Xplor-NIH Tutorial

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outline

1. description, history, installation
2. Scripting Languages: XPLOR, Python, TCL
   - Introduction to Python
3. Overview of an Xplor-NIH Python script
4. Potential terms available from Python
5. IVM: dynamics and minimization in internal coordinates
6. Parallel determination of multiple structures
7. VMD molecular graphics interface
8. line-by-line analysis of an Xplor-NIH script.
9. refinement against solution scattering data.
10. ensemble refinement for a dynamical representation.
11. The PASD facility for automatic NOE assignment

goal of this session:

Xplor-NIH's Python interface will be introduced, described in enough detail such that scripts can be understood, and modified.

Major Contributors

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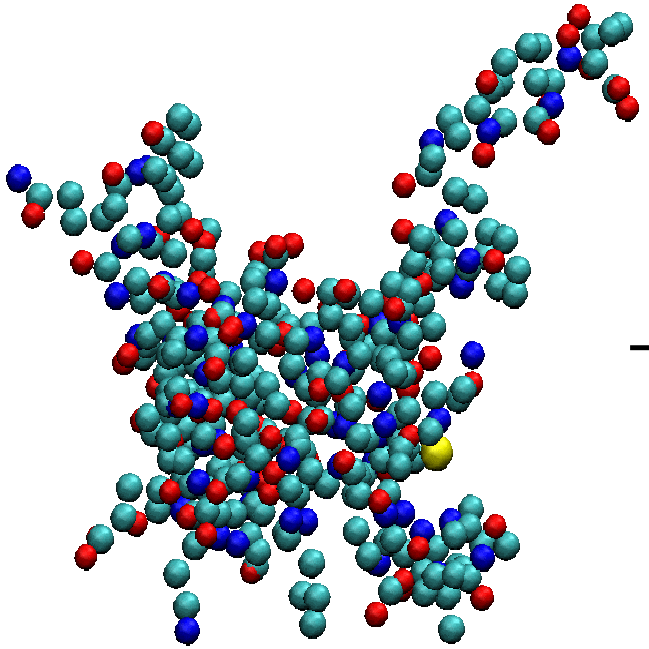
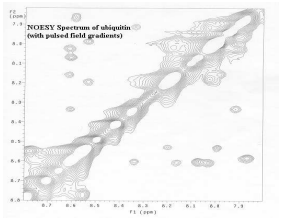
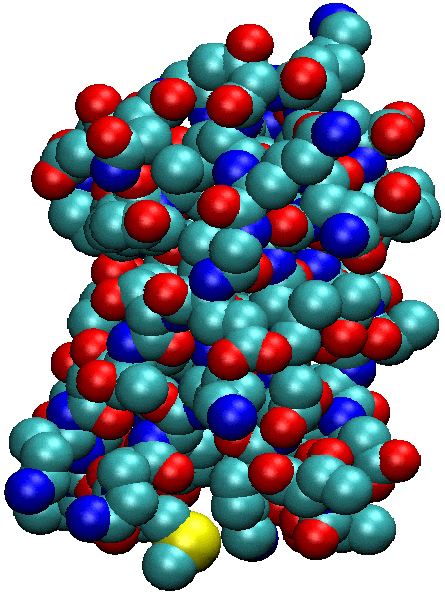
developed in the Imaging Sciences Laboratory, DCB, CIT, NIH

Overview of structure determination





?

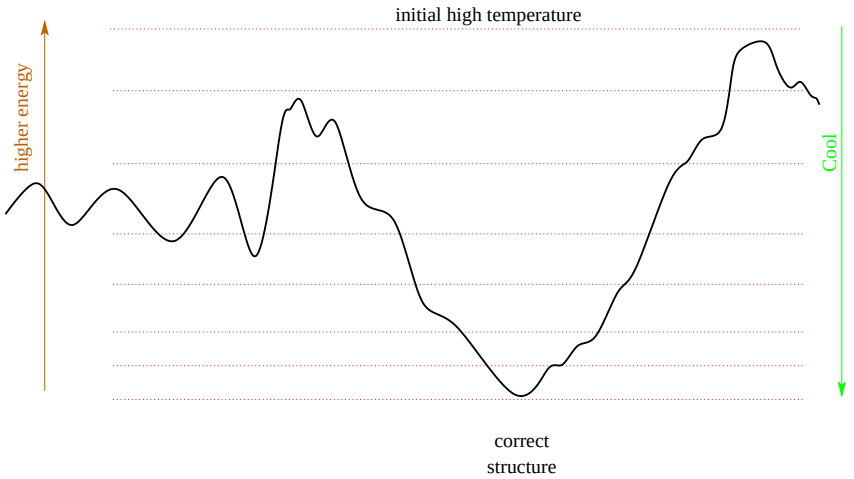


unknown atom positions

correct structure

Minimize energy: $V_{\text{tot}} = V_{\text{noe}} + V_{\text{bond}} + V_{\text{repel}} + \ldots$

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



What is Xplor-NIH?

Biomolecular structure determination/manipulation

- Determine structure using minimization protocols based on molecular dynamics/ simulated annealing.
- Potential energy terms:
  - terms based on input from NMR (and other) experiments: NOE, dipolar coupling, chemical shift data, SAXS, SANS, fiber diffraction, etc.
  - other potential terms enforce reasonable covalent geometry (bonds and angles).
  - knowledge-based potential terms incorporate info from structure database.
- includes: program, topology, covalent parameters , potential energy parameters, databases for knowledge-based potentials, helper programs, example scripts, and high level protocols for structure determination and analysis.
- freely available for non-commercial work. Source code is available.
- For commercial use, please contact `mailto:Charles@Schwieters.org`.

Xplor-NIH Description

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, TCL scripting languages.
- SWIG used to "glue" scripting languages to C++.
- bazaar repository of source code is available online.

Installation

1. download two files from `http://nmr.cit.nih.gov/xplor-nih/`

   a) a -db file: *e.g.* `xplor-nih-2.20-db.tar.gz`

   b) a platform-specific file: *e.g.* `xplor-nih-2.20-Linux_2.4_i686.tar.gz`
2. unpack these files where you wish them to live:

   ```
   zcat xplor-nih-2.20-db.tar.gz | (cd /opt ; tar xf -)
   zcat xplor-nih-2.20-Linux_2.4_i686.tar.gz | (cd /opt ; tar xf -)
   ```
3. perform initial configuration:

   ```
   cd /opt/xplor-nih-2.20
   ./configure -symlinks /usr/local/bin
   ```

   (the `-symlinks` option creates symbolic links in the specified directory for xplor and other commands - it is not necessary.)
4. test the new installation:

   ```
   bin/testDist
   ```

Scripting Languages- three choices

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://nmr.cit.nih.gov/xplor-nih/doc/current/xplor/`

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

Language Examples - printing 1..10

XPLOR

```
eval ($i=1)
while ($i le 10) loop ploop
  display $i
  eval ($i=$i+1)
end loop ploop
```

```
1
2
3
4
5
6
7
8
9
10
```

Python

```
for i in range(1,11):
  print i
```

```
1
2
3
4
5
6
7
8
9
10
```

TCL

```
for {set i 1}
    {$i<11}
    {incr i} {
  puts $i
}
```

```
1
2
3
4
5
6
7
8
9
10
```

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.

