny	18.88	74	222	27.7	850
Love-Royer	24.14	94	282	35.2	1087
Kartha	14.86	109	327	40.8	669
Schiffer	12.07	28	84	10.5	544
Lipscomb-Crawford	22.29	102	306	38.2	1004
Hendrickson	16.72	80	240	29.9	753
Low-Kimball	10.53	191	573	71.4	474
Totals	157.56	1034	3102	386.9	7095

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	Host Core in Megabyte -Hours	Host Disk Space in Track-Months of IBM 3330 Tracks or Equivalent
System Development	204.38	213	639	79.7	24,422
Service Projects	157.56	1,034	3,102	386.9	7,095
Demonstrations	---	137	411	51.2	---
Totals	361.94	1,384	4,152	517.8	31,517

SUMMARY OF GRIP USAGE BY YEAR, 1975-1978

Table IV summarizes the use of the GRIP system for all purposes since we began demonstrations and productive operation on July 15, 1975. We have not tried to estimate the system time spent on development before the beginning of productive operation but know it to be many hundreds of hours. Because we changed from manual to machine logging of GRIP sessions in mid-1976, we believe the true buildup of system usage to be substantially greater than suggested by these data. We have observed that users tend to overestimate the time they spend using GRIP.

Table IV. GRIP Use by Year (Hours)

	1975	1976	1977	1978	Totals
Production	329	581	781	1034	2725
Demonstrations	12	50	161	137	360
Development	297*	186	198	213	894
Totals	638	817	1140	1384	3979

* System development July 15, 1975 through December 31, 1975.

Table V gives the use of the GRIP system by year for each team of biochemists. These teams are identified by their principal investigators. We have re-tallied the GRIP usage for 1975 to obtain a breakdown by user group and have arrived at a total for the year which is about 10 per cent less than that quoted in our previous reports. For the previous total we rounded the durations of the individual sessions; this year we rounded only after calculating the total usage by each group.

TABLE V. -- GRIP PRODUCTIVE TIME (hours)

User	1975	1976	1977	1978	Total
Hermans	7	29	42	13	91
Kim	200	321	105	24	650
Richardson	83	79	91	188	441
Lipscomb	12			102	114
Carter	27	21		4	52
Jensen		46	62		108
Tsernoglou		85	11		96
James			19		19
Low			55	191	246
Davies			85	92	177
Schiffer			35	28	63
Amma			163	35	198
Wright			41		41
Hendrickson			72	80	152
Schevitz				74	74
Love				94	94
Kartha				109	109
Total Hours	329	581	781	1034	2725

Table VI is a list of the biochemists whose research teams have used the GRIP system and their institutions. These are listed in the order of their first use of GRIP. For groups sending more than one biochemist to use our system, the principal investigator is given first and the names of his colleagues follow indented. The institutions of these colleagues are given only where they differ from the principal investigator's. In three cases, Low, Love and Sigler, the principal investigator has made little or no direct use of GRIP himself.

TABLE VI. -- GRIP USERS (1975-1978)

J. Hermans	University of North Carolina
D. R. Ferro	Istituto di Chimica delle Macromolecole
J. E. McQueen	
T. Kuntz	University of California School of Pharmacy, San Francisco
M. Vacatello	Institute of Chemistry, University of Naples, Italy
S. H. Kim	Duke University
J. L. Sussman	
R. W. Warrent	
S. R. Holbrook	
G. M. Church	
D. E. Richardson	Duke University
J. S. Richardson	
E. Getzoff	
J. A. Tainer	
H. C. Taylor	
W. N. Lipscomb	Harvard University
J. L. Crawford	
C. W. Carter	University of North Carolina
R. A. Jones	
L. H. Jensen	University of Washington
K. Watenpaugh	
R. E. Stenkamp	Yale University
D. Tsernoglou	Wayne State University
G. A. Petsko	
M. James	University of Alberta
L. T. J. Delbaere	
G. Brayer	
B. W. Low	Columbia University
A. Sato	
M. Kimball	
D. R. Davies	National Institutes of Health
M. C. Liu	
E. A. Padlan	

M. Schiffer	Argonne National Laboratory
E. L. Amma R. L. Girling R. C. Paslay	University of South Carolina
H. T. Wright	Princeton University
W. Hendrickson	Naval Research Laboratory
P. B. Sigler A. Podjarny R. W. Schevitz	University of Chicago
W. F. Love W. E. Royer	Johns Hopkins University
G. Kartha	Roswell Memorial Institute

DEMONSTRATIONS

Some 137 hours of 1978 resource time were used in demonstrations. During 1978 we have demonstrated GRIP to the following computer scientists.

Mr. John Backus	IBM San Jose
Mr. Steven C. Bruell	Purdue University
Dr. Eric Carlson	IBM San Jose
Mr. Jim Chamberlin	IBM White Plains
Mr. Robert J. Douglas	University of Wisconsin
Prof. Andrei Ershov	Soviet Academy of Sciences, Novosibirsk, Siberia
Prof. Henry Fuchs	University of Texas, Dallas
Dr. Rudiger Hartwig	IBM Heidelberg
Dr. Charles Helvey	Learning Dev. Corp., Nashville
Dr. M. William Krueger	University of Wisconsin
Mr. Mark S. Laventhal	Mass. Inst. Technology
Prof. Ronald Levy	Harvard University
Mr. J. B. Macdonald	Western Electric, Princeton
Mr. William M. McCormack	Syracuse University
Mr. Dale Madden	IBM White Plains
Prof. Akira Nakamura	Hiroshima University, Japan
Mr. James Parkes	University of Florida, Programming Services
Ir. Horst Rettenmaier	Siemans, Munich
Mr. John A. Silvester	U.C.L.A.
Dr. John Szilard	University of Technology, Loughborough, England
Mr. Thomas Tulinsky	Michigan State Univeristy
Mr. Vince Vitagliano	IBM White Plains
Mr. Bruce Weide	Carnegie-Mellon University
Mr. Ed Winn	IBM Raleigh

During 1978 we also demonstrated the system to the following biochemists, chemists, and crystallographers. Also included in this list and the following paragraphs are a number of computer scientists who are primarily engaged in the development of computing systems for biochemical research.

Dr. C. David Barry	Washington University, St. Louis
Prof. H. Berendsen	University of Groningen, Netherlands
Prof. D. M. Crothers	Yale University
Prof. Dorothy Crowfoot-Hodgin	Oxford University, England

Mr. Richard J. Feldmann	National Institutes of Health
Prof. Henry Harbury	Dartmouth Medical School
Dr. Minoru Kanehisa	Johns Hopkins University
Prof. Joseph Kraut	University of California, San Diego
Dr. Robert Ladner	European Molecular Biology Lab, Heidelberg, Germany
Dr. Michael Levitt	Medical Research Council, Cambridge, England
Dr. Bernice Lipkin	National Institutes of Health
Prof. Akeley Miller	Utah State University
Prof. G. N. Ramachandran	Indian Institute of Science
Dr. Georye Reeke	Rockefeller University, New York
Dr. Douglas Richardson	University College, London, England
Dr. Thomas Steitz	Yale University
Dr. Lynn Teneyck	University of Oregon
Dr. Kenneth Thomas	Washington University
Dr. Michele Vacatello	University of Naples, Italy
Dr. David Zipser	Cold Spring Harbor Laboratory

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), 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 chiefly to purchase a large disk to hold user data, a terminal interface and terminal, a suitable camera for recording molecule pictures, and larger air-conditioning equipment.

