

DARESBUURY LABORATORY

INFORMATION QUARTERLY

for

PROTEIN CRYSTALLOGRAPHY

An Informal Newsletter associated with Collaborative Computational Project No. 4
on Protein Crystallography

Number 12

December 1983

Contents

Editor's Note	1
Recent Updates in the CCP Program Suite	3
COMPEDIT - An Aid to Program Maintenance	7
Minutes of a Meeting of Protein Crystallography SRS Users September 1983	15
The Fast at Daresbury	17
Protein Structure and Dynamics A Daresbury Study Weekend November 19 - 20 1983.	19

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Editor's Note

This edition of the Newsletter contains details of various CCP4 activities at Daresbury.

The CCP program suite is continually changing and John Campbell has detailed here the modifications which have been made recently. There is also information for PX users of the synchrotron, in particular an article about the FAST TV Diffractometer.

I imagine most people are aware that a meeting on Protein Structure and Dynamics was held at Daresbury on November 19 -20 1983. There were over 100 participants at this study weekend, including eighteen speakers. Formal proceedings of this meeting will not be issued so the programme, abstracts and list of participants are included here as a record of the meeting. I should like to take this opportunity to thank the participants and most specially the speakers and organisers (Tom Blundell, Mike Sternberg) for making the meeting the success which I believe it was.

Pella Machin

RECENT UPDATES IN THE CCP PROGRAM SUITE

John W Campbell, Daresbury Laboratory

The programs of the CCP suite which have been modified recently are indicated below. Those which require a change to the way the program is currently used are indicated with an asterisk.

<u>Program</u>	<u>Details of the Modification</u>
SFC	Option added for space group $P4_32_12$. Also corrections to cases where the program crashed.
GENSFC	Corrections as for SFC.
*LCFUTILS	Phil Evans version has now been implemented. Missing 's' values are calculated from cell dimensions normally read from the first input LCF file. If there are no cell dimensions in this file then give an extra card at the end of the data control cards with a b c α β γ in Angstroms and degrees.
PASCLCF	There is now an optional scale factor which will be applied to the output intensities. The scale factor, if present, is given on the cell dimensions card following the cell dimensions.
PREDICT	The maximum capacity for residues has been increased from 500 to 800 for all the programs of this set.
PHASE2	The program now allows for input form factors to be spread over more than one card. Certain other minor corrections have also been incorporated.
REFINE2	The program has a new option ~~#~~ SELFH. This is similar to the ~~#~~ SCFHLE option but all reflections are used (subject to the selection of centric terms) instead of reflections being rejected if the sign ambiguity (FHLE/FHUE) is uncertain. One of the alternative estimates of FH is selected for each reflection depending on which is nearest to the calculated FH. The program now also allows for the input of form factors to be spread over several cards.

*LCFDUMP From Phil Evans modified version (handles data from multirecord type LCF files). The CLIST parameter LINES has been replaced by the parameter NREF which specifies the number of reflections to be printed in the second section of the dump. An option is now also present for specifying the first reflection to be listed. There is also an option to set a flag to request the printing of orientation data if present.

*ALMN This now has a maximum cut off limit for F^2 which is specified as an extra item at the end of data card 3. A scale factor for the output map is given on an extra data card at the end of the data control cards. If a file name is given with the new CLIST parameter MAPOUT then a map will be written to this file.

ANSC An option is now available to input the X-ray wavelength. This is given as an extra item at the end of the #CELL card. The code of the program has also been modified to trap certain conditions which may give rise to errors when using the 3-D scaling option.

RFACTOR The label for the phase in the output Fourier Coefficients file has now been corrected and is called PHI.

For all these modifications the relevant corrections have been made to the machine readable versions of the documentation.

Changes Soon to Take Place

The programs AGROVATA and ROTAVATA will be updated to the versions compatible with the post-refinement program and the post-refinement program POSTREF will be implemented.

The Isaacs-Agarwaal structure factor calculation and refinement program will be modified to a version which may be used in conjunction with the Hendrickson-Konnert program. The Hendrickson-Konnert program will be implemented on the NAS AS/7000 so that the combined refinement procedure may be run on the NAS. The possibility of converting both the programs to FORTRAN 77 will be considered so that they may be shifted easily back to the Cray or to other machines.

The program PHARE will soon be included in the program suite. The LCF version with modified input and output specifications is now working and the documentation is being word-processed.

4 October 1983

COMPEDIT - AN AID TO PROGRAM MAINTENANCE

John W Campbell, Daresbury Laboratory

General Description

The programs of the CCP suite though written, in general, in fairly standard Fortran are still not fully portable. This means that, when a program is received from 'off-site' for implementation 'on-site' as part of the CCP program suite at Daresbury, a number of modifications have to be made in order for the program to run on the NAS AS/7000 and perhaps also to make the program comply with CCP standards. Frequently such programs are modified by the 'off-site' suppliers of the programs and the modified versions are sent to Daresbury for implementation. This necessitates the rather tedious and, if fairly extensive changes have been made at Daresbury, time consuming procedure of either making the on-site modifications to the off-site version of the program or vice versa. Apart from being tedious, there is the danger of introducing needless errors at this stage. Also, if the on-site modifications are to be made again to the new version, an accurate record of such modifications must have been kept. The program COMPEDIT has been written to assist in the maintenance of programs supplied as described above. It makes a comparison between three files, automatically performs the edits required and prepares an updated version of the program for use on-site. The three input files to the program are as follows:

1. The old on-site version of the program.
2. The old off-site version of the program (from which (1) was prepared.
3. The new off-site version of the program.

The output file is the new on-site version of the program.

The program is written specifically for Fortran source files. Though it is a powerful tool, it should be used with a certain amount of care as it is not a hundred per cent foolproof. If the on- and off-site modifications are in different parts of the code then the relevant code is automatically transferred to the output file. If such modifications occur in the same area of

the code, then a commented out version of the code from each of the input files is included in the output file and such a section requires manual intervention. When the program is run, a lineprinter listing indicates the code which has been transferred to the output file and also lists the code which has not been used. A summary table at the end of the printer output gives a list of all the subroutines present, details of which input file(s) they were found in, the number of changed sections in each subroutine and the number of such sections requiring manual intervention.

The program COMPEDIT is written in (supposedly standard!) Fortran 77.

Outline of the Method

In the description following, the term subroutine is used to mean a subroutine, function, main program or block data.