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

Introduction to Python

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'''
```

C-style string formatting - uses the % operator

```
s = "a float: %5.2f   an integer: %d" % (3.14159, 42)
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

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

Python is modular

most functions live in separate namespaces called modules

Loading modules - the import statement

```
import sys        #import module sys
sys.exit(0)       #call the function exit in module sys
```

or:

```
from sys import exit  #import exit function from sys into current scope
exit(0)               #don't need to prepend sys.
```

Introduction to Python

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']
```

Introduction to Python

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
```

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

Tools for List Processing:

map - convert one list to another list:

```
map(int, ['1','2','3'])   # apply int() function to list of strings
```

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

lambda - a simple function with no name

```
twoTimes=lambda x: 2*x  # define twoTimes to be a lambda function
twoTimes(3)
```

```
6
```

lambdas are useful when used with map:

```
map(lambda c: 2*int(c), ['1','2','3'])  # convert string list to ints
                                        # with multiplication
```

```
[2, 4, 6]
```

Linear Algebra Facilities in Python

```
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

#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
```

Introduction to Python

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 on the built-in function open
```

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

```
% xplor -py -pydoc -g
```

Xplor-NIH Python module reference:

`http://nmr.cit.nih.gov/xplor-nih/doc/current/python/ref/index.html`

Python in Xplor-NIH

current status: low-level functionality (similar to that of XPLOR script) implemented.

mostly implemented: high-level wrapper functions which will encode default values, and hide complexity.

future: develop repository of still-higher level protocols to further simplify structure determination.

stability: Python interface fairly stable. Small changes possible.

Accessing Xplor-NIH's Python interpreter

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

```
% xplor -py
                        XPLOR-NIH version 2.20

 C.D. Schwieters, J.J.  Kuszewski,       based on X-PLOR 3.851 by A.T. Brunger
 N. Tjandra, and G.M. Clore
 J. Magn. Res., 160, 66-74 (2003).       http://nmr.cit.nih.gov/xplor-nih

python>
```

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

```
% pyXplor
python>
```

or as an extension to an external Python interpreter:

```
 % ( eval `xplor -csh-env` ; python)
Python 2.4.4 (#1, Oct 26 2006, 10:23:26)
[GCC 3.4.3] on linux2
Type "help", "copyright", "credits" or "license" for more information.
>>> import xplorNIH
>>> execfile('script.py')
```

[requires that Python version be consistent between the external interpreter and Xplor-NIH.]

accessing Python from XPLOR: PYTHon command

```
% xplor
                        XPLOR-NIH version 2.20

 C.D. Schwieters, J.J.  Kuszewski,       based on X-PLOR 3.851 by A.T. Brunger
 N. Tjandra, and G.M. Clore
 J. Magn. Res., 160, 66-74 (2003).       http://nmr.cit.nih.gov/xplor-nih

 User: schwitrs     on: khaki     (x86/Linux     )  at:  7-Dec-06 12:37:40
X-PLOR>python           !NOTE: can't be used inside an XPLOR loop!
python> print 'hello world!'
hello world!
python> python_end()
X-PLOR>
```

for a single line: CPYThon command

```
X-PLOR>cpython "print 'hello world!'"   !can be used in a loop
hello world!
X-PLOR>
```

using XPLOR, TCL from Python

to call the XPLOR interpreter from Python

```
xplor.command('''struct @1gb1.psf end
                 coor @1gb1.pdb''')
```

`xplor` is a built-in module - no need to import it for simple scripts.

to call the TCL interpreter from Python

```
from tclInterp import TCLInterp            #import function
tcl = TCLInterp()                          #create TCLInterp object
tcl.command('xplorSim setRandomSeed 778') #initialize random seed
```

Structure Calculation Overview: Script Skeleton

```
import protocol
protocol.loadPDB("model.pdb")     #initialize coordinates

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 ivm import IVM   #configure which degrees of freedom to optimize
dyn = IVM()

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()
    structData.writeStructure(potList)  #write out pdb record to file
                                        # with energies, rmsd's in headers
                                        # a separate .viols file also written

from simulationTools import StructureLoop
StructureLoop(numStructures=100,                        #calculate all structures
              pdbTemplate='SCRIPT_STRUCTURE.sa',        #  in parallel, if desired
              structLoopAction=calcOneStructure).run() #also report stats at end
```

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
   ```

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

- `protocol.initStruct` - load pregenerated .psf file
- `protocol.initCoords` - read pdb file
- `protocol.loadPDB` - read pdb and generate psf info on the fly

To delete atoms whose positions aren't defined (but are in the psf):

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

Loading and Generating Coordinates - details

initial atomic coordinate values: `(x,y,z) = (9999.999, 9999.999, 9999.999)`

these are the values if coordinates are not initialized

a Simulation object contains atom name, position, mass, *etc* and bonding information

The default Simulation is `xplor.simulation`

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

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

each XplorSimulation has a separate XPLOR process associated with it.

to add atoms whose coordinates are not defined:

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

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
```

Atom Selections in Python

use the XPLOR atom selection language, described in the XPLOR manual.

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

```
resid 22:30 and
                 (name CA or name C or name 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.
```

AtomSel objects can be *reevaluated*:

```
xplor.simulation.deleteAtoms("resid 1:2")
sel.reevaluate()
print len(sel)              # prints the correct number of atoms
```

Using potential terms in Python

available potential terms in the following modules:

1. noePot - NOE distance restraints
2. rdcPot - dipolar coupling
3. csaPot - Chemical Shift Anisotropy
4. jCoupPot - ^{3}J-coupling
5. prePot - Paramagnetic relaxation enhancement
6. gyrPot - pseudopotential enforcing correct protein density
7. residueAffPot - contact potential for hydrophobic attraction/repulsion
8. cstMagPot - refine against chemical shift tensor magnitudes
9. planeDistPot - distance between atoms and plane
10. xplorPot - use XPLOR potential terms
11. solnScatPot - potential for solution X-ray and neutron scattering
12. posSymmPot - restrain atomic positions relative to those in a similar structure
13. potList - a collection of potential terms in a list-like object.

all potential objects have the following methods:

instanceName()	-	name given by user
potName()	-	name of potential term, e.g. "RDCPot"
scale()	-	scale factor or weight
setScale(val)	-	set this weight
calcEnergy()	-	calculate and return term's energy

NOE potential term

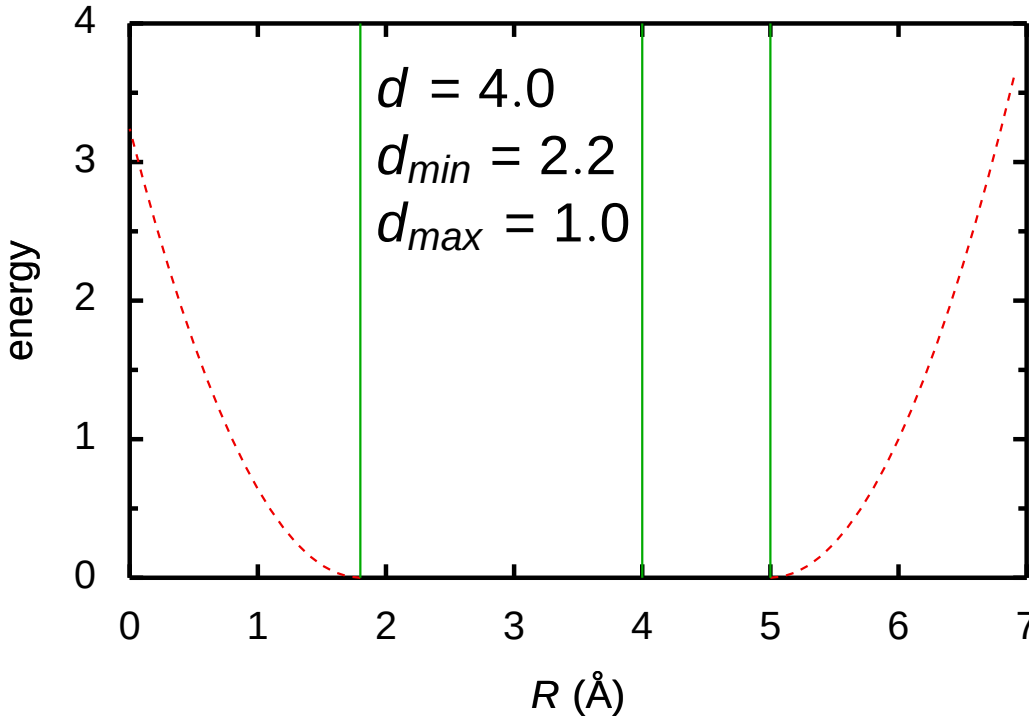
most commonly used effective NOE distance (sum averaging):

$$R = (\sum_{ij} |q_i - q_j|^{-6})^{-1/6}$$

potential energy: piecewise quadratic

"Square potential"

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



XPLOR assignment statement

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

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
```

