Dynamical properties of the calcium pump of sarcoplasmic reticulum: a normal mode analysis

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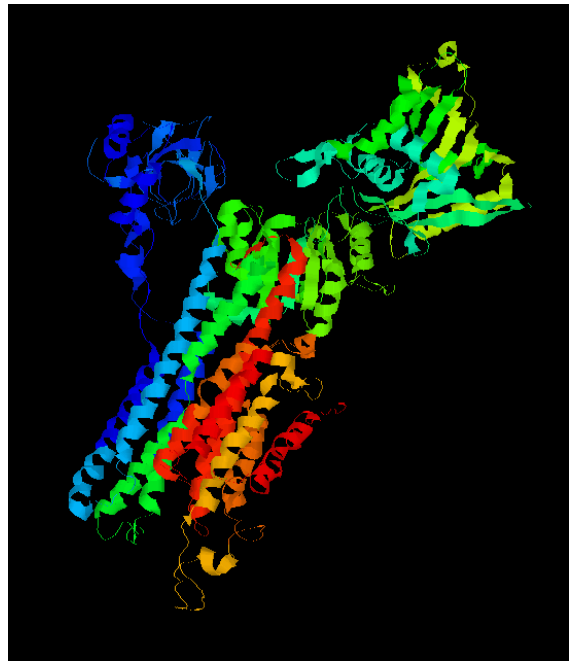
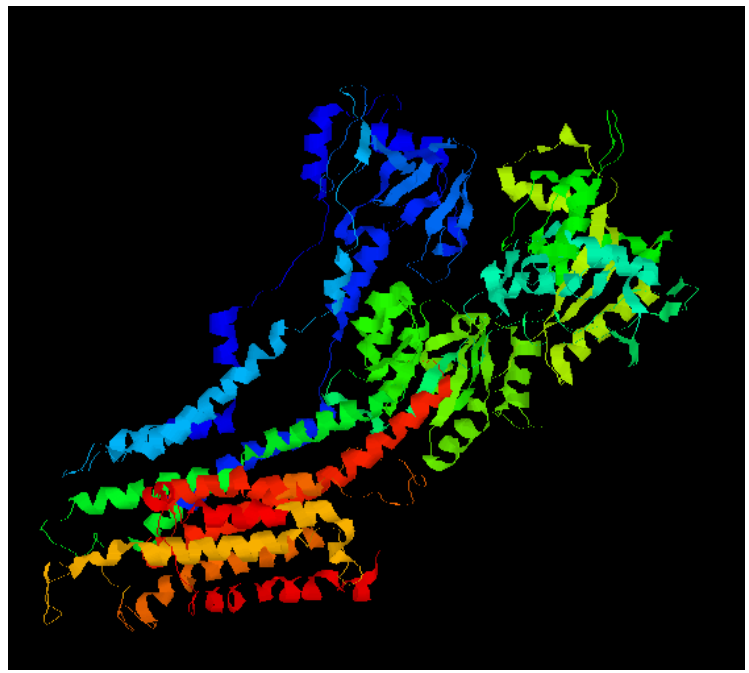
Structures

