PROSA

Version 3.4

User's Manual

Peter Güntert
Institut für Molekularbiologie und Biophysik
Eidgenössische Technische Hochschule
CH-8093 Zürich
Switzerland

December 1994

Contents

Introduction

The program package PROSA ("Processing algorithms") allows to perform the processing steps that lead from the time-domain data furnished by the NMR spectrometer to the multi-dimensional spectrum. Its functions include linear prediction, apodization, Fourier transformation, automatic phase correction, baseline correction, and formatting of the output for easy use with spectrum analysis programs.

The design of the program is simple both because it does not use computer graphics and the complete multi-dimensional data matrix is kept in memory throughout the processing. Therefore the implementation of the program on a variety of different computers is straightforward and time-consuming data processing can be executed in batch mode. The fully processed spectra can then be displayed on a conventional graphics station. In the present implementation, the output of PROSA is compatible with the program package XEASY (Bartels *et al.,* 1994; Eccles *et al.,* 1991).

Since PROSA completely avoids disk storage of intermediate results (i.e., at the outset the time-domain data are read into the computer memory and only the fully processed frequency-domain data are written back onto disk), the computer memory must be sufficiently large to hold the complete data set. On vectorizing computers the program achieves high efficiency because of the complete vectorization of all time-consuming routines, which is facilitated by the fact that identical operations are applied independently to all 1D cross-sections of a data set. PROSA is written in standard FORTRAN-77 and was implemented on a variety of computers.

A description of the program PROSA is given in the following publication:

Güntert, P., Dötsch, V., Wider, G. & Wüthrich K. (1992). Processing of multi-dimensional NMR data with the new software PROSA. *J. Biomol. NMR*, **2**, 619–629.

In this manual literal input is printed in **bold**, other input is printed in *italics*. Optional input is given in square brackets [. . .], and optional input that may be repeated zero or more times is given in curly braces {. . .}. In examples, output from the program PROSA is printed in `typewriter` font.

Comments, suggestions, and reports on bugs are welcome. Please send them to:

Peter Güntert
Institut für Molekularbiologie und Biophysik, HPM G21
Eidgenössische Technische Hochschule
CH-8093 Zürich
Switzerland

electronic mail: guentert@mol.biol.ethz.ch

Installation

Configuration

The program PROSA is delivered either on tape cartridge as a UNIX "tar"-archive or via electronic mail as a uuencoded, compressed, tar-archive file. To extract the individual files from the tape cartridge, use the UNIX command:

tar xvf *tape-device* [1]

which creates in the current directory a subdirectory called **prosa-3.4** that contains the prototype makefile **Makefile.def**, the installation help file **README**, the configuration shell script **configure**, and the subdirectories **help**, **macro**, and **src** for help files, macro files, and source files, respectively.

To extract the individual files from the electronic mail file, use the following sequence of UNIX commands:

uudecode *mail-file*
uncompress prosa-3.4.tar.Z
tar xvf prosa-3.4.tar [2]

The **Makefile** is created from the prototype makefile **Makefile.def** by the shell script **configure** using the UNIX command

./configure [*system*] [3]

that recognizes the following UNIX computer *systems*: **convex**, **hp**, **ibm**, **nec**, **sgi**, **sun**, and **generic**. If no *system* is specified, the script tries to determine the correct system type using the **uname** command or the **HOSTTYPE** environment variable. The script **configure** assumes that the name of the directory where the program PROSA resides is of the form **prosa-***version*. The system-dependent parameters set by the configuration script are shown when the script is executed. The following example is from a Sun-4 computer where the program resides in the directory **/home/guentert/prosa-3.4**:

```
Configuration:
System type      : Sun
Program          : prosa
Makeprogram      : makeprosa
Version          : 3.4
Macro extension  : .pro
Base directory   : /home/guentert/prosa-3.4
RECL unit        : 4 per word
Time routine     : etime(tarray)
Integer length   : 1 per real
Complex data type: complex
Precompiler      : /lib/cpp
Fortran compiler : f77
Compiler options : -c -O
Linker options   :
Libraries        :
```

`RECL unit` denotes the record length unit (in units per word) used in record length specifications in FORTRAN-77 **OPEN** statements for direct access files.[1] The present version of the program PROSA assumes a word length of 32 bit. On computers with variable word length, for example the NEC SX-3, it is essential to choose the correct word length of 32 bit.

Compilation

The executable program **prosa**, the script **makeprosa**, the links for help files, and the default initialization macro **init** are created by

make [4]

By default, a standard memory and workspace size will be used. This, and several other parameters may be changed on the **make** command line (see the **Makefile** for further details). For example:

make MAXS=*memory* **MAXW=***workspace* **PROG=myprosa** [5]

builds a PROSA executable called **myprosa** with the given *memory* and *workspace sizes* (in words). The script **makeprosa** can be used to build additional PROSA executables in other directories. The command

makeprosa *memory workspace* [*executable*] [6]

[1] According to the rules of FORTRAN-77 (Kießling & Lowes, 1987), the record length should be given in words but some compilers assume that it is specified in bytes.

builds a PROSA *executable* with the given *memory* and *workspace* size (in words) in the current working directory. The **makeprosa** command can be used to temporarily create a PROSA executable with optimal memory and workspace usage for the execution of a particular macro (the minimally required memory and workspace sizes for the execution of a macro can be determined with the standard macro **job**; see p. 38).

The **Makefile**, the script **makeprosa**, and the initialization macro **macro/init.pro** are generated from the prototype files **Makefile.def**, **src/makeprosa.def**, and **src/init.pro.def**, respectively. Permanent changes to these files should be made in the corresponding prototype files because they will otherwise be overwritten by **configure** or **make**.

Memory and workspace

Because PROSA does not store intermediate results on disk, the computer memory must be sufficiently large to hold the complete data set throughout the calculation. The *memory* size in words must always be at least as big as the total number of real data points of the current data set. In addition, some processing steps require additional temporary *workspace*. For example, transpositions of the data set (see the command **dimension** on p. 23) need a workspace of at least the size of the transposed plane(s). Thus, for the processing of two-dimensional data sets it is usually advisable to choose equal memory and workspace sizes. For the processing of three- and four-dimensional data sets the workspace size can be significantly smaller than the memory size.

On computers with moderate physical memory size the overall performance of the system can be significantly degraded if the PROSA memory and workspace sizes are chosen much larger than actually necessary. It can therefore be advisable for a given PROSA calculation to temporarily create a PROSA executable with adapted memory and workspace sizes. For the execution of a given *macro* (see p. 37) this can be done as follows:

- Determine the required memory and workspace sizes using the standard macro **job** (see p. 38) and a PROSA executable (called **prosasmall**) with small memory and workspace size:

  ```
  prosasmall

  PROSA, version 3.4 (Sun)

  Memory size   :    300000 words (1171 kbytes)
  Workspace size:    300000 words (1171 kbytes)

  job macro
  (. . .)
  ... Macro "macro" checked.
      The execution of this macro requires memory words of memory
  ```

```
and workspace words of workspace.
*** Error: There are only 300000 words of memory and
    300000 words of workspace available.
(. . .)
```

- Create a temporary executable with the memory and workspace size indicated by **job**:

  makeprosa *memory workspace* **prosatmp** [7]

  Note that only the main program source file (prosa.F) has to be recompiled, not the whole program.
- Use the PROSA executable **prosatmp** for the actual calculation.

The maximal available memory and workspace sizes are given by the functions **maxsize** and **maxwork** (see p. 34). The minimally required memory and workspace sizes required so far in a PROSA calculation are stored in the system variables **usedsize** and **usedwork** (see p. 35). It should be noted that some processing steps, for example linear prediction (see the command **predict** on p. 26), may be inefficient if only the minimally required workspace is available.

Command Interpreter

The program PROSA provides a powerful command line interpreter (comparable to a shell in the UNIX operating system) that allows the use of macros, variables, FORTRAN-77 mathematical and character expressions, control statements (conditionals, loops and jumps), error handling etc. When reading an input command line the command line interpreter executes the following steps:

- Comments, i. e. text following a comment sign "**#**", are discarded.
- The values of variables are substituted from right to left (see p. 16).
- The command line is split in elements (defined as sequences of non-blank characters separated by blank characters). The first element becomes the command name, and the following elements become command parameters.
- If the command name corresponds to a built-in command of the command interpreter (see p. 12), it is executed by the command line interpreter itself.
- Otherwise, if the command name identifies a specific command (see p. 21) unambiguously, the specific command is executed by the program.
- Otherwise, the command line interpreter looks for a macro with the given command name (see p. 19) and, if it is found in the current macro search path (see p. 19), executes it. If no such macro is found, an error occurs.

Special characters

The characters **$ % { } : \ " ' # @** have special meaning in the command line: "**$***variable*" or "**%***variable*" denote the value of the *variable* (see p. 16); The curly braces in "**{$***variable***}**" or "**{%***variable***}**" separate the variable name *variable* from immediately following text; "*label***:**" denotes a label that can be used as address in a **goto** statement (see p. 14); "**\***c*" treats the character *c* literally and allows the use of special characters in normal text, "\" at the end of a line indicates that the statement continues on the following line; **"***text***"** treats *text* as a single parameter, even if it contains spaces; **'***text***'** also treats *text* as a single parameter, but the apostrophes remain part of the text. Apostrophes are used to specify FORTRAN-77 string constants. Text between a comment sign "**#**" and the end of the line is treated as a comment and skipped by the program. Commands that are preceded by "**@**" will only be echoed if the system variable **echo** has the value **full** (see p. 18). "**@**" has a special meaning only if it occurs as the first character of a command.

Built-in commands

There are two kinds of commands in the program: general built-in commands of the command line interpreter, and specific commands (see p. 21). The following is an alphabetical list of all built-in commands of the command line interpreter:

alias [*name statement*]

defines a new alias *name*, i.e. an abbreviation, for the given *statement*. The *statement* may contain an asterisk "*" to indicate where the command line parameters are to be inserted. Without parameters, **alias** gives a list of all currently defined aliases.

Example:

```
alias ? "print \"\%{*}\""
? 5*7
35
```

ask *prompt variable* {*variable*}

writes the string *prompt* to standard output, reads one line from standard input, and assigns from this line strings separated by blanks to the given variables. The command is usually used for interactive input within macros. A *prompt* that contains blanks must be enclosed in double quotes.

Example:

```
ask "First and last point:" begin end
First and last point:
12 45
print "range = $begin...$end"
range = 12...45
```

break

breaks a "do"-loop and is only allowed in macros. The execution of the macro is continued with the first statement following the loop.

command *name*
sequence of statements
end

command

define a new globally visible user-defined command within a macro, i.e. a macro within a macro. User-defined commands defined by **command** statements are called by their *name*, possibly followed by parameters, in exactly the same way as macros. Within a macro, a user-defined command can only be called after it was defined. The statement *command* without parameters gives a list of all user-defined commands, and indicates where they are defined.

do [*variable start end* [*step*]]
sequence of statements
end do

executes a loop within a macro. The loop is executed unconditionally if **do** stands without parameters, i. e. until one of the statements **break, exit, quit** or **return** is encountered, or as a FORTRAN-77 “do”-loop, where the loop counter *variable* and the integer expressions *start, end,* and *step* have the usual meaning.