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
noe.setThreshold( 0.1 )       # violation threshold
print noe.violations()        # number of violations
print noe.showViolations()
```

residual dipolar coupling potential

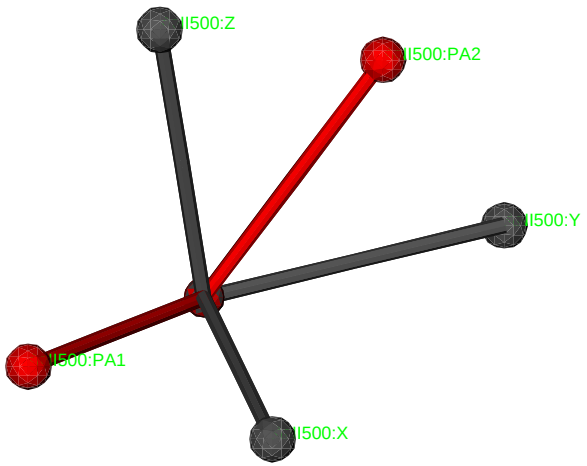
Provides orientational information relative to axis fixed in molecule frame.

$$D^{\mathrm{AB}} = D_a[(3u_z^2 - 1) + \frac{3}{2}R(u_x^2 - u_y^2)] \ ,$$

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

rdcPot (in Python)

- tensor orientation encoded in four axis 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.



→can also be used for paramagnetic pseudocontact shift experiments.

How to use the rdcPot potential

```
from varTensorTools import create_VarTensor, calcTensor
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
```

NOTE: no need to have psf files or coordinates for axis/parameter 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`.

Scaling convention: scale factor of non-NH terms is determined using the experimental error relative to the NH term:

```
scale_toNH(rdcCAHA,'CAHA')   #rescales RDC prefactor relative to NH
```

Use harmonic potential with correct relative scaling

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

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`.

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()
```

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}

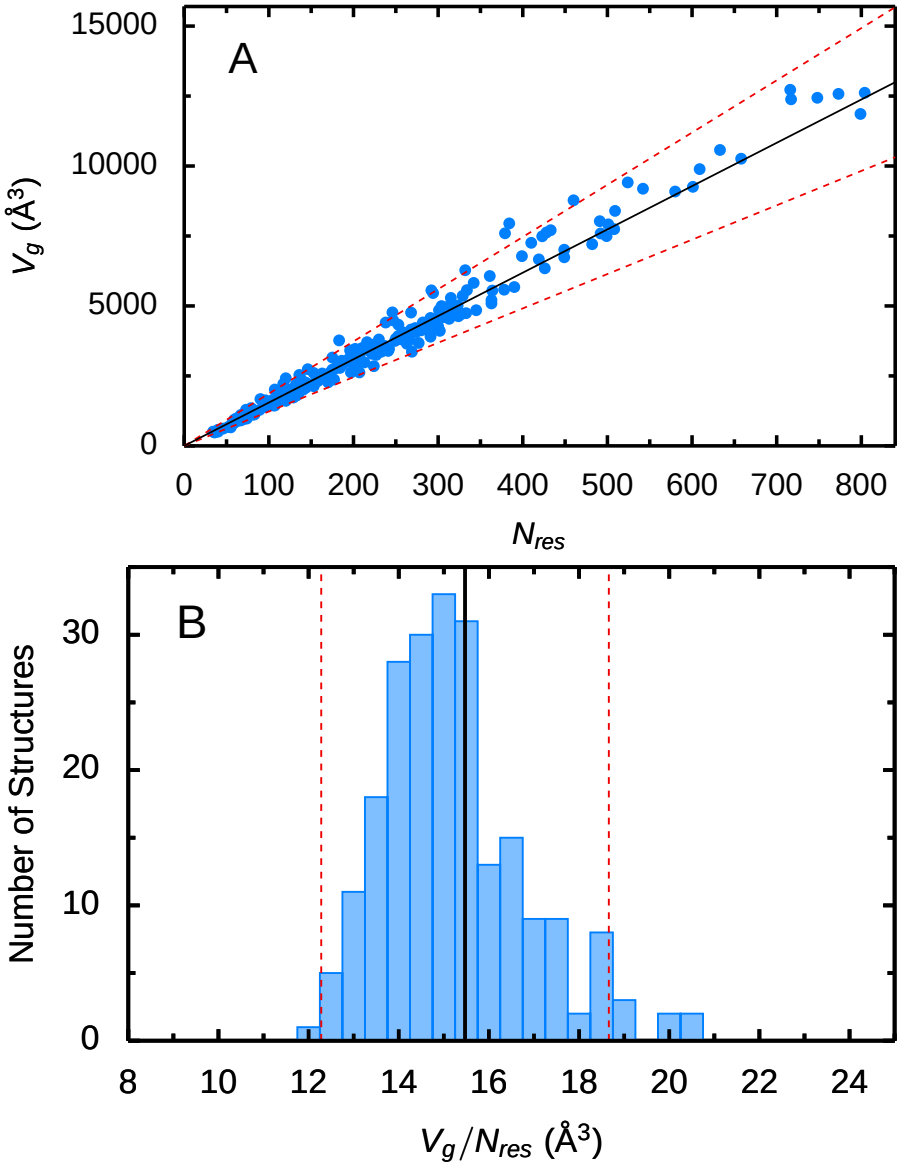
Approximate protein shape as ellipsoidal: gyration tensor:

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

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

Predict

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



The V_g potential

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

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

Example of use of this term:

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

using XPLOR potentials

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

The XPLOR RAMA (torsion angle database) potential

```
import protocol
from xplorPot import XplorPot
protocol.initRamaDatabase()
potList.append( XplorPot('RAMA') )
```

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

Enumeration of XPLOR potential terms

- 3J couplings
- 1J couplings
- ^{13}C shifts
- ^{1}H shifts
- radius of gyration
- chemical shift anisotropy
- conformational database torsion angle potentials
- database residue-residue and base-base positioning potentials
- Generalized Born implicit solvent (Tom Simonson - Strasbourg) .
- PARArestraints module for including paramagnetism-based NMR restraints in refinement (Bertini's group - Italy)
- isac code for floating RDC alignment tensor (Grzesiek's group, Basel)
- vectang pot. for indirect RDC restraints (Michael Nilges' group)
- hbda pot.- empirical bb H-bond relationship
- hbdb pot.- precise database for for bb H-bonds (A. Grishaev. - NIH)

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(rGyr)
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
noe print pot.instanceName()
hnha
COLL
rdcs
```

Implementing a new potential term - in Python

```
from pyPot import PyPot ; from vec3 import norm
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(q1-q2)
        return 0.5 * self.forcec * (dist-self.length)**2
    def calcEnergyAndDerivs(self,derivs):
        energy = self.calcEnergy()
        deriv1 = map(lambda x,y:x*y,
                     [self.forcec * (self.dist-self.length) / self.dist]*3 ,
                     ( self.q1[0]-self.q2[0],
                       self.q1[1]-self.q2[1],
                       self.q1[2]-self.q2[2] ))
        derivs[self.a1.index()] = deriv1
        derivs[self.a2.index()] = -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