D. PREVIOUSLY UNREPORTED PUBLICATIONS BASED ON WORK DONE ON GRIP-75

Ferro, D. R., McQueen, J. E., Jr., McCown, J. T., and Hermans, J., (to be submitted), "Energy Minimizations of Rubredoxin," J. Mol. Biol.

Girling, R. L., Houston, T. E., Schmidt, W. C., Jr., and Amma, E. L., (submitted), "Macromolecular Structure Refinement by Restrained Least Squares and Interactive Graphics as Applied to Sickling Deer Type III Hemoglobin," (abstract), Acta. Cryst.

Holbrook, S. R., Sussman, J. L., Warrant, R. W., Church, G. M., and Kim, S. H., 1977, "RNA-ligand Interactions: (I) Magnesium Binding Sites in Yeast tRNA Phe," Nucleic Acids Research, 4 (8): 2811-2820 (August).

Love, W. E., Fitzgerald, P. M. D., Hanson, J. C., and Royer, R. E., 1978, "Intermolecular Interactions in Crystals of Human Deoxy Hemoglobin A, C, F and S," Proc. the International Meeting on the Development of Therapeutic Agents for Sickle Cell Diseases, 19-20.

Monaco, H. L., Crawford, J. L., and Lipscomb, W. N., 1978, "Three-dimensional Structures of Aspartate Carbamoyltransferase from Escherichia coli and of its Complex with Cytidine Triphosphat," Proc. National Academy of Sciences USA, 75 (11): 5276-5280 (November).

Stenkamp, R. E., Sieker, L. C., Jensen, L. H., and McQueen, J. E., Jr., 1978, "Structure of Methemerythrin at 2.8-A Resolution: Computer Graphics Fit of an Averaged Electron Density Map," Biochemistry, 17 (13): 2499-2504 (June).

Sussman, J. L., Holbrook, S. R., Warrant, R. W., Church, G. M., and Kim, S. H., 1978, "Crystal Structure of Yeast Phenylalanine Transfer RNA: I. Crystallographic Refinement," J. Mol. Biol., 123 (4): 607-630.

PUBLICATIONS ABOUT GRIP-75

Britton, E. G., 1977, A Methodology for the Ergonomic Design of Interactive Computer Graphics Systems, and Its Application to Crystallography, Doctoral Dissertation, University of North Carolina, Chapel Hill.

Britton, E. G., Lipscomb, J. S., and Pique, M. E., 1978, "Making Nested Rotations Convenient for the User," Proc. 1978 ACM SIGGRAPH Conf., Computer Graphics, 12 (3): 222-227 (August).

Brooks, Jr., F. P., 1977, "The Computer 'Scientist' as Toolsmith: Studies in Interactive Computer Graphics," Information Processing 77, B. Gilchrist, ed., North-Holland Pub. Co., Amsterdam, pp. 625-634.

Foley, J. D. and Wright, W. V., 1975. "An Interactive Molecular Graphics System with a Satellite Terminal Closely Coupled to its Host," (Panel discussion position paper), Proc. ACM 1975 Annual Conference, 88-89.

Lipscomb, J. S. (in progress). "Three-dimensional Display of Molecular Models," M. S. Thesis in progress, University of North Carolina, Chapel Hill, North Carolina.

Pique, M. E. (in progress). "Nested Dynamic Rotations for Computer Graphics," M. S. Thesis in progress, University of North Carolina, Chapel Hill, North Carolina.

Wright, W. V., 1971, An Interactive Computer Graphic System of Molecular Studies, Doctoral Dissertation, University of North Carolina, Chapel Hill.

Wright, W. V., 1972, "The Two-Dimensional Interface of an Interactive System for Molecular Studies," ACM SIGPLAN Notices, 7 (10): 76-85 (October).

Wright, W. V., 1976, "An Analysis of the Limitations and Present Use Parameters of GRIP-I Facilities of Display and Analog-Manual Input," GRIP Technical Document No. 1, Department of Computer Science, UNC-Chapel Hill (1 October 1976).

Wright, W. V., 1977, "An Overview of the Current GRIP Implementation," GRIP Technical Document No. 2, Department of Computer Science, UNC-Chapel Hill, (11 July 1977).

OTHER COMMUNICATIONS

As in previous years, the GRIP system was described to many groups and individuals. The highlights were: description of the system by Wright to the national convention of the American Crystallographers Association in Norman, Oklahoma; the keynote speech by Brooks at the IBM conference in Hursley on Advanced Graphics Hardware and Applications; and Wright's participation in a task force sponsored by the National Resource for Computational Chemistry to generate a proposal for that agency's activities in the application of computer graphics to crystallography.

III. RESOURCE EXPENDITURES

A. Summary of Resource Expenditures
Calendar Year 1978
(Parts of Budget Periods RR00898-04, 05, & 05S1)*

1. Personnel	
a. Salaries & Wages	$70,086.90
b. Fringe Benefits	2,857.71
Subtotal	72,944.61
2. Consultant Services	200.00
3. Equipment	
a. Main Resource	---
b. Supporting Equipment	15,541.15
c. Maintenance	8,386.42
Subtotal	23,927.57
4. Supplies	1,994.88
5. Travel	1,421.29
6. Publication Costs	674.01
7. Other	
a. Computer Services	5,306.35
b. Other	447.28
Subtotal	5,753.63
8. Subtotal--Direct Costs	$106,915.99

* The figures presented in this report represent the expenditures from Grant RR-00898 supporting the UNC Molecular Graphics Facility during calendar 1978 and are drawn from the official accounting reports of the University of North Carolina at Chapel Hill. Since neither the -04, -05, nor 05S1 budget periods coincided with calendar 1978, these figures will not be the same as the official reports of expenditures for those budget periods.

