Xplor-NIH: An Introduction

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http://bit.niddk.nih.gov/xplor-nih

Outline

- Intro to structure calculation, Python
- Overview of an Xplor-NIH script
- energy terms
- IVM: dynamics and minimization in internal coordinates
- Miscellaneous topics
  - use of Cryo-EM density maps
  - strict symmetry
  - implicit water potential, including membrane effects
- Live demonstration, including detailed look at a simple Xplor-NIH script

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Many contributions from the community

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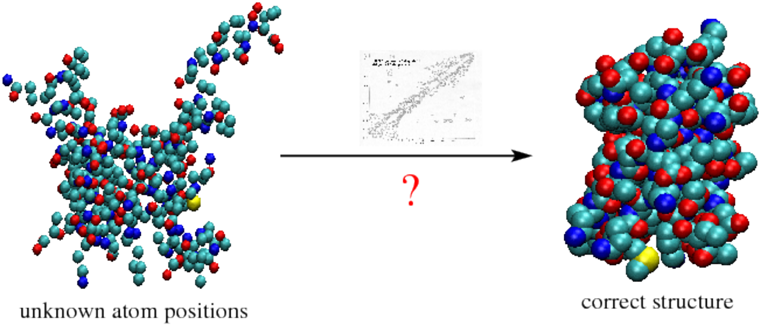
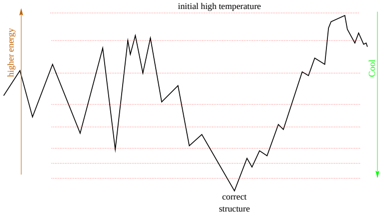
Overview of structure determination



Minimize energy: $V_{\text{tot}} = V_{\text{expt}} + V_{\text{covalent}} + V_{\text{knowledge}} + \ldots$

- molecular dynamics to explore the energy surface.
- slowly decrease the temperature to find the global minimum.
- surface smoothed at high temperature
- restore surface during simulated annealing



Xplor-NIH Description

Originally derived from the X-PLOR program developed by A. Brünger as a fork of the CHARMM molecular dynamics program around 1984.

- freely available for non-commercial work. Source code is available.
- For commercial use, please contact me.

New contributions, additions are encouraged.

Source code of Xplor-NIH:

- original XPLOR Fortran source, with contributions from many groups.
- current work uses C++ for compute-intensive work.
- scripts and much code are written in Python 3.
- SWIG used to “glue” scripting languages to C++.
- bazaar (bzr) repository of source code is available online.

Installation

Easiest way

- download the appropriate installer script (*e.g.* installer-mac-3.4.sh) from
  `http://bit.niddk.nih.gov/xplor-nih/`
- change to the directory where you want Xplor-NIH to be installed, and then execute the file:

  ```
  sh ~/Downloads/installer-mac-3.4.sh
  ```

  This will download and install two .tar.gz files, and configure the executable scripts.

Manual Installation

1. download two files from `http://bit.niddk.nih.gov/xplor-nih/`
   - a -db file: *e.g.* `xplor-nih-3.4-db.tar.gz`
   - a platform-specific file: *e.g.* `xplor-nih-3.4-Linux_x86_64.tar.gz`
2. unpack these files where you wish them to live:
   ```
   zcat xplor-nih-3.4-db.tar.gz | (cd /opt ; tar xf -)
   zcat xplor-nih-3.4-Linux_x86_64.tar.gz | (cd /opt ; tar xf -)
   ```
3. perform initial configuration:
   ```
   cd /opt/xplor-nih-3.4
   ./configure -symlinks /usr/local/bin
   ```
   The optional `-symlinks` argument creates symbolic links in the specified directory for xplor and other commands. It is intended that you specify a directory in your PATH. For instance, if installing in your home directory, you might specify `-symlinks ~/bin`.

Test the new installation

```
bin/testDist
```

Another option: NMRbox .org

Scripting Languages- three in Xplor-NIH

scripting language:

- flexible interpreted language
- used to input filenames, parameters, protocols
- flexible enough to program non compute-intensive logic
- relatively user-friendly

XPLOR language:

strong point:

atom selection language quite powerful.

weaknesses:

String, Math support problematic.

no support for subroutines: difficult to encapsulate functionality.

Parser is hand-coded in Fortran: difficult to update.

XPLOR reference manual:

`http://bit.niddk.nih.gov/xplor-nih/doc/current/xplor/`

NOTE: all old XPLOR 3.851 scripts should run unmodified with Xplor-NIH.

General purpose scripting languages: Python and TCL

- excellent string support.
- languages have functions and modules: can be used to better encapsulate protocols (*e.g.* call a function to perform simulated annealing.)
- well known: these languages are useful for other computing needs: replacements for AWK, shell scripting, *etc*.
- contain extensive libraries with additional functionality (*e.g.* file processing, web access, GUI library, *etc*).
- Facilitate interaction, tighter coupling with other tools.
  - NMRWish has a TCL interface.
  - pyMol has a Python interface.
  - VMD has TCL and Python interfaces.
  - Allow tight integration with CING structure validation suite (currently being implemented).

separate processing of input files (assignment tables) is unnecessary: can all be done using Xplor-NIH.

Introduction to Python 3

assignment and strings

```
a = 'a string'  # <- pound char introduces a comment
a = "a string"  # ' and " chars have same functionality
```

multiline strings - use three ' or " characters

```
a = '''a multiline
string'''
```

f-strings for string formatting - Python expressions inside curly braces

```
api=3.14159; answer=42
s = f"a float: {api:.3} an integer: {answer}"
print(s)
a float:  3.14 an integer: 42
```

raw strings - special characters are not translated

```
a = r'strange characters: \%~!'  # introduced by an r
```

lists and tuples

```
l = [1,2,3]          #create a list
a = l[1]             #indexed from 0 (a = 2)
l[2] = 42            # l is now [1,2,42]
t = (1,2,3)          #create a tuple (read-only list)
a = t[1]             # a = 2
t[2] = 42            # ERROR!
```

Introduction to Python 3

calling functions

```
bigger = max(4,5)  # max is a built-in function
```

defining functions - leading whitespace scoping

```
def sum(item1,item2,item3=0):
    "return the sum of the arguments"  # comment string
    retVal = item1+item2+item3         # note indentation
    return retVal

print(sum(42,1))                   #un-indented line: not in function
```

```
43
```

using keyword arguments - specify arguments using the argument name

```
print( sum(item3=2,item1=37,item2=3) )  # argument order is not important
```

```
42
```

loops - the for statement

```
for cnt in range(0,3):   # loop over the list [0,1,2]
    cnt += 10
    print( cnt )
```

```
10
11
12
```

Introduction to Python 3

Python is modular

most functions live in separate namespaces called modules
Loading modules - the import statement

```
import sys          #import module named sys
sys.version         #return the Python version from the module sys
```

```
'3.7.6 (default, Feb  6 2020, 10:40:17)
[GCC 4.4.7 20120313 (Red Hat 4.4.7-23)]'
```

or:

```
from sys import version  #import version variable into current scope
version                  #don't need to prepend sys.
```

```
'3.7.6 (default, Feb  6 2020, 10:40:17)
[GCC 4.4.7 20120313 (Red Hat 4.4.7-23)]'
```

Introduction to Python 3

In Python objects are everywhere.
Objects: associated functions called *methods*

```
file = open("filename")    #open is built-in function returning an object
contents = file.read()     #read is a method of this object
                           # returns a string containing file contents

dir(file)                  # list all methods of object file
```

```
['__class__', '__delattr__', '__doc__', '__getattribute__',
 '__hash__', '__init__', '__iter__', '__new__', '__reduce__',
 '__repr__', '__setattr__', '__str__', 'close', 'closed', 'fileno'
 'flush', 'isatty', 'mode', 'name', 'read', 'readinto', 'readline'
 'readlines', 'seek', 'softspace', 'tell', 'truncate', 'write',
 'writelines', 'xreadlines']
```

A mapping type: Dictionaries

```
d={}
d['any'] = 4          #elements indexed like arrays
d['string'] = 5       # but the index can be (almost) any type
print( d['string'] )
```

```
5
```

```
list( d.keys() )           #return list of all index keys
list( d.values() )         #return list of all indexed values
```

Tools for List Processing:

List Comprehensions - convert a list to another list

```
stringList=['1','2','3']
[ int(i) for i in stringList ]    # convert list of string to ints
```

```
[1, 2, 3]
```

List comprehension:

- expression within square brackets containing the keywords *for*, *in* and optionally *if*

```
[ 2*int(c) for c in ['3','2','1'] if c!='2' ]
```

```
[6, 2]
```

Introduction to Python 3

interactive help functionality: `dir()` is your friend!

```
import sys
dir(sys)     #lists names in module sys
dir()        # list names in current (global) namespace
dir(1)       # list of methods of an integer object
```

the help function

```
import ivm
help( ivm )          #help on the ivm module
help(open)           # help about the built-in function open
help(sys.exit)       # help about the exit function in the imported sys module
```

browse the Xplor-NIH python library using your web-browser on your local workstation:

```
% xplor -pydoc -b
```

Xplor-NIH Python module reference:

http://bit.niddk.nih.gov/xplor-nih/doc/current/python/ref/index.html

Linear Algebra Facilities in Python

Direct access to efficient C++ routines for matrix/vector manipulation. Includes Numerical Python-like operations.

```
from cdsMatrix import RMat, transpose, inverse
from cdsMatrix import svd, trace, det, eigen

m=RMat([[1,2],       #create a matrix object
        [3,4]])
print( m )

print( m[0,1] )      #element access
m[0,1]=3.14          #element assignment

print( trace(m) )    #matrix trace
print( det(m) )      #determinant
print( transpose(m) )#matrix transpose

print( inverse(m) )  #matrix inverse

print( 0.5*m   )     # multiplication by scalar
print( m+m,m-m )     # matrix addition, subtraction
print( m*m     )     # matrix multiplication
```

```
from cdsVector import CDSVector_double as vector
from cdsVector import norm

v=vector([1,2])      # vectors
print( norm(v) )     # vector norm
print( 2*v,v+v,v-v ) # vector arithmetic
print( m*v )         # matrix multiplication

# 3-dimensional vectors
from vec3 import Vec3, cross, dot, unitVec
v = Vec3(1,2,3)
cross(Vec3(1,0,0),v) ; dot(Vec3(1,0,0),v)
unitVec(v)

# singular value decomposition
r= svd(m)
print( r.u, r.vT, r.sigma )

# eigenvalue decomposition
e= eigen(m)
print( e[0].value()        ) #first eigenvalue
print( list(e[0].vector()) ) #first eigenvector
```

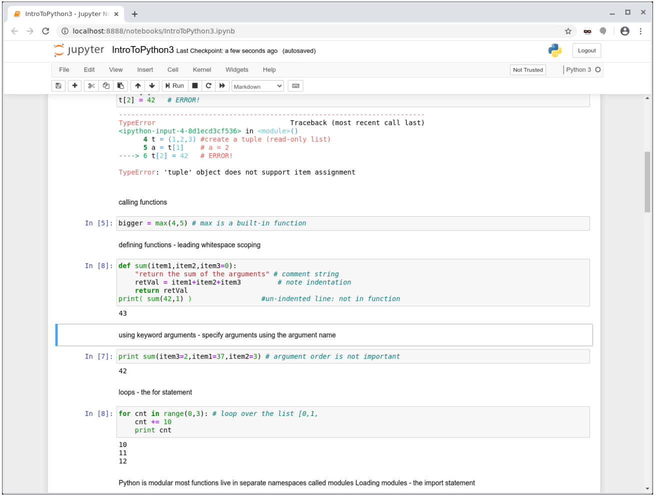
Additional Mathematical Facilities

These modules are distributed with Xplor-NIH.

- cminpack: nonlinear least squares.
- fft: real and complex FFTs.
- moremath: special functions and constants.
- spline: 1-, 2-, and 3- dimensional cubic splines.
- numpy: Numeric Python library is distributed with Xplor-NIH.
- matplotlib: powerful plotting package.

Jupyter Notebook

distributed with Xplor-NIH as the jupyterXplor command



Browser-based interactive environment.