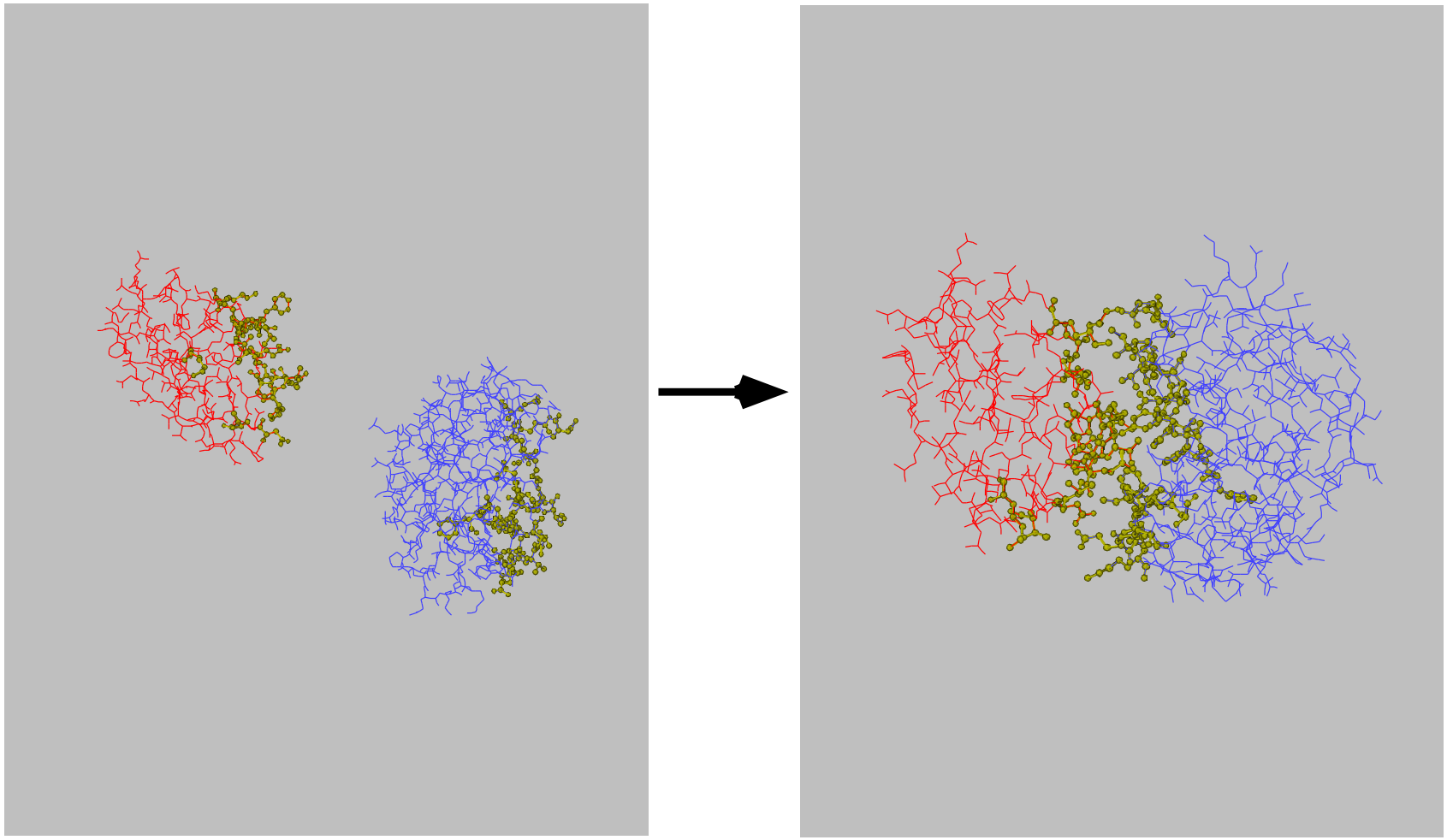
in biomolecular NMR structure determination, many internal coordinates are known or presumed to take usual values:

- 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:
  $N_{\text{torsion angles}} \sim 1/3 N_{\text{Cartesian coordinates}}$
- 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.

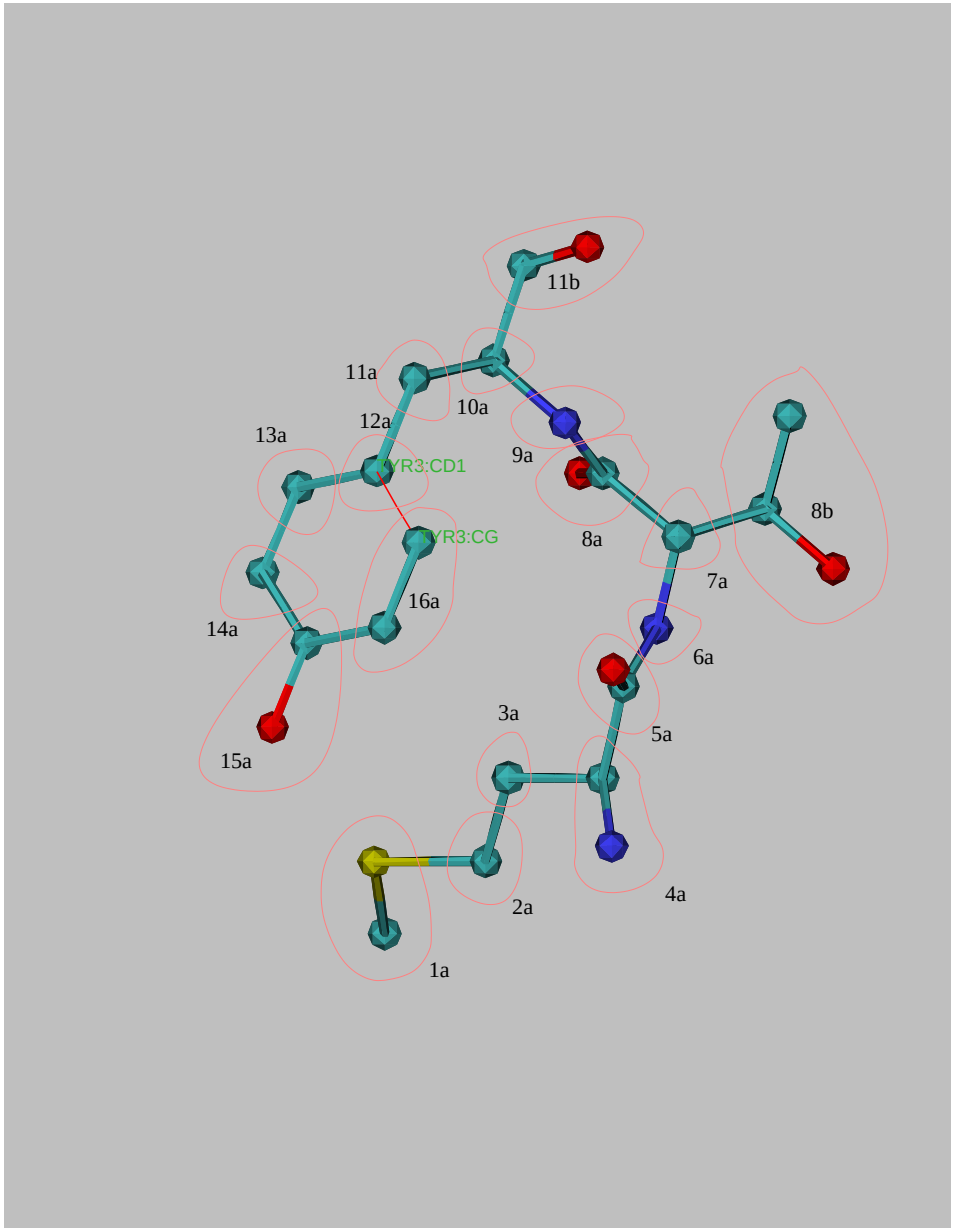
In Cartesian coordinates a is (vector of) atomic accelerations. M is diagonal.

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://nmr.cit.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
                oTensors=listOfVarTensors)          # break proline rings
                                                    # group and setup all remaining
                                                    #  degrees of freedom for
                                                    #  torsion angle dynamics
                                                    #
                                                    # second argument:
                                                    #  topology setup - for RDC
                                                    #  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,
                      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=dyn,             # ivm object used for molecular dynamics
                  rampedParams = coolParams) #list of energy parameters to scale

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

force constants of some terms are geometrically scaled during refinement:

$$k_{\mathrm{NOE}} = \gamma^n k^{(0)}_{\mathrm{NOE}}$$

$\gamma^N = k^{(f)}_{\mathrm{NOE}}/k^{(0)}_{\mathrm{NOE}}$

```
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
              pdbTemplate=pdbTemplate)         # template for output structures
pdbTemplate = 'SCRIPT_STRUCTURE.sa'
#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,
              averageTopFraction=0.5,    # fraction of structures to use
              averageFitSel="not hydro ANI", #atoms used for fitting structures
              averagePotList=potList,    #terms to use to compute of ave. struct
              averageContext=FinalParams(rampedParams),  #force constants used
              averageFilename="ave.pdb", #output filename
              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

computation of multiple structures with different initial velocities and/or coordinates: gives idea of structure precision, convergence of calculation.

