

# **R.E.D. version 1.0**

## **User's Manual and Tutorial**

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## **-I- Why R.E.D. & X R.E.D.?**

The Derivation of RESP <sup>[1, 2]</sup> and ESP <sup>[3]</sup> charges for a new structure is an important step in molecular mechanics simulations based on AMBER force fields. To get such atom-centered charges one proceeds in three steps:

- First, the studied molecule is optimized to determine a stable conformation (using quantum chemistry software).
- Then, this minimized structure is used to calculate a Molecular Electrostatic Potential (MEP) on a three-dimensional grid (using again quantum chemistry software).
- Eventually, this molecular grid is exported into program "RESP", <http://amber.scripps.edu/Questions/resp.html>, which is used to fit atom-centered charges to the electrostatic potential.

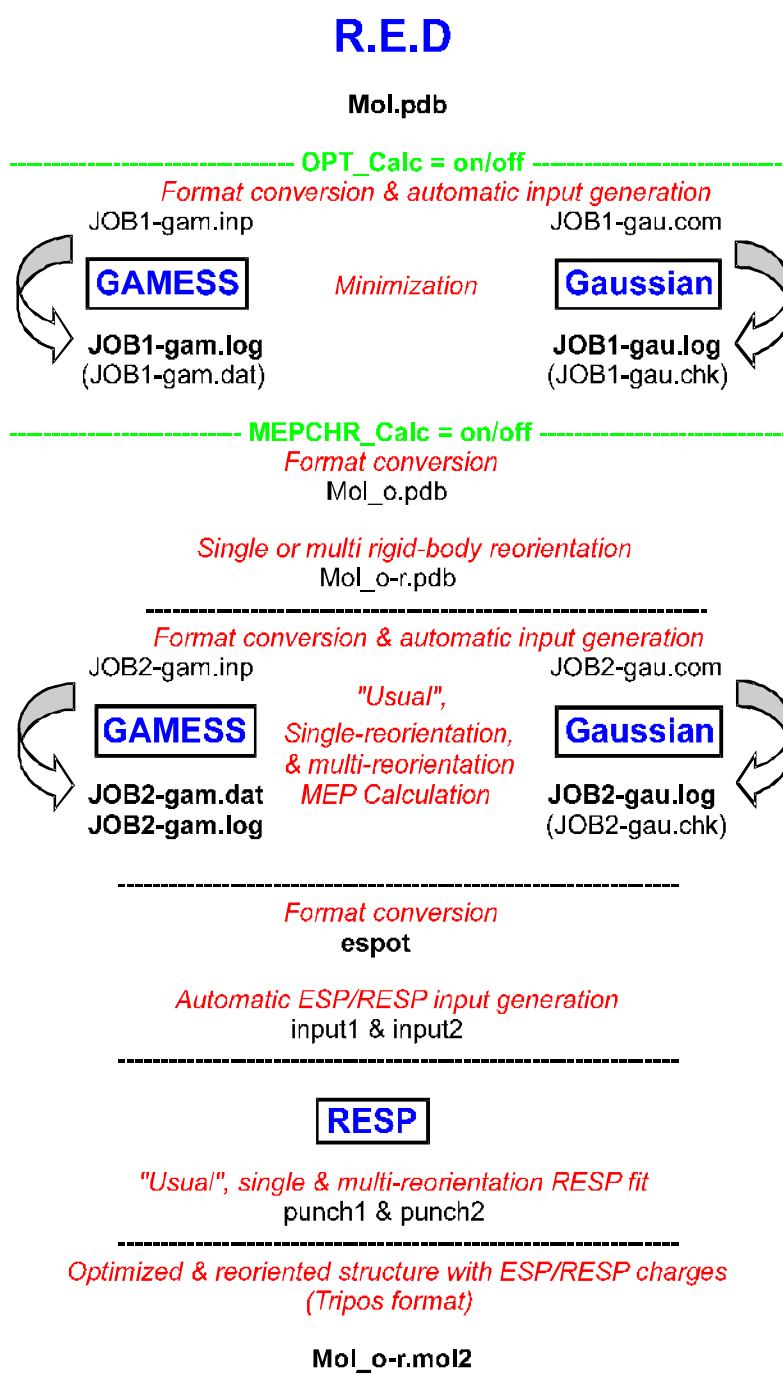
Although this method is now used 'routinely' to determine partial charges for molecules, in our opinion, it suffers from a number of limitations:

- The whole procedure requires several calculation steps involving different programs and various data format conversions between them. Consequently, the procedure is tedious, time-consuming, and numerous errors can be introduced without having a real way to check them.
- Although, in principle, any quantum programs could be used to minimize the starting structure and to calculate the MEP, the "AMBER" developers mainly use program "Gaussian" (<http://www.gaussian.com>) <sup>[4]</sup>, which is a quite expensive proprietary software. Academic program "GAMESS" (<http://www.msg.ameslab.gov/GAMESS/GAMESS.html>) <sup>[5]</sup>, which is provided at no cost and has similar functionality for RESP and ESP charges development as "Gaussian", is not usually used to derive RESP or ESP charges. Indeed, it is known that partial charges obtained with "GAMESS", are 'different' from those calculated using "Gaussian".
- Finally, starting from different sets of Cartesian coordinates for a given molecule, the RESP or ESP partial charges are, in some cases, not reproducible even using program "Gaussian". This makes potential errors in the protocol difficult to detect.

Thus, we developed program **R.E.D.** (Resp Esp charge Derive, <http://www.u-picardie.fr/labo/lbpd/RED/>) to calculate automatically RESP and ESP charges starting from an un-optimized PDB structure. **R.E.D.** sequentially executes (i) either program "GAMESS" or program "Gaussian" to minimize the molecular structure and to compute the corresponding MEP, and then (ii) program "RESP" to fit the atom-centered charges to the grid previously determined (see [Table 1](#) and [Figure 1](#)). Format conversions needed during the procedure and "GAMESS", "Gaussian" and "RESP", inputs are automatically generated by "perl" routines.

**X R.E.D.** is a graphical user-friendly interface to execute **R.E.D.** and modify **R.E.D.** variables.

The role of QM optimization thresholds on the charge values has been studied, and a new RESP fitting procedure based on single- or multi-reorientation(s) has been developed. Our procedure allows to get highly reproducible RESP and ESP charges whatever the QM software is and whatever the starting Cartesian coordinates are. An accuracy in charge reproducibility of 0.0001 e has been achieved. Although such an accuracy is not necessary in molecular mechanics simulation, this allows to define partial charges obtained for a certain molecular orientation, at a given QM theory level, that could be considered as a reference useful for reproducing published data or error checking.