The file comparisons are done on an individual subroutine basis and each file is analysed to find its component subroutines. The subroutine composition may be different in each input file and the order of the subroutines may also be different. The following treatment is carried out for the following cases for a subroutine present in various combinations of the files.

<u>Files</u>	<u>Treatment</u>	
1 2 3	Compare 1 2 3	
1 2 -	Compare 1 2 2	(Take subroutine from input file 1 but indicate changes on the lineprinter output)
1 - 3	Compare 1 1 3	(Take subroutine from input file 3 but indicate changes on the lineprinter output)
1 - -	Take subroutine from input file 1.	
- 2 3	Compare 2 2 3	(Take subroutine from input file 3 but indicate changes on the lineprinter output)
- 2 -	Omit this subroutine	
- - 3	Take the subroutine from input file 3	

The comparison part of the program finds blocks of text up to the next line common to each file. This is done first by examining the next line from each file and, if these match, the required block has been found. If the next lines do not match then a scan is made to find a common set of 'NCHECK' lines (NCHECK is a parameter which may be varied though a value of 5 would normally be satisfactory). If such a common set is found, the first line of this set is taken as the terminating line for the required block. Special adjustments are included when the ends of the subroutines are reached. For each block found, the treatment is as follows in the various cases which may arise:

<u>Case</u>	<u>Treatment</u>
1=2=3	Take the block from input file 1
1=2#3	Take the block from input file 3. (On the lineprinter output only mark the lines with ***3 and print a box containing the code replaced from input files 1 and 2.)
1#2=3	Take the block from input file 1. (On the lineprinter output only mark the lines with ***1 and print a box containing the code replaced from input files 2 and 3.)
1#2#3 (1#3)	The block from each of the three input files is output and printed commented out with the comments starting c***1, c***2 and c***3 respectively. Manual inspection and subsequent editing is required for such a block.
1#2#3 (1=3)	Take the block from input file 1. (On the lineprinter output only mark the lines with ***1***3 and print a box containing the code unused from input file 2.

Example

The example at the end of this article shows some pages of the lineprinter output taken from an update of the program LCFDUMP with modifications made by Phil Evans. The first page of the example shows a section requiring manual intervention. The second page shows the inclusion of on-site modifications and the third page shows the inclusion of off-site modifications. The final page shows the summary table produced at the end of the listing. This indicates for example that 8 sections of the main program were changed and that

one of these changed sections requires manual intervention. The subroutine NTHCHA was only found in input file 1 and the subroutine LCHKHD was only found in input file 3. Both these subroutines were included in the output file.

Summary

The program COMPEDIT can provide a valuable aid in incorporating new off-site modifications into an on-site version of a program. There are however certain problem areas, some of which are indicated below:

- (a) The new sections of off-site code, though correctly incorporated into the output file, may then still require some site specific modifications before the program will run.
- (b) A particular problem would occur if an attempt has been made to solve an identical problem by both on-site and off-site modifications particularly if the two solutions to the problem involve different sections of the code.
- (c) It should of course be noted that it is necessary to have available the relevant files for the comparison and to ensure particularly that the old on-site version was equivalent to the old off-site version (though if the off-site version is older the program may still work).

(shows manual modification required)

```

MAIN
C
C PROGRAM 'LCFDUMP'
C =====
C
C THIS PROGRAM IS USED TO GIVE A FILE DUMP OF A STANDARD 'LCF'
C REFLECTION DATA FILE. THE FILE HEADER INFORMATION IS PRINTED
C FOLLOWED BY A SUMMARY OF THE REFLECTION DATA WHICH GIVES, FOR EACH
C DATA COLUMN, THE FOLLOWING INFORMATION:
C
C COLUMN NUMBER
C SORT ORDER (ASC=ASCENDING, DESC=DESCENDING, BOTH=ALL VALUES THE
C SAME, NONE=NOT SORTED)
C MINIMUM VALUE PRESENT
C MAXIMUM VALUE PRESENT
C AVERAGE VALUE PRESENT
C AVERAGE ABSOLUTE VALUE PRESENT
C NUMBER OF ZERO VALUES PRESENT
C AVERAGE VALUE EXCLUDING ZERO VALUES
C AVERAGE ABSOLUTE VALUE EXCLUDING ZERO VALUES
C COLUMN LABEL
C
C THE SUMMARY CONCLUDES WITH A PRINT OF THE TOTAL NUMBER OF REFLECTIONS
C IN THE FILE
C
C THE SUMMARY IS FOLLOWED BY A COMPACT BUT TABULATED LISTING OF THE
C INDIVIDUAL REFLECTION DATA. THE FIELD WIDTH FOR EACH NUMBER IS
C DETERMINED BY THE MAXIMUM NUMBER OF DIGITS FOR ANY VALUE IN THE FILE
C FOUND FOR A GIVEN COLUMN. THE MAXIMUM NUMBER OF LINES TO BE PRINTED
C MAY BE SPECIFIED TO GIVE A PARTIAL LISTING OF THE DATA.
C
C ONE LINE OF CONTROL DATA IS READ FROM STREAM 5 (OPTIONALLY)
C
C LINMAX,LORFLG,(JHSTRT(1),I=1,3) FREE FORMAT
C
C LINMAX NUMBER OF REFLECTIONS TO LIST = IABS(LINMAX),
C IF LINMAX IS NEGATIVE, THE SUMMARY IS OMITTED,
C WHICH SPEEDS UP THE PROGRAM BY SAVING THE FIRST PASS
C THROUGH THE FILE. IF LINMAX = 0 OR -1, ALL REFLECTIO
C ARE LISTED
C
C LORFLG .GT.0 PRINT ORIENTATION DATA IF PRESENT, =0 DON'T
C
C JHSTRT INDICES HKL OF FIRST REFLECTION TO LIST. IF = 0 0 0,
C START AT BEGINNING.
C
C SPECIFICATION STATEMENTS
C
C===== VAX
C***1---CHECK THIS SECTION---*****
C***1C LOGICAL BATCH_MODE ✓
C***1C=====
C***1C LOGICAL MSTART
C***1C LOGICAL*1 IASC(100),IDESC(100),ARRAY1(1000)
C***2---CHECK THIS SECTION---*****
C***2C LOGICAL BATCH_MODE
C***2C=====
C***2C LOGICAL MSTART
C***2C LOGICAL*1 IASC(100),IDESC(100),ARRAY1(1000)
C***3---CHECK THIS SECTION---*****
C***3C LOGICAL BATCH_MODE
C***3C=====
C***3C LOGICAL MSTART,LCHKHD ✓
C***3C LOGICAL*1 IASC(100),IDESC(100),ARRAY1(1000) ✓
C
C DIMENSION LCK(2),HEAD1(16),IMIN(6),IMAX(6),FORM(66,6)
C DIMENSION F1(66),JHSTRT(3)
C DIMENSION MAXVAL(100),MINVAL(100),SUMVAL(100),NZERO(100)
C DIMENSION ABSVAL(100),IPREV(100),IWIDTH(100),LINBUF(100)
C INTEGER*2 IDATA(100)
C COMMON /LCF1/ICD1,MAXD1,CELL1(6),LRECL1,LTIT1,LABEL1,ARRAY1
C COMMON BLOCK FOR LCF BATCH TITLES AND RECORD LENGTH