B. Expenditure Details
Direct Costs Only

	Effort	Amount
1. Personnel		
Brooks, F. P. (Principal Investigator)	19%	$8,926.00
Fuchs, H.	17%	3,666.66
Leonarz, J.	46%	10,903.91
Graduate Research Assistants:		
Alspaugh, T.	20%	2,150.01
Bittinger, D.	22%	2,300.03
Brown, L.	41%	4,300.01
Cohen, J.	2 %	2,150.01
Jin, G.	26%	2,725.00
Lipscomb, J.	20%	1,999.98
Nackman, L.	6%	649.98
Pique, M.	20%	1,999.98
Siddall, W.	27%	2,800.07
Sierpien, C.	20%	1,999.98
Snipes, H.	20%	1,999.98
Trawick, R.	20%	2,150.01
Voss, D.	39%	4,123.08
Wakabayashi, A.	20%	2,150.01
Williams, T.	61%	6,450.02
Clerical & Technical Support		
Barlowe, N.		218.40
Cornwell, M.		924.00
Dwyer, K.		188.76
Parrott, J.		675.48
Reintjes, P.		4,229.49
Uhlig, J.		406.06
Subtotal --- Direct Salaries		70,086.90
Fringe Benefits		2,857.71
Total Salaries & Wages		72,944.61
2. Consultant Services		
Dr. John Szilard		200.00

3. Equipment		
a. Main Resource		None
b. Supporting Equipment		
	Photodiode Array (Reticon)	833.56
	Terminal (Hewlett-Packard)	1,118.10
	Modem (Expandor)	344.12
	Camera (Olympus)	987.37
	Cable (DEC)	251.69
	Modem (Penril)	1,580.80
	DUP-11 Interface (DEC)	1,654.78
	Air Conditioning Equipment (UNC Phys. Plt.)	1,884.95
	Printer Installation (Versatek)	747.60
	RK06 Disk (DEC)	5,867.79
	Lens (Vivitar)	270.39
	Subtotal	15,541.15
c. Maintenance		
	Contract	5,985.00
	Other	2,401.42
	Subtotal	8,386.42
Subtotal--Equipment		23,927.57
4. Supplies		1,994.88
5. Travel		
Cohen, J.	Conn.	119.32
Siddall, W.	Mo.	43.25
Pizer, S. M.	Conn.	150.02
Brooks, F. P.	DC	164.35
Lipscomb, J.	Mo.	124.39
Pique, M.	Mo.	308.40
Brooks, F.P.	Ok.	275.65
Pique, M.	Mo.	91.64
Pique, M.	DC	38.10
Lipscomb, J.	DC	106.17
Subtotal--Travel		1,421.29
6. Publication Costs		674.01
7. Other		
a. Computer Services		5,306.35
b. Other (Communications)		447.28
Subtotal --- Other		5,753.63
8. Grand Total---Direct Costs		$106,915.99

IV. CORE RESEARCH AND DEVELOPMENT

A. DEVELOPMENT WORK COMPLETED DURING 1978

Head-Motion Parallax Viewing

Several new facilities were developed for the GRIP system during 1978. Of these the most interesting and potentially useful is head motion controlled stereo display. We determine the position of the user's head by having him wear a headband with a small battery-powered light. A horizonal row of photodiodes is mounted just above the display screen. A mask is mounted in front of this row of diodes about one inch away and with its vertical edge in front of the center diode. The shadow cast on the diodes is used to determine the angular position of the user's head relative to the screen, and this information is used to control rotation of the displayed molecule and map about a vertical axis.

Our preliminary experiments with this device have been somewhat surprising. With a 2-D display, when the user moves his head the image appears to rotate in the opposite direction relative to both the user and the display unit. That is, the user perceives the display unit as the fixed object and everything moves relative to it. It is not clear yet whether this is a useful mode of operation, but if it is we probably want to scale the image rotation so that it is several times the angular movement of the user's head relative to the display unit.

With a two-eye stereo display and with careful adjustment of the rotation scale factor relative to the stereo separation angle the image appears to remain fixed in the laboratory space while the user moves around it. We presume that the rotation scale factor necessary for this effect is such that head motion equal to the distance between the user's eyes should cause a rotation equal to the stereo separation angle.

Ramachandran Plots On-Line

We have added a new mode of operation to GRIP which displays a generalized three-dimensional form of a Ramachandran plot showing the conformation of a molecule.

In this plot each amino acid residue of a protein structure is represented by a point. The sequence number of a residue and the dihedral angles associated with the two twistable bonds in its main chain determine the x, y, and z coordinates of its point in our generalized Ramachandran plot. By sighting down the sequence number axis, the GRIP user can identify -helical or -sheet sections of the structure. This facility has already proved useful to our clients, and we are considering its extension to display other conformational parameters of protein structures.

Shaded Molecular Surfaces

M. E. Pique, L. R. Nackman and W. E. Siddall have developed an offline facility for GRIP which generates shaded opaque plots of molecular surfaces using a VERSATEC Printer/Plotter. One GRIP client has found this facility very useful.

On-Line Verb and Menu Definition

During the spring term 1978, a team of students taking a course in software engineering developed a facility for GRIP which enables the user to modify the system verbs and to create new verbs during a working session. This facility has not been integrated into GRIP yet, nor has its usefulness been evaluated.

UNIX

We have purchased an RK06 disk storage unit from the Digital Equipment Corporation and installed it in the UNC computer graphics system. This has enabled us to bring up the UNIX operating system on our PDP11/45 processor. The GRIP system, however, continues to operate under Digital Equipment's DOS, and we have not decided whether we will convert it to run under UNIX, or whether GRI-80 will run under UNIX or another operating system.

Brookhaven Library Data

During 1978 we converted five molecular structures from the Brookhaven library to the GBIP format. The heterogeneous nature of the Brookhaven data required so much hand work on each molecule that we have discontinued this project until a need arises for data on individual molecules.

B. DEVELOPMENT WORK UNDER WAY

Data Tablet Integration

Dirk Voss has undertaken as his master's thesis the integration of our Summagraphics data tablet into the GRIP system as the input device for manual manipulation of molecular structures. This will enable us to replace two joysticks with three input axes each and eight knobs by this single input device, thus improving the tactile continuity of the GRIP man-machine interface. Later we plan to expand the function of the data tablet to replace the light pen, the programmed function keyboard, and the slide potentiometers of the current system.

Users' Manual

We have compiled a number of documents on GRIP to form a first draft of a long-overdue users manual for the current system.

Display Detail Control

We are developing a new menu of verbs to specify the amount of detail of a molecular structure that is displayed. The names given these verbs and the order in which they are invoked by the GRIP user are designed to form an imperative sentence. For example, "HIDE MAINCHAIN" and "SHOW ALL RESIDUES." We believe the use of these new verbs will be more intuitive than those on the current menu.

Enhanced Contouring Functions

We are also developing a new menu for controlling the calculation and display of contour maps. Included are verbs for contouring a region which is larger or smaller than the current view cube without redrawing the molecule at a different scale. Also the addition of a new primitive verb which puts the specifications of an existing map in the

operand stack will enable us to build verbs which can re-contour a map after a change of scale or view center without requiring the user to re-enter this information.

C. DEVELOPMENT WORK PLANNED

In order to solve a number of architectural and implementation problems inherent in the current system, we plan a complete ground-up redesign of the system to be called GRIP-80. The major design objective for this system are:

Architecture

GRIP-80 will retain all functions which have been proved useful in GRIP-75, but some functions may be provided in a new form.

The mathematical model of a molecule will be an abstract linear graph which corresponds more nearly to the physical structures than the tree data structures used in GRIP-75.

On-line modification of molecular constituents and connectivity will be possible.

If technically feasible real-time zoom and pan of the view area will be provided.

The display of electron density data by means of contour maps will be supplemented by a technique for marking the peaks and ridges of high electron density.

A facility for displaying the surface of a molecule as an opaque shaded colored object will be provided but not the real-time animation of such pictures.

Many of the input devices of GRIP-75 will be replaced by a single locator device, probably a data tablet. This will improve the tactile continuity of the GRIP man-machine interface.