Examples:

```
do
    if (filename.eq.' ') break
    . . .
end do
do i 1 10
    print "Iteration $i."
end do
```

error [*filename*] *text* [*option*]

writes the *text* to standard output or into the file with the given *filename* and calls the error handler that is specified with the system variable erract (see p. 18). This statement is suitable to treat errors that occur during the execution of a macro. If the *text* contains blanks it must be enclosed in double quotes. The (default) *option* **append** indicates that the *text* is to be appended to an existing output file *filename*. A new file *filename* will be opened, if necessary. The *option* **close** indicates that the file will be closed after writing the *text*.

eval *variable* = *expression*
variable = *expression*

evaluates the arithmetic or string *expression* according to the rules of FORTRAN-77 and assigns the result to the *variable.* In the short form *variable* = *expression*, without the keyword **eval**, the equal sign must be surrounded by blanks. In contrast to FORTRAN-77 function names must be given in lowercase letters.

Examples:

```
i = 7
sentence = 'A flexible program!'
j = mod(i,4)**2
l = len(sentence)
show i sentence j l
... Variables:
    i        = 7
    sentence = 'A flexible program!'
    j        = 9
    l        = 19
```

exit

returns from a macro to interactive input. Given interactively, it exits from the program.

goto *label*

continues execution of a macro at the first line that begins with the *label*. Jumps into loops (**do** . . . **end do**) or conditionally executed statements (**if** . . . **else** . . . **end if**) are not allowed and can lead to unpredictable results. A *label* may consist of letters, digits, and underscore characters “_”. A label must be followed by a colon.

Example:

```
goto cont
. . .
cont: print "Now at label cont."
```

help [*topic*]

gives on-line help for a given *topic.* With no *topic* given, a list of all available help topics is displayed. On-line help for macros can be included in the macro: **help** *macro* shows all lines of the *macro* that start with “##”.

if (*condition***)** *statement*

executes a logical “if” statement as in FORTRAN-77, i. e. the *statement* is executed if the logical expression *condition* is true. A line with a logical “if” statement must not end with the word “then”. In addition to the possibilities of FORTRAN-77 there are three logical functions: **exist(***variable***)** is true if and only if the *variable* exists; **def(***variable***)** is true if and only if the *variable* exists and has a value different from **NULL**; **file(***filename***)** is true if and only if a file called *filename* exists.

Example:

```
set i=–56
if (i.lt.0) print "$i is negative."
-56 is negative.
```

if (*condition***) then**

sequence of statements

else if (*condition***) then**

sequence of statements

else

sequence of statements

end if

executes a block-”if” statement, as in FORTRAN-77. In addition to the possibilities of FORTRAN-77 there are three logical functions: **exist(***variable***)** is true if and only if the *variable* exists; **def(***variable***)** is true if and only if the *variable* exists and has a value different from **NULL**; **file(***filename***)** is true if and only if a file called *filename* exists.

Example:
```
if (mod(i,2).eq.1) then
     print "$i is an odd number."
else if (def('x') .and. exist('y')) then
     print "The variable x is defined, and the variable y exists."
else if (s.eq.' ') then
     print "The variable s is blank."
end if
```

parameter *variable* {*variable*}

changes the names of the parameters that are passed to a macro; i. e. the parameters **p1**, **p2**, . . . get the names given in the **parameter** statement. The **parameter** statement must precede all other statements in a macro (except **var)** and cannot be used interactively.

print [*filename*] *text* [*option*]

writes the *text* to standard output or into the file with the given *filename.* If the *text* contains blanks it must be enclosed in double quotes. The (default) *option* **append** indicates that the *text* is to be appended to an existing output file *filename*. A new file *filename* will be opened, if necessary. The *option* **close** indicates that the file will be closed after writing the *text*.

quit

exits the program.

return

exits from the current macro and returns to the calling macro or, if the macro was called interactively, to interactive input. Given interactively, **return** exits from the program.

set {*variable*}
set *variable* **=** *value*
variable **:=** *value*

displays or sets *values* of *variables*. If no *variable* is specified, all variables that have values different from **NULL** are displayed. If the names of one or several *variables* are given, the values of these variables are displayed. System variables that must not be changed by the user are marked as "read-only." In the form **set** *variable* = *value* the given *value* (i. e. a string) is assigned to the *variable.* In the short form *variable* **:=** *value*, without the keyword **set**, the "**:=**" sign must be surrounded by blanks.

Examples:
```
set i=456
j := 2 + $i
```

```
set i j
... Variables:
    i = 456
    j = 2 + 456
```

show {*variable*}

displays the values of all or selected *global* variables. If no *variable* is specified, all global variables that have values different from **NULL** are displayed. If the names of one or several global *variables* are given, the values of these variables are displayed. System variables that must not be changed by the user are marked as "read-only." Global variables that are hidden by local variables with the same name are marked as "hidden."

subroutine *name*
sequence of statements
end

define a new user-defined command within a macro, i.e. a macro within a macro. User-defined commands defined by **subroutine** statements are called by their *name*, possibly followed by parameters, in exactly the same way as macros. User-defined commands defined by a **subroutine** statement are local to the current macro (or macros called through it). Within a macro, a user-defined command can only be called after it was defined.

type *name*

displays the macro or user-defined command with the given *name*. Macros in the current path (see the variable **path** on p. 19) can be listed without giving a path; otherwise the path has to be specified.

var *variable* {*variable*}

declares *variables* as local variables of the current macro. In contrast to normal (global) variables, local variables are only visible within the macro where they are declared and within macros that are called via that macro (except when such a macro declares itself a local variable with the same name). The **var** command must precede any other commands in a macro (except the **parameter** command) and cannot be used interactively.

Variables

The command line interpreter allows the use of variables that are similar to shell-variables in the UNIX operating system. A *variable name* consists of up to 20 letters, digits, or underscore characters "_". The *value* of a variable is always a character string (also the results of arithmetic expressions are converted to strings upon assignment to a variable), and is denoted by **$***variable* or *%variable* in the command line.[1] As in FORTRAN-77, parts of character strings may be denoted by **$***variable***(***begin***:***end***)**, where *begin* and *end* are integer expressions that denote the first and last character of the substring, respectively. Numerical values of variables may be formatted according to a given FORTRAN-77 *format* by **$***variable***(***format***)**. The k-th element (elements are separated by commas) of a variable is denoted by **$***variable***(***k***)**, where k is a non-negative integer expression. A variable name that is immediately followed by a letter, digit, or underscore character must be enclosed in curly braces: {**$***variable*}. Examples:

```
set x=4.6                                     Set the variable x.
set y=2.0                                     Set the variable y.
eval sum=x+y                                  Evaluate an expression.
set t=a sum                                   Set the variable t.
set x y sum t                                 Display values.
... Variables:
    x   = 4.6
    y   = 2.0
    sum = 6.60000
    t   = a sum
print "This is $t: $x + $y = $sum"            Use values.
This is a sum: 4.6 + 2.0 = 6.60000
print "This is $t: $x + $y = $sum(F4.1)"      Use FORTRAN-77 format.
This is a sum: 4.6 + 2.0 =  6.6
print "A second $t(3:5)! A third $t(2)!"      Use of substrings.
A second sum! A third sum!
set t(3:)=program                             Assignment to a substring.
print "$t or {$t}me?"                         Use of "{ }".
a program or a programme?
```

The evaluation of the values of variables in the command line goes from right to left. This allows for example the use of "indexed" variables in a loop (assuming **ndim** = 2, **ndata** = 2048, 512):

```
do i 1 ndim
    print "Dimension $i: $ndata(i) points"
end do
Dimension 1: 2048 points
Dimension 2: 512 points
```

System variables are variables that are set and used by the program (not exclusively by the user with **eval**, **set** etc.). The following section gives an alphabetical list

[1] The form *%variable* is preferable for variables that occur in UNIX-shellscripts because it avoids the evaluation by the UNIX shell.

of all system variables.

Write-protected variables cannot be changed explicitly by the user. Only system variables may be write-protected.

Global variables are always visible, except when they are hidden by local variables with the same name. Variables that are not declared in a **var** statement or passed as parameters to a macro are global. In particular, all system variables are global variables.

Local variables exist only within the macro where they are declared, and in macros called from this macro. Local variables must be declared in a **var** statement or passed as parameters to a macro (see p. 37).

The following variables are used by the command interpreter:

echo

determines which commands are echoed, i. e. copied to standard output before execution. The possible settings are:

NULL	(or not set at all) In macros, commands that are not built into the command line interpreter (see p. 21) are echoed; interactively, commands are not echoed.
on	Commands that are not built into the command line interpreter are echoed regardless of whether they occur in macros or interactively.
full	All commands are echoed, and the corresponding line numbers in macros are given.
off	Commands are not echoed.

Labels are not included in the echo; variable substitutions are included in the echo. Statements that are preceded by “@” will only be echoed if the system variable **echo** has the value **full**.

erract

is a variable for error handling. If an error occurs within a macro,[1] the value of **erract** is executed as command. By default the **exit** command is executed, i.e. the program returns to interactive input. Errors that occur interactively are displayed and the program continues with the execution of the next statement

Example: **set erract=chain show ; quit**
With this setting of **erract**, in case of an error a listing of all global variables is given, and the program is stopped. Such error handling can be useful if the program is used non-interactively.

nparam

denotes the number of command line parameters passed to a macro (see p. 19).

[1] Errors that occur interactively are displayed and the program continues with the execution of the next statement.

p1, p2, . . .

denote, by default, the command line parameters of a macro (see p. 19). The names of the command line parameters may be changed at the beginning of the macro with the **parameter** statement (see p. 15)

path

denotes the current search path for macro files. Usually, this variable is initialized in the initialization macro **init** (see p. 19).

Macros

Macros are files containing statements. A macro is called by its name that is identical to its filename except for the extension ".pro" that is required for macro files. Macro files are searched in the directories given in the system variable **path** (see p. 19), or in the explicitly given directory. Command line parameters may be passed into a macro. Within the macro, they are available as local variables that are by default called **p1**, **p2**, . . . These variable names can be changed with the **parameter** statement (see p. 15). The local variable **nparam** denotes the number of command line parameters. Macros can be called from within other macros. On-line help information may be included into a macro as lines that start with two comment signs "##". Such lines are copied to standard output when one requests help about a macro with the command **help** *macro*.

The special macro **init** (created during installation from the file **src/init.pro.def**) is an initialization macro that is automatically executed when the program starts. Typically, this macro sets the system variable **path** (see p. 19) that defines the search path for macro files.

Commands

There are two kinds of commands in the program PROSA: general built-in commands of the command line interpreter (comparable to a shell in the UNIX operating system) that are not specific to the program PROSA[1], and PROSA-specific commands. This chapter gives an alphabetical list of the PROSA-specific commands.