```

(shows inclusion of on-site modifications)

```
DATA HEAD1/'K,FL,LECTION,ON F,FILE ' ' ' ' ' '
* DATA ASC/'ASC '//,DESC/'DESC'//,ANONE/'NONE'//,BOTH/'BOTH'//
```

```
C READ IN MAXIMUM LINES COUNT AND GET FILE NAME
```

```
C C&C&C&C VAX
C OPEN STREAM 5 AS A FILE TO BE DELETED
C OPEN(UNIT=5,STATUS='OLD',DISP='DELETE')
C C&C&C&C
```

```
***1
***1
```

```
-----
:UNUSED SECTION FROM FILES 2 AND 3
:OPEN(UNIT=5,STATUS='OLD',DISP='DELETE')
:C&C&C&C
:-----
```

```
LINMAX=1000000
MSTART=.TRUE.
READ(5,*,END=1)LINMAX,LORFLG,JHSTRT
```

```
C MSTART IS .FALSE. IF STARTING REFLECTION IS GIVEN, 000 = NO REFLECTION
C MSTART=.FALSE.
```

```
DO 2 I=1,3
IF(JHSTRT(I).NE.0) GO TO 1
CONTINUE
```

```
C SET DO1=1 IE LOGICAL NAME LCF11
1 DO1=1
```

```
C CALL DSHAME('DDNAME11',HEAD1(5))
```

```
C NCHLIN=128
```

```
C C&C&C&C VAX
C FIND OUT IF ON-LINE OR NOT, SET LINE LENGTH ACCORDINGLY
```

```
C NCHLIN=80
C IF(BATCH_MODE()) NCHLIN=132
C C&C&C&C
```

```
***1
***1
***1
***1
***1
***1
***1
***1
***1
```

```
-----
:UNUSED SECTION FROM FILES 2 AND 3
:C
:C% NCHLIN=128
:C&C&C&C VAX
:C FIND OUT IF ON-LINE OR NOT, SET LINE LENGTH ACCORDINGLY
:C
:C NCHLIN=80
:C IF(BATCH_MODE()) NCHLIN=132
:C&C&C&C
:-----
```

```
C C INITIALISE COUNTS AND VARIABLES
```

```
C NREFL=0
DO 10 I=1,100
MINVAL(I)=32767
MAXVAL(I)=-32768
SUMVAL(I)=0.0
ABSVAL(I)=0.0
NZERO(I)=0
IASC(I)=.TRUE.
IDESC(I)=.TRUE.
10 CONTINUE
MAXDI=100.0
IDDI=1
```

```
C READ FILE TO COLLECT STATISTICS INFORMATION
```

```
C CALL RFLCF1(2,LOCK,IDATA,.FALSE.)
NCOLS=LRECL1/2
```

```
C STORE RECORD LENGTH FOR S/R RFLCF1
LRECLB=LRECL1
```

```
C PRINT HEADINGS
```

(shows inclusion of off-site modifications)

```

C SKIP INITIAL REFLECTIONS IF REQUIRED
  IF (ISTART) GO TO 434
  IEND=1
436 DO 433 J=1,3
    IF (IDATA(J).NE.JHSTRT(J)) GO TO 432
433 CONTINUE
C FIRST REQUIRED REFLECTION FOUND
434 IEND=1
C
431 IF (IDATA(1).LE.-32000) GO TO 450
    NRF=NRF+1
    DO 440 N=1,NCOLS

```

```

***3
***3
***3

```

```

-----
:REPLACED SECTION FROM FILES 1 AND 2
:431   NRF=NRF+1
:      DO 440 N=1,NCOLS
:-----

```

```

  J=(I-1)*NCOLS+N
  LINBUF(J)=IDATA(N)
440 CONTINUE
  GO TO 480
C
C BATCH HEADER FOUND, PRINT CURRENT LINE 1ST
C
450 II=II-1
    IEND=3
    GO TO 480
C
460 II=II-1

```

```

***3
***3
***3
***3
***3
***3
***3

```

```

-----
:REPLACED SECTION FROM FILES 1 AND 2
:460   II=II-1
:-----

```

```

480 IEND=2
    JJ=NCOLS*II
    LINES=LINES+NRF*LIN
    IF (II.GT.1) WRITE(6,F1)(LINBUF(J),J=1,JJ)
    IF (IEND.EQ.2) GO TO 490
    IF (LINES.GE.LINMAX) GO TO 490
    IF (IEND.NE.3) GO TO 430
    IEND=1
    IF (LCHKHD(IDATA,LURFLG)) GO TO 430
    GO TO 430
C
490 WRITE(6,1007)NRF

```

```

***3
***3
***3
***3
***3

```

```

-----
:REPLACED SECTION FROM FILES 1 AND 2
:      GO TO 430
:490   WRITE(6,1007)NRF
:-----

```

```

  WRITE(6,1008)NREFL
  STOP

```

```

C
C READ AND OUTPUT REFLECTIONS WHEN EACH TAKES MORE THAN ONE LINE
C-----

```

```

C GET RANGE OF COLUMNS TO BE PRINTED ON EACH LINE
C

```

```

500 ILIN=0
    IMIN(1)=1
    ILIN=ILIN+1
    IF (ILIN.GT.1) IMIN(ILIN)=IMAX(ILIN-1)+1
    LWIDTH=0
    IMAX(ILIN)=IMIN(ILIN)-1
520 IMAX(ILIN)=IMAX(ILIN)+1
    IF (IMAX(ILIN).GT.NCOLS) GO TO 550
    J=IMAX(ILIN)
    LWIDTH=LWIDTH+IWIDTH(J)
    IF (LWIDTH.GT.NCHLIN) GO TO 530
    GO TO 520
530 IMAX(ILIN)=IMAX(ILIN)-1
    GO TO 510
550 IMAX(ILIN)=NCOLS
    NLIN=ILIN
C