Commands in GRIP-80 will be specified in prefix order, i.e., operation followed by operands.

The system will be able to prompt the user to guide him through the specification of the operands required by an operation. A description of the function of each verb will also be available to the user at the work station.

The system will distinguish among several types of objects (e.g., atoms, bonds, angles, residues, and peptide units) and will require the user to specify the appropriate type of object for each operand.

The system will store sets of parameters called profiles for controlling display, contouring, manipulation and optimization operations. The user can call these up as needed.

A quick, easy means will be provided for adding new function to the system.

Generalized tools for constrained manipulation will be provided which enable the user to change any aspect of the molecular geometry while holding other features fixed.

The scope of the system will be enlarged by bringing in more types of application data and functions. For example, structure factors, hydrogen bonds, R-factor calculation and optimization, and minimization of the potential energy associated with a structure.

Implementation

Two implementations of GRIP-80 are planned, one in the Department of Computer Science at UNC and one in the IBM/UK Scientific Center in Winchester, England. These implementations will be based on different hardware configurations thus assuring a degree of hardware independence.

In both these systems more functions will be implemented on the dedicated satellite computer than with GRIP-75. In particular all command interpretation and execution of all trival and frequently invoked functions will be done by the satellite computer. A user's data and his private extensions of the system function will be stored on a private disk pack which will be mounted on a drive near the work station.

D. COLLABORATIVE PROJECTS

We consider all our system development which stems from the use of our pilot system by chemists as collaborative work. Almost all the system development during the past year was of this class.

E. SERVICE PROJECTS (A SAMPLING)

1. Amma -- Deer Hemoglobin

Professors Elmer L. Amma and Rowland Girling have used GRIP on four occasions to correct and improve a model of sickling deer hemoglobin, to fit this model to a new electron density map, and to make photographs for publication. 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 -- M603-FAB

Dr. David Davies and his co-worker, Dr.-Eduardo Padlan, of the National Institutes of Health used GRIP to position side-chain and make some minor adjustment to the main-chain of their node of the immunoglobulin M603-FAB. They plan to refine the resulting model using the Connert-Hendrickson program.

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. Since using GRIP, Dr. Hendrickson has carried out an algorithmic refinement of his model and has computed a better elctron density map. He returned to our laboratory in 1978 to inspect and adjust this new map and model.

4. Kim -- Phenylalanine tRNA

Drs. Joel Sussman, Stephen Holbrook, and Wade Warrant, and Mr. George Church working under the direction of Professor Sung-Hou Kim used GRIP in combination with their constrained-restrained least squares method to refine the 3-dimensional structure of Yeast Phenylalanine Transfer RNA.

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 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.

5. Lipscomb -- Aspartate transcarbamylase

Dr. James Crawford, working under Prof. William Lipscomb of Harvard University, used GRIP to obtain an ab initio fit to his electron density map for aspartate transcarbamylase. Alpha carbon coordinates for this molcule were otained from a small-scale map, and a complete model was built using our program. (See Low -- Erabutoxin b.) Dr. Crawford has completed a pass through both the regulator and catalytic units of this molecule using GRIP. He found some errors in the sequence for the catalytic unit. Once these are fixed and the geometry of both units is idealized using Dr. Hermans's REFINE2 program, the model will be put back in GRIP for further adjustment.

6. 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 A 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 methodologies 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 was used as the starting point for further refinement to this structure by Dr. Martha Kimball. Using M. Pique's experimental surface display, Drs. Kimball and Low identified a suspected active site not priviously identified..

7. Richardson -- Superoxide Dismutase

Dr. David and Jane Richardson and their students have used GRIP since September 1975 in their study of suproxide dismutase. An interpretation of one of the subunits of this molecule is finished, and the metal sites of all four subunits have been compared. Mrs. Richardson also used GRIP to make illustrations for one chapter of Principles of Biochemistry by White, Handler, Smith, Lehman, and Hill.

8. Schiffer -- Mcg Bence-Jones

Dr. Marianne Schiffer has used GRIP on two occasions to study and adjust her model of the Bence-Jones dimer. This model of 432 residues was quite advancedwhen it was first place in GRIP, having been completely fit to its map and refined. Dr. Schiffer's map of 2.3 A 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. The manual adjustments whih

Dr. Schiffer made to her model during her first trip to our laboratory reduced its R-factor from 33% to 32%. The results of her second use of GRIP have not yet been evaluated. 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.

APPENDIX A: CRITIQUES FROM GPIP USERS

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

Bldg. 2, Rm. 316

June 7, 1978

Prof. Fred Brooks
Prof. William Wright
Prof. John Leonarz
Computer Service Department
University of North Carolina
Chapel Hill, NC 27541

Gentlemen:

I wish to thank you and your staff for letting me use GRIP and for helping me build an atomic model of the M603 Fab. We had calculated, in 1974, a 3.1 Å electron density function for this molecule from x-ray data, had built a tentative (only about 70% of the amino acid sequence was known at the time) atomic model using a Richard's optical comparator and had measured the coordinates for alpha carbons and carbonyl oxygens. With the almost complete sequence now available and using the measured alpha carbon and carbonyl oxygen coordinates, we had generated coordinates for the rest of the atoms in the structure using Diamond's model idealization program. This was the set of coordinates I started with to build a better model of the ~~model~~ map using GRIP. The side groups, understandably, bore no relation to the map in the beginning and this made fitting of model to map very difficult. This difficulty was compounded by the fact that the electron density function is at 3.1 Å resolution and is not particularly good. With GRIP, I was able to build a complete atomic model of the molecule which closely fits the electron density and, more importantly, obtained a set of atomic coordinates for the more than 3400 non-hydrogen atoms in the structure. Model building and the measurements of coordinates by conventional methods are particularly tedious endeavors and I would estimate that the 11 days I spent with GRIP are equivalent to at least 6 months of work using the old techniques. It is clear that the use of computer graphics in protein model building is the way of the future and we, protein crystallographers, greatly appreciate the effort you have invested in developing GRIP and your letting us use the system.

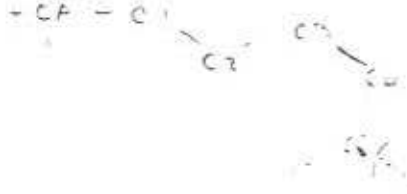
It would be highly desirable to have GRIP developed to the stage where a model can be built from scratch, where residues can be added or deleted and where the identity of the residues can be altered directly on the display. At the present time, knowledge of the sequence is an absolute requirement to be able to fit side group density. In this connection, I would like to suggest a scheme which might be built into the present system and that will allow

fitting an electron density map with or without sequence information. It is not an ab initio model building procedure but it should suffice to get an approximate model that can subsequently be fitted more closely to the map. Suppose the map is of sufficient clarity to trace the course of the backbone but the sequence is not fully determined. A polypeptide chain is now superimposed on the map but for each residue is substituted the following structure:

pseud. sidegroup

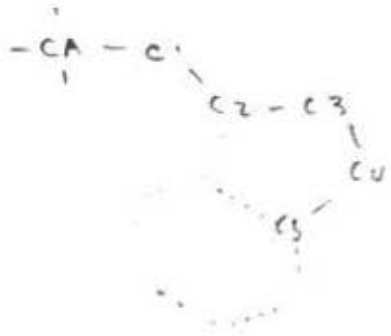
where C1, C2, C3, C4 and C5 are tetrahedral carbons, 1.5 Å apart, and where there is free rotation about each 'sidegroup' bond. This structure can be made to approximate any of the 20 residues. Fitting this pseudo sidegroup structure to electron density corresponding to Gly, Ala, Ser, Cys, Met and Cys is obvious. Further, C3 can be made to approximately represent the OD1 (Diamond nomenclature) in Asp or Asn, and C4 the OE1 in Glu or Gln. Even the aromatic sidegroups can be approximated. For example, by making C1, C2, C3, C4 and C5 coplanar, one can approximately fit density for His:

with C1 ≡ CB, C2 ≡ CG, C3 ≡ ND1, C4 ≡ CE1 and C5 ≡ NE2;
or Phe (Tyr):