```
xplor -parallel -machines <machine file>
```

on a multi-processor computer:

```
xplor -smp <number of CPUs>
```

or, on a Scyld cluster

```
xplor -scyld <number of CPUs>
```

convenient Xplor-NIH parallelization

- spawns multiple versions of xplor on multiple machines via ssh or rsh.
- structure and log files collected in the current local directory.
- robust to crashing compute nodes, crashing XPLOR runs, and the presence of dead nodes

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.

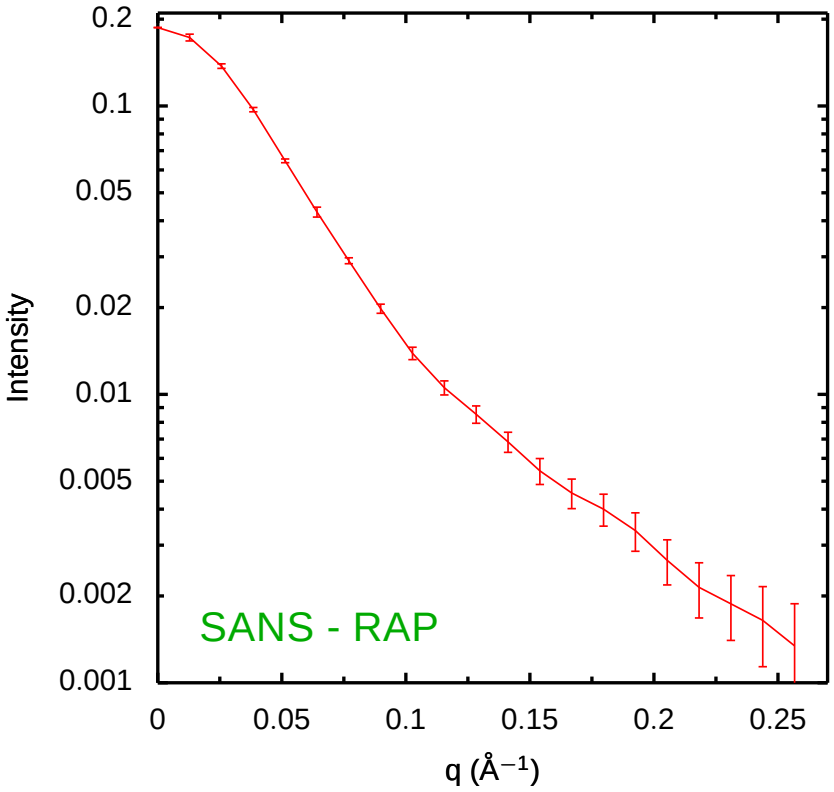
following environment variables set: `XPLOR_NUM_PROCESSES`, `XPLOR_PROCESS`

Solution Scattering Intensity

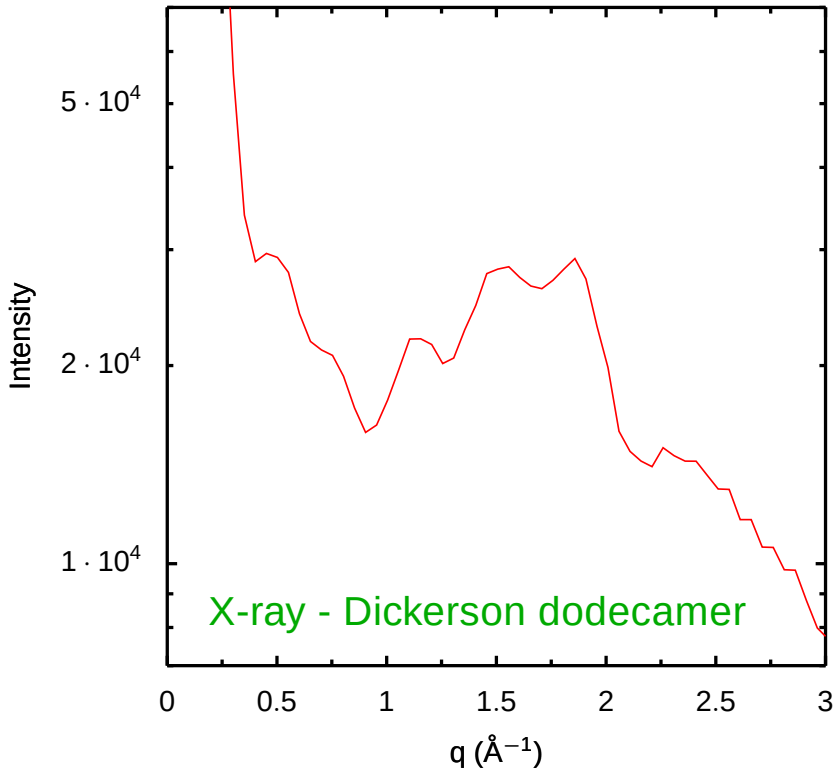
types of experiments:

- 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 NMR



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
->neglect boundary layer contribution<-

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:

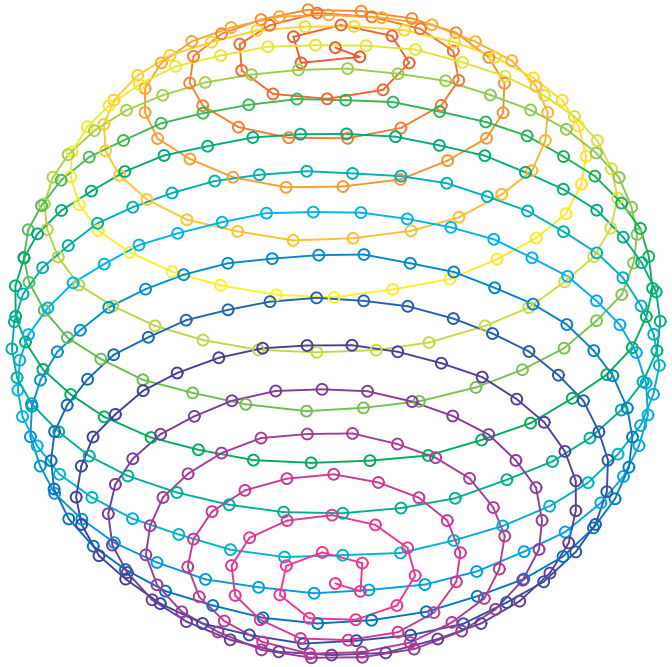
$$I(q) = \sum_{i,j} f_i^{\text{eff}}(q) f_j^{\text{eff}}(q) \text{sinc}(q r_{ij}),$$

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:



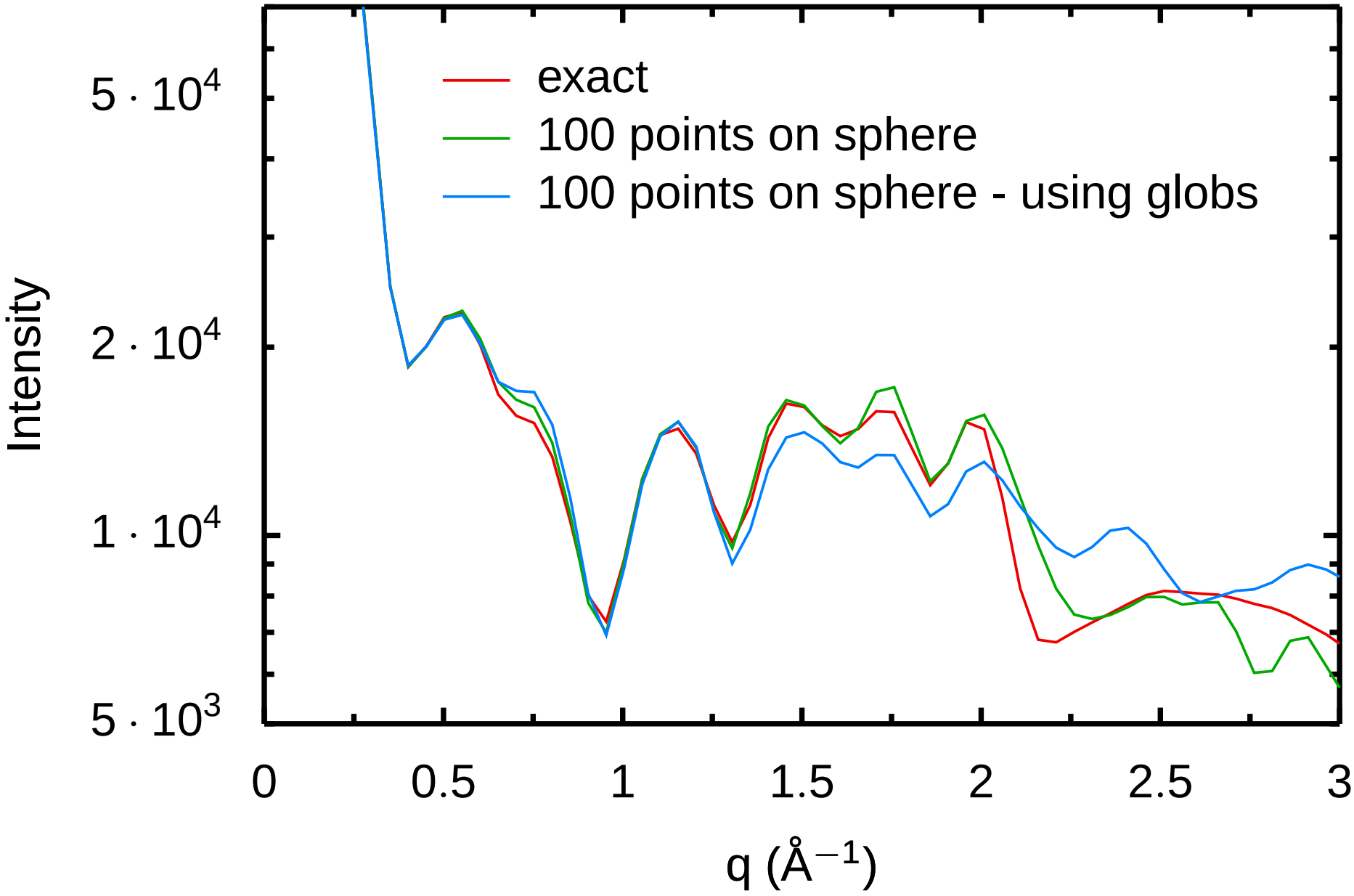
Additionally, combine atoms in "globs":

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

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

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

Calculated intensity for DNA scattering: numerical and globbing approximations:



Refinement against solution scattering data

Refinement target function

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

w_{scat}, ω_j: weight factors

Typically set ω_j to inverse square of error in $I^{\text{obs}}(q_j)$

$I(q)$ typically normalized to $I(0)$.

Efficient computation of $I(q)$ requires uniform spacing in q.

SANS:

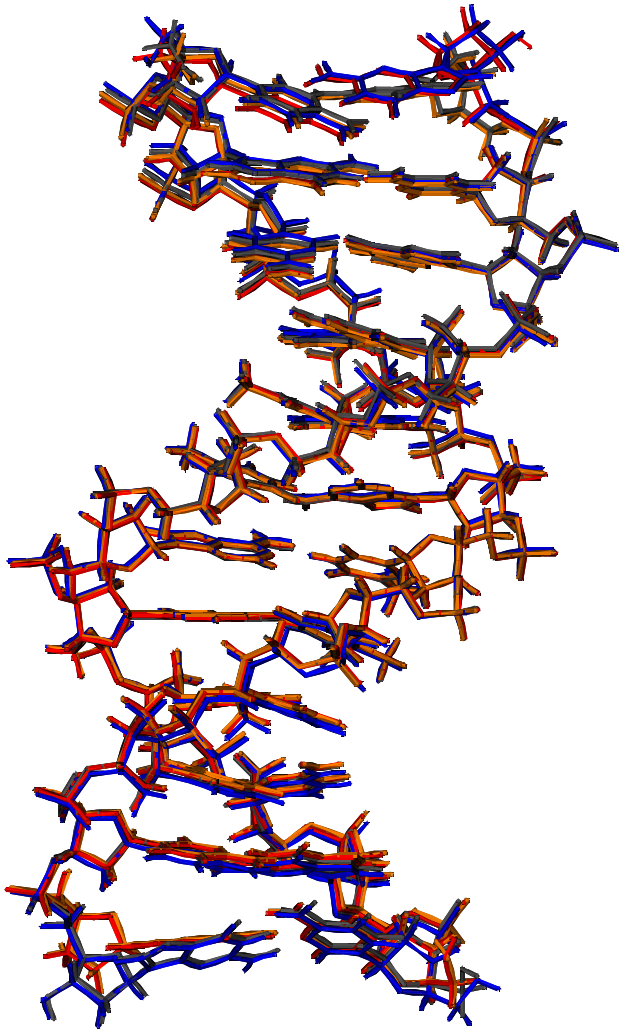
```
from sansPotTools import create_SANSPot, useGlobs
sans = create_SANSPot('sans',"resid 1:323","sans.data",
                      preweighted=False,fractionD2O=1)
sans.setNumAngles(240)
sansPotTools.useGlobs(sans)
rampedParams.append( StaticRamp("sans.calcGlobCorrect()") )
rampedParams.append( MultRamp(1.,100., "sans.setScale( VALUE )") )
```

X-Ray:

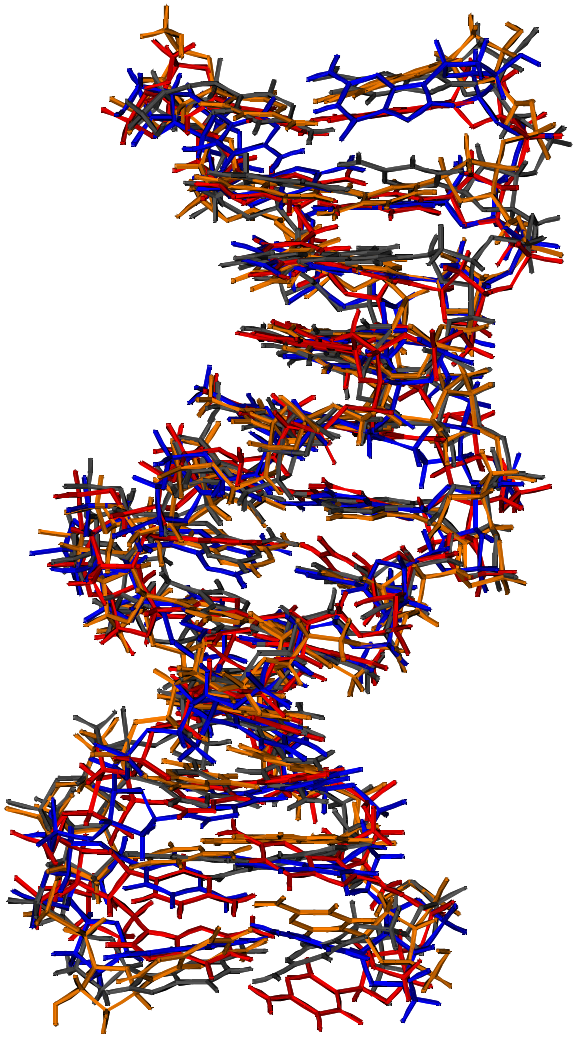
```
from solnXRayPotTools import create_solnXRayPot, useGlobs
xray = create_solnXRayPot('xray61',"not hydro","xray.dat")
xray.setNormalizeIndex(14)
useGlobs(xray)
xray.setNumAngles(100)
#corrects I(q) to the true Debye result
rampedParams.append( StaticRamp("xray.calcGlobCorrect('n2')") )
```

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.

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 $E(\langle D^{\mathrm{AB}} \rangle_e)$.

Energy terms which are ensemble aware: rdcPot, csaPot, noePot, jCoupPot, solnScatPot, distSimmPot, posRMSDPot, potList.

Additional potential terms: RAPPot, ShapePot - restrains 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())
```