*Figure 1: Execution of R.E.D.*

## -II- What is needed to execute R.E.D. & X R.E.D.?

Program **R.E.D.** (~ 1260 line code) is written using the "perl" programming language (<http://www.perl.com>), which presents numerous advantages:

- It is an interpreted language, which does not need to be compiled.
- It is well adapted to extract and format text files.
- It follows the Open Source philosophy and is therefore "freely" available on the Internet.
- It is available on UNIX allowing **R.E.D.** portability on numerous machines (SGI-IRIX, HP-UX, IBM-AIX, SUN-SOLARIS, ALPHA-TRU64/OSF1, PC-LINUX, etc...).
- "perl" functionality can be easily increased using highly flexible modules.

**R.E.D.** used the "**FileHandle**" and "**Math::Trig**" modules that have to be compiled on the system. However, both modules are installed by default with "perl" on our systems (SGI-IRIX and PC-LINUX). If one of these two modules is missing, it can be obtained from the Comprehensive PERL Archive Network (<http://www.cpan.org>).

To check if the two modules are installed on the system, use the following command:

```
find / \( -name FileHandle.pm -o -name Trig.pm \) -print
```

Program **X R.E.D.** is written with the "tcl/tk" programming language (also an interpreted language and "Open Source/Free" software, see <http://www.tcl.tk>) ["tcl/tk" version 8.3 (or newer) has to be installed]. With **X R.E.D.**, the user executes **R.E.D.** in a graphical user-friendly interface and modifies **R.E.D.** variables through this interface.

Below is the procedure that we applied to install "tcl/tk" (version 8.3) under SGI workstations (an older "tcl/tk" version is installed by default under IRIX 6.5):

- REQUIREMENTS: "perl" 5.x ; "tcl" 8.3.5 & "tk" 8.3.5 (or newer).
- HOW TO INSTALL "tcl/tk": Go to <http://www.tcl.tk> and download "tcl" and "tk" sources.

```
gunzip tcl8.3.5-src.tar.gz
tar xvf tcl8.3.5-src.tar
gunzip tk8.3.5-src.tar.gz
tar xvf tk8.3.5-src.tar
```

```
su                                     (password of root)
mkdir /usr/local/tcltk
mv tcl8.3.5 tk8.3.5 /usr/local/tcltk
cd /usr/local/tcltk/tcl8.3.5/unix/
./configure --prefix=/usr/local/tcltk/tcl835
./make
./make install

cd /usr/local/tcltk/tk8.3.5/unix/
./configure --prefix=/usr/local/tcltk/tcl835
./make
./make install
```

Add /usr/local/tcltk/tcl835/bin to "**\$PATH**"

(Use "wish" to execute "tcl/tk")

We tested program **R.E.D.** and program **X R.E.D.** on SGI-IRIX and PC-LINUX ("Red-Hat 7.3", "Red-Hat 9.0" and "Debian 3.0" distributions) workstations ("perl" versions 5.4-5.6 and 5.8, and "tcl/tk" version 8.3), but both programs should work on other UNIX. Although "perl" and "tcl/tk" are also available on Windows and Macintosh workstations, we did NOT test **R.E.D.** and **X R.E.D.** on such Operating Systems. Indeed, the "GAMESS", "Gaussian" and "RESP" programs have to be installed on these OS (see [Table 1](#)).

Under OS UNIX, **R.E.D.** looks for the "GAMESS", "Gaussian" and "RESP" binaries using UNIX command "**which**". Thus, one should update the "**\$PATH**" environment variable (to install these software, see their corresponding documentations). Here is an example of ".cshrc" file (for shell "csh" or "tcsh") needed to execute the "GAMESS", "Gaussian" and "RESP" programs:

```
cat .cshrc

setenv GAUSS_SCRDIR /Big_disk/QM_SCR
setenv g98root /usr/local
source /usr/local/g98/bsd/g98.login

setenv BABEL_DIR /usr/local/babel

set path = ($path /usr/local/babel /usr/local/g98 /usr/local/games /usr/local/resp)
```

Derivation of RESP & ESP charges	Use	Type of software
UNIX (Neither Win32 nor Mac OS)	Operating System	Proprietary or "Free"...
Gaussian <sup>[4]</sup>	QM software (Minimization & MEP)	Academic software Copyrighted
And/or GAMESS <sup>[5]</sup>	QM software (Minimization & MEP)	Proprietary software Copyrighted
RESP (Part of AMBER) <sup>[6,7]</sup>	Fitting software for atom-centered charges	Academic software Copyrighted
<b>R.E.D.</b>	Auto. & reproducible charge derivation	Academic program Copyrighted
"perl" (at least version 5.4) (For <b>R.E.D.</b> execution)	Interpreted programming language	Open Source software "Free"
<b>X R.E.D.</b> (optional)	Graphical user interface for <b>R.E.D.</b>	Academic program Copyrighted
"tcl/tk" (at least version 8.3) (For <b>X R.E.D.</b> execution)	Interpreted programming language	Open Source software "Free"

**Table 1: "What is needed to Derive RESP & ESP charges?"**

**R.E.D.** and **X R.E.D.** are available at no cost (but are copyrighted) for academic users on the Internet, <http://www.u-picardie.fr/labo/lbpd/RED/>, after signing a license (other users can also get a license, contact us in this case).

### -III- How to use R.E.D. & X R.E.D.?

#### GENERAL VARIABLES AVAILABLE IN R.E.D.

Either program "GAMESS" (<http://www.msg.ameslab.gov/GAMESS/GAMESS.html>) or program "Gaussian" (versions 2003, 1998 or 1994) (<http://www.gaussian.com>) has to be installed. The user has to set the **R.E.D.** variable "\$QM\_SOFT" = "GAMESS" or = "GAUSSIAN" (see the MAIN PROGRAM section, line 1235) according to the QM software installed on the system. Whatever the "GAMESS" version is, the same binary names i. e. "rungms", "ddkick.x" and "gamess.\$i.x" ("i", number between "01" and "09" which is recognized and executed by **R.E.D.**) are used. In the case of running various versions of program "Gaussian", different names of binaries has to be used ("g94" for "Gaussian" 1994, "g98" for "Gaussian" 1998 and "g03" for "Gaussian" 2003). Thus, **R.E.D.** automatically checks which binary is installed and run the one that is found. We only tested **R.E.D.** with "GAMESS" and "Gaussian" 1998. However, "Gaussian" 2003 or "Gaussian" 1994 can also be executed by **R.E.D.** Actually, the latest "Gaussian" version detected is executed by **R.E.D.**

Testing the QM software installed before executing it in **R.E.D.** is a good idea!