```

SUMMARY TABLE

NAME	FILE1	FILE2	FILE3	NO. OF CHANGES	MANUAL EDITS	COMMENTS
MAIN	Y	Y	Y	8	1	NORMAL COMPARISON
CCLDUM	Y	Y	Y	1	0	NORMAL COMPARISON
WRTLIN	Y	Y	Y	0	0	NORMAL COMPARISON
FMTGET	Y	Y	Y	2	0	NORMAL COMPARISON
FGMPR	Y	Y	Y	0	0	NORMAL COMPARISON
HEADXX	Y	Y	Y	0	0	NORMAL COMPARISON
GTCFLD	Y	Y	Y	0	0	NORMAL COMPARISON
CPYCHA	Y	Y	Y	0	0	NORMAL COMPARISON
PLLCF1	Y	Y	Y	0	0	NORMAL COMPARISON
PTLCF1	Y	Y	Y	0	0	NORMAL COMPARISON
NTHCHA	Y	N	N	0	0	SUBROUTINE TAKEN FROM FILE 1
LCHKHD	N	N	Y	0	0	SUBROUTINE TAKEN FROM FILE 3

NUMBER OF LINES IN COMPARISON BLOCKS WAS 5

COMPEDIT Example
(shows summary table)

SCIENCE AND ENGINEERING RESEARCH COUNCIL

DARESBUURY LABORATORY

Meeting of Protein Crystallography Users of SRS on September 24th 1983 at Daresbury Laboratory.

Twenty one users were present:

	A C Bloomer	MRC, Cambridge	N Marks	Daresbury
	G C Ford	Sheffield	H Muirhead	Bristol
	A Geddes	Leeds	M Papiz	Keele
	I D Glover	Birkbeck	L Sawyer	Napier, Edinburgh
	J Habash	Keele	D Stuart	Oxford
Secretary	M Harding	Liverpool	B Sutton	Oxford
Chairman	J R Helliwell	Daresbury	R Todd	Oxford
	F Koerber	Leeds	H Watson	Bristol
	A Liljas	Uppsala, Sweden	J White	Sheffield
	B Loder	MRC, Cambridge	A Wonacott	Imperial
	P Machin	Daresbury		

1. John Helliwell reported on the present equipment on station 7.2 (oscillation camera), on which no further development is planned. Users are concerned that Andrew Thompson is leaving shortly and that there will be no new appointment to his position. Technical support at this level is valued by all users; the presence of one person familiar with the current detailed operation of the equipment substantially increases the amount of data that can be collected in the normal short sessions of a few shifts; without such help much time can be wasted on fairly trivial operations. The SRFC should be asked to consider this again.
2. Pella Machin reported on current facilities at DL for intensity measurements. They start with the Joyce Loebel Scandig - 3 Scanner; then Alan Wonacott's 'MOSCO' program with synchrotron geometry is available either

a) on the VAX 11/75, using magtape input, from films already scanned

or

b) on the DG NOVA, using input direct from the scanner.

In either case the final stages (agrovata etc) are done by CCP4 programs on the AS7000.

Some discussion of levels of accuracy achievable with photographic data ensued - in the best cases RSYM ~ 1% seems to have been achieved, but this is not 'normal' (why not?!). More consideration should be given to absorption corrections (an ion chamber behind the crystal now allows measurements for an empirical correction at the SRS), and perhaps profile fitting.

3. Miroslav Papiz reported on the development and testing (in the Netherlands) of the FAST System - kappa goniostat, TV area detector, control system and LSI 11 - and of the software for its use. For the user, Phase 1 will be equivalent to the use of 'MOSCO' on films.

4. The wiggler station, 9.3: John Helliwell reported that commissioning of the FAST System will start as soon as it is delivered (November). From January, station 9.3 will be used primarily for the second oscillation camera. Unfortunately, at present, there is no money available at DL for film for the period January - March 1984. "FAST" trials will take place initially off line on an x-ray set and then on the beamline. Regular calibration of the TV detector on the beamline requires a conventional x-ray generator; this needs to be purchased. The need for an introductory course for users of FAST was recognised - if Nonius do not provide a suitable course in the UK then it will be necessary to arrange one at DL. There will be problems with large data volumes, many mag tapes per crystal and an overwhelmed VAX especially if the majority of users decide to do initial processing at DL. Interestingly, this seems to be the intention in contrast to the present relatively lower level of demand for film processing at DL. Hence, it can already be foreseen that a "protein crystallography VAX" needs to be funded. Rapid data transfer between the area detector and such a VAX will be very important (ethernet). The accuracy that has been achieved with FAST so far in intensity measurements on rhodanase crystals is about 2 - 3% (R_{sym} on I for the strongest reflections) - Papiz and Helliwell unpublished work.
5. John Helliwell summarised the achievements so far in anomalous dispersion studies and enquired of the demand for more facilities - is there a need for a dedicated station? The demand from users at the meeting was not great. A program is available for calculating dispersion correction (f' and f'') at any wavelength, for any element.
6. John Helliwell reported on crystal cooling equipment and on a design study (Oxford Instruments) for a liquid He cryostat. This could be developed if it were useful for a particular project.

THE FAST AT DARESBUY

M Z Papiz, J Helliwell (Daresbury Laboratory)

The FAST TV Diffractometer is in the final stages of development. It is expected that MRC Laboratory Cambridge and Daresbury Laboratory will receive delivery from ENRAF-NONIUS before the end of 1983.

The FAST is unlikely to be made available immediately on delivery because it is thought essential to the user community that the FAST is commissioned thoroughly before routine use is made of it. Even after commissioning it is likely that the first users will find themselves in the 'suck it and see' branch of science; although it is hoped that any experience gained by the Laboratory during the period of commissioning will help to make initial experiences of the FAST less painful.

The FAST system will be placed in the PX Wiggler station and will join the Arndt-Wonacott camera purchased in addition to the one on beam line 7.2. The two systems will be readily interchangeable and will offer the choice of film or TV detector to anyone using the PX Wiggler station. The storage of FAST data will be handled by a RL02 10 mB portable disk, 67 mB Winchester disk, Kennedy tape drive (125 ips, 800/1600 bpi) and a possible direct link to the local VAX which has large disk space available for PX users.