Accessing Xplor-NIH's Python interpreter

from the command-line: use the -py flag:

```
% xplor -py

                       XPLOR-NIH version 3.4
 C.D. Schwieters, J.J.  Kuszewski,       Progr. NMR Spectr. 48, 47-62 (2006).
 N. Tjandra, and G.M. Clore              J. Magn. Res., 160, 66-74 (2003).
 http://bit.niddk.nih.gov/xplor-nih      based on X-PLOR 3.851 by A.T. Brunger
python>
```

or, the pyXplor executable - a bit quieter- and can be used as a complete replacement for the python command:

```
% pyXplor
python>
```

To run a script:

```
% xplor script.py
```

or, as an extension to an external Python interpreter:

```
% ( eval `xplor -csh-env` ; python3)
```

or

```
% xplor -sh -c python3
```

```
Python 3.7.6 ...
>>> import xplorNIH, xplor
>>> xplor.execfile('script.py')
```

[for extension: Python version must be same in external interpreter and Xplor-NIH.]

Structure Calculation Overview: Script Skeleton

```
import protocol
protocol.loadPDB("model.pdb")     #initialize coordinates
from ivm import IVM   #configure which degrees of freedom to optimize
dyn = IVM()
coolParams=[] # a list which specifies potential smoothing

# set up potential terms from NMR experiments, covalent geometry,
# and knowledge-based terms
# initialize coolParams for annealing protocol for each energy term
from simulationTools import AnnealIVM
coolLoop=AnnealIVM(dyn,...)      #create simulated annealing object

def calcOneStructure( structData ):
    """ a function to calculate a single structure """
    # [ randomize velocities ]
    # [ perform high temp dynamics ]
    dyn.run()
    # [ cooling loop ]
    coolLoop.run()
    # [ final minimization ]
    dyn.run()

from simulationTools import StructureLoop
StructureLoop(numStructures=100,                 #calculate 100 structures
              structLoopAction=calcOneStructure, #using this function
              doWriteStructures=True,            #then write to pdb file
              pdbTemplate='SCRIPT_STRUCTURE.sa'  #using this template
             ).run()                             # a .viols file also written
```

StructureLoop handles parallel structure calculation, and optional structural statistics calculation and regularized mean structure calculation.

Loading and Generating Coordinates

PSF file - contains atomic connectivity, mass and covalent geometry information. This information must be present before coordinates can be loaded.
generate via external helper scripts

1. seq2psf - generate a psf file from primary sequence
   ```
   % seq2psf file.seq
   ```
2. pdb2psf - generate a psf file from a pdb file
   ```
   % pdb2psf file.pdb
   ```
3. More involved: most modified and nonstandard residues and small molecules.

Within the Python scripting interface (in the `protocol` module)

- `protocol.initStruct` - load pregenerated .psf file. Not necessary for standard residues.
- `protocol.initCoords` - read pdb file using the current PSF. It also reads mmCIF files.
- `protocol.loadPDB` - read pdb or mmCIF and generate psf info on the fly. Also fixes-up input coordinates (naming, symmetric sidechains, disulfide bonds, BIOMT records). It can also delete atoms whose coordinates are not present.

To write out a PDB file, use `protocol.writePDB("file.pdb")` or `protocol.writeCIF("file.cif")`

Loading and Generating Coordinates - details

A Simulation object contains atom name, position, mass, *etc* and bond info.
Default Simulation: `xplor.simulation`

A completely separate PSF can be loaded by creating a new XplorSimulation.

Each XplorSimulation has its own XPLOR process.

```
from xplorSimulation import XplorSimulation
new_xsim = XplorSimulation()
import protocol
protocol.initStruct('other.psf',simulation=new_xsim)
```

Initial atomic coordinate values:
`(x,y,z) = (9999.999, 9999.999, 9999.999)`
these are the values if coordinates are not initialized.

To delete atoms:

```
xplor.simulation.deleteAtoms("not known")
```

To add atomic coordinates if some are not defined:

```
from protocol import addUnknownAtoms
addUnknownAtoms()
```

These coordinates will have proper covalent geometry.
To correct covalent geometry (bonds, angles and impropers):

```
from protocol import fixupCovalentGeom
fixupCovalentGeom('resid 30:50')  # this may cause significant changes in
                                  # the selected atomic positions
```

Topology and Parameters

Topology specifies how residues and (small) molecules are connected.
Parameters specify force constants, bond lengths, atomic radii, *etc*.
For modified or artificial residues or small molecule ligands, may need to generate new topology and parameters, using:

- PRODRG

  `http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg`
- ACPYPE

  `http://bio2byte.be/acpype/`
- An existing PDB (for small molecules) Generate Topology/Parameters using `eginput/PSF_generation/genLigand.py`. Currently, needs help with planar regions.
- From a PDB search `http://rcsb.org/` (for small molecules and non-canonical residues) It will provide an mmCIF file, from which .top, .par files can be generated using `eginput/PSF_generation/genLigandCif.py`.

Topology Entry for Alanine

```
residue ALA
  group
    atom N   type=NH1 charge=-0.36 end
    atom HN  type=H   charge= 0.26 end
  group
    atom CA  type=CT  charge= 0.00 end
    atom HA  type=HA  charge= 0.10 end
  group
    atom CB  type=CT  charge=-0.30 end
    atom HB1 type=HA  charge= 0.10 end
    atom HB2 type=HA  charge= 0.10 end
    atom HB3 type=HA  charge= 0.10 end
  group
    atom C   type=C   charge= 0.48 end
    atom O   type=O   charge=-0.48 end

  bond N  HN
  bond N  CA   bond CA HA
  bond CA CB   bond CB HB1
  bond CB HB2   bond CB HB3
  bond CA C
  bond C  O

  improper HA  N   C  CB  !stereo CA
  improper HB1 HB2 CA HB3 !stereo CB
end
```

For water refinement (see Water Refinement below), alternate topology and parameters are required. Please see the examples.

Atom Selections in Python

The atom selection language is enhanced over that of XPLOR- described in the module documentation.

```
from atomSel import AtomSel
sel = AtomSel('''resid 22:30 and
                 (name CA C N)''')
print( sel.string() )        #AtomSel objs remember their selection string
resid 22:30 and
                 (name CA C N)
```

AtomSel objects can be used as lists of Atom objects

```
print( len(sel) )                    # prints number of atoms in sel
for atom in sel:                     # iterate through atoms in sel
   print( atom.string(), atom.pos() )# prints atom string, and its position.
```

Order of atoms in an AtomSel is the PSF order unless specified:

```
AtomSel("resid 2 or resid 1",
        ordered=True          )
```

Atomwise AtomSel operations:

```
import atomSel
sel2 = AtomSel('name C')
atomSel.intersection(sel,sel2)
atomSel.union(sel,sel2)
atomSel.notSelection(sel)
```

Saving Atom Selections

Named atom selections- saved when nameSelection is called

```
import atomSelLang
atomSelLang.nameSelection("nTerminus",
                          "resid 1:140")
atoms = AtomSel('recall nTerminus')
```

Abbreviations - re-evaluated each time AtomSel is called.

```
# denoted by square brackets
atoms = AtomSel("[protein]")
import atomSelLang
print(atomSelLang.abbreviations())
```

File Formats: mmCIF and NEF

PDB replacement format for atomic coordinates: mmCIF

```
loop_
_atom_site.group_PDB
_atom_site.id
_atom_site.type_symbol
_atom_site.label_atom_id
_atom_site.label_alt_id
_atom_site.label_comp_id
_atom_site.label_asym_id
_atom_site.label_entity_id
_atom_site.label_seq_id
_atom_site.pdbx_PDB_ins_code
_atom_site.Cartn_x
_atom_site.Cartn_y
_atom_site.Cartn_z
_atom_site.occupancy
_atom_site.B_iso_or_equiv
_atom_site.Cartn_x_esd
_atom_site.Cartn_y_esd
_atom_site.Cartn_z_esd
_atom_site.occupancy_esd
_atom_site.B_iso_or_equiv_esd
_atom_site.pdbx_formal_charge
_atom_site.auth_seq_id
_atom_site.auth_comp_id
_atom_site.auth_asym_id
_atom_site.auth_atom_id
_atom_site.pdbx_PDB_model_num
ATOM 1      N N    . MET A 1 1  ? -14.136 1.321   3.616   1.00 0.93 ? ? ? ? ? ? 1
ATOM 2      C CA   . MET A 1 1  ? -13.451 0.063   4.032   1.00 0.36 ? ? ? ? ? ? 1
ATOM 3      C C    . MET A 1 1  ? -11.981 0.360   4.336   1.00 0.36 ? ? ? ? ? ? 1
ATOM 4      O O    . MET A 1 1  ? -11.557 1.499   4.315   1.00 0.64 ? ? ? ? ? ? 1
ATOM 5      C CB   . MET A 1 1  ? -13.532 -0.987  2.924   1.00 1.26 ? ? ? ? ? ? 1
ATOM 6      C CG   . MET A 1 1  ? -14.934 -0.967  2.313   1.00 1.15 ? ? ? ? ? ? 1
ATOM 7      S SD   . MET A 1 1  ? -15.215 0.142   0.911   1.00 1.64 ? ? ? ? ? ? 1
```

NMR Exchange Format (NEF)

Contains

- sequence, molecular identity
- chemical shifts
- NOE peak lists
- Derived Restraints
  - distance
  - dihedral
  - RDC

```
save_nef_chemical_shift_list_bmrb21.str
   _nef_chemical_shift_list.sf_category                nef_chemical_shift_list
   _nef_chemical_shift_list.sf_framecode               nef_chemical_shift_list_bmrb21.
   _nef_chemical_shift_list.atom_chemical_shift_units  ppm

   loop_
      _nef_chemical_shift.chain_code
      _nef_chemical_shift.sequence_code
      _nef_chemical_shift.residue_name
      _nef_chemical_shift.atom_name
      _nef_chemical_shift.value
      _nef_chemical_shift.value_uncertainty

     A   10   HIS   C      175.19    0.4
     A   10   HIS   CA     56.002    0.4
     A   10   HIS   CB     30.634    0.4
     A   10   HIS   CD2    119.578   0.4
     A   10   HIS   HA     4.687     0.02
     A   10   HIS   HBX    3.106     0.02
     A   10   HIS   HBY    3.201     0.02
     A   10   HIS   HD2    7.067     0.02
```

Using potential terms in Python

Available potential terms in the following modules:

- noePot - NOE distance restraints
- rdcPot - dipolar coupling
- sardcPot - RDCs in steric alignment media - J.-r. Huang and S. Grzesiek
- rdcCorrPot - fit RDCs without alignment tensor - C. Camilloni and M. Vendruscolo
- csaPot - Chemical Shift Anisotropy
- cstMagPot - refine against chemical shift tensor magnitudes and orientations
- jCoupPot - ^{3}J-coupling
- prePot - Paramagnetic relaxation enhancement
- diffPot - refine against rotational diffusion tensor
- relaxRatioPot - refine directly against NMR relaxation data
- solnScatPot - potential for solution X-ray and neutron scattering
- planeDistPot - distance between atoms and plane
- gyrPot - pseudopotential enforcing correct protein density
- residueAffPot - contact potential for hydrophobic attraction/repulsion
- xplorPot - use old XPLOR potential terms
- posSymmPot - restrain atomic positions relative to those in a similar structure
- potList - a collection of potential terms in a list-like object.

All potential objects have the following methods:

instanceName()	-	name given when created
potName()	-	name associated w/ potential type, e.g. "RDCPot"
scale()	-	scale factor or weight (force constant). Set with setScale(val).
calcEnergy()	-	calculate and return term's (scaled) energy

Distance Restraints - the NOE potential term

Most commonly used effective NOE distance R is computed:

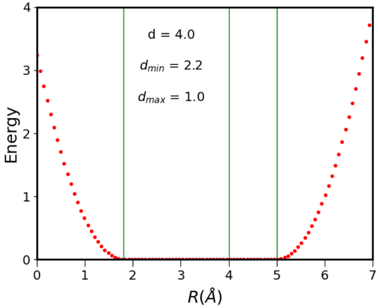
$$R = \left(\sum_{ij} |q_i - q_j|^{-6} + \sum_{ij} |q'_i - q'_j|^{-6}\right)^{-1/6}$$

"sum averaging" - usually effectively picks out the shortest interatomic distance.