- 1) Bound Ca^{2+}



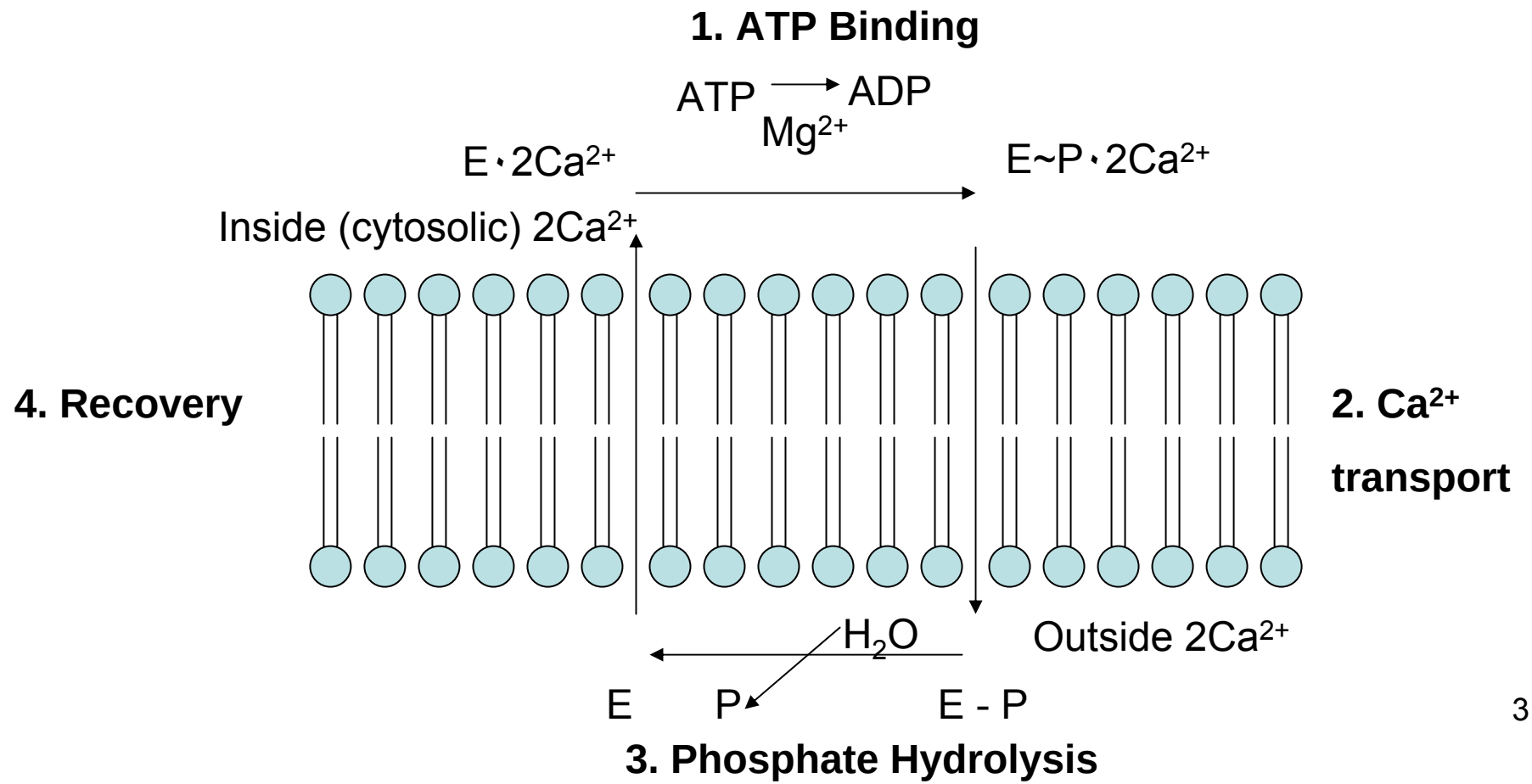
- 2) Dissociated Ca^{2+}



```
$ wc -l *.pdb
   8534 1IWO-A-domain.pdb
   8534 1IWO-B-domain.pdb
  16206 1IWO.pdb
   8268 1eul.pdb
  41542 total
```