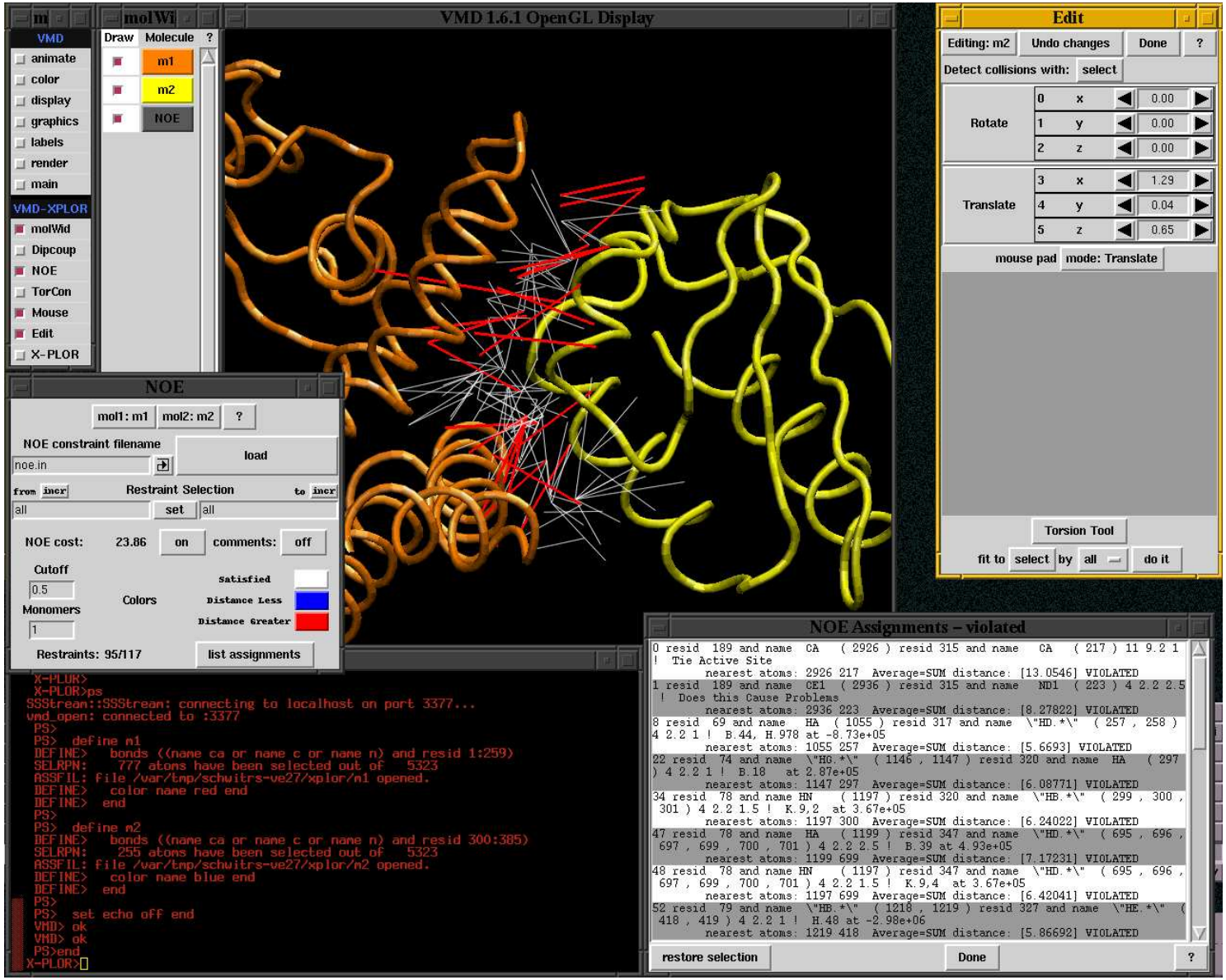
and crystallographic temperature factor for atom j in terms of q_{ij}, it's position in ensemble i, and it's ensemble-averaged value q_j