Many commands are applied to the *active dimension* of the data set. When a data set is read the first dimension, i. e. the dimension along which the data are stored sequentially in the data file, becomes the active dimension. Later on, the user can change the active dimension by suitable transpositions with the command **dimension** (see p. 23). The non-active dimensions are referrred to as *passive dimensions*.

abs

replaces the data in the active dimension by its absolute value, $s \to |s|$.

autophase *width threshold height overlap* $\phi_1^{\max}$ {*option*}

determines constant and linear phase correction parameters and performs an automatic phase correction (see p. 53). The parameters have the following meaning:

width	Maximal half-width (in data points) of peaks in the power spectrum (default: 10 data points).
threshold	Threshold to determine the extent of peak regions: In a peak region intensities must exceed *threshold* times the noise level and 10% of the maximal height (default: 2).
height	Minimal intensity of acceptable signal maxima with respect to the noise level: acceptable signal maxima in the absolute value spectrum must exceed the product of *height* times the noise intensity (parameter κ on p. 53).
overlap	Maximal number of acceptable signals that involve a common frequency coordinate (parameter ν on p. 53).
$\phi_1^{\max}$	maximal absolute value of the linear phase correction parameter ϕ_1, i. e. ϕ_1 will be chosen such that $|\phi_1| \le \phi_1^{\max}$; $\phi_1^{\max} = 0$ indicates that only a constant phase correction will be determined.

[1] These are the commands **ask, break, do, error, eval, exit, goto, help, if, parameter, print, quit, return, set, show, type** and **var** (see p. 12).

option	= **apply**	The phase correction will be determined and applied.
	= **determine**	The phase correction will be determined but not applied.
	= **complex**	Signals will be searched in the real and imaginary parts of the passive dimensions.
	= **real**	Signals will only be searched in the real parts of the passive dimensions. This option is useful if the phases in the passive dimensions are already approximately correct.
	= **global**	The global maximum of the target function in the range $-\phi_1^{\max} \le \phi_1 \le \phi_1^{\max}$ will be used to determine the linear phase correction parameter ϕ_1.
	= **local**	The local maximum of the target function with the smallest absolute value $\lvert\phi_1\rvert$ will be used to determine the linear phase correction parameter ϕ_1.
	= **symmetrize**	Symmetrized signal regions are used for the phase determination. Signal regions are symmetrized such that the absolute value spectrum becomes symmetric with respect to the peak maximum.
	= **info**	Information about every peak used for the phase determination is displayed.
	= **equal**	All signals have the same weight.
	= **sqrt**	Signals are weighted with the square root of their intensity.
	= **proportional**	Signals are weighted with their intensity.

The options **apply**, **complex**, **global** and **equal** are set by default.

The values determined for the constant and linear phase correction parameter are assigned to the system variables **phi0** und **phi1** (see p. 35).

complex

converts $2n$ real data points in the active dimension into n complex data points by considering subsequent real data points r_{2k-1} and r_{2k} as the real and imaginary parts, respectively, of complex numbers $z_k = r_{2k-1} + i\, r_{2k}$ $(k = 1, \ldots, n)$. If the number of real data points is odd, the imaginary part of the last complex data point will be set to zero. Complex data remain unchanged.

complexify

converts n real data points r_k in the active dimension into n complex data points $z_k = r_k$ with vanishing imaginary parts. Complex data remain unchanged.

conjugate

takes the complex conjugate of the complex data in the active dimension. Real data remain unchanged.

dimension *active dimension* {*dimension*}

transposes the data matrix such that *active dimension* becomes the active dimension. If additional dimensions are given the requested order of dimensions is obtained by suitable transpositions of the data set.

Examples:	**dimension 2**	# transposes the data set such that # dimension 2 becomes active
	dimension 1 2 3	# restores the original order of # dimensions of a 3D data set

flatten flatt *n* τ *function* {*function*}
flatten derivative *n* τ *function* {*function*}
flatten iterative [{*region*} **-**] *function* {*function*}
flatten manual [{*region*} **-**] *function* {*function*}

flattens the baseline of the real frequency domain data in the active dimension (see p. 54). The parameters have the following meaning:

method	can be **flatt** or **derivative** and specifies the method for the determination of pure-baseline regions—either the FLATT method (Güntert & Wüthrich, 1992; see p. 54) or the method of Dietrich *et al.* (1991) that relies on a smoothed derivative of the spectrum.
n	When using the FLATT method, *n* indicates the half-width of the line segments that are fitted to the data (see Eq. [24] on p. 54). When using the derivative method, *n* indicates the half-width of the smoothing of the spectrum (Güntert *et al.,* 1992).
τ	is a threshold for the determination of pure-baseline regions. With $\tau = 1$ the program recognizes about one third of the data points as pure-baseline regions, higher values of τ yield larger regions of pure-baseline (see p. 55; Güntert *et al.,* 1992).
region	When using the iterative method or the manual selection of pure-baseline regions, a *region* can be given in one of the following formats (*m* and *n* denote integer expressions):

n	denotes the data point *n,*
m **..** *n*	includes the data points $m, m+1, \ldots, n$,
m **..**	includes the data points $m, m+1, \ldots$ up to the last data point,
.. *n*	includes the data points $1, 2, \ldots, n$,
*	stands for all data points.

On the command line, a minus sign separates the last *region* from the first base function.

function denotes a base function that is used to represent the baseline distortions. Any integer or real FORTRAN-77 expression can be used to specify these functions; a lowercase **k** denotes the data point *k*.

ft [*N*] [n_b] [n_e] [ϕ_0] [ϕ_1] {*option*}

executes a Fourier transformation in the active dimension. With real input data a real Fourier transformation is performed, with complex input data a complex Fourier transformation is performed. After Fourier transformation the data are always complex. Prior to Fourier transformation the data are zero-filled to *N* complex data points. *N* must be a power of 2. If *N* is not specified, the program zero-fills up to the next power of 2, if necessary. Upon request, i.e. if n_b and n_e are given, the program retains only the strip consisting of the frequency-domain data points $n_b, \ldots, n_e$. If phase correction parameters ϕ_0 and ϕ_1 are given, the program performs a phase correction according to Eq. [23] and discards the imaginary parts of the data. Given the *option* **full,** the phase correction parameters ϕ_0 and ϕ_1 refer to the full spectral width; otherwise, with the default *option* **strip**, they refer to the strip of data points $n_b, \ldots, n_e$.

ift [*N*] [n_b] [n_e]

executes a complex, inverse Fourier transformation. Before the inverse Fourier transformation the data are symmetrically zero-filled to *N* complex data points. If *N* is not specified, the program zero-fills up to the next power of 2, if necessary. Upon request, i.e. if n_b and n_e are given, the program retains only the strip consisting of the time-domain data points $n_b, \ldots, n_e$.

multiply *factor* [*start end* [*step*]]

multiplies the data in the active dimension with a constant or variable *factor.* The *factor* may contain a lowercase **k** that denotes an index that runs over the data points in the active dimension from *start* to *end* with the given *step*. *Factor* must be a integer, real, or (in the case of complex data) complex FORTRAN-77 expression. All data points are multiplied if *start, end,* and *step* are omitted. If only *start* is specified, the data point *start* will be multiplied. The default *step* is 1.

```
Examples: multiply 0.05                   # scale data
          multiply 0.5 1                  # multiply first data point by 1/2
          multiply -1 2 $n 2              # change sign of every second
                                          # point
          multiply cos($pi/(2*$n)*(k-1))  # cosine window function
```

plot *format filename base factor* n_+ n_-
x-size x-offset x-margin x-labels x-tics y-size y-offset y-margin y-labels y-tics

{*option*}

creates a contour plot of the spectrum. The parameters have the following meaning ($\alpha = x, y$):

format	Plot files can be written in the following *formats*:

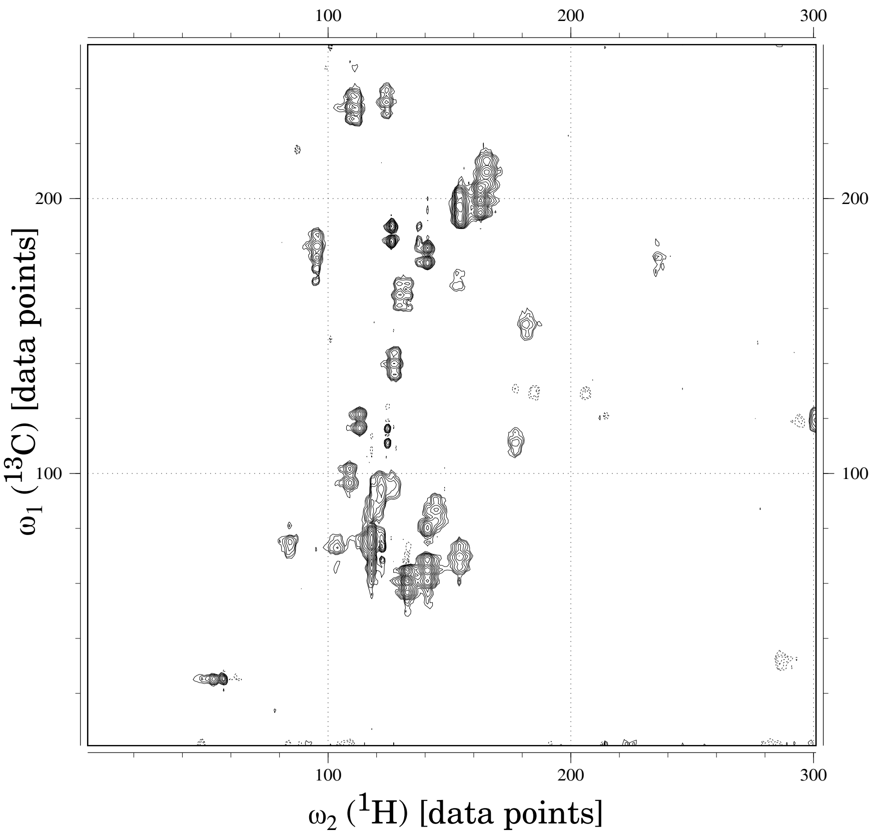
format	Language	Plotter/Printer	Paper size
hp7550a	HP-GL	HP 7550A	A3
hp7550a/a4	HP-GL	HP 7550A	A4
hp7596a/a0	HP-GL	HP 7596A	A0
hp7596a/a1	HP-GL	HP 7596A	A1
hp7596a/a2	HP-GL	HP 7596A	A2
hp7596a/a3	HP-GL	HP 7596 A	A3
postscript	Postscript	Postscript printer	A4

filename	Name of the output plot file. For a spectrum with several planes, a separate plot file called *filename.k* is written for every plane *k*.
base	Height of the lowest contour line. Default value: 5 times the noise level.
factor	Factor between the heights of adjacent contour lines. Default value: $\sqrt{2}$.
n_+	Maximal number of positive contour levels. Default value: 12.
n_-	Maximal number of negative contour levels. Default value: 12.
α-*size*	Size of the plot (excluding margins) in the α-dimension in cm.
α-*offset*	Offset in cm from the reference point in α-dimension.
α-*margin*	Margin width in α-dimension in cm.
α-*labels*	Label spacing in α-dimension, given in spectral units (ppm, if the spectrum is calibrated, otherwise data points). This parameter also determines the grid size if the *option* **grid** is set.
α-*tics*	Spacing for tics in α-dimension, given in spectral units (ppm, if the spectrum is calibrated, otherwise data points).

option

= **grid**	overlays the spectrum with a grid,
= **nogrid**	does not draw a grid,
= **margin**	surround the spectrum with a labelled margin,
= **nomargin**	does not draw a margin,
= **eject**	ejects the plot,
= **noeject**	does not eject the plot.