The software will be made available in three stages. Immediately, the FAST can be used as film for which the MOSCO (Bristol, Oxford-version) program suite has been supplied as part of the system. However there is no reason why it should not be possible for the digitised images to be taken away and processed in the users home laboratories.

The second stage of software will use the FAST digital facility of masking out regions of the detector not receiving incident diffracted beams of radiation; in this way contributions from background radiation are minimized. Data will be collected by a continuous crystal rotation method thus eliminating partially recorded reflections and the need to scale together several 'film packs' of data; however it still will be necessary to collect data at various exposure times to capture the full dynamic range of intensities (detector dynamic range $\approx 100:1$).

The third stage of software development will include inclination crystal geometry and the continual refinement and updating of crystal and FAST parameters for data collection.

It will be feasible in the last two stages of software to produce integrated intensities as the data is collected, however it may be more sensible to produce three dimensional profiles of each reflection and to leave the production of integrated intensities to a more leisurely moment elsewhere. The three dimensional profile has the advantage that errors made during data collection have less serious consequences and time can be spent extracting the best integrated intensities from the data.

On my last visit to Enraf-Nonius, Delft data were collected, as part of the FAST acceptance tests, from a rhodonase crystal (C2, $a = 156 \text{ \AA}$, $b = 49 \text{ \AA}$, $c = 42 \text{ \AA}$, $\beta = 98.6^\circ$). The data were processed at Daresbury on the VAX using the Alan Wonacott version of MOSCO. R_{sym} 's (in intensities) of the strongest reflections were 2%. The distribution of R_{sym} with I confirmed the estimate of dynamic range of the detector as 100:1. The data suggest that the detector performs no worse than film in terms of peak to noise ratio with the added advantage of greater radiation sensitivity as witnessed in the exposure times of 250 sec per $2-5^\circ$ rotation (c.f. film $\approx$ 2-3 hours) on a sealed Cu tube source.

These preliminary results bode well for the second and third phases of software since they utilize the advantageous design features of the FAST outlined above. Additional improvements in the accuracy of data can be made by collecting at $\lambda = 1.08 \text{ \AA}$ to minimize absorption errors. At other wavelengths absorption errors can be corrected by the Huber method. These considerations therefore suggest that the FAST will prove to be superior to film.

SERC DARESBURY LABORATORY

PROTEIN STRUCTURE AND DYNAMICS: STUDY WEEKEND
19-20 NOVEMBER 1983

PROGRAMME

Saturday, 19th November

TIME	TOPIC	SPEAKER	CHAIRMAN
<u>X-RAY DIFFRACTION</u>			
9.00 - 9.15	Introduction	Dr. M. Sternberg (Birkbeck, London)	Dr. M. Sternberg
9.15 - 10.00	X-ray crystallography and protein dynamics	Prof. Sir David Phillips (Oxford)	
10.00 - 10.15	Analysis of temperature factors in Avian Pancreatic polypeptide.	Mr. I. Glover (Birkbeck, London)	
10.15 - 10.30	Anisotropic x-ray refinement of rigid group vibrations in protein structures	Dr. D. Moss (Birkbeck, London)	
10.30 - 11.00 COFFEE			
11.00 - 11.45	Domain motion in proteins	Prof. R. Hüber (Munich)	
11.45 - 12.00	General Discussion		
12.00 - 13.30 LUNCH			
<u>NEUTRON DIFFRACTION</u>			Prof. Sir David Phillips
13.30 - 14.15	Inelastic scattering from biomolecules: principles and prospects	Dr. H. Middendorf (King's London)	
14.15 - 14.30	Inelastic neutron scattering studies of vibrational modes in proteins	Dr. S. Cusack (ILL Grenoble)	
14.30 - 14.45	General Discussion		
<u>THEORETICAL STUDIES</u>			
14.45 - 15.45	Molecular Dynamics simulations on proteins.	Prof. M. Karplus (Harvard)	
15.45 - 16.15 TEA			
16.15 - 17.00	Normal mode analysis of proteins	Dr. M. Levitt (Weizmann, Israel)	Prof. R. Hüber
17.00 - 17.15	Normal mode analysis of proteins	Mr. I. Haneef (Birkbeck, London)	
17.15 - 17.30	Computer graphics and molecular dynamics	Dr. A.J. Morffew (IBM, Winchester)	
17.30 - 17.45	Dielectric models and protein dynamics	Mr. N.K. Rogers (Oxford)	
17.45 - 18.00	Structural changes and helix movement in insulin and haemoglobin	Dr. R. Hubbard (York)	
18.00 - 18.15	General Discussion		
18.15 Coach departs for Hotel			
19.30 DINNER			

Sunday, 20th November

TIME	TOPIC	SPEAKER	CHAIRMAN
<u>SPECTROSCOPIC METHODS</u>			
9.15 - 10.00	NMR and protein dynamics	Dr. C. Dobson (Oxford)	Prof. M. Karplus
10.00 - 10.45	Protein dynamics by optical spectroscopy	Dr. P. Bayley (NIMR, London)	
10.45 - 11.15	COFFEE		
11.15 - 11.30	Functional mobility of calcium trigger proteins	Dr. B. Levine (Oxford)	
11.30 - 12.15	Information on normal modes and molecular dynamics from Raman spectroscopy	Prof. R. Hester (York)	
12.15 - 12.45	General Discussion		
12.45 - 14.15	LUNCH		
<u>DISCUSSION AND CONCLUSION</u>			
14.15 - 14.45	Molecular mechanics and real life - an introduction to the discussion	Dr. A. Cooper (Glasgow)	Prof. T. Blundell
14.45 - 15.45	GENERAL DISCUSSION		
15.45	TEA and CLOSE OF MEETING		

MODELLING VIBRATIONS IN PROTEIN IN PROTEINS FROM X-RAY DATA -
A SIMPLE MECHANICAL APPROACH

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ABSTRACT

The methods used for describing vibrations in proteins on the basis of X-ray observations are those which have been found to be appropriate to the investigation of small molecules. In this latter case the intra-molecular motion is often small compared to the inter-molecular motion. Furthermore the overall degree of mobility is usually rather restricted so that more extensive X-ray observations are possible than for most proteins. For small molecules most workers have used a statistical approach whereby a generalised harmonic distribution (usually with only the second moment non-zero) is fitted to the X-ray data. This may subsequently be interpreted in mechanistic terms.