One has also to install program "RESP" (<http://amber.scripps.edu/Questions/resp.html>) which can be downloaded at no cost from the official AMBER Internet site (<http://amber.scripps.edu>) <sup>[6, 7]</sup>. Then, one selects which type of charges has to be computed. **R.E.D.** gives the opportunity to calculate RESP charges (used in Cornell *et al.* type force field) <sup>[8-11]</sup> and ESP charges (used in the 'old' Weiner *et al.* force field) <sup>[12, 13]</sup>. The variable "\$CHR\_TYP" has to be set = "RESP" or = "ESP" (see the MAIN PROGRAM section, line 1240) according to the user choice.

Testing program "RESP" before executing it in **R.E.D.** is a good idea!

Two types of input can be provided for **R.E.D.**:

- The user ONLY provides a starting (un-optimized) PDB file (read carefully the section related to this file below) and set the **R.E.D.** variables "\$OPT\_Calc" = "ON" and "\$MEPCHR\_Calc" = "ON" (see the MAIN PROGRAM section, lines 1237-1238). In this case, **R.E.D.** first executes either program "GAMESS" or program "Gaussian" to minimize the starting PDB structure and to calculate the MEP, and then program "RESP" to fit the atom-centered charges to the grid. This is the default way in **R.E.D.**

- The user may have already optimized the starting structure. For example, minimization of the structure may be performed on a powerful computer (on a computer cluster for instance) while MEP calculation and RESP fit may be launched on a 'simple' workstation. In this case, the minimization output of "GAMESS" or "Gaussian" (**R.E.D.** variable "\$JOB\_OPT"; see the MAIN PROGRAM section, line 1246) can be provided as input to **R.E.D.** by setting the **R.E.D.** variables "\$OPT\_Calc" = "OFF", leaving "\$MEPCHR\_Calc" = "ON". **R.E.D.** automatically extracts the minimized Cartesian coordinates found in the corresponding output (the minimized Cartesian coordinates found after the words "EQUILIBRIUM GEOMETRY LOCATED" for program "GAMESS" and the geometry called "Standard orientation" found after the words "Stationary point found" for program "Gaussian").

*Remark:* In this last case, the user ALSO has to provide the starting PDB file (Read carefully the section related to this file below).

See the "*Tutorial*" for more information.

### **EXECUTION OF R.E.D.**

If **X R.E.D.** is NOT executed, the "**XRED**" variable in **R.E.D.** must be set to "**OFF**" in order to execute **R.E.D.** ("**XRED**" = "**OFF**" by default, MAIN PROGRAM section, [line 1234](#)). After having selected the correct variables for its execution, one uses simply one of the following commands to launch **R.E.D.**:

```
perl RED.pl                               or
perl RED.pl > RED.log                     (To save the RED ".log" file; see Figure 2)
                                           (Typical use: DISTANT workstation/computer cluster)
nohup perl RED.pl > RED.log &
```

### **EXECUTION OF X R.E.D.**

As **X R.E.D.** executes **R.E.D.**, both programs have to be present in the working directory. If **X R.E.D.** is executed, the "**XRED**" variable in **R.E.D.** must be set to "**ON**" in the **R.E.D.** source code (MAIN PROGRAM section, [line 1234](#)) otherwise the variables selected in the **X R.E.D.** graphical interface will NOT be taken into account!!!

```
wish XRED.tcl                               (Typical use: LOCAL workstation; see Figure 3)
wish8.3 XRED.tcl
["wish8.3" has to be used instead of "wish" if two versions of the binary 'wish' (tcl/tk) are
installed on the machine]
```

Thus, once **X R.E.D.** has been executed, one has simply to select the appropriate variables from the graphic interface. The variables available in **R.E.D.** (See the MAIN PROGRAM section, [lines 1235-1246](#)) are NO LONGER used. When **X R.E.D.** is executed, a file "**RED.cfg**" is generated with the variables needed by **R.E.D.** for its execution.

### **MISCELLANEOUS**

One has to provide the formal charge (or total charge) of the studied molecule and set the **R.E.D.** variable "**\$CHR\_VAL**" to the correct value (default "**\$CHR\_VAL**" = "0").

A title for the studied molecule can also be provided by the variable "**\$TITLE**" (default "**\$TITLE**" = "**MOL**") which is used in the "GAMESS", "Gaussian" and "RESP" inputs.

The name of the directory in which the calculations are saved once they are done, can also be modified through the variable "**\$DIR**" (default "**\$DIR**" = "**CHRCalc**"). Moreover, this directory is incremented automatically if a previous one does already exist.



```

RED
-----
*   Welcome to R.E.D   *
  Resp Esp charge Derive

MOLECULE = EtOH
CHARGE   = RESP
-----

* Selected re-orientation(s) *

      1   5   8
      8   5   1

* Software checking *

rungms           [ OK ]
gamess.00,x      [ OK ]
ddkick,x         [ OK ]
resp             [ OK ]

The structure is being minimized ...           [ OK ]
  See the file "JOB1-gam.log"

MEP is/are being calculated ...                 [ OK ]
  See the file(s) "JOB2-gam(X).dat"

Charges are being calculated ...                [ OK ]
  See the "punch2" file

  A Tripos Mol2 file has been created.

*****

Execution time: 0 h 0 m 57 s
Done, press Enter to exit.

*****
      R.E.D. (version 1.0) was written by:

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(1) Faculte de Pharmacie, Amiens, France
(2) Accelrys Inc., San Diego, USA

  Thanks for using this program.

*****

```

Figure 2: Typical ".log" file generated by **R.E.D.**

### **EXECUTION OF "GAMESS" & "GAUSSIAN" IN PARALLEL IN R.E.D.**

Program "GAMESS" and program "Gaussian" can also be executed 'in parallel' in **R.E.D.** [using several processors on the same motherboard (Shared Memory Based, SMP systems) or using several 'nodes' having a unique processor]. Indeed, "GAMESS" uses 'replicated and distributed' memory while "Gaussian" uses 'shared' memory (through the UNIX library 'shmem') or 'virtual shared' memory (through Linda, <http://www.lindaspace.com/about/index.html>). The **R.E.D.** source code can be easily modified to execute both QM software in parallel (The "perl" commands which execute program "GAMESS" or program "Gaussian" have to be modified: see [lines 421 & 696](#) for "GAMESS" and [lines 445 & 730](#) for "Gaussian" in **R.E.D.**).