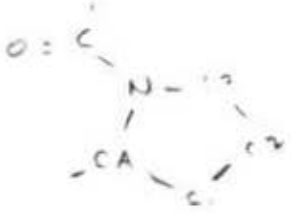
with C1 ≡ CB, C2 ≡ CG, C3 ≡ CD1, C4 ≡ CE1 and C5 ≡ CZ;

or Trp:



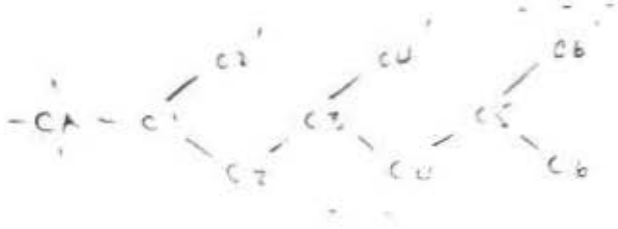
with C1 ≡ CD, C2 ≡ CG, C3 ≡ CD1, C4 ≡ NE1, and C5 ≡ CE2;

or by making C1, C2, C3 and C4 nearly coplanar, one can fit Pro:



with C1 ≡ CB, C2 ≡ CG, C3 ≡ CD and C4 coinciding with N.

The pseudo sidegroup structure can only give the approximate location of the corresponding atoms but it should suffice to enable an idealization program to generate a proper sidegroup (or the rest of it) for later refitting. Val, Thr, Ile, Leu and Arg could be better fitted by adding more atoms to the pseudo sidegroup, thus:



C2' can be used to better fit density corresponding to Val, Thr or Ile; C4' for Leu; and the planar C4, C5, C6 and C6' for Arg.

If an ab initio model building procedure is going to be incorporated into GRIP, I would suggest that the user be able to invoke helices or extended chains of any desired length - it is much easier to bodily fit a stretch of preformed helix rather than twist a stretch of residues into a helical configuration.

At the present stage, GRIP is more suited to fitting a model to electron density map rather than to building a model to fit the map.

Builders require a large view of the map and model (to see the interrelation between the different parts), whereas fitters can work with a much more limited view (since the model already closely fits the density function). Stereo viewing is a great help to builders, yet buffer requirements for stereo run counter to those for large view display. Fitters, on the other hand, especially those who are working with difference maps, would benefit from the ability to display map features contoured in the conventional manner - in the final stages of fitting, the density gradient becomes very important.

A complete documentation of the operation and consequences of the various commands is highly desirable. In addition, "tricks" that permit a more efficient usage of the system should be documented. (I learned from Bill Sidall, for example, the trick of contouring as large a part of the map as feasible and then isolating smaller regions by adjusting the scale - the object here being to save time since contouring takes a long time to accomplish.) I would suggest that the experiences of all those who have used the system be pooled and made available to potential users.

Listed below are some personal experiences and suggestions:

(1) Operations, which detach and translate groups (e.g. FITRES, FITCO, FITRANGE), necessarily build in distortions. It is not easy to assess the departure from ideality by simply looking at the image on the screen, nor is it practical to demand the values of the bond lengths and angles after each fitting. After becoming more familiar with the system, I noticed that the residues I had fitted during the first two days were frequently distorted and had to be subjected to extensive refitting. I also noted that the 'toothpick' works in laboratory space and that distortions from using the 'toothpick' can be avoided by properly positioning the model before applying the rotation (e.g. end on view of the bond for clockwise and counterclockwise rotations, etc.)

A display of the instantaneous values of the length of the "rubber" bond (at least) would be very useful. Furthermore, idealization at the preliminary steps of building should probably be very restrictive (i.e., CA should be made strictly tetrahedral, the peptide link planar, etc.)

(2) The TWSTBOND can have dire consequences on atoms that are outside the viewing area. Potential users should be warned of this.

(3). FITRANGE and TWSTRANGE can cause the lengthening of a bond which is difficult to reverse, if at all. I ended up saving the file prior to using either of these verbs.

(4) One should be able to specify the atom (bond) about which rotation is accomplished in TWSTRANGE (or FITRANGE) - this becomes desirable when fitting backwards from the C- to the N-terminus.

(5) In FITRANGE or TWSTRANGE, the operation is not confined to the light-penned atom; certain atoms appear to be linked to each other (list dependent?). This should be documented.

(6) The image on the screen sometimes changes hand and in my case this led to confusion in the beginning. This is compounded by the inability to rotate the view a full 360°. I was once so confused that I started flipping the peptides around. This was before I realized that I can display all the surrounding residues (by invoking SHOW) to see more clearly the interrelation between neighboring segments. The cause of this image reversal should be documented and a warning given to potential users.

(7) While the side group rotations are disabled in proline, the ring can be broken in two and the individual parts moved separately (with FITRANGE). This should be documented.

(8) After several hours of use, a part isolated by FITRES, FITCO, etc. may start to drift; I found that holding the 'toothpick' at an extreme position for a second and then relasing would usually stop this drift.

(9) Also after several hours of work, the air in the room would seem drier with frequent 'sparking' between fingers and light pen causing a noticeable disturbance in the system. (I may have crashed the system once by such sparking.) Would a humidifier help here?

Trouble-shooting procedures should also be documented. For example, I learned from Dirk Voss that, if the screen does not respond to the light pen, one should try increasing the tube intensity. Further, I learned from Carol Serpien that, if the system does not respond to "mode" selection, one should try pulling on the buttons.

I am sure I share with other users the hope that you will continue to improve GRIP. Of course, as it now stands, GRIP is already a fantastic system.

Finally, I wish to thank the graduate students, in particular Bill Siddall, Dirk Voss and Carol Serpien, who were always around to help when I ran into problems. I also wish to express my deep appreciation for the patience of Bill Siddall who got me started, did all the idealizations and file handling and without whose help I would not have accomplished as much.

Yours truly,

Eduardo A. Padlan

HARVARD UNIVERSITY

DEPARTMENT OF CHEMISTRY

12 Oxford Street
Cambridge, Massachusetts 02138
U.S.A.

June 19, 1978

Dr. F.P. Brooks
Chairman, Dept. of Computer Science
U. of N. Carolina
Chapel Hill, N.C.