An alternative is to reduce the number of parameters in the model by imposing a priori mechanical constraints (prejudices) on the vibrations. In the simplest case the motion may be described as deriving from librations about covalent bonds in the molecule. This allows the number of independent parameters to be dramatically cut to less than that required for the independent isotropic model. We have derived simple and approximate mathematical expressions necessary to represent and refine this model in reciprocal space and intend to investigate its usefulness in the near future.

AN ANALYSIS OF THE TEMPERATURE FACTORS FROM THE REFINEMENT OF APP

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ABSTRACT

Avian Pancreatic Polypeptide, (aPP), is a small, 36 residue, polypeptide (MW=4250) with some hormonal characteristics isolated from the turkey pancreas. It was crystallised, monoclinic C2, $a=34.18\text{\AA}$, $b=32.92\text{\AA}$, $c=28.45\text{\AA}$, $\beta=105.26^\circ$. The structure was initially solved using a single Hg derivative with anomalous scattering to $2.1\text{\AA}$ (1). The structure comprises of a polyproline helix, residues 1-8, with prolines at positions 2, 5 and 8, which packs against an α helix residues 14 to 32. Residues 9-13 form a β turn. The C-terminal residues are oriented away from the tightly packed globular core of the molecule. A modified tangent formula was used to extend the native phases to $1.4\text{\AA}$ (2). Native data was then collected to $0.98\text{\AA}$ was then used to refine the model using restrained least squares methods. The initial stages of the refinement, using isotropic temperature factors, indicated that the thermal motion of atoms within the polyproline helix and the α helix was significantly less than those in the β turn. The C terminal region showed a high degree of flexibility. The effect of water molecules on the main chain hydrogen bonding gives rise to a partially sequence specific distortion of geometry in the exposed residues of the α -helix.

In the later stages of the refinement, six parameter anisotropic thermal parameters were used. The anisotropic thermal parameters indicate the possibility of concerted motion within aromatic residue side chains and in densely packed regions of the structure(3) and greater anisotropy of motion in the main chain atoms compared to those of the side chains.

- (1) Blundell, T.L., Pitts, J.E., Tickle, I.J., Wood, S.P., and Wu, C-W (1981) P.N.A.S. USA 78 4175-4179.
- (2) Tickle, I.J. (1981) Proc Symp Refinement, Daresbury Lab, Science Research Council, U.K.
- (3) Glover, I.D., Haneef, I., Pitts, J.E., Wood, S.P., Moss, D., Tickle, I.J. and Blundell, T.L. (1983) Biopolymers 22, 293-304.

ANISOTROPIC X-RAY REFINEMENT OF RIGID GROUP VIBRATIONS
IN PROTEIN STRUCTURES

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ABSTRACT

The data-to-parameter ratio in protein structure refinements is usually not sufficiently favourable to allow free anisotropic refinement of thermal parameters. By making reasonable assumptions about the atomic motions, the number of parameters may be reduced.

Some groups of atoms in protein structures exhibit dynamic or static disorder which may be approximated by rigid body displacements. Notable among such groups are the planar rings of phenylalanine, tyrosine, histidine and tryptophan residues where non-rigid body vibrations have mean square amplitudes ($< 0.01 \text{ \AA}^2$) much smaller than observed in protein crystal structures ($> 0.1 \text{ \AA}^2$).

We have applied the TLX approximation to the refinement of several rings in ribonuclease at $1.45 \text{ \AA}$ resolution, including the catalytically important His-119 where relative site occupancy has also been refined to allow for anharmonic nature of the disorder.

DOMAIN MOTION IN PROTEINS

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ABSTRACT

Large scale informational changes related to flexibility of proteins are discussed for a few selected examples: trypsinogen-trypsin, alpha 1 proteinase inhibitor, immunoglobulins, citrate synthase.

The structural background and the functional implications are reported.

INELASTIC SCATTERING FROM BIOMOLECULES:
PRINCIPLES AND PROSPECTS

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ABSTRACT

High-resolution neutron spectroscopy can provide unique spatio-temporal information on diffusive processes and low-frequency collective modes in biomolecules. By energy-analysing scattered neutrons at a number of angles, familiar diffraction methods are extended to give data on molecular motions in the form of dynamical structure factors $S(Q, \omega)$. Following an introduction to quasi-elastic and inelastic neutron scattering from the viewpoint of biophysical and bio-chemical applications, an outline of the interpretation of $S(Q, \omega)$ in terms of time correlation functions for co-operative as well as diffusive processes is presented. Expressions for the main types of motion contributing to neutron spectra from biomolecular samples between 0.0001 and 1000 cm^{-1} are discussed and illustrated by measured spectra. Whereas high-frequency features are mainly due to small groups, the transition to motions involving larger structural elements is observed for energy transfers $\lesssim kT$, together with increasing contributions from diffusive processes below about 10 cm^{-1} . These can, in favourable cases, be separated. A key quantity characterising the dynamics is given by the hydrogen-weighted density-of-states function $Z(\omega)$. Changes in $Z(\omega)$ describe the way in which the various degrees of freedom of biomolecules in processes such as hydration, ligand binding and catalysis are repartitioned, and integrals over $Z(\omega)$ relate to thermodynamic properties. The prospects for developing this application of neutron scattering are examined, and a number of problem areas offering considerable scope for future work are identified.

INELASTIC NEUTRON SCATTERING STUDIES OF VIBRATIONAL
MODES IN PROTEINS

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ABSTRACT

The prediction of inelastic neutron scattering spectra (INSS) of proteins from models of protein dynamics will be discussed, with special reference to the calculation of the INSS of pancreatic trypsin inhibitor from the normal mode analysis of Brooks and Karplus. A comparison with preliminary experimental data on PTI will be given and problems in the interpretation of INSS of proteins will be discussed.

NORMAL MODE ANALYSIS OF PROTEINS

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ABSTRACT

Normal mode analysis is an established technique for investigating the thermal motional properties of molecules. However, such analyses have so far been hindered by the daunting task of diagonalizing a large matrix, where large amounts of computer time and storage are required.