For parallel execution of QM software, take a look at the 'RED-SMP.pl' code provided as example with "\$NP" = "4" (where "\$NP" is the number of 'SMP' processors). Moreover, the minimization and MEP input(s) in the **R.E.D.** code may also have to be slightly updated [modifying the "**MWORDS**" and "**MEMDDI**" values for "GAMESS" inputs, and modifying the number of processors "N" ("**%NProc**" = "N" or "**%NProcLinda**" = "N") and increasing the "**%Mem**" value for "Gaussian" inputs]. See the respective "GAMESS" and "Gaussian" documentations for more information for their execution in parallel.

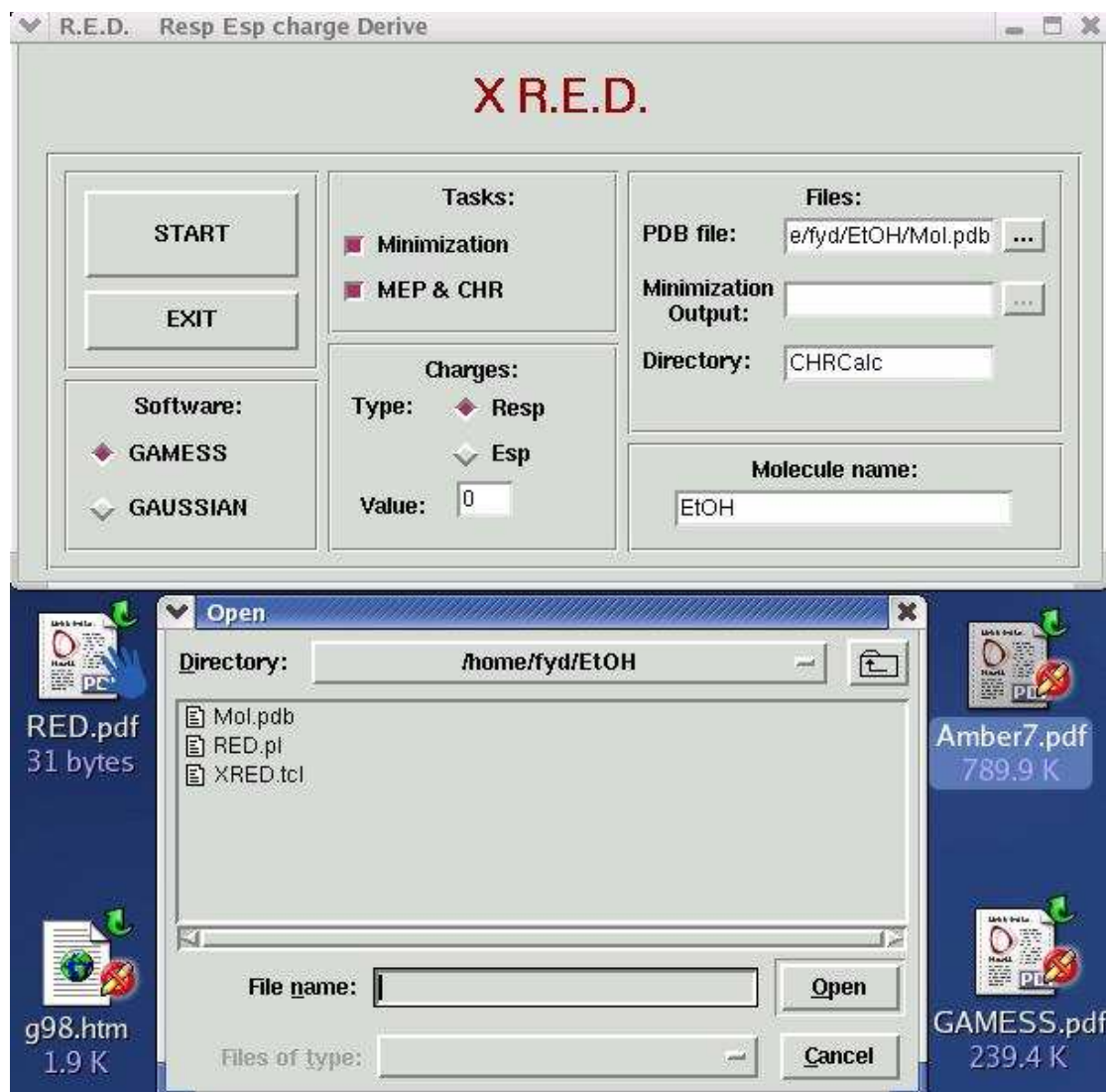


Figure 3: X R.E.D. graphical user-friendly interface

## -IV- Inputs automatically generated by R.E.D.

As **R.E.D.** sequentially executes program "GAMESS" or program "Gaussian" and program "RESP", it also automatically generates the inputs needed by these three programs. Although the default keywords of these inputs set up in **R.E.D.** should be correct for most of the simulations, the user has still the opportunity to modify some inputs generated by **R.E.D.** (See the "GAMESS", "Gaussian" and "RESP" documentations in this case).

*Minimization accuracy is important for getting reproducible RESP and ESP charges that are independent of the QM software and initial (un-minimized) structure used. See information below regarding minimization thresholds selected in program "GAMESS" and in program "Gaussian". Following keywords have been chosen based on a study of 17 structures and more than 100 orientations...*

### "GAMESS" INPUT FOR MINIMIZATION

To modify this input, see lines 403-413 in **R.E.D.** Below is a description of some specific keywords used by **R.E.D.**, which values can be modified:

- For structure minimization, **R.E.D.** uses by default the "6-31G\*" basis set.
- "QMTTOL" = "1.0E-08", "ITOL" = "30", "ICUT" = "20", "INTTYP" = "HONDO" (just to increase accuracy, see "GAMESS" documentation for information about them).
- A SCF convergence criterion of  $10^{-8}$  ("CONV" = "1.0E-08") is used (as in "Gaussian").
- A minimization threshold of "OPTTOL" = "1.0E-06" is used to set up minimization accuracy. *Be aware that decreasing this value might change charge values in "some" cases (Use even "OPTTOL" = "1.0E-07", if you can afford it!)*