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

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

Ensemble Feature: ensemble calculations can be parallelized by specifying the `-num_threads` option to the `xplor` command.

VMD interface



vmd-xplor screenshot

Use VMD-XPLOR to

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

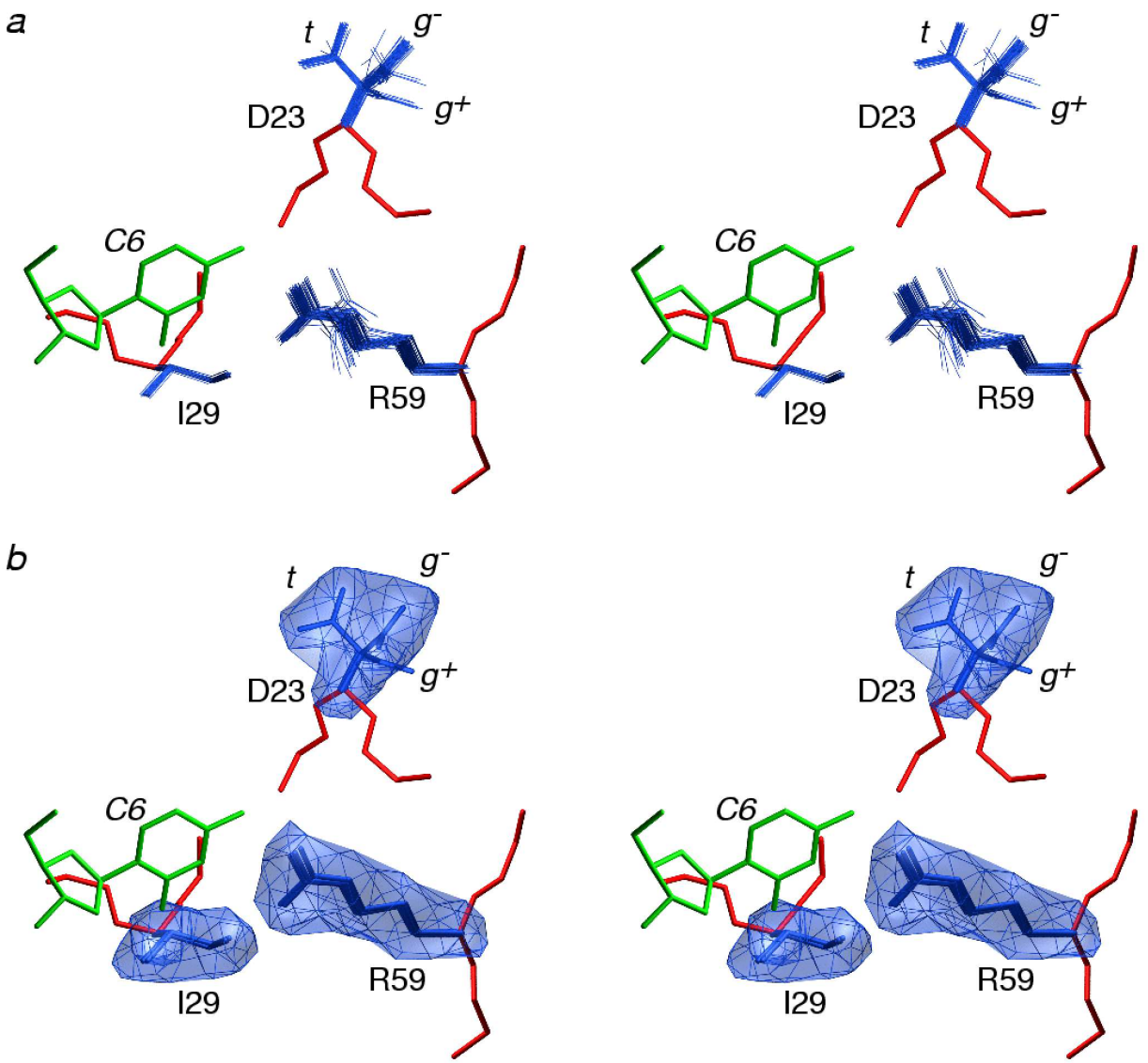
command-line invocation of separate Xplor-NIH and VMD-XPLOR jobs:

```
% 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 or name c or name n") )
label = vmd.makeObj("label")
label.labels( AtomSel("name ca") )
```

Graphical Representation of ensembles



intelligently convert ensemble of structures into a probability distribution.

Convenience Scripts

pdb2psf - generate a psf from a PDB file. Convenient when working from the PDB database.

```
% pdb2psf 1gb1.pdb
```

creates 1gb1.psf. Please send us pdb files which fail.