The Lanczos method has been widely used in engineering for finding the eigen-pairs for large symmetric matrices. We have used this technique to calculate the normal modes of a small protein (aPP, 580 atoms). This method is particularly useful for calculating a small region of the vibrational spectrum. We have exploited this to calculate the low frequency modes for aPP. Further, the computational cost is substantially reduced when a "good guess" for the modes exists.

The technique clearly lends itself to investigating the changes in vibrational modes caused by structural changes in proteins, such as those that might occur on substrate binding or those that exist amongst related structures.

One is able to extend the vibrational analysis for very large structures (proteins, several thousand atoms) by using sparse force constant matrices, or calculating these matrices for a smaller number of "grid points" on the protein.

COMPUTER GRAPHICS AND MOLECULAR DYNAMICS

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ABSTRACT

Recently, research groups interested in molecular dynamics and other time dependent research, have turned to computer graphics to view their results. In collaboration with Professor Blundell's group at Birkbeck College, we have been experimenting with new techniques to analyse this kind of data. This paper is in three parts:

- Atom trajectories and conformation comparisons
- Computer graphics animation techniques
- Representations to highlight features of interest

One method of displaying atom trajectories that has been developed at UKSC is to start with a standard bond drawing of the first conformation. The atom trajectories are plotted on top of this representation. The trajectories are then coupled to a tablet and pen in such a way that when the pen is moved in a particular direction, the trajectories are stretched out in the same direction. Both directions are with respect to a marked point. Comparison of conformations can be carried out by coupling frames to the pen in a way that allows them to be superimposed. This has been useful for comparing two or three conformations.

Animation techniques can be divided into two classes, vector and raster techniques. Vector refresh systems can be used for animation by storing successive frames on a disk or in memory. These frames can then be streamed to the device at a rate that creates smooth motion on the screen. At the same time, the researcher is able to alter the viewpoint using i/o devices. Raster refresh systems allow two types of animation. First, by keeping a set of conformations in the memory planes and using a hardware pan feature. Second, by loading a complex picture and selectively turning vectors on and off using a colour look-up table.

Although the animated bond diagrams may provide the researcher with some insight, we have found that particular features of interest can be highlighted by animating other representations. A simple case is to use the virtual bond representation to see the overall molecular motion. Distance matrices can be used to examine which atoms are moving co-operatively and Balasubramanian plots can be used to examine the changes in dihedral angles. A static derivative of this last representation has been found very convenient for examining the way a pair of angles has changed during a simulation.

DIELECTRIC MODELS AND PROTEIN DYNAMICS

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ABSTRACT

The atoms of a globular protein are in constant thermal motion and so are the charges or partial charges associated with them. Hence the field arising from the distribution of charges is a fluctuating field and a function of position and time.

Electrostatic calculations have used three main models of the dielectric, namely the uniform, distance dependent and cavity models. The cavity model attempts to describe the markedly different dielectric responses of the solvent and the protein. Given such a model, we have examined:

- a) The properties of a fluctuating field within such a cavity, e.g. higher order field generation, resonant fields, the frequency dependence of the solvent dielectric response and the significance of magnetic effects; and
- b) The consequences of the cavity model for the currently used models of the dielectric in molecular dynamics simulations.

References

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C.H. Paul, J. Mol. Biol., 155 (1982) 53-62.

STRUCTURAL CHANGE AND HELICAL MOVEMENT IN INSULIN AND HAEMOGLOBIN

The York Crystallographic Laboratory

presented by Rod Hubbard

There is a great deal of interest in protein mobility and its role in protein function. Molecular motions in proteins are beginning to be calculated with encouraging success but proper treatment of the phenomenon is still a formidable task. The series of pig insulin crystals in which the hormone assumes different conformations has provided examples of real structural changes which can be largely understood in terms of crystal contacts and the constitution of the solvent. These studies showed that the molecule's changes in structure were achieved mainly through simple movements of helix elements. (Chothia et al., 1983, Nature, 302, 500-505). A further example of this kind of behaviour in insulin is seen in the crystal structure of a modified monomeric insulin (despenta-peptide (B30-B26) insulin; DPI). There are two molecules of DPI in the crystal's asymmetric unit and we find there are significant variations in their structures produced by relative adjustments of the helices whose internal organisation is scarcely altered.

Haemoglobin crystallised in the T state from PEG has been shown to bind oxygen at the two crystallographically independent α haems. Comparison of the deoxygenated and α oxygenated molecules reveals distinct structural changes, the most notable of which is the translation of the F helix.

We acknowledge discussions with and ideas from C. Chothia, A. Lesk and D. C. Hodgkin.

NMR AND PROTEIN DYNAMICS

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ABSTRACT

Methods by which NMR spectroscopy can provide information about the dynamical behaviour of proteins will be described. Examples of the different methods will be taken from studies of hen egg white lysozyme, and particular emphasis will be placed on (i) correlation of NMR data with results from x-ray diffraction studies and molecular dynamics simulations and (ii) approaches to the study of unfolding and folding.

PROTEIN DYNAMICS BY OPTICAL SPECTROSCOPY

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ABSTRACT

This paper considers two optical spectroscopic techniques - circular dichroism and fluorescence - which have been widely used for the study of protein conformation in solution. Specifically, the scope and limitations of rapid observations with chiroptical techniques and the use of time-resolved fluorescence studies are reviewed in relation to the dynamic properties of proteins.

1. The use of chiroptical observations with conventional rapid reaction techniques (stopped-flow; temperature, pressure and electric field jump; flash photolysis) has been reviewed⁽¹⁾. Time resolution has generally been restricted to 10^{-3} s by flow methods or 10^{-6} s by relaxation methods. Recent developments suggest that the range of temperature jump techniques may well be extended to 10^{-8} to 10^{-9} s and flash methods in principle can be extended to the subnanosecond range. The CD signal resolution under optimal conditions is given by

$$\delta\Delta A \propto (\sigma_{\lambda} \cdot P_0 \cdot TC)^{-1/2}$$

where σ_{λ} is the efficiency of photon detection, P_0 the incident light power and TC the time constant. Time resolution can thus be improved only with increased power: conventional sources (used to date) achieve a few μ watts; lasers or synchrotron sources are potentially applicable, and the limits will be set by sample photo-damage. Modulation frequencies (conventionally 50 kHz) can be enhanced to ~ 100 MHz using Pockels cells. Double beam techniques and chiral photobleaching, performed with repetitive relaxation methods, offer further possibilities. However the method remains an absorption technique and depends on the magnitude of $\Delta A (= A_L - A_R) = \Delta \epsilon \cdot c \cdot l$. Practically ΔA may be 10^{-4} with $\delta\Delta A \sim 10^{-5}$ ($P_0 \cdot 10^{-5}$ watt at $TC = 10^{-3}$ s). Thus the experimental realisation of the detection of small changes in ΔA at short time constant ($< 10^{-3}$ s) is very challenging.