### "GAUSSIAN" INPUT FOR MINIMIZATION

To modify this input, see lines 437-440 in **R.E.D.** Here are some particular keywords used by **R.E.D.**, which can be modified:

- For structure minimization, **R.E.D.** uses by default the "6-31G\*" basis set.
- A SCF convergence criterion of  $10^{-8}$  "SCF(Conver=8)" is used (default in Gaussian).
- A minimization threshold of "Tight" is used to set up minimization accuracy. *Be aware that decreasing this value might slightly change charge values in "some" cases (Use even "VTight", if you can afford it!)*

*Remark:* It is evident that getting minimized structures with these thresholds might be difficult in some particular cases. For small molecules (DMSO, EtOH or MeOH for instance), this should be straightforward with "GAMESS" or "Gaussian" inputs generated by **R.E.D.** However, achieving such criteria of convergence for larger structures might be more difficult. In this case, the best choice would be to run the "GAMESS" or "Gaussian" optimization without **R.E.D.** and modifying the optimization input, 'manually'. Once one gets the minimization output (with the correct minimized set of Cartesian coordinates at the demanded threshold), one is ready to use it as input in **R.E.D.** specifying the two following **R.E.D.** variables: "\$OPT\_Calc" = "OFF" and "\$MEPCHR\_Calc" = "ON" (see sections III and VI of this manual for more information).

Applying the "Tight" (or "VTight") minimization convergence criterion in program "Gaussian" may cause difficulties in some circumstances: Use "SCF(Conver=8)" (decreasing the SCF threshold to "SCF(Conver=6)" makes the optimization even more difficult) and update the optimization keywords calculating the Hessians on the first structure or at each minimization step:

```
#P HF/6-31G* OPT=(Tight,CalcFC) SCF(Conver=8) Test      or
#P HF/6-31G* OPT=(Tight,CalcAll) SCF(Conver=8) Test
```

In the case of minimization using program "GAMESS", we have not encounter any problem with getting "OPTTOL" = "1.0E-06" optimized structures for any molecule size. One simply has to use a huge numbers of optimization steps in "GAMESS" and to be patient. However, it might be better to calculate the Hessians every ten steps (for instance) using the following keywords:

```
$STATPT      HESS=CALC IHREP=10                      $END
$FORCE       METHOD=ANALYTIC VIBANL=.F.              $END
```

*Remark:* A reason for this difference between the two QM software might be, that program "Gaussian" checks four thresholds (maximum Force, RMS Force, maximum displacement and RMS displacement) while program "GAMESS" checks only two (maximum Gradient and RMS Gradient).

### "GAMESS" & "GAUSSIAN" "INPUTS FOR MEP CALCULATION"

For RESP charge derivation (used in Cornell *et al.* type force fields), the "6-31G\*" basis set has to be used to calculate the MEP. On the contrary, for ESP charges (used in the Weiner *et al.* force field) the "STO-3G" basis set was used. See [lines 670-682](#) & [716-720](#) in the **R.E.D.** source code. *However, these lines should not be modified for classical RESP and ESP charge Derivation!*

### "RESP" INPUTS

The RESP input files are automatically created based on the PDB atom names (read the section V in this manual related to this file). Thus, these input files can NOT be modified before running **R.E.D.** However, the user can run manually "RESP" once **R.E.D.** execution is done by modifying the inputs generated by **R.E.D.** and using the following commands:

For RESP charges:

```
resp -0 -i input1 -e espot(x) -o output1 -p punch1 -q qout \
      -t qout1 -w qwts -s esout
resp -0 -i input2 -e espot(x) -o output2 -p punch2 -q qout1 \
      -t qout2 -w qwts -s esout                                "x" positive integer
```

For ESP charges:

```
resp -0 -i input1 -e espot(x) -o output1 -p punch1 -q qout \
      -t qout1 -w qwts -s esout                                "x" positive integer
```

In the last case, the ESP charges have to be manually made equivalent *at posteriori* to the fit (see file "punch2" for examples).

## -V- The starting (un-optimized) PDB file

### ATOM NAMES

**R.E.D.** automatically generates input(s) for program "RESP" based on the atom names found in the starting PDB file. The user must follow three simple rules to manually modify these atom names before running **R.E.D.**:

-1- The methyl (CH<sub>3</sub>) and methylene (CH<sub>2</sub>) carbons must have the atom name "CT" (or "T"). The other atoms must bear the **letter** of their corresponding **chemical element** [i. e. "C" for the other carbons (in C=O, CH, C=C etc...), "O" for oxygen, "H" for hydrogen etc...] as atom names.

*Remark:* Do NOT use "HO1" (often found in PDB files for an hydrogen linked to an oxygen); Use "H1" instead because the chemical element of hydrogen is obviously "H" and not "HO" otherwise **R.E.D.** may complain...

-2- The **same number** must be added to this/these letter(s) for equivalent atoms. Consequently, non-equivalent atoms (hydrogen, carbon or heteroatom) must have a unique atom name.

-3- Each hydrogen link to a carbon must have the **same number as this carbon**.

Thus, equivalent atoms must have the same atom names [same letter(s) and number(s)] in the starting PDB file. Such convention is NOT very convenient to visualize the molecule studied in some graphic interfaces. For instance, "InsightII" (<http://www.accelrys.com>) renames automatically the atoms, which have the same name in a PDB file to differentiate them. Thus, these 'user-modified' atom names are only necessary in the starting PDB file. The "Tripos" and PDB files generated after minimization, MEP and charge calculations have more 'classical' atom names [the atom name is then composed of the chemical symbol (even for the methyl and methylene carbons) and a number which is automatically incremented].

See the "Tutorial" for more information.

### REORIENTATION(S)

*As minimization accuracy, molecular orientation affects MEP (i.e. the number of MEP points is different if the orientation of the minimized structure changes) <sup>[14, 15]</sup> and consequently the RESP and ESP charges. Molecular orientation 'can' be controlled in program "GAMESS" using the molecular principal axes (keyword "COORD" = "CART") and in program "Gaussian" by placing the center of nuclear charge at the origin (keyword "Symmetry"). However, as both software do NOT apply the same algorithm, the molecular orientation of the minimized structure is different in both of those software. To solve this problem, a re-orientation algorithm has been introduced in **R.E.D.** which 'can' be applied to the minimized structure just before calculating the MEP. This allows to get highly reproducible charges whatever the QM software or the starting structure is.*

The algorithm applied for structure re-orientation is a simple rigid-body re-orientation (see <http://www.srl.gatech.edu/education/ME6175/notes/xforms.html> for more information). The first two atom numbers define the ( $O, X$ ) axis; the third one allows defining the ( $O, X, Y$ ) plan, the  $Z$ -axis being automatically defined as  $X \times Y$ .