I have just finished a very successful stay in Chapel Hill to use your department's graphics facility and would like to comment on the GRIP system and the work I accomplished.

I am working on the protein aspartate transcarbamylase, an allosteric enzyme that catalyzes the reaction of carbamoyl-phosphate with aspartic acid to form carbamoylaspartate and phaophate and is the first step unique to the pyrimidine biosynthetic pathway. The molecule is a 310,000-dalton complex of 6 copies of each of 2 unique polypeptide chains, called the regulatory (R) chain and the catalytic (C) chain. The crystal form I am currently working with is in space group P321 with 2 copies of the R and C chains in the asymmetric unit and with the inhibitor CTP bound to the enzyme. An initial 2.8 Å map was calculated using multiple isomorphous replacement phases. This map was then averaged about the non-crystallographic 2-fold so that the final map had only one unique R and C chains. The quality of the map differs dramatically between different regions. Over the majority of the map, the quality is very good with all side chain peaks and many carbonyl peaks apparent. However in approximately one-fourth of the map, including 100 of the 152 residues of the R chain, the density is weaker, gaps are apparent in the main chain, and the side chain peaks are obscured by noise peaks. In addition the sequence is either unknown or incorrect for about 75 out of 300 residues of the C chain.

At Harvard I had measured α-carbon coordinates for all the residues from a crude Kendrew model built in a Richard's box. The main goal for my work at the graphics facility was to accurately build the 275 residues for which both the map is good and the sequence reliable, and to accurately build as much of the main chain as I could where the sequence is unreliable. I am pleased to report that I was easily able to accomplish this goal in the ten days I spent at U.N.C. I found the system very adequate for my task and for the most part have only praise for the system and for the people there who helped me, in particular Bill Siddall.

I would like to make a few suggestions which would make the system even better. The most serious defect is the lack of a user's manual which could be sent to a user before arriving at Chapel Hill. I know that I wasted at least half a day learning all the key words available and I never did learn all the options available. Second, since our sequence is not accurate or complete, it would have been very useful to be able to edit residues with the system; that is to add, delete, or change residues. As it was, I could not test any sequence changes and those which I knew were obvious I have to make at Harvard. Third, I would suggest putting in verbs to build a model α-helix between given residue numbers and rotate and translate this helix as a unit. I'm sure for our resolution map a model helix would be better than my building. Finally, it would be useful for comparing structures or maps if two atom files could be displayed at one time and if two maps that are displayed could be rotated and translated relative to each other. I had no need for such features this time but would find them useful when we get maps of other forms of the molecule.

In closing I would like to say that I thoroughly enjoyed my stay in Chapel Hill and certainly hope I can return again soon.

Sincerely,

James L. Crawford

THE UNIVERSITY OF CHICAGO
DEPARTMENT OF BIOPHYSICS AND THEORETICAL BIOLOGY
CUMMINGS LIFE SCIENCE CENTER
920 EAST 58TH STREET
CHICAGO · ILLINOIS 60637

June 26, 1978

Professor Fred Brooks
Department of Computer Science
University of North Carolina
Chapel Hill, N. C.

Dear Professor Brooks:

Thanks for the help. We don't know yet the bottom line of the model building effort but it is clear that Podjarny and Schevitz, persons of high technical standards, found the persons at the facility cordial and technically first-rate, and the facility very well conceived and managed.

I hope our experience, which is detailed in the enclosed report, helps you to further develop your fine program. Please feel free to solicit further testimonials if necessary.

Thanks again.

Sincerely,

Paul B. Sigler

Paul B. Sigler
Professor

PBS:dw

Report on the use of the graphic facility at the computation center at the University of North Carolina

Molecule: yeast $tRNA_f^{Met}$

Map: 4.5 A MIR map with direct methods and density modification.

Users: A. D. Podjarny, R. Schevitz

Date: June 1-4 and June 13-21, 1978

The laboratory of macromolecular crystallography at The University of Chicago, Department of Biophysics, has been working for several years in the structure of a eukaryotic initiator tRNA, $tRNA_f^{Met}$. Due basically to weak high resolution intensities the structure has been particularly difficult to solve, and only in the past six months has a map suitable for model interpretation emerged.

Large portions of the structure of tRNA are helical and have a very definite structure. Also, the structure of this initiator tRNA should be locally similar to that of $tRNA^{Phe}$, though significant overall differences might be expected. Therefore, a system which allows the superimposition of large portions of the map with substructures of known stereochemistry is highly desirable.

A Richards box was set up and a physical model was built, but though it followed most of the map it did not have satisfactory stereochemistry. This was traced back to the problem of lack of rigidity in the secondary structure and a failure to maintain the known stereochemistry.

To overcome this problem, a static graphic system was implemented in Chicago. The hardware consisted of a 370/195 computer with storage tube CRT terminals (Tektronix 4010 or 4014) or refresh graphic terminals (HP2648A). The software consisted in a set of Fortran IV subroutines originally developed at

Columbia University, at the laboratory of Professor Cyrus Leventhal, and adapted for map interpretation at the Weizmann Institute, Rehovot, Israel, and then in Chicago, by A. D. Podjarny

Using this static system, several portions of the $tRNA^{Phe}$ molecule were rotated and translated as rigid bodies in order to fit the electron density map. We also had located by means of heavy atom markers the positions of three residues in the unit cell, which were used to anchor the interpretation. Some bond angle changes were made in regions between rigid substructures, but in general that was not the case. We ended with a model that fit most of the density, but the static system was clearly not flexible nor fast enough to accurately fit the density. The task was made even more difficult by the fact that only three views of the map and molecule were available, and only one if a large portion was displayed.

It was at this moment that we approached the North Carolina facility. Our main purpose was to implement a more flexible system that would let us make a better fit of the map and also have a better view of the current interpretation. As several other alternatives were feasible, we wanted to be able to compare them in a real time system. The reason for choosing the North Carolina facility was that (a) the previous experience in dealing with tRNA and (b) that it was available to NIH supported scientists.

We sent our map and molecule in advance, and one of us (A. D. Podjarny) arrived at Chapel Hill for the first of two sessions on June 1st and stayed for four days. The system was practically ready to go, and it was possible to start working within half an hour of arrival. We regard this fact as a proof of excellent support of the visiting researchers, and this was reaffirmed during the whole stay. In three days, from the first to the fourth of June, a complete pass on the molecule was done. The general strategy used was to move rigid

substructures as rigid bodies, and then independently fit the connecting residues. The map was good enough to locate the rigid substructures, but not for locating individual residues. In the three loop regions of the molecule, the strategy consisted of fitting the whole range of the loop by individual bond rotations; the range to be fitted was advanced by one residue in order to access sequential bonds, until it was completed. A new verb (Fitrngno) was created in order to define more easily the range to be fitted. In general, this worked quite smoothly; the only problem was the need to break helical portions in two strands, because of a software limitation which made it impossible to simultaneously fit residues not connected in sequence.