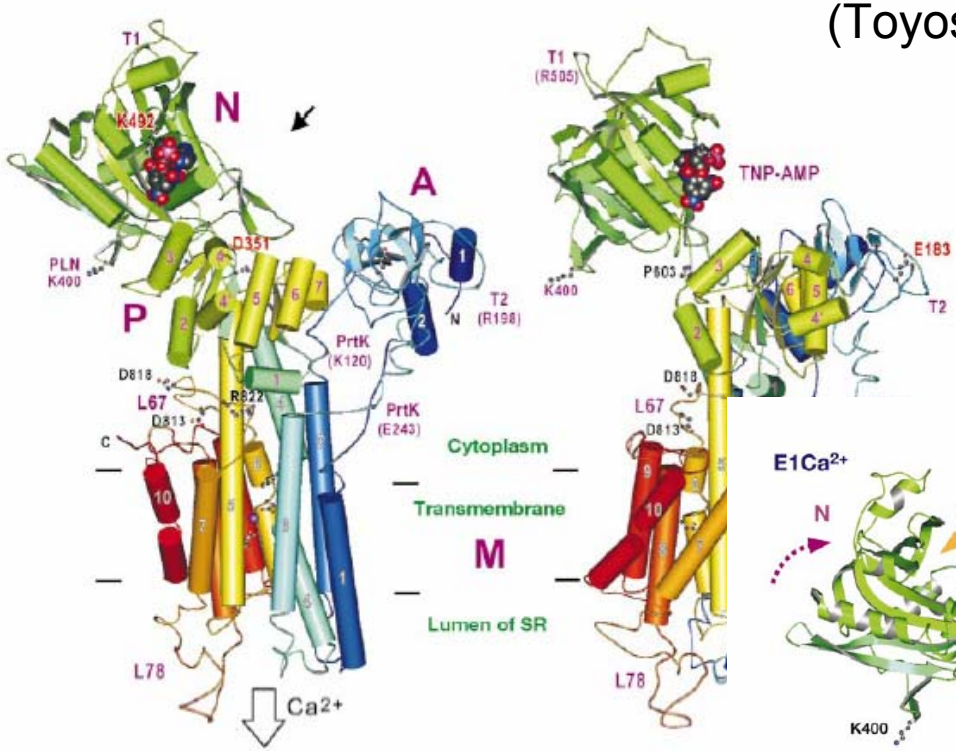
Ca^{2+}-ATPase

- Cytosolic [Ca^{2+}] effects muscle contraction, neurotransmitter release, glycogen breakdown, and oxidative metabolism.
- The concentration is maintained by Ca^{2+}-ATPase, as transported across the plasma membrane, the endoplasmic reticulum, and the mitochondrial inner membrane.

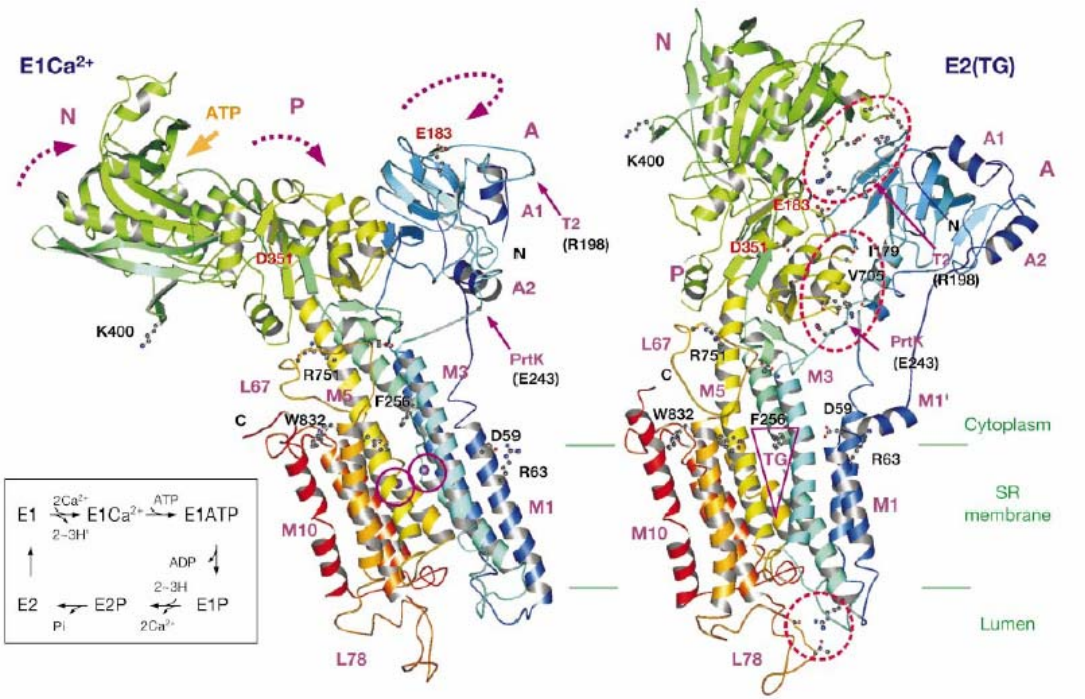


Three regions of 1EUL

(Toyoshima, 2000)



(Toyoshima, 2002)



On right: absence of Ca2+, presence of thapsigargin (TG)

Three regions of 1IWO

Normal Mode Math

$$[M]\{\ddot{x}\} + [K]\{x\} = \{0\} \tag{1}$$

$[M]$:= mass matrix of macromolecule

$[K]$:= stiffness matrix; second derivatives of potential energy of molecule

$\{x\}$:= displacement vectors of all atoms from their equil. positions

$\{\ddot{x}\}$:= second derivatives w.r.t. time

Let $\{x\} = \{\chi \sin(\omega t)\}$; χ are normal mode variables, ω are circular frequency variables.

$$\{[K] - \omega^2[M]\}\{\chi\} = 0 \tag{2}$$

Solving this Eq. yields natural frequencies and corresponding normal mode vectors. The harmonic dynamics of macromolecular system are fully described thus.