If **re-orientation** information is provided in the starting PDB file, the minimized structure is automatically re-oriented according to the three **atom numbers** (not atom names!) given. To re-orient a structure, one has to use a particular format: after PDB keyword "**REMARK**" and add the keyword "**REORIENT**" (on the same line) i. e.:

```
REMARK REORIENT atm_nb$i atm_nb$j atm_nb$k
```

(where  $i$ ,  $j$  and  $k$  are the atom numbers).

Thus, a MEP is calculated using the minimized and re-oriented Cartesian coordinates (instead of the Cartesian coordinates provided directly at the end of the minimization output).

If ' $X$ ' sets of **three atom numbers** ( $X$  positive integer) are given [i. e. for example: "**REMARK REORIENT atm\_nb\$i atm\_nb\$j atm\_nb\$k | atm\_nb\$k atm\_nb\$j atm\_nb\$i | atm\_nb\$k atm\_nb\$i atm\_nb\$l | atm\_nb\$l atm\_nb\$i atm\_nb\$k**" ( $i$ ,  $j$ ,  $k$ , and  $l$  are atom numbers), pipe character "|" is used as separator between two different orientations], the set of minimized Cartesian coordinates obtained from the QM software output is re-oriented ' $X$ ' times [i. e. ' $X$ ' set of re-oriented (and minimized) Cartesian coordinates are generated] and used to calculate ' $X$ ' MEPs. An ' $X$ '-orientation fit (or multi-orientation RESP fit) is then applied using RESP ( $X = 4$  is the example just above). **This allows to average the differences in charge values caused by a particular orientation using several orientations.**

*Remark:* For numerous re-orientations, the corresponding format can be provided (on several lines), each one starting by the keywords "**REMARK REORIENT**" i. e.:

```
REMARK REORIENT atm_nb$i atm_nb$j atm_nb$k | atm_nb$k atm_nb$j atm_nb$i
```

```
REMARK REORIENT atm_nb$k atm_nb$i atm_nb$l | atm_nb$l atm_nb$i atm_nb$k
```

```
REMARK REORIENT atm_nb$l atm_nb$j atm_nb$k | atm_nb$k atm_nb$j atm_nb$l
```

Etc ...

If no **re-orientation** information is provided in the starting PDB structure, the Cartesian coordinates obtained from the minimization (using either program "GAMESS" or program "Gaussian") are directly used to calculate the MEP and the RESP or ESP charges (this was the 'usual' way to derive charges before **R.E.D.**). In this case, the charges calculated by program "GAMESS" or program "Gaussian" are 'different' and thus NOT perfectly reproducible!

See the "*Tutorial*" for more information.



## CONNECTIONS

If atom **connections** are available in the starting PDB file (through the PDB keyword "**CONNECT**"), a Tripos file ("Mol2" format, <http://www.tripos.com/custResources/mol2Files/>) with minimized Cartesian coordinates and RESP or ESP charges is also generated by **R.E.D.**

The Tripos format is convenient since the Cartesian coordinates, charges, and atom types are present in this single file. We do use Tripos format within "InsightII/Discover" (<http://www.accelrys.com>) to run molecular mechanics calculations with AMBER force fields converted by AmberFFC (<http://www.u-picardie.fr/labo/lbpd/AmberFFC/>)<sup>[16]</sup> and to automatically calculate force field parameters using FFParmDev (<http://www.u-picardie.fr/labo/lbpd/FFParmDev/>, program still under development).

*Restrictions:* In the first version, program **R.E.D.** generates only the atom names in the Tripos "atom type" column. Moreover, as **R.E.D.** does NOT verify connections, the user must check carefully the connections provided. A simple way to generate safely and automatically a starting PDB structure with correct connections is to execute program BABEL (<http://www.eyesopen/babel/index.html>).

To convert a PDB file without connection information included, into a PDB file with connections, use the following commands:

```
babel -ipdb Mol-noconnect.pdb -opdb Mol-connect.pdb
ln -s Mol-connect.pdb Mol.pdb (if "$MOL_START" = "Mol.pdb" in R.E.D.)
```

*Remark:* Since only single bond connections are available in the PDB format ([http://www.rcsb.org/pdb/docs/format/pdbguide2.2/guide2.2\\_frame.html](http://www.rcsb.org/pdb/docs/format/pdbguide2.2/guide2.2_frame.html)), only this type of bond is printed in the Tripos format by **R.E.D.** Thus, if the user wants to display double or triple bonds in a graphic user interface such as "InsightII" or "Sybyl" (<http://www.tripos.com>), the last column of the "**@<TRIPOS>BOND**" section must be manually modified (<http://www.tripos.com/custResources/mol2Files/>). However, since only single bonds are used in molecular mechanics, missing double or triple bonds in the Tripos format generated by **R.E.D.** do NOT cause problem for simulations within "InsightII".

If no atom **connection** is provided in the starting PDB file, the RESP and ESP charges are only available in the RESP "punch2" file and no Tripos file is generated (as usual RESP use).

See the "*Tutorial*" for more information.

## -VI- Tutorial

### 1) First example: dimethylsulfoxide without re-orientation 'RESP' fit

- Below is a starting PDB file (i. e. not minimized) needed for the execution of **R.E.D.**:

```
REMARK File name = dms0-noconnect.pdb; Directory name = /usr/people/fyd/DMSO
ATOM      1  CT1  DMSO   1      3.8750  0.6780 -8.4170  1.00  0.00
ATOM      2  H1   DMSO   1      3.8000  1.6900 -8.0760  1.00  0.00
ATOM      3  H1   DMSO   1      3.4060  0.0260 -7.7110  1.00  0.00
ATOM      4  H1   DMSO   1      3.3890  0.5830 -9.3660  1.00  0.00
ATOM      5  S2   DMSO   1      5.3590  0.2930 -8.5590  1.00  0.00
ATOM      6  O3   DMSO   1      5.4600 -1.0590 -9.0140  1.00  0.00
ATOM      7  CT1  DMSO   1      6.0550  0.4300 -7.1930  1.00  0.00
ATOM      8  H1   DMSO   1      7.0870  0.1630 -7.2900  1.00  0.00
ATOM      9  H1   DMSO   1      5.9800  1.4410 -6.8530  1.00  0.00
ATOM     10  H1   DMSO   1      5.5820 -0.2190 -6.4850  1.00  0.00
```

=> The two carbons are equivalent and belong to a methyl group.