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.

tang.py - collect and average protein torsion angle values.

```
% eginput/protG/tang.py -psf=[psf file] [pdb files] >average.info
```

also:

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

pairRMSD.py - report pairwise RMSD

targetRMSD - report RMSD to a reference structure

calcTensor.py - calculate an alignment tensor and report back-calculated RDC values given one or more structures

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

mleFit - fit an ensemble of structures based on similarity using a maximum likelihood algorithm

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

Putting it together: a full script

Full script for refining protein G from a random extended chain, using NOEs, RDCs, Jcoup data.
`http://nmr.cit.nih.gov/xplor-nih/doc/current/python/anneal.py.html`

Also available in the Xplor-NIH distribution in as `eginput/protG/anneal.py`

The PASD facility for automatic NOE assignment

developed by John Kuszewski

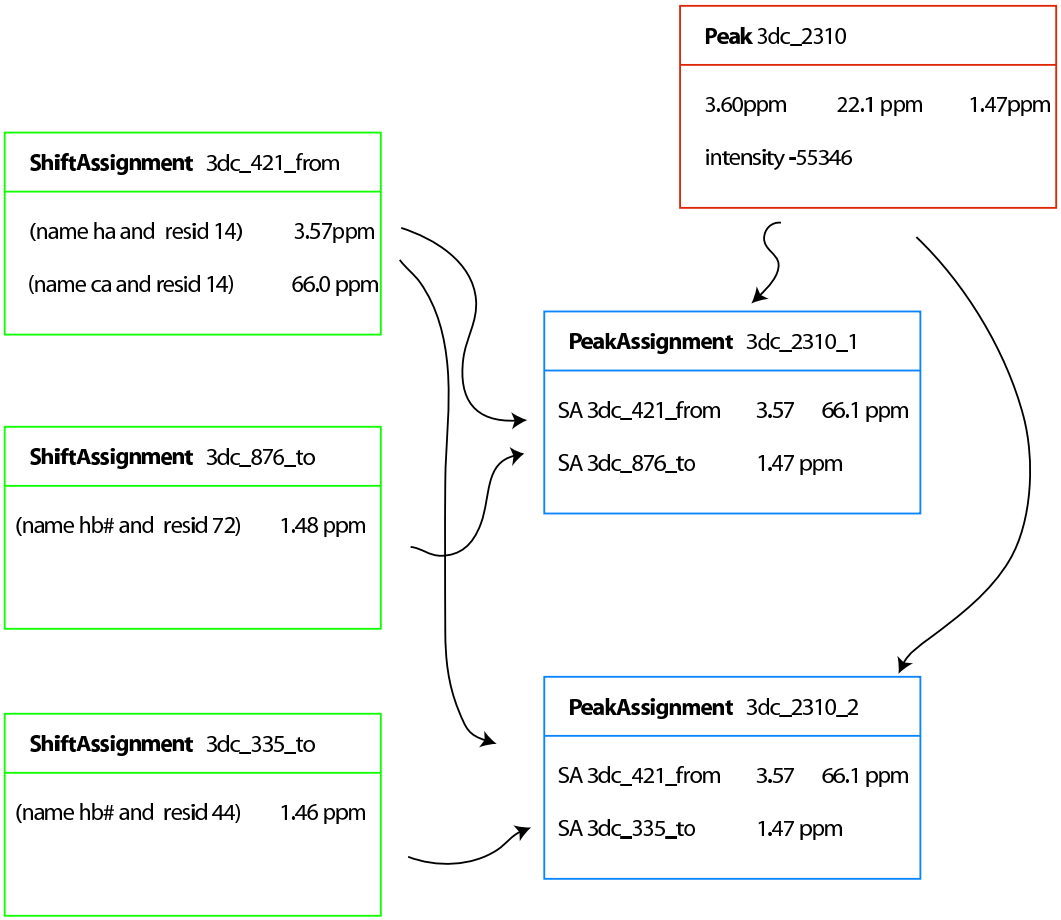
sometimes referred to as Marvin

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.
- 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).

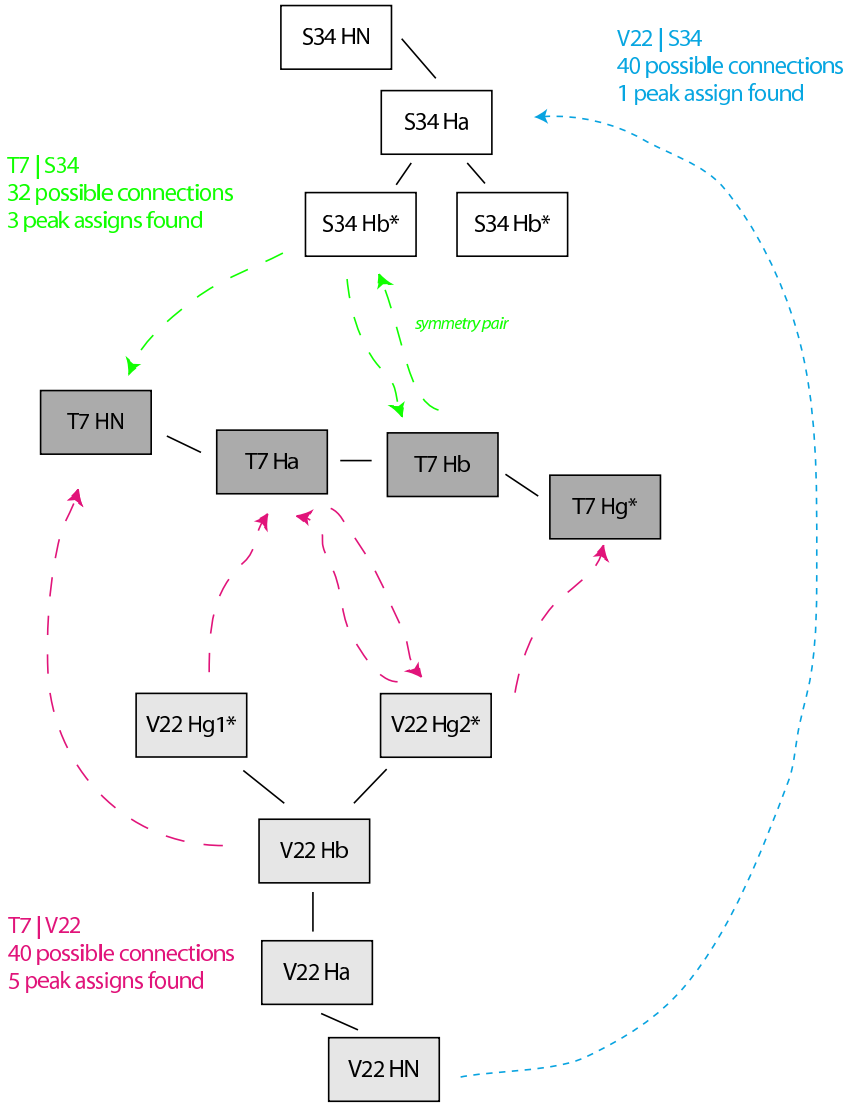
distances calculated using these selections using $1/r^6$ summing.

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

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

λ_i: likelihood of assignment i:

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

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

Δ_i - violation of assignment i

D_v - tunable parameter

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

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

assignment i is activated if random num between 0..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
incorrect restraint should have all likelihoods near 0
correct restraint should have one assignment with a likelihood near 1.

results:

- Published results for six proteins. 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 obtained.
- 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, pipp, xeasy.

Where to go for help

online:

`http://nmr.cit.nih.gov/xplor-nih/`	-	home page
xplor-nih@nmr.cit.nih.gov	-	mailing list
`http://nmr.cit.nih.gov/xplor-nih/faq.html`	-	FAQ
`http://nmr.cit.nih.gov/xplor-nih/doc/current/`	-	current Documentation, including the XPLOR manual

subdirectories within the xplor distribution:

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

Python:
M. Lutz and D. Ascher, "Learning Python, 2nd Edition" (O'Reilly, 2004);
`http://python.org`

TCL:
J.K. Ousterhout "TCL and the TK Toolkit" (Addison Wesley, 1994);
`http://www.tcl.tk`

Please complain! and suggest!