The interpretation brought from Chicago was sustained, with minor changes for better fit of the density, for 56 out of 75 residues. For the other 19, a better interpretation was found. The most important advantages of this system over the static graphics system were (a) the ability to look at a continuous range of views, (b) the ability of real time fitting in a much shorter time and (c) the display of solid type contours.

A very important constraint of this crystal structure are the symmetry elements, in particular the dense network of dyad axes. In regions where the density encroaches on the dyad the intermolecular contacts were quite accurately defined. The first approach to displaying the symmetry was to display the symmetry axis, and that was enough for the first page.

On June 13th Podjarny and Schevitz arrived at Chapel Hill for a second session. A strategy to display symmetry related molecules was discussed with one of the members of the supporting team (Bill Siddall) and it was agreed that the best was to generate a file with a pair of superimposed molecules and then rotate one of them on-line into the desired symmtry related position. While this was being done, A. D. Podjarny corrected some stereochemistry that had been incorrectly placed during the first pass and Richard Schevitz looked at

the current interpretation and tried all the possible alternatives that seemed apparent. It should be stressed here that while A. D. Podjarny had previous experience with graphic systems, Richard Schevitz had not. Therefore, his opinion as a newcomer to the field must be of particular interest to the facility. He found the system easy to use, though designed for better quality maps. In particular, he found that it was not well suited for fast screening of different alternatives within reasonable time.

The net result of Dr. Schevitz's trials was that no alternative could be found that made a better fit of the density and had a comparable stereochemistry. Therefore, the current interpretation was upheld as the most likely one.

Meanwhile, the symmetry related procedure was operational and it was used to investigate the packing in the contact regions. The packing is quite tight, but very reasonable. Small adjustments have to be made to the residues which are involved in the packing.

A set of photographs was taken in Kodak 3-X film using the Olympus OM2 Camera with a Vivitar zoom lens set at 200 mm. The automatic exposure facility of the Olympus was used, presetting the f-stop at f-8. This meant a speed of 1/8 of a second with automatic normal exposure and of 1/30 of a second with the -2 option. This film was developed at Chicago and the result was satisfactory. Prints are being made to decide which exposure (Standard auto or -2 auto) worked best.

The system suited our needs, but some modifications would improve its performance when dealing with substructures of known stereochemistry. It would be very useful to be able to rotate and translate as a rigid body a portion of the molecule which forms an independent structural unit in three dimensions but is not connected in sequence; in our case, the candidates are the helical segments of the molecule, whose stereochemistry is very well defined.

When treating the loops, we found the need to accept a given conformation and advance our fitting region by one residue in order to have access to the following bond angles. This combination of verbs could be improved if it were combined in a single very that would accept the given confirmation, advance by one residue and then start fitting again. It would also be useful to have the option of deciding which bond angles are connected to the fitting knobs, instead of having them connected to the available ones in sequence.

Besides being useful for manual fitting, a system of this kind could be improved if it were also served as an interface for a higher level of feedback than visual fitting. For example it would be advantageous to call for the calculation of a standardized numerical indicator of the "goodness of fit" between the model and density and a short (one or two cycles) computational refinement or adjustment of the model to maximize the fit. The idea is to be able to invoke (albeit with much longer delays) a computed fit (refinement) after visual adjustment. Various other graphic estimates of validity could also be invoked as high level feedback. For example the investigator could call for a phase calculation besed on the well fit part of the model. A new map of a test region of the density (generally not used in the model calculation) would then be displayed and compared with the original density to see if the phasing had been improved. Such packages are sufficiently well understood and appreciated by the 'structural community' to be useful standard tools.

HARVARD UNIVERSITY

DEPARTMENT OF CHEMISTRY

12 Oxford Street
Cambridge, Massachusetts 02138
U.S.A.

September 15, 1978

Dr. F.P. Brooks, Jr.
Chairman, Dept. of Computer Science
U. of N. Carolina at Chapel Hill
New West Hall
Chapel Hill, N. C. 27514

Dear Dr. Brooks,

I would like to report on my visit to Chapel Hill to use the computer graphics facility during the week of August 14. This was my second visit to use GRIP and was as successful as my first visit in terms of accomplishing what I wanted to do.

During my first stay in May, 1978, I fit approximately 275 of the 450 residues of the enzyme aspartate transcarbamylase from a 2.8 Å electron density map averaged around a non-crystallographic 2-fold axis. After two cycles of Diamond real space refinement starting with the coordinates I brought back from N. Carolina, a structure factor calculation based on 60% of the atoms in the protein gave a crystallographic R-factor of 44.1%. I calculated a new map from observed structure factors and phases which were the MIR phases combined with the calculated phases. This map was not averaged around the non-crystallographic axis and I brought to N. Carolina two maps including both copies of the polypeptide chains.

At N. Carolina I accomplished four things. First, I compared the Diamond-refined coordinates with the original map to see that the refinement had adjusted the atom positions correctly. Second, I compared the original map with the new maps to see if there was any improvement in the quality of the map. There was a definite improvement in the poorest regions as best indicated by the more apparent coiling and chain connectivity in several ill-defined helices. Third, I compared the electron density of both copies of the polypeptide chains with each other to see how closely the molecule obeys the non-crystallographic symmetry. All of the protein appears to obey the symmetry with the possible exception of some of the side chains in the region of the map which is still too poor to even tell which peaks are side chains and which are noise. Fourth,

I built the main chain for most of the remaining residues, bringing the total number of atoms built to 75% of the protein. The side chains not yet built include all those in poor areas of the map and a stretch of about 70 residues for which there is no accurate sequence.

The improvements to the GRIP system which I would have found most useful during my visit were the same as those I commented on after my first visit. These suggestions include a comprehensive user's manual, an editor within the GRIP system for changing the sequence, an easy way to make a model α-helix in a specified portion of the polypeptide chain, and a way to display two maps so that one can be rotated relative to the other and so that one of the slide knobs could be used to alternately see one or the other of the maps.

In the future I hope to improve the quality of the map by more cycles of refinement and structure factor calculations and to determine a better sequence before returning to use GRIP to build the remaining portions of the molecule.

Sincerely,

James L. Crawford

THE JOHNS HOPKINS UNIVERSITY · BALTIMORE, MARYLAND 21218

THE THOMAS C. JENKINS DEPARTMENT
OF BIOPHYSICS

9 October 1978

Dr. William V. Wright
The University of North Carolina at Chapel Hill
Dept. of Computer Science
New West Hall 035A
Chapel Hill, N.C. 27514

Dear Dr. Wright,

This letter is to report on my use of the GRIP-75 molecular graphics system this summer in the refinement of the sickle cell hemoglobin structure.

I had two purposes for coming down to Chapel Hill to use the GRIP-75 system. One was to refit the most poorly fit residues in the structure to improve the overall agreement between the model and the x-ray data. The other purpose was examine and refit regions of contact between neighboring hemoglobin molecules.