Potential Energy: piecewise quadratic

"Square potential"

$$V(R) = \begin{cases} (R - d - d_{max})^2 & \text{for} R > d + d_{max} \\ (R - d + d_{min})^2 & \text{for} R < d - d_{min} \\ 0 & \text{in between} \end{cases}$$

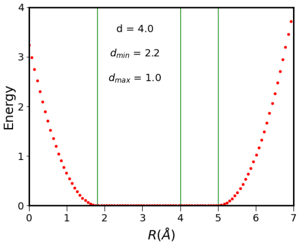


XPLOR assignment statement

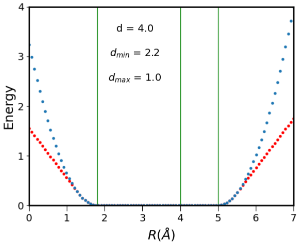
```
assign (resid 2 and  name HA )(resid   19 and  name HB# ) 4.0 2.2 1.0 !
or     (resid 6 and  name HA )(resid   27 and  name HB# ) !ambiguity
```

NOE potential term

creating an NOEPot object:




```
from noePotTools import create_NOEPot
noe = create_NOEPot("noe","noe_all.tbl")
#noe.setPotType("soft")    #uncomment if bad NOE
                           #restraints may be present
```

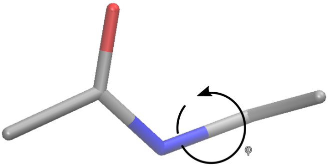



```
from noePotTools import create_NOEPot
noe = create_NOEPot("noe","noe_all.tbl")
noe.setPotType("soft")     #comment-out when no bad NOE
                           #restraints are present
```

Use:

```
print( noe.instanceName() )   # prints 'noe'
print( noe.potName() )        # prints 'NOEPot'
noe.setAveExp(5)              # change exponent for 1/r^6 sum
                              #   a reduced value reduces barriers

print( noe.rms() )            # the rmsd from the allowed distance range
```

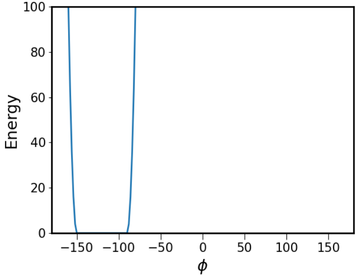
Restraints on Dihedral Angles



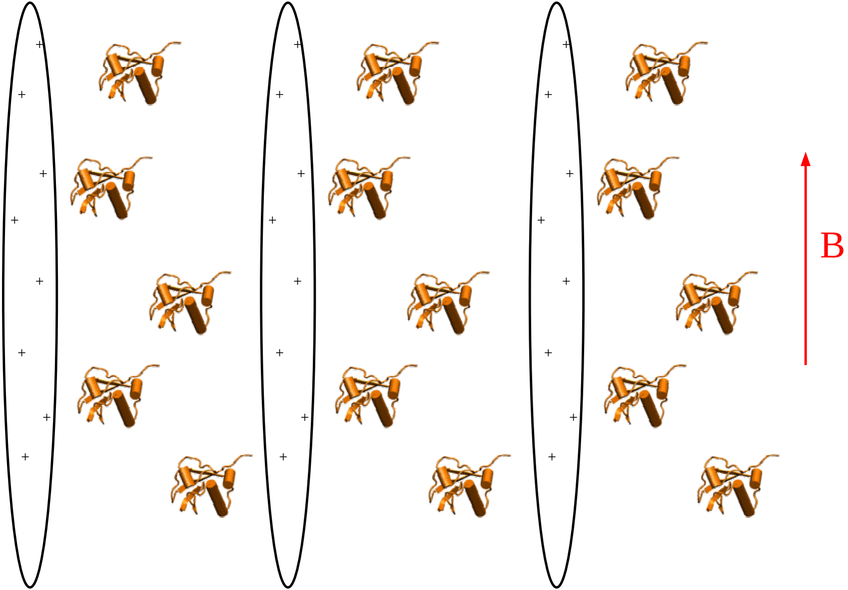
Given a restraint table generated by *e.g.* TALOS, with restraints specified as

```
assign (resid   10 and name c )   (resid   11 and name n )
       (resid   11 and name ca)   (resid   11 and name c )  1.0 -120.0 30.0 2
```

```
from dihedralPotTools import create_DihedralPot
dihePot = create_DihedralPot('dihePot',
                             "dihed_g_all.tbl")
```



Residual Dipolar Couplings



partial alignment in aligning medium

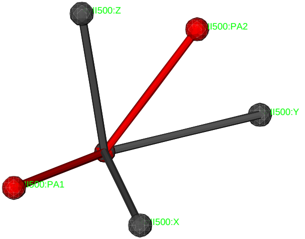
Residual Dipolar Couplings

$$\delta^{\text{RDC}}_{\text{calc}} = D_a[(3u_z^2 - 1) + \frac{3}{2}R(u_x^2 - u_y^2)] \,,$$

u_x, u_y, u_z- projection of bond vector onto principal axes of an alignment tensor.
D_a, R- measure of axial and rhombic tensor components.

`rdcPot` - used for RDCs in solution and ssNMR dipolar couplings

- tensor orientation encoded in four axis pseudo-atoms
- allows Da, R to vary: values encoded using extra atoms.
- reads both SANI and DIPO XPLOR assignment tables.
- allows multiple assignments for bond-vector atoms - for averaging.
- allows ignoring sign of D_a (optional)
- can (optionally) include distance dependence: $D_a \propto 1/r^3$.
- tensor values can be computed using SVD.



$\rightarrow$ is also used for paramagnetic pseudocontact shifts (PCS).

How to use the rdcPot potential

```
from varTensorTools import create_VarTensor, calcTensor, calcTensorOrientation
ptensor = create_VarTensor('phage')    #create a tensor object

ptensor.setDa(7.8)           #set initial tensor Da, rhombicity
ptensor.setRh(0.3)
ptensor.setFreedom('varyDa, varyRh') #allow Da, Rh to vary

from rdcPotTools import create_RDCPot
rdcNH = create_RDCPot("NH",oTensor=ptensor,file='NH.tbl')

calcTensor(ptensor)    #calc tensor parameters from current structure
                       #using SVD

calcTensorOrientation(ptensor)  #calc tensor orientation (with fixed Da, Rh)
                                #from current structure using SVD
```

NOTE: no need to introduce psf files or coordinates for alignment tensor pseudo-atoms: this is automatic.

analysis, accessing potential values:

```
print( rdcNH.rms(), rdcNH.violations())# calculates and prints rms, violations
print( ptensor.Da(), ptensor.Rh() )    # prints these tensor quantities
rdcNH.setThreshold(0)                  # violation threshold
print( rdcNH.showViolations() )        # print out list of violated terms
from rdcPotTools import Rfactor
print( Rfactor(rdcNH) )                # calculate and print a quality factor
```

RDCPot: additional details

using multiple media:

```
btensor=create_VarTensor('bicelle')
rdcNH_2 = create_RDCPot("NH_2",tensor=btensor,file='NH_2.tbl')
#[ set initial tensor parameters ]
btensor.setFreedom('fixAxisTo phage') #orientation same as phage
                                      #Da, Rh vary
```

multiple expts. single medium:

```
rdcCAHA = create_RDCPot("CAHA",oTensor=ptensor,file='CAHA.tbl')
```

`rdcCAHA` is a new potential term using the same alignment tensor as `rdcNH`.

Normally, experiments are normalized to NH Da values.

```
from rdcPotTools import scale_toNH
scale_toNH(rdcCAHA,'CAHA')   #rescales RDC prefactor relative to NH
                             # includes gyromagnetic ratios and
                             # bond lengths
```

Sign convention: the default is to consider the ^{15}N gyromagnetic ratio to be positive, *i.e.* the sign of NH experiments is flipped in the input tables. If you do not follow this convention, place the following at the *beginning* of your script:

```
from  rdcPotTools import correctGyromagneticSigns
correctGyromagneticSigns()
```

Scaling convention: scale factor of non-NH terms frequently uses error relative to the NH term:

```
rdcCAHA.setScale( (5/2)**2 )
#  inverse error   ^^^  in expt. measurement relative to that for NH
```

Note: the square well potential is only used for nonbonded (e.g. H-H) experiments.

RDCs in Steric Alignment Media

When alignment is due solely to molecular shape.

```
from sardcPotTools import create_SARDCPot
sardc = create_SARDCPot("saRDC","NH.tbl")
```

J.-R. Huang and S. Grzesiek, “Ensemble calculations of unstructured proteins constrained by RDC and PRE data: a case study of urea-denatured ubiquitin,” J. Am. Chem. Soc. **132**, 694-705 (2010).

- Important for ensemble calculations where RDCPot leads to underdetermined alignment tensors.
- Input tables are the same format used in RDCPot.

One can extract a traditional Xplor-NIH alignment tensor:

```
from varTensorTools import saupeToVarTensor
from sardcPotTools import saupeMatrix

#generate a VarTensor representation of the SARDC alignment tensor
medium = saupeToVarTensor( saupeMatrix(sardc),dmax )

print( medium.Rh() )  #print rhombicity
```

Chemical Shift Anisotropy potential

Provides additional orientational information from the full chemical shift tensor from measurements in an aligning medium.

$$\Delta\delta = \sum_{i,j} A_i \sigma_j \cos^2(\theta_{i,j})$$

A_i - a principal moment of the alignment tensor
σ_j - a principal moment of the CSA tensor
$\theta_{i,j}$ - angle between the i^{th} orientation tensor principal axis and the j^{th} CSA tensor principal axis.

How to use the csaPot potential

```
from csaPotTools import create_CSAPot
csaP = create_CSAPot(name,oTensor=tensor,file='csaP.tbl')

csaP.setDaScale( val )     # s.t. can be used with RDC alignment tensor
csaP.setScale( forceConstant )
calcTensor(tensor)         #use if the structure is approximately correct
```

NOTE: `create_CSAPot` uses built-in values for the chemical shift tensor. Alternate values can be specified by modifying `csaPotTools.csaData`.
→can be used with ssNMR CSA or chemical shift tensor data.

J-coupling potential

Karplus relationship

$$^3J = A\cos^2(\theta + \theta^*) + B\cos(\theta + \theta^*) + C,$$

θ is a torsion angle, defined by four atoms.
A, B, C and θ^* are set using the COEF statement in the j-coupling assignment table (or using object methods).

Use in Python

```
from jCoupPotTools import create_JCoupPot
  # set Karplus parameters while creating the potential term.
jCoup = create_JCoupPot("hnha","jna_coup.tbl",
                        A=15.3,B=-6.1,C=1.6,phase=0)
```

analysis:

```
print( Jhnha.rms() )
print( Jhnha.violations() )
print( Jhnha.showViolations() )
```

Paramagnetic Relaxation Enhancement

$$\Gamma = S_{AB}(\tau_c) r_{AB}^{-6},$$

r_{AB} - distance between paramagnetic center and amide proton.
$S_{AB}(\tau_c)$ - function of correlation time τ_c.

```
from prePotTools import create_PREPot
pre = create_PREPot("pre","file.tbl",
                    eSpinQuantumNumber=2.5,
                    freq=500,          # Larmor frequency in MHz
                    tauc=3.0,          # correlation time in ns
                    fixTau=True)
potList.append(pre)
```

- restrain directly against PRE values, not converted distances.
- uses modified Solomon-Bloembergen Eq. which can account for tag motion and multiple tag conformations.
- simultaneously determine correlation time.
- or refine against the correlation between observed and back-calculated: independent of the constant prefactor.

Example in `eginput/pre/refine/newRefine.py`
Iwahara, *et. al.* JACS 126, 5879 (2004).