The options **grid**, **margin** and (except for the HP 7596A plotter) **eject** are set by default.



Contour plot produced with the PROSA command **plot** *of part of a [^{13}C-^{1}H] COSY spectrum of a complex between the* Antennapedia(C39S) *homeodomain and a DNA 14-mer (Qian* et al.*, 1993). The spectrum shows correlations between ^{13}C and ^{1}H atoms of aromatic rings. The default values were used for all parameters of the* **plot** *command.*

The default values for the parameters α-*size*, α-*offset*, α-*margin*, α-*label*, and α-*tics* depend on the *format* and are chosen such that the plot makes good use of the available paper size. To use the default value for a parameter, an asterisk “*” may be specified.

power

replaces the data in the active dimension by its squared absolute value (power spectrum): $s \rightarrow |s|^2$.

predict *method m n* [k_b k_e]

calculates complex data points in the active dimension using linear prediction (see p. 52) with the following parameters:

method	must be **lpsvd** in the present version of PROSA, i. e. the linear prediction coefficients are calculated with singular value decomposition.
m	Number of linear prediction coefficients in Eq. [16].
n	Number of predicted complex data points. For positive *n*, *n* addition-

	al data points are appended at the end; for negative *n,* the $\|n\|$ first data points are replaced with data points obtained from linear prediction. The latter possibility is used for the correction of baseline distortions that are caused by errors in the first time-domain data points (see p. 52).
k_b, k_e	specify the range of data points used for the determination of linear prediction coefficients in Eq. [16]. If k_b and k_e are not specified, the program uses all available data points for the determination of linear prediction coefficients.

project *n*

projects the data along the *last* dimension. If $n = 0$, the projection is given by the data point with the largest absolute value ("skyline projection"). For a natural number *n*, the projection *p* of the data points s_k along the last dimension is computed according to

$$p = \sqrt[n]{\sum_k \operatorname{sgn} s_k \, |s_k|^n}\,. \qquad [8]$$

re

replaces complex data in the active dimension by its real part. Real data remain unchanged.

read *format filename* {n_k [**c**]}
read *format filename* **combine** [f_1] [f_2]

reads a file with time- or frequency-domain data. In the first form, data in memory are overwritten; in the second form, a linear combination of the data in memory and in the input file is formed. The second form of the **read** statement is not allowed for files in **vnmr** format. The parameters have the following meaning:

format	= **real**	serial data file containing real numbers,
	= **integer**	serial data file containing integers,
	= **swap**	serial data file containing integers with reversed byte ordering,
	= **text**	data file that can be read with FORTRAN-77 list-directed input,
	= **easy** or **xeasy**	submatrix 8- or 16-bit data file of the program XEASY (Bartels *et al.*, 1995),
	= **vnmr**	data file in the format of the Varian VNMR program (Varian Associates Inc., 1993).
filename	Name of the input data file. In the case of the format **easy** two files will be read; a XEASY parameter file called *filename*.**3D.param** and the data file *filename*.**3D.8** or *filename*.**3D.16**, respectively, and the system variables for calibration will be set according to the XEASY	

	parameter file.
n_k	Number of real or complex data points to read in dimension k. If a "**c**" follows the number n_k, complex data are read, otherwise real data are read. n_k has to be specified with the *format*s **real**, **integer**, **swap**, **text,** and (optionally) **vnmr**. If the number of points is not given for a file in **vnmr** format, the program assumes a two-dimensional data set with complex data in both dimensions and one FID per trace, and extracts the number of data points from the file header. In the case of the *format* **easy** the corresponding numbers are read from the XEASY parameter file, and real data are read from the data file. In its present version the program can handle 2D, 3D, and 4D data sets. A one-dimensional data set is formally treated as a two-dimensional data set with a single row, i. e. by setting $n_2 = 1$.
f_1	Linear combination coefficient for data in memory. Default value: 1.
f_2	Linear combination coefficient for data read from input file. Default value: –1, i. e. by default the difference between the data in memory and in the input file is formed.

real

converts n complex data points z_k in the active dimension into $2n$ real data points r_l according to $r_{2k-1} = \mathrm{Re}\, z_k$ and $r_{2k} = \mathrm{Im}\, z_k$ $(k = 1, \ldots, n)$. Real data remain unchanged.

reduce *region* {*region*}

reduces the data matrix to the specified *regions*. The first *region* corresponds to the active dimension, the second *region* corresponds to the second dimension etc. If the number of *regions* is less than the number of dimensions of the data set, all data points will be used from the remaining dimensions. Data points outside the specified *regions* are discarded. A *region* can be given in one of the following formats:

n	denotes the data point n,
m **..** n	includes the data points $m, m+1, \ldots, n$,
m **..**	includes the data points $m, m+1, \ldots$ up to the last data point,
.. n	includes the data points $1, 2, \ldots, n$,
*	stands for all data points.

reverse

reverses the order of (real or complex) data points in the active dimension:

$$s_1, s_2, \ldots, s_n \rightarrow s_n, s_{n-1}, \ldots, s_1 . \qquad [9]$$

n denotes the number of data points in the active dimension.

select {*region*}

selects the specified *regions* from the complete the data matrix. The first *region* corresponds to the active dimension, the second *region* corresponds to the second dimension etc. If the number of *regions* is less than the number of dimensions of the data set, all data points will be used from the remaining dimensions. Data points outside the specified *regions* remain in memory, and the complete data set can be restored by a **select** statement without parameters, provided that the size of the selected data was not changed. All PROSA statements can be applied to the selected portion of the data in exactly the same way as for the complete data set.

A *region* can be given in one of the following formats:

n	denotes the data point *n,*
m **..** *n*	includes the data points $m, m+1, \ldots, n$,
m **..**	includes the data points $m, m+1, \ldots$ up to the last data point,
.. *n*	includes the data points $1, 2, \ldots, n$,
*	stands for all data points.

n and *m* always refer to the complete data set; the **select** statement cannot be used recursively.

Examples:	**select 100..200 50..80**	# selects the points $100, \ldots, 200$ in the # active and the points $50, \ldots, 80$ in the # second dimension
	select * * 20	# selects plane 20 of a 3D spectrum
	select	# uses again all data

shift *m*

shifts the data in the active dimension circularly by *m* points to the right:

$$s_1, s_2, \ldots, s_n \rightarrow s_{n-m+1}, s_{n-m+2}, \ldots, s_n, s_1, s_2, \ldots s_{n-m} \quad . \qquad [10]$$

n denotes the number of data points in the active dimension. A circular shift by m' data points to the left is achieved with $m = -m'$ or $m = n - m'$. *m* is an integer expression that is interpreted *modulo n* and in which a lowercase **k** may denote an index that runs over all data points in the *passive* dimensions.

Example: **shift n/2-nint(real(n-1)/(ndata(perm(2))-1)*(k-1)+1)**

In a two-dimensional spectrum with a diagonal through the lower left and upper right corners, this command shifts the diagonal to the centre of the spectrum. Subsequently, the diagonal may be removed using the **smooth** command (Friedrichs *et al.,* 1991).

smooth *n function* [*m*] {*option*}

smooths the data in the active dimension by computing the moving average s'_k over the n preceding and n following data points s_k, that are weighted with the given *function*. In the *function* f_k a lowercase **k** stands for the index that runs from $-n$ to n.

$$s'_k = \frac{f_{-n}s_{k-n} + \ldots + f_0 s_k + \ldots + f_n s_{k+n}}{f_{-n} + \ldots + f_0 + \ldots + f_n} \qquad [11]$$

The following options are possible:

option	= **extrapolate**	computes the $m \geq n$ data points in the border regions by quadratic extrapolation of the smoothed data,
	= **circular**	assumes periodic data to smooth the border regions,
	= **linear**	uses only the available data points for smoothing in the border regions (e. g. for the smoothed data point 2 the data points $1, 2, 3, \ldots, 2+n$),
	= **replace**	replaces the data by the smoothed data,
	= **subtract**	subtracts the smoothed from the original data.

The parameter *m* has only a meaning with the option **extrapolate**. By default, the options **extrapolate** and **replace** are set.

Example: **smooth 20 cos(0.5*$pi/19*k) 22 extrapolate subtract**

is a method to suppress signals with zero frequency, for example the residual water signal. The data are extrapolated in the border regions over more data points than used in the smoothing in order to avoid using the first two data points which are often corrupted. This method is conveniently implemented in the macro **suppress** (see p. 39)

status [**max**] [**full**] [**silent**] [**data**]

displays information about the size and organization of the current data set. With the option **max**, also the maximum absolute value is calculated and assigned to the system variable **max**. With the option **full**, also the maximum absolute value and the noise level are calculated and assigned to the system variables **max** and **noise**, respectively. The option **silent** suppresses the display, which is useful if the system variables **max** and **noise** should be updated silently. With the option **data** the data are written to standard output (if less than 2048 numbers).

write *format filename* {*region*}

writes part or all of the data into the output file called *filename*. The parameters have the following meaning:

format	= **real**	serial data file containing real numbers,
	= **integer**	serial data file containing integers,
	= **swap**	serial data file containing integers with reversed

		byte ordering,
	= **text**	text file written with FORTRAN-77 format (1PE12.4),
	= **easy8** or **xeasy8**	submatrix 8-bit data file for the program XEASY,
	= **easy16** or **xeasy16**	submatrix 16-bit data file for XEASY.

filename Name of the output data file. In the case of the formats **easy8**, **xeasy8**, **easy16**, **xeasy16**, **easy**, and **xeasy** two files will be written; a XEASY parameter file called *filename***.3D.param** and the data file *filename***.3D.8** or *filename***.3D.16**, respectively. The calibration entries of the parameter file are set to the corresponding values of the system variables for calibration, **delta**, **w0**, and **ppmmax** (see p. 33/35), if possible. Otherwise the spectrum will be treated as "uncalibrated" by XEASY.

region Regions of the data set that are written into the output file. The first *region* corresponds to the active dimension, the second *region* corresponds to the second dimension etc. If the number of *regions* is less than the number of dimensions of the data set, all data points will be used from the remaining dimensions. A *region* can be given in one of the following formats:

n	denotes the data point *n*,
m **..** *n*	includes the data points $m, m+1, \ldots, n$,
m **..**	includes the data points $m, m+1, \ldots$ up to the last data point,
.. *n*	includes the data points $1, 2, \ldots, n$,
*	stands for all data points.

Optionally, a *region* may be followed by "**r**" or "**i**" to indicate that in the given dimension only the real or imaginary part, respectively, of a complex data set should be written into the output file.