2. Real-time fluorescence measurements^(2,3) resolve many of the uncertainties of steady state measurements. They can provide direct measurement and multiple lifetimes are resolved as $F(t) = \sum a_i \cdot e^{-t/\tau_i}$ where $\sum a_i = 1$. A mean lifetime is defined as $\langle \tau \rangle = \sum a_i \tau_i$. Polarised measurements yield the real-time anisotropy

$$A(t) = \frac{V(t) - gH(t)}{V(t) + 2gH(t)} \quad \text{with } V, H = \text{vertical and horizontal components and } g \text{ a small correlation factor}$$

For isotropic protein motion $A(t) = A_0 \cdot e^{-t/\phi}$ and multiple probe motions may then be represented:

$$A(t) = A_0 \cdot \sum f_j \cdot e^{-t/\phi_j} \quad (\sum f_j = 1).$$

Experimentally the use of pulsed nanosecond flash lamps and pulsed lasers (mode-locked, synchronously pumped, cavity dumped, frequency doubled, etc.), plus the increasing use of synchrotron radiation allows subnanosecond phenomena to be investigated with time resolution ranging from psec to nsec depending on the detection system. For such processes, deconvolution of observed signal versus the lamp profile is mandatory, the events at shortest time are critical in determining parameters such as A_0 . Amongst the many examples, the following parameters are particularly useful: (a) Lifetimes, (b) Time-resolved fluorescence emission spectra, (c) Selective Dynamic Quenching, (d) Static Quenching, (e) Oxygen Quenching of Trp fluorescence in proteins, (f) Time-resolved fluorescence anisotropy of tryptophan in proteins, (g) Segmental Flexibility of Immunoglobulin.

Conclusions: Time-resolved fluorescence studies, (and particularly the real-time anisotropy) offer unique information on the dynamics of fluorophores (in the excited state). The least ambiguous case is where a single intrinsic fluorophore exhibits a single fluorescence lifetime but more than one rotational correlation time; where it exhibits multiple lifetimes, time-resolved emission is to be suspected and the anisotropy is correspondingly more difficult to assign; multiple lifetimes and non-interconnecting species may indicate microheterogeneity, and multiplicity of anisotropic components may then indicate heterogeneous dynamics. Thus, fast motions are undoubtedly observable; their magnitudes, origins and potential biological significance remain to be established.

References

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2. Badea, M. and Brand, L. (1979) Methods in Enzymology, 61, 378-425.
3. Yguerabide, J. (1972) Methods in Enzymology, 26, 498-578.

FUNCTIONAL MOBILITY OF CALCIUM TRIGGER PROTEINS

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ABSTRACT

Inside the cell the role of calcium in regulating activity is mediated by a set of structurally related receptor proteins. Physiological response occurs upon transmission of the binding event through the receptor protein contacts with the partner subunit(s) of the cellular system activated. Studies using NMR and circular dichroism spectroscopy have identified common structural features for the trigger proteins. The analysis of the movements connecting calcium binding with the parts of the proteins that relay information and the mechanism of antagonism of information transfer by drugs is related to the mobility within structure that allows transmission to occur.

INFORMATION ON NORMAL MODES AND MOLECULAR DYNAMICS
FROM RAMAN SPECTROSCOPY

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ABSTRACT

Vibrational modes involving chemical bond stretching, inter-bond angle deformation or torsional motion all occur in the wavenumber range $10\text{--}4000\text{ cm}^{-1}$. The whole of this range is routinely and conveniently accessible to standard commercial Raman spectrometers. Such instruments can be used to probe the normal modes of vibration of molecules in the solid state, in crystalline or amorphous form, and in aqueous solutions equally readily. Thus the technique can provide a link between the well-defined single crystal state, about which x-ray crystallography can provide such a wealth of structural detail, and the many other conditions in which proteins and other biological molecules exist and which are inaccessible to the x-ray probe.

The time scale corresponding to internal molecular vibrational motion is ca. $10^{-13}\text{--}10^{-14}$ s. Since large-scale molecular motions, such as the diffusion or gross rotation of a globular protein, for example, or the unwinding of a double helix, are relatively very slow, the Raman spectrum provides a 'snap shot' picture which may be used to good advantage in molecular dynamics studies.

The use of pulsed laser light sources for Raman spectroscopy provides a degree of control for time-resolved spectroscopic studies of biological processes. Nanosecond and picosecond pulsed lasers are readily available today and even femtosecond laser pulses have been generated, although these are of limited interest to vibrational spectroscopists due to the loss of information resulting from uncertainty broadening effects at the shortest times ($1\text{ fs} \approx 300\text{ cm}^{-1}$).

Recent applications of Raman, resonance Raman, and time-resolved methods to some proteins as solids and in a variety of aqueous solution conditions will be described. These will include some enzyme-substrate reaction intermediate studies (e.g. papain and alcohol dehydrogenase), deligation reactions of haemoglobins and photoisomerization of rhodopsins. Normal mode analyses will be related to the use of Raman spectra for determining secondary structure in proteins and in nucleic acids. Recent data relating to conformational flexibility in DNA and some oligonucleotides also will be presented.

MOLECULAR MECHANICS AND REAL LIFE - AN INTRODUCTION
TO THE DISCUSSION

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ABSTRACT

I will attempt to summarise the various views of protein dynamics and try to stimulate subsequent discussion by making some (possibly) outrageous comments, under the following broad headings:

- 1) Do we all mean what we think we mean by "protein dynamics"?
Are we too constrained by, or devoted to, particular mechanical models? What hidden assumptions might bias interpretation of experimental data?
- 2) Are the rest of us wasting our time when it can all be done so much better by computer simulation?
Are Newton's Laws enough? Does quantum theory play a role? How does molecular dynamics compare with thermodynamics? Is molecular dynamics bound to miss all the interesting bits of a protein's fluctuations?
- 3) Do dynamics have function?
Are there any aspects of protein function for which dynamics offers the only explanation? Can dynamic processes provide alternative explanations of apparently well-understood phenomena? I will suggest some examples.

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