Solvent Paramagnetic Relaxation Enhancement data

Empirical (but fast!) relationship:

$$\Gamma_{\mathrm{sPRE}} \approx A S_{\mathrm{Acc}} + B$$

with effective surface area:

$$S_{\mathrm{Acc}} \approx \left(\sum_i r_i^{-2} \right)^{-1}$$

r_i is distance of amide proton in question to a heavy atom, and the sum is over all heavy atoms.

```
from nbTargetPotTools import create_NBTargetPot, calibrate
psol = create_NBTargetPot('psol','file.tbl',restraintFormat='xplor')
#psol.setPotType("correlation")
calibrate(psol)    # determine A and B by fit to experiment
potList.append(psol)
```

also used to describe solvent NOE.
Wang, *et. al.* J. Magn. Res. 221, 76 (2012).

sPREs using PSolPot - a more rigorous approximation

$$\Gamma_{\text{sPRE}} \sim \int_{V_e} dv\; k' 1/r^6,$$

integral over all excluded volume.
r is distance from dv to the nucleus of interest
k' is constant prefactor

Divergence Theorem converts to a surface integral:

$$\Gamma_{\text{sPRE}} = -k'/3 \int_S ds\; \mathbf{n} \cdot \mathbf{r}/|\mathbf{r}|^6,$$

n is the outward pointing surface normal
r is distance from surface to nucleus

Co-solute-excluded surface represented by triangular patches.
Surface integral becomes a sum over triangles.

```
from psolPotTools import create_PSolPot
psol = create_PSolPot("psol",file='sPRE.tbl')
psol.setRmin(0.1)
psol.setThreshold(0)
psol.setProbeRadius(4.0)
psol.setTargetType("correlation")
potList.append(psol)
```

H. Kooshapur, C. Schwieters and N. Tjandra, *Angew. Chem.* **57**, 3519 (2018).
Zhou Gong, C.D. Schwieters and Chun Tang, *Methods* **148**, 48 (2018).

Use of Relaxation Data in Structure Calculation

Yaroslav Ryabov

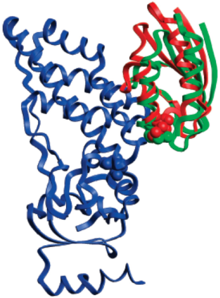
Ratio of transverse to longitudinal relaxation rates: $\rho = R_2/R_1$ contains information on bond vector orientation relative to a diffusion tensor.
The diffusion tensor can be computed from atomic coordinates.
Thus: relaxation data can be used to obtain bond vector and overall shape information.

```
# read in relaxation data from file relax.dat
# data format specified by tablePattern
from diffPotTools import readInRelaxData, mergeRelaxData, make_ratio
relax_data = readInRelaxData("relax.dat",
                             pattern=tablePattern,
                             verbose=True)

from diffPotTools import make_ratio
for item in relax_data: make_ratio(item)

from relaxRatioPotTools import create_RelaxRatioPot
pot =create_RelaxRatioPot('relax',
                          data_in = relax_data,
                          freq = freq,              #spectrometer freq
                          temperature =temperature, # expt. temp.
                         )
```

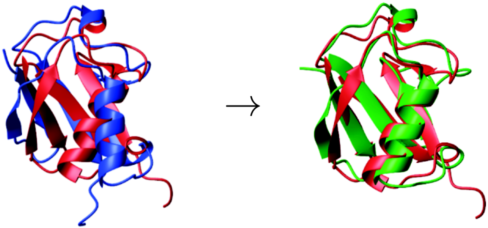
The term iteratively determines and excludes outliers.

Use of Relaxation Data in Structure Calculation

Used for docking



Improves structures with sparse restraints:



- temperature is an approximate fit parameter and should usually be optimized (facilities included).
- outliers are determined automatically, and updated regularly during a calculation

docking:
Y. Ryabov, G.M. Clore, C.D. Schwieters, *J. Am. Chem. Soc.* **132**, 5987 (2010).

single domain:
Y. Ryabov, C.D. Schwieters, and G.M. Clore, *J. Am. Chem. Soc.* **133**, 6154 (2011).

Gyration Volume potential - a pseudopotential

NOE distance restraints: approximate, loose.

Result: determined structures are too loosely packed.

But: Proteins pack to a constant density of 1.43 ± 0.03 g cm^{-3}

Approximate protein shape as ellipsoidal: gyration tensor:

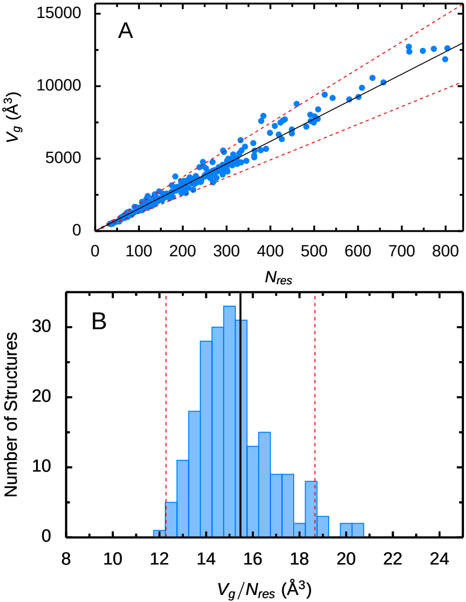
$$G = \frac{1}{N}\sum_{i=1}^{N} \Delta q_i \otimes \Delta q_i,$$

gyration volume

$V_g \equiv 4/3\pi\sqrt{|G|}$

Predict

$$V_g \approx V_g^{res} N_{res},$$



The V_g potential

$$E_{gyr} = w_{gyr}\left(w_{gyr}^{(1)}E_p(V_g - V_g^{res};0) + w_{gyr}^{(2)}E_p(V_g - V_g^{res};\Delta V_g)\right)$$

$$E_p(x,\Delta x) = \begin{cases} (x-\Delta x)^2 & \text{for } x > \Delta x \\ (x+\Delta x)^2 & \text{for } x < -\Delta x \\ 0 & \text{otherwise} \end{cases}$$

Example of use of this term:

```
from gyrPotTools import create_GyrPot
gyr = create_GyrPot('Vgyr','not resname ANI')
potList.append(gyr)
```

Reference: C.D. Schwieters and G.M. Clore, *J. Phys. Chem. B* **112**, 6070-6073 (2008).

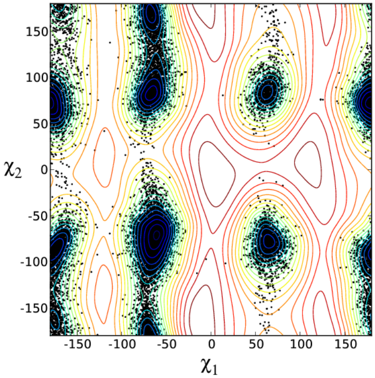
Can also restrain radius of gyration

```
from gyrPotTools import create_RgyrPot
gyr = create_RgyrPot("Rgyr","known",
                     targetRg=35,
                     rangeRg=0.7) #zero energy for Rg=34.3 .. 35.7
```

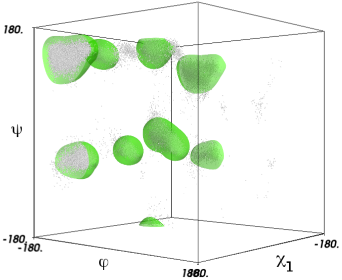
The TorsionDB potential

Improved dihedral angle potential of mean force based on observed protein structures.

Histidine



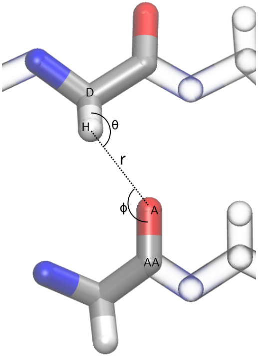
Valine

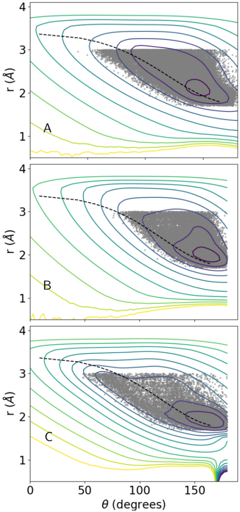



```
from torsionDBPotTools import create_TorsionDBPot
torsionDB=create_TorsionDBPot('torsionDB',
```

The HBPot term

Improved hydrogen bonding potential of mean force based on observed protein structures. Includes backbone and sidechain donors/acceptors.



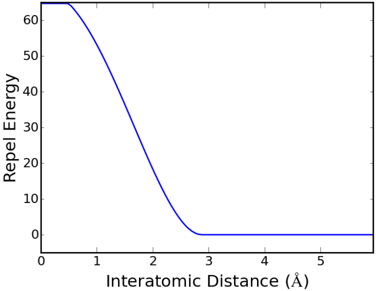



```
from hbPotTools import create_HBPot
hb = create_HBPot('hb')
hb.setScale(2.5)
potList.append( hb )
```

Schwieters *et. al.*, Protein Science 29, 100-110 (2020).

The Default Nonbonded Potential

Piecewise quartic, purely repulsive term




```
from repelPotTools import create_RepelPot, initRepel
repel = create_RepelPot('repel')
potList.append(repel)
```

Additional term used because TorsionDB only covers torsion angles involving heavy atoms:

```
# Selected 1-4 interactions.
import torsionDBPotTools
repel14 = torsionDBPotTools.create_Terminal14Pot('repel14')
potList.append(repel14)
```

using old XPLOR energy terms

The XPLOR non-bonded potential

```
import protocol
from xplorPot import XplorPot
protocol.initNBond(repel=1.2)      #specify nonbonded parameters
vdw = XplorPot('VDW')

print( vdw.violations()     )      #print number of overlapping atom pairs
print( vdw.calcEnergy()     )         #term's energy
print( vdw.potName()        )         # 'XplorPot'
print( vdw.instanceName()   )         # 'VDW'
```

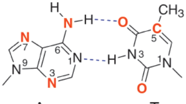
all other access/analysis done from XPLOR interface.

All parameters for the nonbonded term are listed in the XPLOR manual.

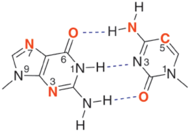
For nucleic acids, the XPLOR ORIE potential: database-derived basepair packing (translation and orientation)

References: J. Kuszewski *et al* , J. Am. Chem. Soc. ***123***, *3903-3918 (2001); Clore & Kuszewski,* J. Am. Chem. Soc. ***125***, *1518-1525 (2003).*

→Important for proper base-stacking separation.




```
xplor.command("@dna_positional.setup") #external setup to
                                       #specify base-pairing
potList.append( XplorPot("ORIE") )
rampedParams.append(
    StaticRamp("potList['ORIE'].setScale(0.2)") )
```



Other commonly used XPLOR terms: BOND, ANGL, IMPR.

using old XPLOR potentials

other terms have no Python helpers, and must be configured via the XPLOR interface.

XRay Diffraction

```
xplor.command(r'''
xref
    .
    .
    .
end
''')
potList.append( XplorPot("xref") )
```

Fiber XRay Diffraction

```
xplor.command(r'''
fiber
    .
    .
    .
end
''')
potList.append( XplorPot("xref") )
```

References: R.C. Denny *et al* , Fibre Diffr. Rev ***6****, 30-33 (1997) ; H. Wang and G. Stubbs,* Acta Cryst ***A49****, 504-513 (1993).*

Collections of potentials - PotList

potential term which is a collection of potentials:

```
from potList import PotList
pots = PotList()
pots.append(noe); pots.append(Jhnha); pots.append(gyr)
pots.calcEnergy()                                    # total energy
```

nested PotLists:

```
rdcs = PotList('rdcs')                  #convenient to collect like terms
rdcs.append( rdcNH ); rdcs.append( rdcNH_2 )
rdcs.setScale( 0.5)                     #set overall scale factor
pots.append( rdcs )
for pot in pots:                        #pots looks like a Python list
    print( pot.instanceName() )
```

```
noe
hnha
Vgyr
rdcs
```

Implementing a new potential term - in Python

```
from pyPot import PyPot ; from vec3 import norm, Vec3
class BondPot(PyPot):
    ''' example class to evaluate energy, derivs of a single bond'''
    def __init__(self,name,atom1,atom2,length,forcec=1):
        ''' constructor - force constant is optional.'''
        PyPot.__init__(self,name) #first call base class constructor
        self.a1 = atom1       ; self.a2     = atom2
        self.length = length; self.forcec = forcec
        return
    def calcEnergy(self):
        self.q1 = self.a1.pos() ; self.q2 = self.a2.pos()
        self.dist = norm(self.q1-self.q2)
        return 0.5 * self.scale() * self.forcec * (self.dist-self.length)**2
    def calcEnergyAndDerivList(self,derivs):
        energy = self.calcEnergy()
        deriv1 = self.forcec * (self.dist-self.length) / self.dist *
                 (self.q1 - self.q2)
        derivs[self.a1] += self.scale() * deriv1
        derivs[self.a2] -= self.scale() * deriv1
        return energy
    pass
```

to use:

```
p = BondPot('bond',AtomSel('resid 1 and name C')[0],
                   AtomSel('resid 1 and name O')[0], length=1.5)
```

The IVM (internal variable module)

Used for dynamics and minimization

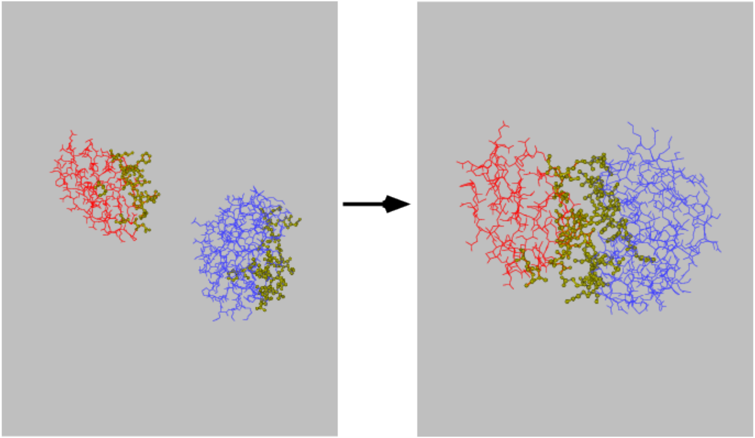
in biomolecular NMR structure determination, many internal coordinates are known or presumed to take usual values:

- bond lengths, angles- take values from high-resolution crystal structures.
- aromatic amino acid side chain regions - assumed rigid.
- nucleic acid base regions - assumed rigid.
- refinement against RDC data can't distort covalent geometry.
- non-interfacial regions of protein and nucleic acid complexes (component structures may be known- only interface needs to be determined)

Can we take advantage of this knowledge (find the minima more efficiently)?