Variables

The command line interpreter of the program PROSA allows the use of variables that are similar to shell-variables in the UNIX operating system (see p. 16). The following is a list of all system variables specific to the program PROSA. System variables associated with the command line interpreter are explained on p. 16–19.

check

determines whether PROSA statements that change the data matrix are only checked for errors or actually executed. Statements are executed if **check** is not set or equal to **NULL**, otherwise, i. e. if one or several **check** options are set, statements are checked for different types of errors without doing the calculation. The following options are possible:

memory	Insufficient memory or workspace size is an error.
file	Input data files that do not exist, or output data files that cannot be opened or created result in an error.
command	All other errors (syntax errors, for instance) are reported.

The option **command** is always active. To determine the memory and workspace size required for the execution of a macro it is useful *not* to set the option **memory**, and to examine after the test the system variables **usedsize** and **usedwork**. If, during the execution of a macro, a new data file is written and later read again, the option **file** should not be set because the attempt to test the existence of the file results in an error that would not occur if the macro is really executed (not just tested). The most convenient way to test macros before execution is to use the standard macro **job** (see p. 38).

delta(k)

denotes the time or frequency increment between two data points in dimension k (in seconds for the time-domain, in Hertz for the frequency-domain). If a data file in the format of the program XEASY (Bartels *et al.,* 1994; see p. 27) is read, the system variables **delta(k)** are set according to the values in the XEASY parameter file, and updated during Fourier transformation.

dim

denotes the active dimension and is write-protected.

icmplx(*k*)

equals 1 if the data in dimension *k* are real, and 2 if the data in dimension *k* are complex. This variable is write-protected.

m

denotes the product of the numbers of real data points in the passive dimensions and is write-protected.

max

denotes the maximal absolute value of the data and is write-protected. **max** is only calculated and assigned with the statement **status full**.

maxsize and **maxwork**

denote the available memory and workspace sizes in words (see p. 9), respectively, and are write-protected.

n

denotes the number of real or complex data points in the active dimension and is write-protected.

ndata(*k*)

denotes the number of real or complex data points in dimension *k* and is write-protected. **ndata($dim)** is equivalent to **n**.

ndim

denotes the number of dimensions and is write-protected.

noise

denotes the noise level and is write-protected. **noise** is only calculated and assigned with the statement **status full**. An estimate of the median of the absolute values of the data points is used for the noise level.

perm

denotes the current order of dimensions and is write-protected. **perm(1)** is equivalent to **dim** and denotes the active dimension.

phi0 and **phi1**

denote the constant (ϕ_0) and linear (ϕ_1) phase correction parameters (see Eq. [23]). **phi0** and **phi1** are calculated and assigned with the statement **autophase** but can also be set by the user.

pi

has the value **3.141593** and is write-protected.

ppmmax(*k*)

denotes the chemical shift (in ppm) of the first data point in dimension *k*. If a data file in the format of the program XEASY (Bartels *et al.,* 1994; see p. 27) is read, the system variables **ppmmax(*k*)** are set according to the values in the XEASY parameter file.

timing

is a system variable to control the reporting of CPU times. CPU times are given for all commands (except those that are built into the command line interpreter) that need more seconds of CPU time than the value of **timing** indicates.

usedsize and **usedwork**

denote the *used* memory and workspace sizes in words. At the beginning of a PROSA session both variables have the value 0. The execution of every subsequent statement increases these variables according to the necessary memory and workspace sizes. The variables **usedsize** and **usedwork** can also be altered explicitly by the user.

w0(*k*)

denotes the spectrometer frequency (in MHz) in dimension *k*. If a data file in the format of the program XEASY (Bartels *et al.,* 1994; see p. 27) is read, the system variables **w0(*k*)** are set according to the values in the XEASY parameter file.

Macros

This chapter gives an alphabetical list of the standard macros that are provided with the program PROSA. The general initialization macro **init** is explained in the chapter on the command line interpreter (see p. 19).

dummycal

sets the system variables **delta(*k*)**, **ppmmax(*k*)** and **w0(*k*)** (see p. 33/35) to default values (**delta(*k*)** = **w0(*k*)** = 1000.0 and **ppmmax(*k*)** = *number of data points in dimension k*). It thus avoids that XEASY treats the spectrum as "uncalibrated."

cflatt flatt n τ *baseset* m [n_0 n_b] [ϕ_1]
cflatt derivative n τ *baseset* m [n_0 n_b] [ϕ_1]
cflatt iterative [{*region*} **-**] *baseset* m [n_0 n_b] [ϕ_1]
cflatt manual [{*region*} **-**] *baseset* m [n_0 n_b] [ϕ_1]

("convenient FLATT") flattens the baseline in the frequency-domain of the active dimension (see p. 23) using standard base function sets. It thus provides a convenient interface to the **flatten** command (see p. 23). The parameters n, τ and *region* have the same meaning as for the **flatten** command. The other parameters define the base function set:

baseset denotes the set of base functions used to represent baseline distortions. The following choices are possible:

baseset	Base functions ($t = (k - n_b + 2)/n_0$, $k = 1, \ldots, n$)
cft	$1, \cos 2\pi t, \sin 2\pi t, \ldots, \cos 2\pi t(m-1), \sin 2\pi t(m-1)$
rft	$1, \cos \pi t, \sin \pi t, \ldots, \cos \pi t(m-1), \sin \pi t(m-1)$
cftw	same as **cft**, plus Lorentzian functions to account for contributions from the water line
rftw	same as **rft**, plus Lorentzian functions to account for contributions from the water line
polynom	polynomial of order m

The methods **cft** and **rft** use trigonometric functions that correspond to the first m data points after complex and real Fourier transformation, respectively. The additional Lorentzian functions used in the basesets **cftw** and **rftw** assume that the water signal is located in the middle of the spectrum (before a possible strip transform).

m	determines the number of base functions used to represent baseline distortions. There will $2m - 1$ base functions with the methods **cft** and **rft**, $2m + 1$ base functions with the methods **cftw** and **rftw**, and m base functions with the method **polynom**.
n_0, n_b	specifies that the present data in the active dimension represent a strip out of a total of n_0 data points starting at data point n_b. By default, the values $n_0 = n$ and $n_b = 1$ are used (n denotes the number of data points in the active dimension).
ϕ_1	denotes the linear phase correction parameter used for phase correction.

The parameters n_0, n_b and ϕ_1 are not allowed when using the *baseset* **polynom**.

hilbert

performs a Hilbert transformation (Ernst, 1969) in the active dimension. Real data are converted to complex data such that the real part remains unchanged and the Kramers-Kroning relations are fulfilled.

im

replaces complex data in the active dimension by its imaginary part. Real data remain unchanged.

job *macro* {*parameter*}

checks for errors in the *macro* without executing the actual calculation and, provided that there is no error, executes the macro afterwards. The value of the system variable **check** (see p. 33) determines the type errors that are detected. By default, i. e. if **check** has the value **NULL** when the macro is called, **check** will be set to **command file**. If no error is detected, the memory and workspace sizes necessary for the execution of the macro are displayed and the execution of the macro is started if sufficient memory and workspace is available. In case of an error, the values of all global variables are listed and the program is stopped. The *macro* must not contain statements such as **quit** that stop the program. **job** is particularly useful to execute macros in batch jobs.

phase ϕ_0 [ϕ_1]

applies a phase correction according to Eq. [23] and the given constant (ϕ_0) and linear (ϕ_1) phase correction parameters. The values of ϕ_0 and ϕ_1 must be given in degrees. If the phase correction parameters are known, it is in general more efficient to use them together with the Fourier transformation (see p. 24) than to call the macro **phase**.

reduceppm *region* {*region*}

works as the statement **reduce** (see p. 28), except that the *regions* must be specified in ppm units instead of points. This macro can only be used if the system variables **delta(*k*)**, **ppmmax(*k*)** and **w0(*k*)** (see p. 33/35) are set.

savequit {*filename*}

displays the values of all global variables, writes the current data in **real** format into the file called *filename* (by default, **savequit.out**) and stops the program. This macro is a useful error handler for long calculations.

Example: **set erract=savequit**
sets **savequit** as error handling routine.

scale *method intensity*

scales the data such that in the case *method* = **max** the maximal absolute intensity and in the case *method* = **noise** the noise level is set to the given *intensity*. The default *intensity* is 500’000 for the maximal absolute intensity and 100 for the noise level, respectively.

selectppm {*region*}

works as the statement **select** (see p. 29), except that the *regions* must be specified in ppm units instead of points. This macro can only be used if the system variables **delta(*k*)**, **ppmmax(*k*)** and **w0(*k*)** (see p. 33/35) are set.

suppress [*weight* [*n*]]

suppresses signals of zero frequency (the water line, for instance) by subtracting smoothed time-domain data from the original time-domain data using the statement **smooth** (see p. 30). The smoothed data are calculated from the original data according to Eq. [11]. The following *weights* are possible:

weight	weighting function	name
cos	$f_k = \cos(\pi k/(2(n+1)))$	cosine weighting
gauss	$f_k = e^{-4(k/n)^2}$	Gaussian weighting
equal	$f_k \equiv 1$	equal weighting

window *type* {*parameter*}

applies commonly used window functions (DeMarco & Wüthrich, 1976; Ernst *et al.,* 1987):

type	*parameter*	window function	name
cos	–	$\cos(\pi t/2)$	cosine window
cos2	–	$\cos(\pi t/2)^2$	cosine squared window
exp	L	$e^{-\pi L n \Delta t}$	exponential line broadening
gauss	$L\ G$	$e^{-\pi L n \Delta t(1-t/2G)}$	Lorentz-Gauss transformation
hamming	–	$0.54 + 0.46\cos\pi t$	Hamming window
hanning	–	$0.5 + 0.5\cos\pi t$	“Hanning” window
sin	ϕ	$\sin(\phi - (\phi - \pi)t)$	shifted “sine-bell”
sin2	ϕ	$\sin(\phi - (\phi - \pi)t)^2$	shifted “squared sine-bell”

The symbols in the table have the following meaning:

$t = (k-1)/n$	, where $k = 1, \ldots, n$ runs over all n data points in the active dimension.
L	denotes the line broadening in Hertz.
G	denotes the maximum of the Lorentz-Gauss window function at $t = G$.
Δ	denotes the time increment in seconds, i. e. the value of the system variable **delta(active)** (see p. 33).
ϕ	denotes the shift of the sine-bell or squared sine-bell in degrees (for example, **window sin 90** is equivalent to **window cos**).

Examples

This chapter illustrates the use of PROSA with some practical examples of the processing of 2D and 3D data sets. The program can be used in three different ways:

- The user may enter statements interactively.
- The program can execute a sequence of statements contained in a macro file. This strategy is shown in two examples (see p. 41–45 and 46–47).
- A customized user interface may be created with the help of macros and the “ask”-command.