=> Atom name "CT1" (or "T1").

=> The six hydrogens are equivalent and linked to a carbon atom "CT1".

=> Same number as carbon atoms (i. e. "H1").

=> Sulfur and oxygen atom names: just have a number different from "1".

- No connection and no re-orientation information is provided in this PDB file.

=> No Tripos Mol2 file is generated and the "RESP" type charges are ONLY available in file "punch2".

=> No re-orientation is carried out: The minimized Cartesian coordinates is directly used to calculate the MEP. In this case, the orientation of the minimized structure is simply the orientation selected by the QM software (see the corresponding inputs generated).

=> Two warnings are printed in the **R.E.D.** ".log" file:

```
WARNING: No re-orientation found in the PDB file
The molecule will NOT be re-oriented !
```

```
WARNING: No connection found in the PDB file
The Tripos Mol2 file will NOT be generated !
```

- Below are examples of **R.E.D.** variables (see section "MAIN PROGRAM" in **R.E.D.**)

```
$XRED      = "Off";           # X RED is not used to execute RED
$QMSOFT    = "GAMESS";      # GAMESS is used

$OPT_Calc  = "On";          # The minimization is done
$MEPCHR_Calc = "On";        # The MEP and charges are calculated

$CHR_TYP   = "RESP";        # RESP charges are calculated
$CHR_VAL   = "0";           # Total charge of DMSO = 0

$TITLE     = "DMSO";        # Just a title used each time it is possible
$DIR       = "CHRCalc";     # Directory name (where the data are saved)
$MOL_START = "dms0-noconnect.pdb"; # Starting PDB File
# (Must be in the working directory)
$JOB_OPT   = "JOB1.log";    # NOT used since $OPT_Calc = "ON"
```

Now, program **R.E.D.** can be executed : `perl RED.pl > RED.log &`

DMSO is minimized, and using the minimized Cartesian coordinates directly obtained from the QM software, MEP and charges are calculated (the ".log" file is saved in RED.log).



## 2) Second example: Ethanol with single re-orientation 'RESP' fit

- Below is a starting PDB file needed for the execution of **R.E.D.**:

```
REMARK REORIENT 1 5 8
REMARK File name = EtOH.pdb; Directory name = /usr/people/fyd/EtOH
ATOM 1 CT1 EtO 1 3.1080 0.6530 -8.5260 1.00 0.00
ATOM 2 H1 EtO 1 2.8150 -0.3490 -8.7610 1.00 0.00
ATOM 3 H1 EtO 1 2.5170 1.0150 -7.7110 1.00 0.00
ATOM 4 H1 EtO 1 2.9560 1.2780 -9.3810 1.00 0.00
ATOM 5 CT2 EtO 1 4.5970 0.6740 -8.1320 1.00 0.00
ATOM 6 H2 EtO 1 4.7480 0.0490 -7.2770 1.00 0.00
ATOM 7 H2 EtO 1 5.1870 0.3120 -8.9470 1.00 0.00
ATOM 8 O3 EtO 1 4.9880 2.0120 -7.8180 1.00 0.00
ATOM 9 H4 EtO 1 5.9160 2.0250 -7.5720 1.00 0.00
CONNECT 1 2 3 4 5
CONNECT 2 1
CONNECT 3 1
CONNECT 4 1
CONNECT 5 1 6 7 8
CONNECT 6 5
CONNECT 7 5
CONNECT 8 5 9
CONNECT 9 8
MASTER 0 0 0 0 0 0 0 0 0 9 0 9 0
END
```

=> The two carbons are not equivalent and belong to a methyl and methylene groups.

=> Different atom names "CT1" and "CT2"

=> The three hydrogens of the methyl have the same number as the methyl carbon, i. e. "H1" and the two hydrogens of the methylene bears the same number as the methylene carbon, i. e. "H2".

- Connection information is provided in the starting PDB file.

=> The charges are available in the "punch2" and Tripos Mol2 files.

- A unique re-orientation is demanded ("REMARK REORIENT 158" in the starting PDB file).

=> The minimized Cartesian coordinates are re-oriented and used in MEP calculation.

- Below are examples of **R.E.D.** variables (see the "MAIN PROGRAM" section):

```
$XRED = "Off"; # X RED is not used to execute RED
$QMSOFT = "GAUSSIAN"; # GAUSSIAN is used this time

$OPT_Calc = "On"; # The minimization is done
$MEPCHR_Calc = "On"; # The MEP and charges are calculated

$CHR_TYP = "RESP"; # RESP charges are calculated
$CHR_VAL = "0"; # Total charge of Ethanol = 0

$TITLE = "Ethanol"; # Just a title
$DIR = ""; # No directory name is provided
# The data are saved in the working directory
$MOL_START = "EtOH.pdb"; # Starting PDB File
$JOB_OPT = "JOB1.log"; # NOT used since $OPT_Calc = "ON"
```

Now, **R.E.D.** can be executed : `perl RED.pl`

(The ".log" file is this time simply printed in the working terminal; see [Figure 2](#))

Molecule EtOH is first minimized. Then, the set of minimized Cartesian coordinates is re-oriented once according to the three atom numbers provided. Finally, MEP and charges are calculated using the set of re-oriented Cartesian coordinates.