- can take larger MD timesteps (without high freq bond stretching)
- configuration space to search is smaller:
  $N_{\text{torsion angles}} \sim 1/9\ N_{\text{Cartesian coordinates}}$
- don't have to worry about messing up known coordinates.

Hierarchical Refinement of the Enzyme II/ HPr complex



active degrees of freedom are displayed in yellow.

MD in internal coordinates is nontrivial

Consider Newton's equation:

$$F = Ma$$

for MD, we need a, the acceleration in internal coordinates, given forces F.
Problems:

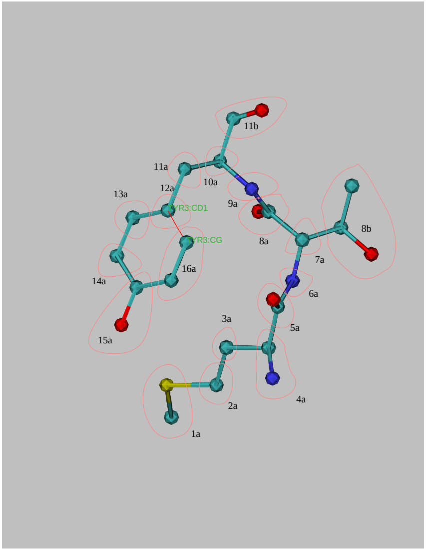
- express forces in internal coordinates
- solve the equation for a.

In Cartesian coordinates a is (vector of) atomic accelerations. M is diagonal. In internal coordinates M is full and varies as a function of time: solving for a scales as $N^3_{\text{internal coordinates}}$.

Solution: comes to us from the robotics community. Involves clever solution of Newton's equation: The molecule is decomposed into a tree structure, a is solved for by iterating from trunk to branches, and backwards.

Xplor-NIH implementation: C.D. Schwieters and G.M. Clore; J. Magn. Reson. 152, 288-302 (2001).
A copy of the IVM paper with some corrections is available at
`http://bit.niddk.nih.gov/xplor-nih/doc/intVar.pdf`

Tree Structure of a Molecule



atoms are placed in rigid bodies, fixed with respect to each other.

between the rigid bodies are “hinges” which allow appropriate motion

rings and other closed loops are broken- replaced with a bond.

Topology Setup

torsion angle dynamics with fixed region:

```
from ivm import IVM
integrator = IVM()                             #create an IVM object
integrator.fix( AtomSel("resid 100:120") )     # these atoms are fixed in space
integrator.group( AtomSel("resid 130:140") )   # fix relative to each other,
                                               # but translate, rotate in space

from protocol import torsionTopology
torsionTopology(integrator)                    # group rigid side chain regions
                                               # break proline rings
                                               # group and setup all remaining
                                               #  degrees of freedom for
                                               #  torsion angle dynamics
                                               #
                                               #  topology setup of pseudoatoms
                                               #  e.g. alignment tensor atoms:
                                               #  - tensor axis should rotate
                                               #    only - not translate.
                                               #  - only single dof of Da and Rh
                                               #    parameter atoms is significant.
```

IVM Implementation details:

other coordinates also possible: e.g. mixing Cartesian, rigid body and torsion angle motions.

convenient features:

- variable-size timestep algorithm
- will also perform minimization
- facility to constrain bonds which cause loops in tree.

full example script in `eginput/gb1_rdc/refine.py` of the Xplor-NIH distribution.

Dynamics with variable timestep

```
import protocol
bathTemp=2000
protocol.initDynamics(ivm=integrator ,         #note: keyword arguments
                      bathTemp=bathTemp,
                      initVelocities=True,  # Maxwell-dist. velocities
                      finalTime=1,          # use variable timestep
                      printInterval=10,     # print info every ten steps
                      potList=pots)

integrator.run()          #perform dynamics
```

High-Level Helper Classes

AnnealIVM: perform simulated annealing

```
from simulationTools import AnnealIVM
anneal= AnnealIVM(initTemp =3000,       #high initial temperature
                  finalTemp=25,         #final temperature
                  tempStep =25,         # temperature increment
                  ivm=integrator ,      # ivm object used for molecular dynamics
                  rampedParams = coolParams) #list of energy parameters to scale

anneal.run()  # actually perform simulated annealing
```

Force constants of some terms are geometrically scaled during refinement: during simulated annealing step *n* of *N*, the force constant is

$$k^{(n)} = \gamma^n k^{(0)}$$

- $k^{(0)}$ and $k^{(N)}$ - initial and final force constants
- $\gamma^N = k^{(N)}/k^{(0)}$

```
from simulationTools import MultRamp     #multiplicatively ramped parameter
coolParams=[]
coolParams.append( MultRamp(2,30,        #change NOE scale factor
                            "noe.setScale( VALUE )") )
```

StructureLoop: calculate multiple structures

```
from simulationTools import StructureLoop
StructureLoop(structureNums=range(10),          # calculate 10 structures
              structLoopAction=calcStructure,   # calcStructure is function
              doWriteStructures=True,           # write out structures
              pdbTemplate=pdbTemplate)          # template for structure files

pdbTemplate = 'SCRIPT_STRUCTURE.pdb'     # (the default value)
#SCRIPT -> replaced with the name of the input script (e.g. 'anneal.py')
#STRUCTURE -> replaced with the number of the current structure
```

`StructureLoop` also helps with analysis:

```
from simulationTools import StructureLoop, FinalParams
StructureLoop(structureNums=range(10),
              structLoopAction=calcStructure,
              pdbTemplate=outFilename,
              pdbFilesIn="file_*.pdb"     # specify input files
              doWriteStructures=True,     # after calcStructure, write structure, viols
              averageTopFraction=0.5,     # fraction of structures to use
              averageFitSel="not hydro", # atoms used for fitting structures
              averagePotList=potList,     # terms to use to compute of ave. struct
              averageContext=FinalParams(rampedParams),  #force constants used
              averageFilename="ave.pdb", # filename for average structure
              genViolationStats=True,     # generate a .stats file with
                                          #  energy/violation/structure stats
             ).run()
```

`StructureLoop` transparently takes care of parallel structure calculation.

Parallel computation of multiple structures

Structures have different initial conditions: for structure precision, convergence.

Launch options for:

- a multi-processor computer:
  `xplor -smp <number of CPUs>  ...`
- a cluster running PBS: [Supported implementations: PBSPro and Torque]
  `pbsxplor -l nodes=<number of nodes>  ...`
- a cluster running SLURM: [use this on Biowulf at NIH]
  `slurmXplor  -ntasks < num > [options] script.py`
- a Scyld cluster
  `xplor -scyld <number of CPUs>  ...`
- manual node specification
  `xplor -parallel -machines <machine file>  ...`

Convenient Xplor-NIH parallelization

- spawns multiple versions of Xplor-NIH on multiple machines via ssh or rsh.
- structure and log files collected in the current local directory.
- robust to crashing cluster nodes, crashing Xplor-NIH processes.

requirements:

- ability to login to remote nodes via ssh or rsh, without password
- shared filesystem which looks the same to each node
- fully populated /bin and /usr/bin directories.

following environment variables set: `XPLOR_NUM_PROCESSES, XPLOR_PROCESS`

Integrative Approaches to Structure Calculation

Combine multiple sources of data

Combine NMR data with

- Solution Scattering - SAXS, SANS
- Cryo-EM
- X-ray crystallogaphy
- Fiber Diffraction
- EPR
- AFM

Solution Scattering Intensity

types of experiments:

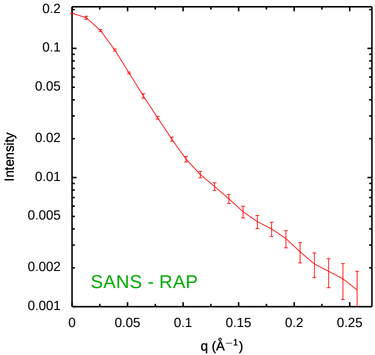
- small-angle X-ray scattering (SAXS)
- wide (or large) angle X-ray scattering (WAXS)
- Neutron scattering (SANS)

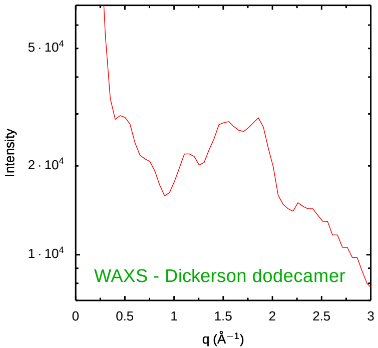
Provides information on overall molecular shape, size

→ complementary to solution NMR

→ very similar sample conditions

Example Spectra:





Calculating Scattering Intensity

Sum over all atoms: point-source scatterers

$$A(\mathbf{q}) = \sum_j f_j^{\text{eff}}(q) e^{i\mathbf{q}\cdot\mathbf{r}_j},$$

scattering vector amplitude: $q = 4\pi \sin(\theta)/\lambda$

$\theta = 0$ is the forward scattering direction

effective atomic scattering amplitude: $f_j^{\text{eff}}(q) = f_j(q) - \rho_s g_j(q)$
$f_j(q)$: vacuum atomic scattering amplitude
$\rho_s g_j(q)$: contribution from excluded solvent
$-$>boundary layer contribution can be optionally included

Difference between neutron and X-ray calculation: different $f_i^{\text{eff}}(q)$

Measured intensity

$$I(q) = \langle |A(\mathbf{q})|^2 \rangle_\Omega$$

$\langle \cdot \rangle_\Omega$: average over solid angle

Closed form solution: the Debye formula:

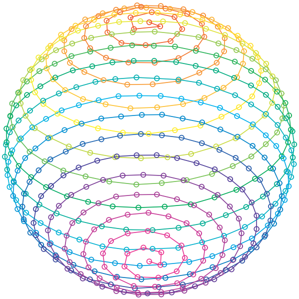
$$I(q) = \sum_{i,j} f_i^{\text{eff}}(q) f_j^{\text{eff}}(q) \text{sinc}(q r_{ij})$$

↑ sum is over all pairs of atoms. Expensive!

Scattering Intensity Approximations

Instead, compute $A(\mathbf{q})$ on a sphere and integrate over solid angle numerically.