The first example shows the data processing of a 2D [^{1}H,^{1}H]-NOESY data set of the basic pancreatic trypsin inhibitor (BPTI). The PROSA commands are printed in **bold**, and comments are printed in Helvetica.

```
set timing=1        Show the CPU time for commands that take more than1s of CPU time
read swap /files/nmr/vd/ser 1024c 100c
                    The time-domain data consist of 1024 complex data points in the first
                    (aquisition) dimension, and 100 complex data points in the second, in-
                    direct dimension. The data are stored as integer numbers with inverted
                    byte-ordering in a serial file called /files/nmr/vd/ser (see p. 27).
status                                        Show the size and organization of the data
suppress cos 30
                    The residual water signal (that has frequency zero in the aquisition di-
                    mension) is suppressed using the macro suppress (see p. 39).
print
print “-------- Dimension 1 --------”            Processing of the aquisition dimension
print
multiply 0.5 1                                           Scale the first data point by 1/2
window cos                                                     Cosine window (see p. 40)
ft 1024             Fourier transformation with zero-filling to 1024 complex data points
status                                        Show the size and organization of the data
print
print “-------- Dimension 2 --------”               Processing of the second dimension
print
dimension 2                              Transposition that activates the second dimension
```

```
multiply -1 2 $n 2            Change sign of every second data point ("States-TPPI")
window cos                                                Cosine window (see p. 40)
ft 256                 Fourier transformation with zero-filling to 256 complex data points
print
print "--------  Phase correction --------"
print
dimension 1                                    Re-activate the aquisition dimension
autophase 10 2.0 10.0 10 0                      Automatic phase correction (see p.21)
re                                   Discard imaginary part of the aquisition dimension
dimension 2                            Transposition that activates the second dimension
autophase 6 2.0 6.0 10                          Automatic phase correction (see p.21)
re                                                              Discard imaginary part
print
print "--------  Baseline correction --------"
print
dimension 1                             Transposition that activates the first dimension
cflatt cft 10 6.0 3
                   Baseline correction using the FLATT method (see p.23/37/54) with a
                   half-width of 10 data points and a threshold parameter τ = 6 for the de-
                   termination of pure-baseline regions. The basis functions that are used
                   to represent the baseline distortions are the (5) trigonometric functions
                   that correspond to the first 3 time-domain data points.
dimension 2                            Transposition that activates the second dimension
cflatt cft 6 6.0 3                             Baseline correction in the second dimension

dimension 1 2                                      Restore original order of dimensions
dummycal
scale noise 100                                      Scale data to a noise level of 100
status full                  Show the size, noise level, and maximal intensity of the data
write easy16 /home/vd/noesy
                   Write an output spectrum file /home/vd/noesy.3D.16 and a parameter
                   file /home/vd/noesy.3D.param for XEASY.
```

Assuming that the above sequence of PROSA commands is stored in a macro file called **noesy.pro**, the data processing is executed as follows (the statements are displayed before execution in the form *macro: statement*; informative output of the program starts with "..." and is indented):

```
prosa
PROSA, version 2.4 (Sun)

Memory size    :   4456448 words (17408 kbytes)
Workspace size:    4202496 words (16416 kbytes)

... Ready.
job noesy
... Checking the macro "noesy":
```

```
job: noesy
```

(Output from the check phase is omitted.)

```
... "noesy" checked. The execution of the macro requires
    1052672 words of memory and 1049088 words of workspace.
job: noesy
noesy: read swap /files/nmr/vd/ser 1024c 100c
... File "/files/nmr/vd/ser" read.
    (CPU time: 3.8 s, total CPU time: 4.3 s)
noesy: status
... Occupied memory      :    410000 words (9 %)
    Dimension 1          :      1024 complex points
    Dimension 2          :       100 complex points
    Order of dimensions  : 1 2
noesy: suppress cos 30
suppress: smooth 30 cos(0.5*3.141593/(30+1)*k) 30+3 extrapolate
          subtract
... Smoothed data with extrapolated border regions subtracted.
    (CPU time: 16.2 s, total CPU time: 20.8 s)

--------  Dimension 1  --------

noesy: multiply 0.5 1
... Data multiplied.
noesy: window cos
window: multiply cos(3.141593/(2*1024)*(k-1))
... Data multiplied.
noesy: ft 1024
... Complex Fourier transform performed.
    (CPU time: 4.0 s, total CPU time: 25.8 s)
noesy: status
... Occupied memory      :    410000 words (9 %)
    Dimension 1          :      1024 complex points
    Dimension 2          :       100 complex points
    Order of dimensions  : 1 2

--------  Dimension 2  --------

noesy: dimension 2
... New order of dimensions: 2 1
noesy: multiply -1 2 100 2
... Data multiplied.
noesy: window cos
window: multiply cos(3.141593/(2*100)*(k-1))
... Data multiplied.
noesy: ft 256
... Complex Fourier transform performed.
    (CPU time: 8.7 s, total CPU time: 36.4 s)

--------  Phase correction --------

noesy: dimension 1
... New order of dimensions: 1 2
```

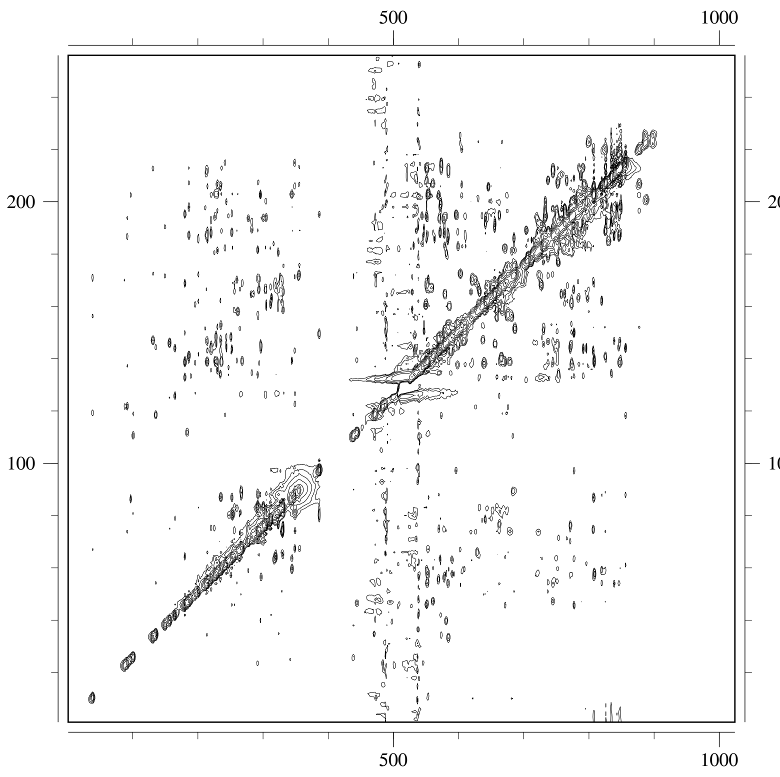
```
    (CPU time: 2.7 s, total CPU time: 39.1 s)
noesy: autophase 10 2.0 10.0 10 0
... Noise standard deviation : 2.249E+04
    Number of peaks used     :       199
    Constant phase correction:     -25.2 deg
    Standard deviation       :      28.5 deg
    Automatic phase correction applied.
    (CPU time: 2.5 s, total CPU time: 41.7 s)
noesy: re
... Real part kept, imaginary part discarded.
noesy: dimension 2
... New order of dimensions: 2 1
noesy: autophase 6 2.0 6.0 10
... Noise standard deviation : 1.875E+04
    Number of peaks used     :       414
    Constant phase correction:      63.3 deg
    Linear phase correction  :    -129.0 deg
    Standard deviation       :       8.2 deg
    Automatic phase correction applied.
    (CPU time: 5.1 s, total CPU time: 47.9 s)
noesy: re
... Real part kept, imaginary part discarded.

--------  Baseline correction --------

noesy: dimension 1
... New order of dimensions: 1 2
noesy: cflatt cft 10 6.0 3
cflatt: flatten flatt 10 6.0 1 sin(6.135924E-03*(-1+k))
        cos(6.135924E-03*(-1+k)) sin(1.227185E-02*(-1+k))
        cos(1.227185E-02*(-1+k))
... Average size of baseline regions: 63.2 %
    Minimal size of baseline regions: 48.2 %
    Baseline corrected.
    (CPU time: 19.9 s, total CPU time: 69.0 s)
noesy: dimension 2
... New order of dimensions: 2 1
noesy: cflatt cft 6 6.0 3
cflatt: flatten flatt 6 6.0 1 sin(2.454370E-02*(-1+k))
        cos(2.454370E-02*(-1+k)) sin(4.908740E-02*(-1+k))
        cos(4.908740E-02*(-1+k))
... Average size of baseline regions: 84.0 %
    Minimal size of baseline regions: 55.5 %
    Baseline corrected.
    (CPU time: 17.3 s, total CPU time: 87.4 s)
noesy: dimension 1 2
... New order of dimensions: 1 2
noesy: dummycal
noesy: scale noise 100
scale: status full
... Occupied memory      :    262400 words (6 %)
    Dimension 1          :      1024 real points
    Dimension 2          :       256 real points
    Order of dimensions  : 1 2
```

```
        Maximal magnitude    :  1.64E+07
        Noise magnitude      :  3.40E+03
        (CPU time: 1.0 s, total CPU time: 89.3 s)
    scale: multiply (100)/3404.98
    ... Data multiplied.
    noesy: status full
    ... Occupied memory      :    262400 words (6 %)
        Dimension 1          :      1024 real points
        Dimension 2          :       256 real points
        Order of dimensions  : 1 2
        Maximal magnitude    :  4.81E+05
        Noise magnitude      :  1.00E+02
        (CPU time: 1.1 s, total CPU time: 90.7 s)
    noesy: write easy16 /home/vd/noesy
    ... File "/home/vd/noesy.3D.16" written.
        (CPU time: 4.9 s, total CPU time: 95.7 s)
    ... Ready.
```

The resulting spectrum is shown in the Figure.



Contour plot produced with the PROSA command **plot** *of part of a NOESY spectrum of BPTI. The data set was processed as described above.*

As an example for the processing of higher-dimensional datas sets, the following shows the data processing of a three-dimensional ^{15}N-correlated [^{1}H, ^{1}H] NOESY data set:

read swap /tmp/SFS/angio.3d 1024c 150c 16c

The time-domain data consist of 1024 complex data points in the first (aquisition) dimension, 150 complex data points in the second dimension, and 16 complex data points in the third dimension. The data are stored as integer numbers with inverted byte-ordering in a serial file called **/tmp/SFS/angio.3d** (see p.27).

suppress cos 30

The residual water signal (that has frequency zero in the aquisition dimension) is suppressed using the macro **suppress** (see p. 39).

print	
print "-------- Dimension 1 --------"	Processing of the aquisition dimension
print	
multiply 0.5 1	Scale the first data point by 1/2
window cos	Cosine window (see p. 40)
ft 2048 1 1024	

The data are zero-filled to 2048 complex data points prior to Fourier transformation, and only the left half of the resulting spectrum is retained (see p.24).

print	
print "-------- Dimension 2 --------"	Processing of the second dimension
print	
dimension 2	Transposition that activates the second dimension
multiply -1 2 $n 2	Change sign of every second data point (“States-TPPI”)
window cos	Cosine window (see p. 40)
ft 256 1 256 90 -180	

The data are zero-filled to 256 complex data points prior to Fourier transformation, a constant phase correction of 90˚ and a linear phase correction of –180˚ are applied, and only the real part of the spectrum is retained (see p.24).