### 3) Third example: N-Methyl-Acetamide (Trans)

#### -I- Multi-orientation 'RESP' fit

- Below is a starting PDB file needed for the execution of **R.E.D.**:

```
REMARK REORIENT 1 5 7 | 7 5 1 | 5 6 7 | 7 6 5
REMARK REORIENT 1 7 9 | 9 7 1
REMARK File name = Mol.pdb; Directory name = /usr/people/fyd/NMeAcetamide
ATOM 1 CT1 NMA 1 2.0000 1.0000 0.0000 1.00 0.00
ATOM 2 H1 NMA 1 1.6360 -0.0270 0.0000 1.00 0.00
ATOM 3 H1 NMA 1 1.6360 1.5140 0.8900 1.00 0.00
ATOM 4 H1 NMA 1 1.6360 1.5140 -0.8900 1.00 0.00
ATOM 5 C2 NMA 1 3.5220 1.0000 0.0000 1.00 0.00
ATOM 6 O3 NMA 1 4.1440 -0.0600 0.0000 1.00 0.00
ATOM 7 N4 NMA 1 4.1200 2.1940 0.0000 1.00 0.00
ATOM 8 H4 NMA 1 3.5610 3.0350 0.0000 1.00 0.00
ATOM 9 CT5 NMA 1 5.5630 2.3280 0.0000 1.00 0.00
ATOM 10 H5 NMA 1 6.0200 1.3380 0.0000 1.00 0.00
ATOM 11 H5 NMA 1 5.8770 2.8730 -0.8900 1.00 0.00
ATOM 12 H5 NMA 1 5.8770 2.8730 0.8900 1.00 0.00
CONNECT 1 2 3 4 5
CONNECT 2 1
CONNECT 3 1
CONNECT 4 1
CONNECT 5 1 6 7
CONNECT 6 5
CONNECT 7 5 8 9
CONNECT 8 7
CONNECT 9 7 10 11 12
CONNECT 10 9
CONNECT 11 9
CONNECT 12 9
MASTER 0 0 0 0 0 0 0 0 0 12 0 12 0
END
```

=> The carbon name of the carbonyl group: Atom name is NOT "CT2" but "C2".

=> The three hydrogens in the two methyl groups have the same number as their methyl carbon, i. e. "H1" and "H5" (the two methyl groups are not equivalent).

- Connection information is provided in the starting PDB file (a Tripos Mol2 file is generated).

- Six different orientations are requested: The minimized Cartesian coordinates are re-oriented six times and for each orientation a MEP is calculated. RESP type charges are derived using a six-orientation fit.

- Below are examples of **R.E.D.** variables:

```
$XRED = "off"; # X RED is not used to execute RED
$QMSOFT = "GAMESS"; # GAMESS is used

$OPT_Calc = "On"; # The minimization is done
$MEPCHR_Calc = "On"; # The MEP and charges are calculated

$CHR_TYP = "RESP"; # RESP charges are calculated
$CHR_VAL = "0"; # Total charge of NMeAcetamide = 0

$TITLE = "MOL"; # Just a title
$DIR = "TEST"; # Directory name (where the data are saved)
$MOL_START = "./Mol.pdb"; # Starting PDB File
$JOB_OPT = "JOB1-gam.log"; # NOT used since $OPT_Calc = "ON"
```

Now, **R.E.D.** can be executed: `perl RED.pl > RED.log`

*-2- Multi-orientation 'ESP' fit*

- Use the same PDB file as for the example "*-1- Multi-orientation 'RESP' fit*"

- Below are **R.E.D.** variables to be used (see MAIN PROGRAM section):

```
$XRED   = "Off";           # X RED is not used to execute RED
$QMSOFT = "GAMESS";       # GAMESS is used

$OPT_Calc   = "Off";       # The minimization is ALREADY done
                        # See "-1- Multi-orientation 'RESP' fit"
$MEPCHR_Calc = "On";      # The MEP and charges are calculated

$CHR_TYP = "ESP";         # ESP charges are calculated this time
                        # (Only useful for the "old" Weiner et al. FF)
$CHR_VAL = "0";          # Total charge of NMeAcetamide = 0

$TITLE = "MOL";          # Just a title
$DIR   = "TEST";         # Directory name (where the data are saved)
                        # The directory name is automatically incremented
                        # i.e. TEST, TEST-1, TEST-2, etc...

$MOL_START = "./Mol.pdb"; # Starting PDB File (un-optimized structure)
                        # Must be provided each time R.E.D. is executed

$JOB_OPT  = "TEST/JOB1-gam.log"; # Obtained from "-1- Multi-orientation 'RESP' fit"
```

Now, **R.E.D.** can be executed : `perl RED.pl > RED.log`

Six orientations are requested: The minimized Cartesian coordinates are re-oriented six times and for each orientation a MEP is calculated. 'ESP' type charges are, this time, calculated using a six-orientation fit.

*Remark:* After having done the third example of this tutorial, the "*Multi-orientation 'RESP' fit*" data are available in directory "**TEST-1**" and the "*Multi-orientation 'ESP' fit*" data in directory "**TEST**"!

#### 4) Applications:

##### a) "Multi-RED" run

For each molecule one is interested in deriving RESP or ESP charges, a particular "RED.pl" script with specific variables needed has to be prepared (for instance, for this tutorial which presents four examples, four different "RED.pl" scripts have to be prepared). Let's name these scripts "RED-DMSO.pl", "RED-EtOH.pl", "RED-NMeAcetamide-RESP.pl" and "RED-NMeAcetamide-ESP.pl", respectively. Finally, a "Multi-RED" script (see below) has to be created that will execute automatically these four scripts.

```
cd                                # Goes in the $HOME directory
touch Multi-RED                   # Creation of an empty file
chmod +x Multi-RED                # Make it executable
vi Multi-RED                       # Edition

i                                  (Insertion mode)
cd DMSO
perl RED-DMSO.pl > RED-DMSO.log
cd ../EtOH
perl RED-EtOH.pl > RED-EtOH.log
cd ../NMeAcetamide
perl RED-NMeAcetamide-RESP.pl > RED-NMeAcetamide-RESP.log
perl RED-NMeAcetamide-ESP.pl > RED-NMeAcetamide-ESP.log
cd ..
echo "All Done!"
:wq!                               (Save and quit "vi")
```

The four "RED-molecule.pl" scripts are executed sequentially using:

```
./Multi-RED                       # Execution
```

##### b) Execution of "RED.pl" with "XRED.tcl"

- A unique **R.E.D.** variables has to be set to "ON" (the others are no longer used).

```
$XRED = "On";                      # XRED is, this time, used to execute RED
```

- Now, program **X R.E.D.** can be executed: `wish8.3 XRED.tcl`

- Use the graphical interface (see [Figure 3](#)) to select the variables needed, the starting PDB file, etc...

## -VII- How to reference R.E.D.?

A. Pigache, P. Cieplak & F.-Y. Dupradeau, Automatic and highly reproducible RESP and ESP charge derivation: Application to the development of programs RED and X RED, Submitted to the 227<sup>th</sup> ACS National Meeting, Anaheim, CA, March 28 – April 1, **2004** (and manuscript in preparation).

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