Points are selected quasi-uniformly on the sphere using the Spiral algorithm:



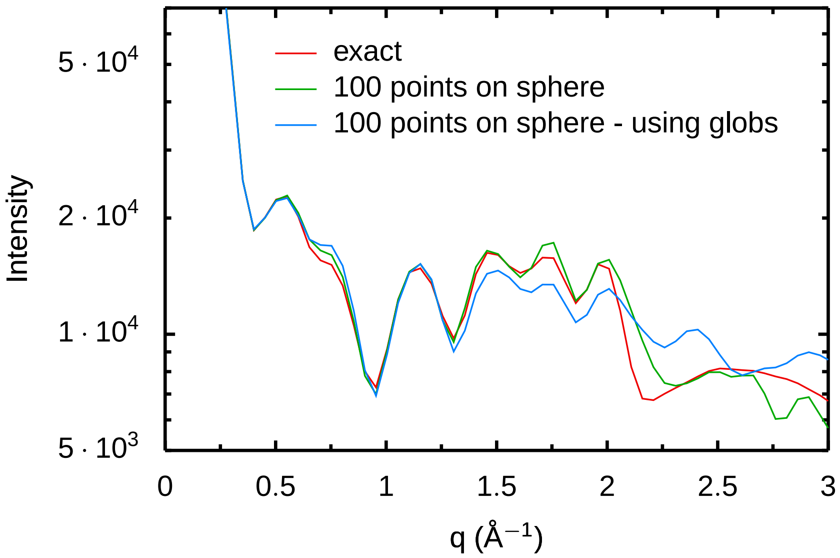
Additionally, combine atoms in "globs":

$$f_{\text{glob}}(q) = [\sum_{i,j} f_i^{\text{eff}}(q) f_j^{\text{eff}}(q) \text{sinc}(q r_{ij})]^{1/2},$$

Correct globbing, numerical integration errors with a multiplicative q-dependent correction factor c_{correct}:

$$I(q) = c_{\text{correct}}(q) I_{\text{approx}}(q),$$

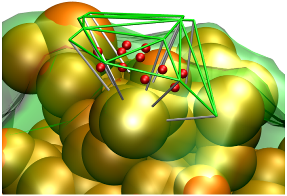
Calculated intensity for DNA scattering: numerical and globbing approximations:



Xplor-NIH

Boundary layer contribution

Bound water contributes to the scattering amplitude.
Model as a layer of uniform thickness around the molecular structure with density ρ_b.



- Use the Varshney[a] algorithm to efficiently generate an outer surface: roll solvent molecule over atoms whose radii are increased by r_b.
- Inner surface is generated using the points and surface normals.
- Each voxel defined by the tesselization procedure contributes to the scattering amplitude:

$$\sum_k f^{\text{sph}}(q; r_k) e^{i\mathbf{q}\cdot\mathbf{y}_k},$$

with

$$f^{\text{sph}}(q; r_k) = \rho_b 4\pi/q^2[\sin(qr_k)/q - r_k \cos(qr_k)]$$

[a] A. Varshney, F.P. Brooks, W.V. Wright, *IEEE Comp. Graphics App.* **14**, 19-25 (1994)

Alternate fit procedure available if buffer subtraction is significant source of error.

Determining Solvent Scattering Parameters

as in Crysol[1] three parameters are fit

Effective atomic scattering amplitude:

$$f_j^{\text{eff}}(q) = f_j(q) - \rho_s g_j(q),$$

$f_j(q)$: vacuum atomic scattering amplitude
ρ_s: bulk solvent electron density
amplitude due to excluded solvent:

$$g_j(q) = s_V V_j \exp(-\pi q^2 V_j^{2/3}) \times \exp[-\pi (q r_m)^2 (4\pi/3)^{2/3} ({s_r}^2 - 1)]$$

V_j: atomic volume
r_m: is the radius corresponding to the average atomic volume
s_V, s_r: scale factors to be fit.

Bound solvent scattering amplitude

$$f^{\text{sph}}(q; r_k) = \rho_b 4\pi/q^2 [\sin(q r_k)/q - r_k \cos(q r_k)]$$

ρ_b: boundary layer electron density
r_k: radius corresponding to voxel volume.

three parameters are fit using a grid search.
For SANS: one additional parameter: isotropic background added to calculated $I(q)$.

Solution Scattering of Rigid Bodies

For atoms within a rigid body, the relative atom positions do not change, so after an initial calculation the corresponding contribution to the scattering amplitude can be computed without referring to atomic positions. If $\mathbf{r}'_j$ is the atomic position of atom j after displacement of the rigid body with initial posistion given by $\mathbf{r}_j$, then

$$\mathbf{r}'_j = R\mathbf{r}_j + \Delta\mathbf{r},$$

where R and $\Delta\mathbf{r}$, respectively, describe the rotation and translation of the rigid body, the corresponding rigid body scattering amplitude is:

$$A_{\text{rigid}}(\mathbf{q};\{\mathbf{r}\}) = e^{i\Delta\mathbf{r}\cdot\mathbf{q}} A^0_{\text{rigid}}(\mathbf{q}';\{\mathbf{r}\}),$$

where $\{\mathbf{r}\}$ denotes the dependence on the set of initial atomic coordinates and

$$\mathbf{q}' = R^T\mathbf{q}.$$

In practice, $A_{\text{rigid}}(\mathbf{q};\{\mathbf{r}\})$ is computed using a spline over a spherical surface of constant q to evaluate $A^0_{\text{rigid}}(\mathbf{q}';\{\mathbf{r}\})$, the scattering amplitude at initial atomic position, but rotated scattering vector amplitude, $\mathbf{q}'$.
The use of this expression yields vast speedups when a calculation can be decomposed into a small number of rigid bodies, as it becomes independent of the number of atoms.

Refinement against solution scattering data

Refinement target function

$$E_{\rm scat} = w_{\rm scat} \sum_j \omega_j (I(q_j) - I^{\rm obs}(q_j))^2,$$

$w_{\rm scat}$, ω_j: weight factors
Typically set $\omega_j = 1/\Delta I^{\rm obs}(q_j)^2$ - inverse square of error. $\rightarrow$ so $E_{\rm scat} \sim \chi^2$.

- Correction factor $c_{\rm correct}$ periodically recomputed.
- Non-zero scattering contribution from surface-bound solvent- periodically computed, effect included in $c_{\rm correct}$
- Rigid subunits' scattering contribution computed very efficiently during dynamics, minimization.
- Buffer background subtraction can be included using `solnScatPotTools.fitSolventBuffer`.
- SANS data is also supported

Example Xplor-NIH SAXS setup

```
from solnXRayPotTools import create_solnXRayPot
import solnXRayPotTools
xray=create_solnXRayPot('xray',
                        experiment='saxs.dat',
                        numPoints=26,
                        normalizeIndex=-3,preweighted=False)

xrayCorrect=create_solnXRayPot('xray-c',
                               experiment=saxs.dat',
                               numPoints=26,
                               normalizeIndex=-3,preweighted=False)

solnXRayPotTools.useGlobs(xray)
xray.setNumAngles(50)
xrayCorrect.setNumAngles(500)
potList.append(xray)
crossTerms.append(xrayCorrect)

#corrects I(q) for globbing, small angular grid and
#  includes solvent contribution corrections
from solnScatPotTools import fitParams
rampedParams.append( StaticRamp("fitParams(xrayCorrect)") )
rampedParams.append(StaticRamp("xray.calcGlobCorrect(xrayCorrect.calcd())"))
```

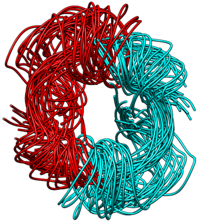
Example Xplor-NIH SANS setup

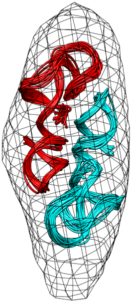
bound-solvent contribution frequently much less important

```
from sansPotTools import create_SANSPot
import sansPotTools
sans=create_SANSPot('sans',
                    experiment='sans.dat',
                    numPoints=20,
                    fractionD2O=0.41,
                    fractionDeuterated=1.,
                    altDeuteratedSels=[("resid 601:685",0.)],
                    cmpType="plain",
                    normalizeIndex=-3,preweighted=False)

sansPotTools.useGlobs(sans)

sans.setNumAngles(80)
sans.setScale(40)
potList.append(sans)

#correct using the Debye equation
rampedParams.append( StaticRamp("sans.calcGlobCorrect('n2')") )
```

Cryo Electron Microscopy Data

Detailed molecular size and shape information!

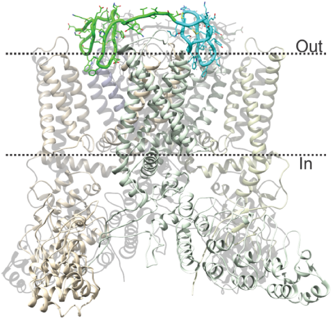


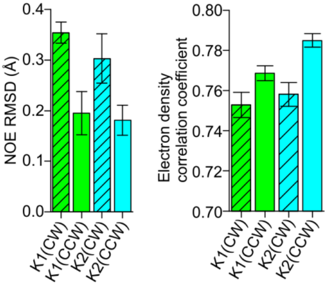


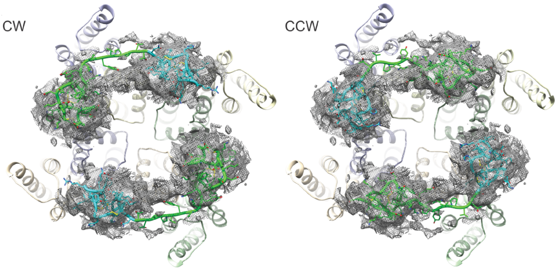

```
from atomDensity import DensityGrid
Dmap = DensityGrid()
Dmap.readCCP4('map1.ccp4',verbose = True)
from probDistPotTools import create_probDistPot
prob = create_probDistPot("prob",Dmap,"not pseudo and not name H*",
                          potType = "correlation")
```

Gong *et. al.* Plos One **10**, e0120445 (2015)

Cryo EM + NMR: TRPV1 with bound double-knot toxin

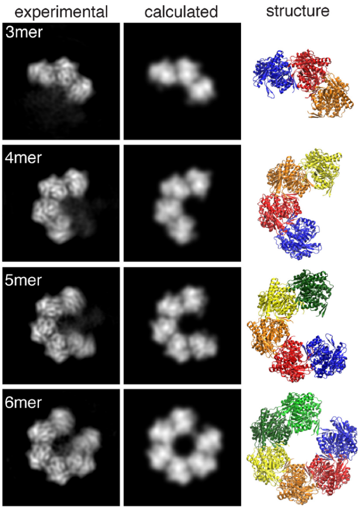






Bae *et. al.* eLife, **5**, e11273 (2016).

Using 2D Cryo-EM images

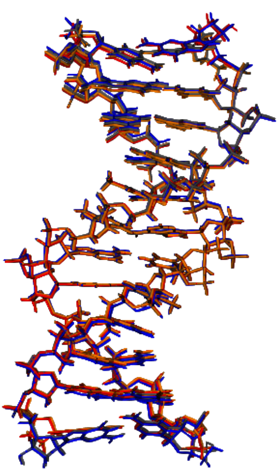


Wälti *et. al.*, J. Mol. Biol. 433, 167322 (2021).

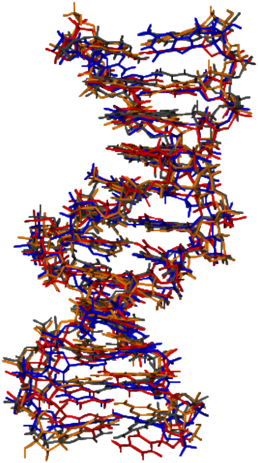
```
from densityGridTools import create_DensityGrid
map = create_DensityGrid('file.mrcs',slice=0,
                         verbose=True)

from probDistPotTools import create_probDistPot
probDistPot=create_probDistPot("prob-proj",map,
                               "not PSEUDO",
                         rotateCenterMap=True,
                         potType="cross_correlation'
                         mapType="projection")
```

Refinement against an ensemble

Refinement of DNA 12-mer using NOE, RDC and X-ray scattering data



four calculated structures



One four-membered ensemble

Refinement against an ensemble

```
esim = EnsembleSimulation('ensemble',3)  #creates a 3-membered ensemble
```

creates two extra copies of the current atom positions, velocities, *etc*.
Ensemble members don't interact, except with explicit potential terms.
Ensemble Features:

- Heterogeneous ensembles of mixed species can be treated.
- Ensemble calculations can be parallelized by specifying the `-num_threads` option to the `xplor` command.
- Care is taken to ensure data locality on Linux NUMA hardware.

Energy terms:

AvePot- average over the ensemble with no intra-ensemble interactions.

```
from avePot import AvePot
aveBond=AvePot(XplorPot,'bond') # ensemble averaged bond energy
```

aveBond's energy is $\langle E_{\mathrm{BOND}} \rangle_e$ averaged over the ensemble.