dimension 1	Re-activate the aquisition dimension
autophase 14 2.0 8.0 8 0	Automatic phase correction (see p.21)
re	Discard imaginary part of the aquisition dimension
print	
print "-------- Dimension 3 --------"	Processing of the third dimension
print	
dimension 3	Transposition that activates the third dimension
multiply -1 2 $n 2	Change sign of every second data point (“States-TPPI”)
predict lpsvd 5 16	

Append 16 complex data points by linear prediction with 5 coefficients (see p.26).

window cos2 Cosine squared window (see p. 40)
ft 32 Fourier transformation with zero-filling to 32 complex data points
autophase 4 2.0 6.0 5 Automatic phase correction (see p.21)
re Discard imaginary part

print
print "-------- Baseline correction --------"
print
dimension 1 Activate the aquisition dimension
cflatt cft 10 6.0 3 2048 1

Baseline correction using the FLATT method (see p. 23/37/54) with a half-width of 10 data points and a threshold parameter $\tau = 6$ for the determination of pure-baseline regions. The basis functions that are used to represent the baseline distortions are the (5) trigonometric functions that correspond to the first 3 time-domain data points. To correctly generate these basis functions the command must be provided with the information that the present data constitutes a strip taken out of the complete size of 2048 complex points starting at data point 1 (cf. the command **ft 2048 1 1024** above; see p. 37).

dimension 2 Activate the second dimension
cflatt cft 4 6.0 3 Baseline correction in the second dimension
dimension 3 Activate the third dimension
cflatt cft 3 6.0 3 Baseline correction in the third dimension
dimension 1 2 3 Restore original order of dimensions
dummycal Set dummy calibration parameters for XEASY
write easy16 /tmp/SFS/vd/hxnoe3d

Write an output spectrum file **/tmp/SFS/vd/hxnoe3d.3D.16** and a parameter file **/tmp/SFS/vd/hxnoe3d.3D.param** for XEASY.

Algorithms

Spectrum file formats

The program PROSA supports various formats for the storage of time- or frequency-domain data. The formats are summarized in the following table:

format	order	bits/point	coding	number format
real	serial	32	binary	real
integer	serial	32	binary	integer
swap	serial	32	binary	integer[a]
text	serial	13×8 [b]	ASCII	real
xeasy8	submatrix	8	binary	logarithmic
xeasy16	submatrix	16	binary	real
vnmr[c]	serial	16 or 32[d]	binary	integer or real[e]

[a] With inverted byte-ordering.
[b] On output. On input, the number of bits/point is variable.
[c] Only for input.
[d] Depending on the status bit S_32 in the file header.
[e] Depending on the status bit S_FLOAT in the file header.

For all formats except **vnmr** the data file contains only the data points themselves without any headers or other information. For the formats **xeasy8** and **xeasy16** a separate parameter file accompanies the data file (see below).

In a *serial* data file the data points of the first dimension (usually the acquisition dimension) are stored sequentially. Next, the data are ordered by the second dimension etc. For example, the data points s_{kl} ($k = 1, \ldots, m$; $l = 1, \ldots, n$) of a two-dimensional data set with m points in the first dimension and n points in the second dimension are stored in the following order:

$$s_{11}, \ldots, s_{m1}, s_{12}, \ldots, s_{m2}, \ldots, s_{1n}, \ldots, s_{mn} \qquad [12]$$

For complex data, the real part is stored followed by the imaginary part.

In a *submatrix* data file (formats **xeasy8** and **xeasy16**) the data set is split into submatrices with sizes as given in the accompanying parameter file (Bartels *et al.,* 1995; Xia & Bartels, 1993). Within each individual submatrix the data are ordered as in a serial file, and the submatrices as a whole are ordered in the same way as the data points of a serial file. On output, the program PROSA uses for a data set with n real data points in a given dimension a submatrix size of $n/8$ (but at least 1) in the corresponding dimension. If necessary, the submatrix size in the active dimension is increased to the next integer multiple of 4 or 2 for the formats **xeasy8** or **xeasy16**, respectively, in order to align the submatrices at word boundaries. On input, the submatrix size in the active dimension must be an integer multiple of 2 or 4 for the formats **xeasy8** or **xeasy16**, respectively. For the submatrix sizes in the other dimensions there is no such condition.

In the format **real** the data are stored in the binary floating point number format of the given computer with one real data point per (32 bit) word. Data files written with **real** format contain the data with full accuracy but are machine dependent. In the format **integer** the data are stored in the binary integer number format of the given computer with one real data point per (32 bit) word. In principle **integer** format files are also machine dependent but because most computers use the same binary representation of integer numbers, such files can in general be used on various computers. In the format **swap** the data are stored with inverse byte-ordering in the binary integer number format of the given computer with one real data point per (32 bit) word, i. e. the 4 bytes 1–2–3–4 of an integer number are stored in the order 4–3–2–1. This format is useful to read integer data from a computer that uses inverse byte-ordering with respect to the given machine but otherwise identical representation of integers. For example, Bruker X32 computers use inverse byte-ordering when compared to Sun, Convex, and NEC-SX3 computers. **Text** is a simple ASCII format using the FORTRAN-77 format (1PE12.4) for output and list-directed ("free format") FORTRAN-77 input. This format is primarily designed to store small (1D) data sets for use with simple simulation programs or graphics programs such as GNUPLOT. The format **xeasy8** uses a logarithmic representation of the data with 1 byte per real data point (Bartels *et al.*, 1995; Xia & Bartels, 1993). For a given data point s_k the program first determines the integer l that minimizes the expression

$$\left| |s_k| - \sqrt{2}^l \right| \qquad [13]$$

(i. e. $l \approx {}_{\sqrt{2}}\log|s_k|$) and then stores in one byte

$$\begin{aligned} e_k &= \min(l+1, 47), \qquad &\text{if } s_k \geq 0; \\ &= 95 - \min(l, 47), \qquad &\text{if } s_k < 0. \end{aligned} \qquad [14]$$

This format can represent numbers approximately in the range $-\sqrt{2}^{47} \leq s_k \leq \sqrt{2}^{46}$, i. e. $-1.2\ 10^7 \leq s_k \leq 8.4\ 10^6$ with a relative error of less than 20%. The format **xeasy16** uses a 16 bit floating point format with the "exponent" e_k given by Eq. [14] in the lower valued byte and the mantissa

$$a_k = 721\frac{|s_k|}{\sqrt{2}^l} - 615, \qquad \text{if } l \neq 0 \qquad [15]$$

($a_k = 0$ if $l = 0$) in the higher valued byte (Bartels *et al.,* 1995; Eccles *et al.,* 1991; Xia & Bartels, 1993). This format can represent numbers in the same range as the format **xeasy8** but with a relative error of less than 1%.

A **xeasy8** or **xeasy16** format data set consists of a data file *file***.3D.8** or *file***.3D.16**, respectively, and a parameter file *file***.3D.param** that contains information about the type, size, and organization of the data file. A parameter file written by PROSA for a two-dimensional data set has the following entries:

`Version ......................`	`1`	(always 1 in PROSA)
`Number of dimensions ..........`	`2`	(ndim)
`16 or 8 bit file type .........`	`16`	(8 for xeasy8, 16 for xeasy16)
`Spectrometer frequency in w1 ..`	`600.0`	(w0(2))
`Spectrometer frequency in w2 ..`	`600.0`	(w0(1))
`Spectral sweep width in w1 ....`	`10.0`	(delta(2)/w0(2) * ndata(2))
`Spectral sweep width in w2 ....`	`10.0`	(delta(1)/w0(1) * ndata(1))
`Maximum chemical shift in w1 ..`	`10.0`	(ppmax(2))
`Maximum chemical shift in w2 ..`	`10.0`	(ppmax(1))
`Size of spectrum in w1 ........`	`256`	(ndata(2))
`Size of spectrum in w2 ........`	`301`	(ndata(1))
`Submatrix size in w1 ..........`	`32`	(see above)
`Submatrix size in w2 ..........`	`38`	(see above)
`Permutation for w1 ............`	`2`	(ω_1-dimension)
`Permutation for w2 ............`	`1`	(ω_2-dimension)
`Folding in w1 .................`	`RSH`	(always RSH in PROSA)
`Folding in w2 .................`	`RSH`	(always RSH in PROSA)
`Type of spectrum ..............`	`?`	(always ? in PROSA)

Given in parentheses are the corresponding PROSA system variables (see p. 33–35). The parameter files for three- and four-dimensional data sets contain similar entries for the additional dimensions.

Data files in **vnmr** format start with a 32 byte file header followed by blocks consisting of a block header and sequentially stored data (Varian Associates, 1993). The organization of the data is extracted from the file header, block headers are simply skipped by the program PROSA.

Noise level

Several PROSA statements (for example **autophase** and **plot**) use a noise level to automatically adapt parameters to the scaling of data. The program PROSA uses an approximation of the median (Press *et al.,* 1986) of the absolute value of the real data points to estimate the noise level. To improve the efficiency of the noise level calculation, only about 10% or 1% of the data points are taken into account for the noise level calculation if the data set includes more than 10^5 or 10^6 data points, respectively.

Linear prediction

In PROSA, linear prediction (Olejniczak and Eaton, 1990; Stephenson, 1988; Zhu and Bax, 1990) is used to reduce effects caused by discrete Fourier transformation of truncated time-domain signals, i.e., primarily line broadening and the appearance of sidelobes. The linear prediction method is based on the assumption that a data point s_k can be written as a linear combination of the m preceding data points $s_{k-m}, \ldots, s_{k-1}$:

$$s_k = \sum_{l=1}^{m} a_l s_{k-l} . \qquad [16]$$

For a superposition of at most m damped oscillations with amplitudes A_α, phases ϕ_α, frequencies ω_α and dampings Γ_α that is sampled in time steps Δt,

$$s_k = \sum_{\alpha} A_\alpha e^{i\phi_\alpha} e^{-(\Gamma_\alpha + i\omega_\alpha)k\Delta t} , \qquad [17]$$

this assumption is fulfilled, and the zeros $z_1, \ldots, z_m$ of the polynomial

$$1 - \sum_{l=1}^{m} a_l z^l \qquad [18]$$

are related to the frequencies and damping factors by $z_\alpha = e^{(\Gamma_\alpha + i\omega_\alpha)\Delta t}$. The linear prediction coefficients $a_1, \ldots, a_m$ are determined by application of Eq. [16] to the measured data using singular value decomposition (Barkhuijsen *et al.,* 1987; Kumaresan and Tufts, 1982; Press *et al.,* 1986). Since each individual coefficient represents one frequency component, it is necessary to have an approximate estimate of the number of frequencies included in the time domain signal, and additional coefficients are needed to account for the noise. Singular value decomposition uses an overdetermined system of equations, and the maximal number of coefficients can be as high as one half of the number of complex data points. To ensure that the predicted signal is stable, the roots of the characteristic polynomial [18] of the linear prediction coefficients are calculated. Following conventional use of linear prediction, PROSA takes all roots into account and guarantees a stable predicted signal by reflecting the roots z about the unit circle, $z \to z/|z|^2$, if necessary (Press *et al.,* 1986). Although this procedure incorporates noise into the predicted data, the results do usually not differ significantly from those obtained when putative noise roots are eliminated, because the noise roots usually lead to rapidly decaying components of small intensity in the predicted data (Stephenson, 1988).