I brought several electron density maps of the type which we call "fragment delta-r". These maps remove desired portions of the model from the phase calculation, so as not to bias their placement in the density map. From standard difference maps, I determined the most poorly fit 54 residues (about 5% of the structure) and calculated a fragment delta-r map for these residues. Using GRIP-75, I was able to fit 46 of these to good density. In addition, I refit about 90 residues which are involved in various contact regions in the crystal structure. Calculation of new difference electron density maps, using the new model coordinates in the calculations, demonstrate that the new positions are much more accurate than the previous ones. Another indication of the improvement of the overall structure was the decrease in the 'R value' (a measure of the discrepancy between the model and the x-ray data) from .392 to .387.

Since we are concerned with the molecular aggregation of sickle cell hemoglobin, I was most interested in studying the contact regions between neighboring hemoglobin molecules in the crystal. Of particular interest is the structural adjustments which may be necessary for the formation of a strong contact. Using a fragment delta-r map of the "Wishner Contact", which involves the mutant residue, it was observed that the "donor" side of the contact must undergo significant rearrangement for the contact to be formed. In a paper, soon to be sent for publication, we

propose that the ability of a hemoglobin to gel is determined by the pliability of this region of the hemoglobin. This proposal is a direct result of observations made using the GRIP-75 molecular graphics system.

The GRIP-75 system was very well suited for my purposes. It not only allowed me to refit residues much faster than would have been possible with our system, but also enabled me to observe molecular contacts better than I had ever been able to in the past. The accomplishments were well worth the effort of preparing my data for the system and traveling south in the heat of the summer.

Sincerely,

William E. Royer, Jr.

HARVARD UNIVERSITY

DEPARTMENT OF CHEMISTRY

12 Oxford Street
Cambridge, Massachusetts 02138
U.S.A.

November 23, 1978

Professor Fred Brooks
Department of Computer Sciences
University of North Carolina
Chapel Hill, N.C. 27514

Dear Fred:

I would like to express my great appreciation for the advances that we were able to make in solving the structure of the large regulatory enzyme aspartate transcarbamylase with the use of the graphics system that your people have developed over recent years. The ability to reorient the molecule within contours, and especially to view specific regions in various orientations was very critical in this study and has allowed more firm conclusions than we could possibly have reached if we had done all of the work here. Thus your graphics system has provided us with a more searching and powerful technique than I believe is available anywhere else in the world for the study of protein and nucleic acid structures.

I hope to be writing to you within the next several months, and perhaps after that, with respect to studies of a totally different enzyme conformation of aspartate transcarbamylase. I also hope that the graphics system will be available for us to map out the complete structure of carboxypeptidase A complexed with the protein inhibitor from the potato, a study which we now have very well under way. Therefore I shall be writing again soon about establishing definite dates for these studies in collaboration with your computer graphics group.

Sincerely yours,

Bill Lipscomb

William N. Lipscomb
Abbott and James Lawrence Professor
of Chemistry

L:G

College of Physicians & Surgeons of Columbia University | New York, N.Y. 10032

DEPARTMENT OF BIOCHEMISTRY

630 West 168th Street

December 11, 1978

Professor Frederick P. Brooks, Jr.
Chairman, Department of Computer Science
University of North Carolina at Chapel Hill
New West Hall
Chapel Hill, North Carolina 27514

Dear Professor Brooks:

As you know, I had hoped to be able to come down with Dr. Kimball when she returned this time to continue refinement studies on our molecule using the GRIP Molecular Graphics System. Unfortunately several circumstances, including a broken arm, have delayed my work here. I am further hampered at this time by ad hoc University meetings tomorrow and on Wednesday and Thursday mornings, all of which I ought to attend.

I know there is to be a site visit from the National Institutes of Health concerned with the funding of the System and I wish I might be there to speak of my concerned and enthusiastic interest.

I write with a sense of urgent need to tell you how important the Chapel Hill Computer Graphics facility has been to us in our work on erabutoxin:- a protein snake venom neurotoxin.

The structure of erabutoxin was initially established by working with electron-density maps without even a Richard's box. Having worked with the latter there is no doubt that chain tracing is significantly easier with the Graphics System. By far the most important and significant advantages of the GRIP system, its unique and outstanding features, are however, in my view the flexible approach to model fitting and refinement which it incorporates, and, even more fundamental, the development of an understanding of structure-function relationships which the display system promotes.

As I wrote to Dr. Baker in February of this year, our initial Chapel Hill refinement run established most of the intra-molecular interactions, both those of the dominant β structure, the multiple β turns, as well as the complex of intra-molecular bonding.

After the initial Chapel Hill run I was able to propose a probable reactive site region for the toxins and to describe reactive groupings within it. As I think you know,

erabutoxin was the first three-dimensional toxin structure established and it may be considered as a prototype for the whole class of nearly fifty snake venom curaremimetic postsynaptic neurotoxins for which sequences have been determined.

Subsequent refinement runs have extensively exploited two capabilities of your system,

1. The display of Ramachandran angles during fitting. Without the ability to calculate Ramachandran angles the choices made in chain fitting on occasion lead to improbable and essentially "forbidden" values. This can now be easily avoided. This is a very important new development.

2. The map we are working with is not a high resolution map; there are always some ambiguities in fitting side-chain groups. The ability to calculate, at will, distances between non-convalently bonded atoms helps to avoid overcrowding.

3. Some of the reactive groupings in this molecule involve hydrophobic residues. Hydrophobic interactions are not vectorial. In defining hydrophobic regions overall fit must be considered.

Van der Waal's interactions are most elegantly characterized by the visual examination of the shaded-surface representations of the molecule which your new development for showing Van der Waal's packing radii provides. This is an excellent study aid. Such interactions could only be worked out more painstakingly and slowly by other means.

The System has further capabilities. In particular it can be used to display intermolecular packing between adjacent molecules. The information about intermolecular interactions, so easily established by display, has been totally unexpected. It suggests to us possible modes of interactions between toxins and/or receptors when the toxins bind to the subunits of the receptor molecule. It also provides provocative insight into the possible nature of the problems of crystallization experienced with some toxins.

Use of the Computer Graphics System is, even after several experiences, dependent on the organized critical help of the Director, his associates and students. Although I have not worked myself for long periods at Chapel Hill, Dr. Kimball tells me that the support she has received has been exemplary. It has involved not only technical help and instrumental instruction but also the critical interchange of ideas concerning problems and their possible solutions.

Finally in simple terms of my own research interests and the budget which supports them.

1. There are many studies which it has been possible to make on the Computer Graphics System which we could have made only with very great difficulty, with the loss of enormous amounts of time and several thousand research dollars from my own research budget.

2. The kind of interactive study which is possible with the Computer Graphics System provokes ideas and promotes ways of thinking about inter-and intra-molecular interactions which come less easily with other kinds of model building studies.

3. In terms of our own needs, the facility at Chapel Hill is ideal.

The Department of Computer Science in developing this system has interacted so strongly and positively with the protein crystallographers who use it, that more fruitful developments have been achieved and may be expected than could have been hoped for even if each protein crystallographer had, at great expense, had an individual computer graphic system in his or her own laboratory.

A system which is intrinsically well-developed, but also responsive to needed modification rather than "locked-in", and one which is also under the direction of experts both in computer science and the needs of the crystallographers must, be definition, be the best possible.

Yours sincerely,

Barbara W Low.

BWL:rd

Barbara W. Low
Professor of Biochemistry