Refinement against an ensemble

Most NMR observables must be averaged appropriately- AvePot is not appropriate- it only averages ensemble energies.
For example, the appropriate RDC value is $\langle D^{\mathrm{AB}} \rangle_e$ averaged over the ensemble.
The resulting energy is then $E(\langle D^{\mathrm{AB}} \rangle_e)$.
Most Python energy terms will do proper ensemble averaging. Old XPLOR terms will not.

Additional potential terms: RAPPot, ShapePot - restrain atom positions within an ensemble - so members don't drift too far apart.
Example: restrain the positions of C_α atoms to be the same in all members of the ensemble.

```
from posRMSDPotTools import RAPPot
rap = RAPPot("ncs","name CA") # create term
rap.setScale( 100.0 )
rap.setPotType( "square" )    # harmonic potential has a flat region
rap.setTol( 0.3 )             # 1/2-width of flat region
```

Can also refine against bond-vector order parameter for ensemble of size N_e, with unit vector u_i along the appropriate bond vector in ensemble member i

$$S^2 = \frac{1}{2N_e^2}\sum_{ij}(3\cos(u_i \cdot u_j)^2 - 1)$$

[can use data from e.g. relaxation experiments.]

```
from orderPot import OrderPot
orderPot = OrderPot("s2_nh",open("nh_s2.tbl").read())
```

and crystallographic temperature factor for atom j in terms of q_{ij}, it's position in ensemble i, and it's ensemble-averaged value q_j

$$B_j = 8\pi^2/N_e \sum_i |q_{ij} - q_j|^2$$

```
from posRMSDPotTools import create_BFactorPot
bFactor = PotList("bFactor")
```

Ensemble Weights (populations)

Setting ensemble weights

```
esim = EnsembleSimulation('ensemble',3)
esim.setWeights( [0.2,0.1,0.7] ) # set weights for all ensemble members
noe = NOEPot('noe')
noe.setEnsWeights( [0.2,0.1,0.7] ) # set weight for only this NOE term
```

Ensemble Weights can be optimized during structure calculation.

```
from ensWeightsTools import create_EnsWeights
ensw = create_EnsWeights('ensw')
ensw.setWeights([0.2,0.2,0.6])  # set the initial/target weights

from sardcPotTools import create_SARDCPot
rdc = create_SARDCPot("RDC",restraints=stericRDC)
rdc.addEnsWeights(ensw) # specify that this potential term use this set
                        # of ensemble weights
```

different energy terms can have different ensemble weights - different experimental conditions

```
potList.append(ensw) #potential term biases the weights towards
                     # initial values
```

Using the EnsWeights energy term avoids situations with zero ensemble weight.

Symmetric Multimers

Maintain C_2 Symmetry

"NCS" term - keep dimer subunits identical

```
from posDiffPotTools import create_PosDiffPot
diNCS = create_PosDiffPot("diNCS", "segid A", "segid B")
potList.append(diNCS)
```

Distance symmetry to enforce C_2 symmetry

```
from distSymmTools import create_DistSymmPot, genDimerRestraints
from selectTools import minResid, maxResid

dSymm = create_DistSymmPot('dSymm')
for r in genDimerRestraints(segids=['A','B'],
                            resids=range(minResid(),maxResid(),10)):
    dSymm.addRestraint(r)
    pass
dSymm.setShowAllRestraints(True)
potList.append(dSymm)
```

Proper NOE distance calculation for SUM averaging subunit ambiguous restraint: the nMono setting

```
noe.setNMono(2)
```

Alternative: Strict Symmetry

Duplicate coordinates of protomer using rigid body rotation/translation.

- fewer degrees of freedom.
- fewer interactions to calculate.
- don't have to balance energy terms.

Example for symmetric dimer with C_2 symmetry.

```
from symSimulation import SymSimulation
symSim = SymSimulation('symSim',
                       subSel="all") # atoms to duplicate

from math import pi
from vec3 import Vec3, rotVector
symSim.addCopy(rotVector(Vec3(0,0,1),pi), #rotation of 180 deg about z
               segidSuffix="B")           #second subunit has segid B

symSim.symOp(0).segidSuffix='A'           #first subunit has segid B
```

Packing of two subunits specified by protomer center of mass position.

Refinement in Explicit Solvent

Nederveen AJ, *et. al.*, "RECOORD: a recalculated coordinate database of 500+ proteins from the PDB using restraints from the BioMagResBank," Proteins, **59**, 662-672 (2005).

	No Water	Explicit Water
Violation analysis		
RMS distance restraint violations (Å)	0.04 ± 0.06	0.04 ± 0.05
# Consistent violations > 0.5 Å[b]	0.3 ± 1.5	0.1 ± 0.6
RMS dih. restr. violations (degrees)	0.5 ± 0.7	0.5 ± 0.5
# Bumps per 100 residues[c]	11 ± 9	10 ± 7
WHAT_CHECK Z-scores		
2^{nd} Generation packing quality	−4.1 ± 1.9	−2.5 ± 2.0
Ramachandran plot appearance	−4.6 ± 1.2	−3.4 ± 1.4
χ_1/χ_2 Rotamer normality	−0.9 ± 1.3	−0.9 ± 1.0
Backbone conformation	−3.4 ± 2.6	−3.8 ± 2.7
DSSP secondary structure analysis		
Helical content	22.3 ± 20.2	25.6 ± 22.2
Sheet content	14.6 ± 13.1	17.8 ± 15.0
Secondary structure content[d]	69.3 ± 10.9	73.7 ± 9.0
PROCHECK results		
Most favored regions	69.0 ± 13.1	76.1 ± 11.3
Allowed regions	26.0 ± 9.9	19.6 ± 8.5
Generously allowed regions	3.7 ± 3.2	2.5 ± 2.1
Disallowed regions	1.3 ± 1.4	1.8 ± 1.8
Precision NMR ensemble[e]		
Backbone RMSD (Å)	2.9 ± 3.2	2.9 ± 3.1
Well-ordered RMSD (Å)	1.0 ± 1.4	1.1 ± 1.4
Backbone RMSD ORG (Å)[f]	3.7 ± 3.9	3.7 ± 3.8
Well-ordered RMSD ORG (Å)	1.4 ± 1.7	1.5 ± 1.6
Circular variance	0.05 ± 0.05	0.05 ± 0.03

Refinement Protocol:

1. solvate with water
2. heat system while restraining protein heavy atoms
3. high temperature dynamics
4. simulated annealing

```
import waterRefineTools
waterRefineTools.refine(potList=potList, coolingParams=rampedParams)
```

example in `eginput/gb1_rdc/wrefine.py`

Calculations Using Implicit Solvent: EEFx

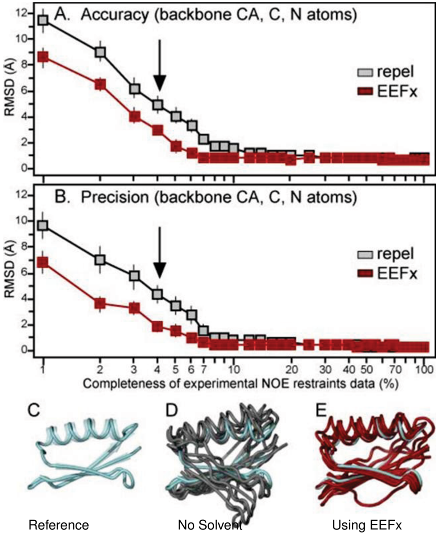
The EEFx Implicit Solvent Model

Y. Tian, *et. al.*, "A Practical Implicit Solvent Potential for NMR Structure Calculation ," J. Magn. Res. **243**, 54-64 (2014).

Can be used at all stages of structure determination.

Example in `eginput/gb1_rdc/refine_eefx.py`

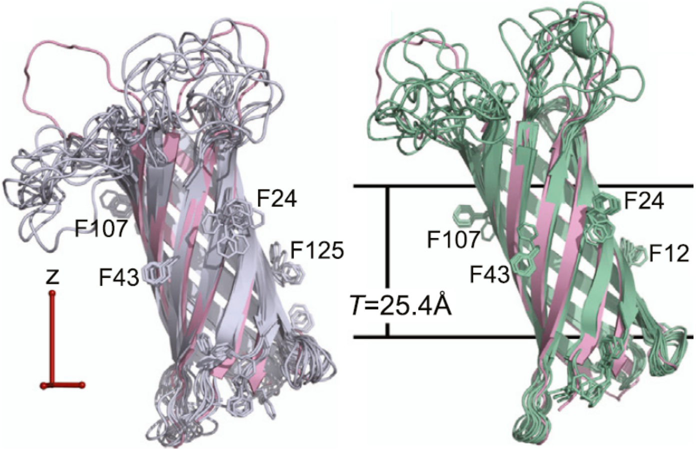
Now also includes implicit membrane potential.




```
from eefxPotTools import create_EEFxPot, param_LK
eefxpot=create_EEFxPot("eefxpot","not name H*",paramSet=param_LK)
```

Implicit Solvent with a membrane: EEFx

Ye Tian, C.D. Schwieters, S.J. Opella and F.M. Marassi, “A Practical Implicit Membrane Potential for NMR Structure Calculations of Membrane Proteins,” Biophys J. 109, 574-585 (2015).

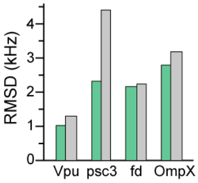


No Membrane   Using EEFx+membrane

Example in `eginput/eefx/membrane`

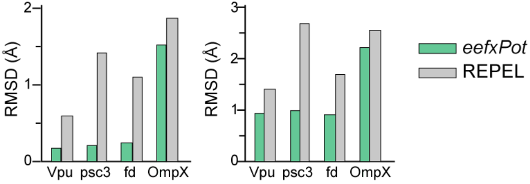
EEFXPot improves accuracy, precision and conformation of membrane protein structures

Structural Accuracy
HN DC cross validation

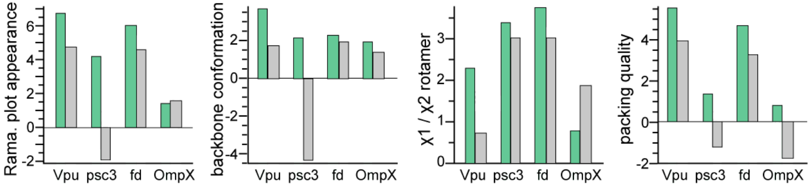


Sructural precision
(CA, C, N atoms)

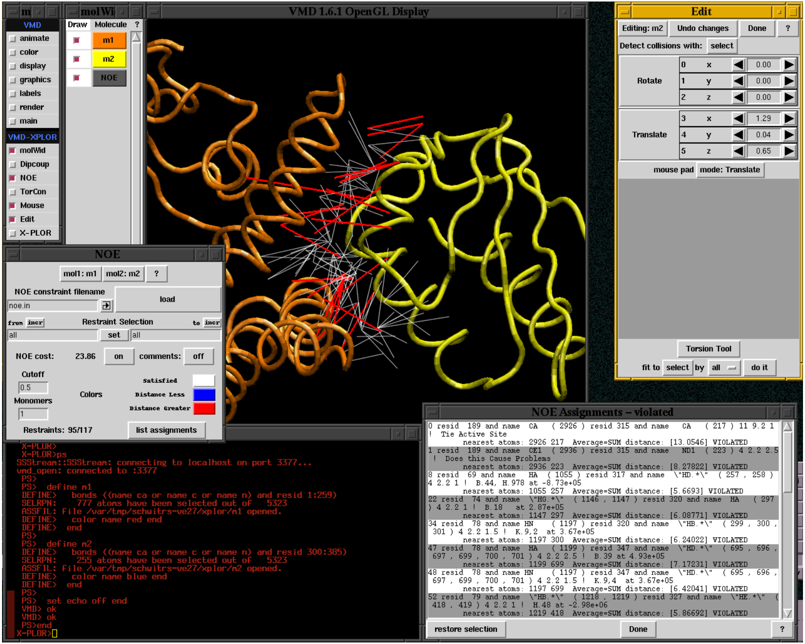
(heavy atoms)



Structural quality - WHAT-IF validation parameters



VMD interface: VMD-XPLOR



- visualize molecular structures
- visualize restraint info
- manually edit structures
- generate publication-quality figures

load multiple files at once

```
% vmd-xplor refine*.pdb
```

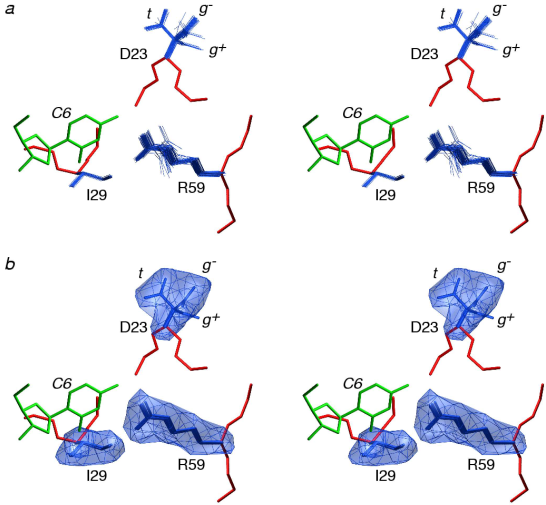
command-line invocation of separate Xplor-NIH and VMD-XPLOR jobs:

```
% vmd-xplor -port 3359 -noxplor
% xplor -port 3359 -py
```

Xplor-NIH snippet to draw bonds between backbone atoms, and labels:

```
import vmdInter

vmd = VMDInter()
x = vmd.makeObj("x")
x.bonds( AtomSel("name ca c n") )
label = vmd.makeObj("label")
label.labels( AtomSel("name ca") )
```

Graphical Representation of ensembles



`atomProb`: intelligently convert ensemble of structures into a probability distribution.