Since baseline distortions are primarily caused by errors in the measurement of the first few time-domain data points (Otting *et al.*, 1986), the backward linear prediction implemented in PROSA can be used to restore this corrupted part of the signal, and is thus also a suitable method for baseline correction (Marion and Bax, 1989). When compared with baseline correction procedures that work in the frequency-domain (Dietrich *et al.*, 1991; Güntert and Wüthrich, 1992; Pearson, 1977), an advantage of this method is that the baseline correction is performed at the beginning rather than at the end of the data processing, which may improve the results of other processing steps that rely on having a flat baseline.

Automatic phase correction

The automatic phase correction routine developed for PROSA determines the constant and linear phase correction parameters ϕ_0 and ϕ_1 by first searching the 1D cross-sections of the power spectrum for strong, well separated peaks. Then, in the phase-sensitive spectrum, the sum $S(\phi_0, \phi_1)$ of the difference between the squared real and imaginary parts of the normalized integral over the peak region in the phase-corrected spectrum, $\hat{I}_p$,

$$S(\phi_0, \phi_1) = \sum_p [(\mathrm{Re}\hat{I}_p)^2 - (\mathrm{Im}\hat{I}_p)^2] \qquad [19]$$

with

$$\hat{I}_p = \frac{I_p}{|I_p|} e^{-i(\phi_0 + \phi_1 \omega_p)} \qquad [20]$$

is maximized. I_p denotes the integral over the region of the peak p in the spectrum before phase correction, and ω_p denotes the normalized position of peak p $(0 \le \omega_p \le 1)$. The summation runs over all the peaks that were found to be acceptable for the purpose of the phase correction (see below). The maximum of $S(\phi_0, \phi_1)$ is obtained by selecting the linear phase correction ϕ_1 such that the function [21],

$$s(\beta) = \sum_p \left(\frac{I_p}{|I_p|}\right)^2 e^{-2i\beta\omega_p} , \qquad [21]$$

has its maximum absolute value at $\beta = \phi_1$. The constant phase correction is given by

$$\phi_0 = \frac{1}{2} \arg s(\phi_1) , \qquad [22]$$

where arg s is the argument of the complex number s. Because $s(\beta)$ has in general multiple local maxima, PROSA determines ϕ_1 by a one-dimensional grid search with a step size $\Delta\beta$ (usually, $\Delta\beta = 1^\circ$). The phase-corrected spectrum $\hat{s}_1, \ldots, \hat{s}_n$ is obtained from the original spectrum $s_1, \ldots, s_n$ by

$$\hat{s}_k = s_k e^{-i(\phi_0 + \phi_1 t)} \qquad \text{with} \qquad t = \frac{k-1}{n-1} . \qquad [23]$$

The method can be used for spectra containing both positive and negative peaks (but of course not for anti-phase multiplets).

In practice, to determine peak positions in 1D cross-sections of the power spectrum, the program first identifies all local maxima that are more than κ times above the noise level of the power spectrum (typically, $\kappa = 10$). For each maximum the corresponding boundaries for peak integration are set at the first data points on either side that are lower than either 10% of the maximal peak intensity or twice the noise level. To decide which of the maxima correspond to suitable peaks for use in the automatic phase correction, the following criteria are checked: (i) The width of the integration area must be smaller than a predetermined value u. (ii) To exclude overlapping peaks the average intensity in the regions of width $u/4$ adjoining the integration area to the left and to the right must be either below 10% of the maximal peak intensity or below twice the noise level. (iii) An upper limit, v, is imposed on the number of peaks that may have the same coordinates along one frequency axis (typically, $v = 20$–50). If v is exceeded, the program will only retain the v highest peaks so that the phasing cannot be dominated by one or several small spectral regions. The results of the automatic phase correction do not depend critically on the selection of the three parameters κ, u, and v. We found that the value of κ should be decreased for spectra with low signal-to-noise ratio, that the maximal peak width, u, should account for the increased line widths in power spectra and that v can be reduced when a spectrum contains a large number of peaks. Usually the number of one-dimensional peaks included for the phase correction of a 3D spectrum is of the order 1000, which renders the method robust against instabilities that might arise if only a small number of peaks were used, be it because of low signal-to-noise ratio, poor digital resolution, peak overlap, or occasional inclusion of artifactual peaks.

Baseline correction

Baseline correction in the frequency domain is performed for each 1D cross-section by first identifying regions of "pure-baseline" (Güntert and Wüthrich, 1992) and then subtracting a function which is best-fitted to the pure-baseline regions. The pure-baseline regions are identified either with modified versions of the FLATT procedure (Güntert and Wüthrich, 1992) or with the "derivative method" of Dietrich *et al.* (1991). For a data point k with intensity s_k, both methods yield a parameter, p_k, which becomes small if the data point k is located in a pure-baseline region, and large otherwise:

$$\text{FLATT:} \qquad p_k = \min_{a,b} \sum_{l=-n}^{n} (s_{k+l} - a - bl)^2, \qquad n \geq 1 \qquad [24]$$

$$\text{Derivative method:} \quad p_k = (s_{k+\max(1,n)} - s_{k-n})^2, \qquad n \geq 0 \qquad [25]$$

In Eq. [24], p_k is determined by fitting a straight line, $a + bl$, to a stretch of $2n + 1$ data points centered about the data point k. For the data points near the boundaries of the 1D cross section, where p_k is not defined by Eq. [24], p_k has the same value as the nearest data point inside the definition range. A minimal width for pure-baseline regions (Güntert and Wüthrich, 1992) is ensured by smoothing p_k:

$$\text{FLATT:} \qquad \bar{p}_k = \min(p_{k-n/3}, \ldots, p_{k+n/3}) \qquad [26]$$

Derivative method: $\bar{p}_k = \max(\min(p_k, \max(p_{k-1}, p_{k+1})), \min(p_{k-1}, p_{k+1}))$ [27]

The definition of $\bar{p}_k$ for the derivative method implies that any point of which both neighbors belong to pure-baseline regions will also belong to it, and that there is no point in a pure-baseline region for which both neighboring points do not belong to it (Dietrich *et al.,* 1991). A cutoff, p_c, is then defined such that $\bar{p}_k \leq p_c$ for one third of the data points and $\bar{p}_k > p_c$ for two thirds of the data points, and all data points k that satisfy the relation $\bar{p}_k \leq \tau p_c$ are considered as pure baseline, where τ is a user-defined parameter (typically, $\tau = 4$). Any linear combination of functions that can be written as FORTRAN-77 arithmetic expressions may be used to represent the baseline distortions. Usually, we use the constant and the trigonometric functions corresponding to the first m time-domain data points (see Eq. [4] in Güntert and Wüthrich, 1992). The linear least-squares fit to the pure-baseline regions is solved by standard techniques using singular value decomposition (Press *et al.,* 1986).

References

Barkhuijsen, H., De Beer, R. & van Ormondt, D. (1987). Improved algorithm for noniterative time-domain model fitting to exponentially damped magnetic resonance signals. *J. Magn. Reson.* **73,** 553–557.

Bartels, C., Xia, T., Güntert, P., Billeter, M. & Wüthrich, K. (1995). The program XEASY for computer-supported NMR spectral analysis. *J. Biomol. NMR*, in preparation.

DeMarco, A. & Wüthrich, K. (1976) Digital filtering with a sinusodial window function: An alternative technique for resolution enhancement in FT NMR. *J. Magn. Reson.* **24,** 201–204.

Dietrich, W., Rüdel, C. H. & Neumann, M. (1991). Fast and precise automatic baseline correction of one- and two-dimensional NMR spectra. *J. Magn. Reson.* **91,** 1–11.

Eccles, C., Güntert, P., Billeter, M. & Wüthrich, K. (1991). Efficient analysis of protein 2D NMR spectra using the software package EASY. *J. Biomol. NMR*, **1,** 111–130.

Ernst, R. R. (1969). Numerical Hilbert transform and automatic phase correction in magnetic resonance spectroscopy. *J. Magn. Reson.* **1,** 7–26.

Ernst, R. R., Bodenhausen, G. & Wokaun, A. (1987). *The principles of nuclear magnetic resonance in one and two dimensions,* Clarendon, Oxford.

Friedrichs, M. S., Metzler, W. J. & Mueller, L. (1991). Removal of diagonal peaks in two-dimensional NMR spectra by means of digital filtering. *J. Magn. Reson.* **95,** 178–183.

Güntert, P. & Wüthrich, K. (1992). A new procedure for high-quality baseline correction of two- and higher-dimensional NMR spectra. *J. Magn. Reson.* **96,** 403–407.

Güntert, P., Dötsch, V., Wider, G. & Wüthrich K. (1992). Processing of multi-dimensional NMR data with the new software PROSA. *J. Biomol. NMR*, **2,** 619–629.

Kießling, I. & Lowes, M. (1987). *Programmierung mit FORTRAN-77,* Teubner, Stuttgart.

Kumaresan, R. & Tufts, D. W. (1982). Estimating the parameters of exponentially damped sinusoids and pole-zero modeling in noise. *IEEE Transactions on Acoustics, Speech, and Signal Processing,* **ASSP-30,** 833–840.

Marion, D. & Bax, A. (1989). Baseline correction of 2D FT NMR spectra using a simple linear prediction extrapolation of the time domain data. *J. Magn. Reson.* **83,** 205–211.

Olejniczak, E. T. & Eaton, H. L. (1990). Extrapolation of time-domain data with linear prediction increases resolution and sensitivity. *J. Magn. Reson.* **87,** 628–632

Otting, G., Widmer, H., Wagner, G. & Wüthrich, K. (1986). Origin of t_1 and t_2 ridges in 2D NMR spectra and procedures for suppression. *J. Magn. Reson.* **66,** 187–193.

Pearson, G. A. (1977). A general baseline-recognition and baseline-flattening algorithm. *J. Magn. Reson.* **27,** 265–272.

Press, W. H., Flannery, B. P., Teukolsky, S. A., Vetterling, W. T. (1986). *Numerical Recipes. The art of scientific computing,* Cambridge University Press, Cambridge.

Qian, Y. Q., Otting, G., Billeter, M., Müller, M., Gehring, W. & Wüthrich, K. (1993). NMR spectroscopy of a DNA complex with the uniformly ^{13}C-labeled *Antennapedia* homeodomain and structure determination of the DNA-bound homeodomain. *J. Mol. Biol.* **234,** 1070–1083.

Stephenson, D. S. (1988). Linear prediction and maximum entropy methods in NMR spectroscopy. *Prog. NMR Spectrosc.* **20,** 515–626.

Varian Associates Inc. (1993). *User programming VNMR 4.3,* Palo Alto, California.

Wüthrich, K. (1986). *NMR of Proteins and Nucleic Acids,* Wiley, New York.

Xia, T. H. & Bartels, C. (1993). *XEASY. ETH Automated Spectroscopy for X Window Systems. User Manual,* Institut für Molekularbiologie und Biophysik, ETH Zürich.

Zhu, G. & Bax, A. (1990). Improved linear prediction for truncated signals of known phase. *J. Magn. Reson.* **90,** 405–410.