The PASD facility for automatic NOE assignment

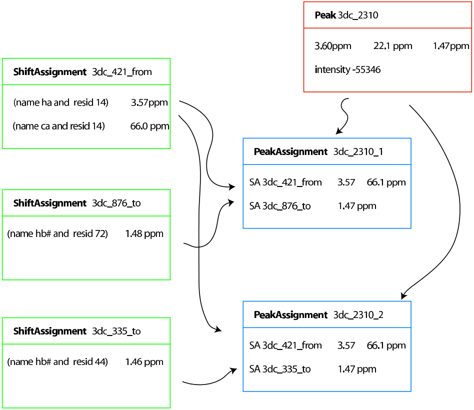
developed by John Kuszewski

Main Features:

- initial assignment likelihoods set by topological network of interconnected distance restraints.
- probabilistic selection of good NOE assignments
- for a given NOE peak, multiple possible assignments are simultaneously enabled during initial passes.
- inverse (repulsive) NOE restraints are used, consistent with the current set of active assignments.
- soft linear NOE energy.
- during structure calculation assignment likelihoods slowly change from relying on prior data to reflecting structures.
- successive passes of assignment calculation are not based on previously determined structures.
- in addition to NOE data, TALOS dihedral restraints are used.

The PASD facility

each NOE cross-peak generates a Peak



each Peak can have zero or more Peak Assignments

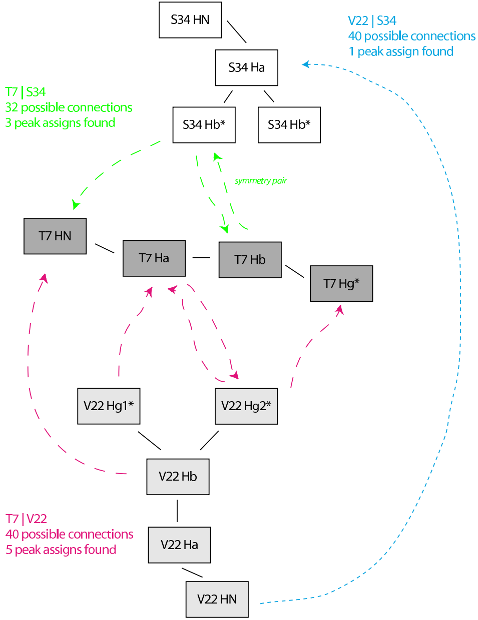
each Peak Assignment contains a from- and a to- Shift Assignment - selections of one or more atoms (containing generally indistinguishable atoms such as stereo pairs).
distances calculated between these selections using $1/r^6$ summing.

The PASD facility

Initial Likelihoods

network analysis:
mark as likely assignments between residues with more interconnecting assignments.

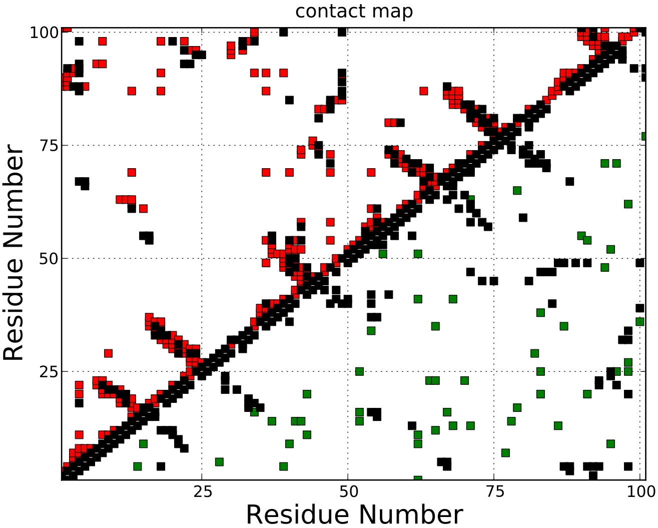
Primary sequence filter:
when there are multiple choices, always choose intra-residue assignment. Long range assignments get zero initial likelihood.



The PASD facility

Initial Likelihoods

network analysis: a contact map



The PASD facility

each assignment is activated or deactivated based on combination of current distance violations and prior likelihoods.

λ_i: likelihood of assignment *i*:

$$\lambda_i = w_0 \lambda_{pi} + (1 - w_0)\lambda_{vi}$$

λ_{pi} - prior likelihood fraction of good structures from previous calculation pass in which assignment *i* is satisfied.

λ_{vi} - instantaneous likelihood [$= \exp(-\Delta_i^2/D_v^2)$]

Δ_i - violation of assignment *i*

D_v - tunable parameter

$w_0 = 1 \ldots 0$ - relative weight of λ_{pi} to λ_{vi}

assignment *i* is activated if random num between $0 \ldots 1$ is smaller than λ_i

Entire collection of assignments is accepted or rejected using a Monte Carlo criterion, based on the NOE energy. Activation/deactivation of assignments is continued until Monte Carlo acceptance.

PASD Assignment optimization protocol

pass 1:

- start with collapsed structure with random torsion angles
- Linear NOE pot used.
- Inverse NOE potential used.
- high temp 1: 4000K
  - activation/deactivation carried out 10 times using only prior likelihoods.
  - only C_α nonbonded repulsion is enabled.
- high temp 2: 4000K
  activation/deactivation carried out 10 times using equally weighted prior and instantaneous likelihoods ($w_0 = 0.5$).
- cooling: 4000 $\rightarrow$ 100K
  64 assignment activation/deactivation steps, with decreasing D_v
  w_0 reduced from 0.5 $\rightarrow$ 0.
- prior likelihoods regenerated from top 10% of structures.

pass 2:

- quadratic NOE potential used.
- high temp: 4000K
  assignment, single activated assignment chosen at 10 intervals, based solely on the pass 2 prior likelihoods.
- cooling: 4000 $\rightarrow$ 100K
  assignments selected, restraints activated/deactivated 64 times w_0 reduced 0.5 $\rightarrow$ 0. force constants increased.

The PASD facility

Final Assignment:

- final likelihoods are computed for each assignment from top 10% of structures.
- incorrect restraint should have all likelihoods near 0
- correct restraint should have one assignment with a likelihood near 1.

Results:

- Demonstrated successfully on proteins with over 210 residues.
- method can tolerate about 80% bad NOE data.
- failure is clearly indicated by a low value of resulting NOE coverage: the number of long-range high-likelihood assignments per residue. [a value > 2]
- poor structural precision may mean that the algorithm failed, or that only subregions have been determined.
- regardless, high-likelihood assignments are very likely to be correct.

input formats supported: nmrdraw, nmrstar (including combined version 2.1), pipp, xeasy, Sparky, and NEF.

Example updated Python scripts using NEF input can be found in `eginput/PASD/nef` in the Xplor-NIH distribution.

Helper Programs

getBest - Helper to print out file names associated to the best structures resulting from a particular Xplor-NIH calculation. It can also create symbolic links to these files.

pdb2psf - generate a psf from a PDB file.

seq2psf - generate a psf file from primary sequence.

```
% seq2psf -segname PROT -startresid 300 -protein protG.seq
```

creates protG.psf with segid PROT starting with residue id 300.

torsionReport - collect and average protein torsion angle values.

```
% torsionReport -psf=[psf file] [pdb files] >average.info
```

aveStruct - average structures and report per-atom RMSD to the mean-unregularized.

targetRMSD - report RMSD to a reference structure

pairRMSD.py - report pairwise RMSD

mleFit - fit an ensemble of structures based on similarity using a maximum likelihood algorithm - no need to specify atom selection.

findClusters - find clusters of similar structures within an ensemble.

domainDecompose - given an ensemble of structures, find regions of structural similarity, using maximum-likelihood fitting.

Helper Programs

calcTensor - calculate an SVD alignment tensor and report back-calculated RDC values given one or more structures. Can create plot of observed vs. calculated RDCs.

calcETensor - calculate an ensemble of SVD alignment tensors from an ensemble of structures and observed RDC values. The tensors are underdetermined.

calcDaRh - calculate estimates of D_a and rhombicity given only RDC values (no structures) - using a maximum likelihood approach.

calcSARDC - Predict RDCs in steric alignment media from bond vector orientation and molecular shape, and compare with observed values. Can create plot of observed vs. calculated RDCs.

calcSAXS - given a structure, calculate a SAXS or SANS curve, optionally comparing with experiment. Can also compute optimal excluded solvent parameters (including boundary layer contribution).

calcPRE - Compute and optionally plot PRE values given a molecular structure and a restraint list.

calcPSol - Compute the solvent PRE given a molecule structure and a restraint list.

detChirality - Determine the chirality of centers in the specified PDB file.

Helper Programs

calcSA - Compute solvent-accessible surface area for the specified atoms or residues.

ramaStrip - plot selected backbone angles in a 2D map showing likely Ramachandran regions for the given residue types.

hbScore - score input PDB files bases on the correctness of their hydrogen-bonding geometry.

contactMap - plot a contact map for the specified structures.

convertTalos - Generate Xplor-NIH dihedral restraints from TALOS+ or TALOS-N output. These tables are more appropriate than those produced by TALOS itself.

analyzeRepel - Analyze structures for RepelPot nonbonded clashes.

evalDihedrals - Print out fit of given dihedral restraints to the specified structures.

evalDists - Print out fit of given distance restraints to the specified structures.

evalCovalent - Print out Covalent violations in the specified structures.

calcPr - given a structure, compute the pairwise distance distribution due to adding MTSL tags at the specified residues.

calcTrace - compute the DEER trace arising from signal due to adding a pair of MTSL tags added at the specified residues.

Helper Programs

scriptMaker - Graphical tool to generate Xplor-NIH scripts (written by Alex Maltsev).

idleXplor - Integrated development environment, including an editor.

energyPlot - plot various energies as the structure calculation progresses.

plotLinear,plotLog - Create 2D plot from columnar data.

ens2pdb - convert ensemble of structures into a MODEL-separated pdb for submission.

Where to go for help

online:

Home Page	http://bit.niddk.nih.gov/xplor-nih/
Mailing List	mailto:xplor-nih@list.nih.gov
FAQ	http://bit.niddk.nih.gov/xplor-nih/faq.html
Current Documentation	http://bit.niddk.nih.gov/xplor-nih/doc/current/
Tutorial	http://bit.niddk.nih.gov/xplor-nih/doc/current/python/tut.pdf
Hands-on Examples	http://bit.niddk.nih.gov/xplor-nih/xplor-nih-tutorial.tgz

subdirectories within the Xplor-NIH distribution:

eginputs	-	newer complete example scripts
tutorial	-	repository of older XPLOR scripts
helplib	-	help files
helplib/faq	-	frequently asked questions

Python:

M. Lutz, “Learning Python, 5th Edition” (O’Reilly, 2013);

http://python.org

Please complain! and suggest!