Approximate potential energy function by harmonic modes around minimum energy conformation. By diagonalizing the Hessian matrix of mass-weighted second derivatives of the potential energy arrive at analytical solution to equations of motion.

Eigenvectors are the normal modes; eigenvalues are the squares of the associated frequencies.

Software Tools for Normal Modes

- MMTK – NormalModes.py
- Tinker

  http://starship.python.net/crew/hinsen/MMTK/

  – pdbxyz.f
  – vibrate.f
  – sizes.i (10000->30000)

  http://dasher.wustl.edu/tinker/
- AMBER – nmode
  – 122 Fortran files
  – 17K lines

2377 bio01a@wart /gt/lib1/Library/amber7/exe> ./nmode

usage: nmode [-O] -i nmdin -o nmdout -p prmtop -c inpcrd
-r restrt -ref refc -v vecs -t tstate -l lmode -e expfile

Nmode script

```
#!/bin/csh -f

# Sample Run Nmode Script

set AMBER1=/gt/lib1/Library/amber7/
/bin/rm nmode.in
/bin/rm nmode.out
/bin/rm heme.vecs
set DIR=$AMBER1
#
cat << eof > nmode.in
Test of normal modes on heme
&data ntrun=1, cut=12.0 , drms=12.0, nvect
=255, &end
eof
#
$DIR/exe/nmode -O \
-i nmode.in \
-o nmode.out \
-c min2.xyz \
-v heme.vecs || goto error
/bin/rm -f nmanal.out
cat << eof > nmanal.in
normal mode analysis, rms fluctuations
&data
ntrun = 1, nvect=255, iend=255,
pcut = 1e-3,
&end
eof
$DIR/exe/nmanal -O -i nmanal.in \
-v heme.vecs \
-o nmanal.out || goto error
exit(0)
error:
echo "Failure: run.nmode check .out and
retry"
exit(1)
```

http://www.bioinfo.no/tools/normalmodes

Deformation Energies

Deformation energies

A number of 200 normal modes have been calculated for your structure, 20 have been kept for the analysis.

Normal mode index	Deformation Energy
7	134.221838224
8	252.279288901
9	344.292696219
10	503.726451178
11	537.070029852
12	927.866556353
13	795.259193386
14	1228.03770082
15	1329.00229421
16	1862.39033308
17	2155.85457904
18	2094.99246412
19	2682.35044576
20	2683.31640169

elapsed time (normal modes calculations) 236.86 seconds

Deformation energies = average deformation energy per residue for each mode

a deformation energy is associated with every atom; low values characterize rigid regions, whereas high values indicate flexible regions. A low average deformation energy thus indicates a mode with large rigid regions, which has a good chance of describing domain motions.

Although the energy scale for the deformation energies is arbitrary (see Analysis of domain motions in large proteins" by K. Hinsen, A. Thomas, and M.J. Field for a detailed discussion), it is nevertheless an absolute scale independent of the specific protein. This means that deformation energy values can be compared between proteins and, in the case of a normal mode based analysis, between modes. Example of typical multi-domain proteins: first mode (#7) of the SERCA1 Ca-ATPase had an average deformation energy of 134.2, lysozyme: 2378.5, MscL homologue (1msl.pdb): 794.97. On the other hand, a trypsin (1ANB) has a deformation energy of 5881.7 for mode 7.

Normalized Squared Atomic Displacements & Vector Field

Normalized Squared Atomic Displacements & Vector Field

The normalized squared atomic displacements and vector fields are calculated for modes 7 to 12.

Normal Mode index	Plot file (pdf)	Raw data at the (x,y) format	Vector Field at the VMD format
mode7	pdf plot	raw data	vmd file
mode8	pdf plot	raw data	vmd file
mode9	pdf plot	raw data	vmd file
mode10	pdf plot	raw data	vmd file
mode11	pdf plot	raw data	vmd file
mode12	pdf plot	raw data	vmd file
all modes from 7 to 12	pdf plot	raw data	pdb for visualization of vector field

Normalized squared atomic displacements:

square of the displacement of each Calpha atom, normalized so that the sum over all all residues is equal to 100. Highest peaks on the plots thus correspond to the most displaced regions. One should look for cluster of peaks, those identify significantly big regions. Isolated peaks reflect local flexibility and are not relevant.(see Reuter et al., Biophys. J., 2003)

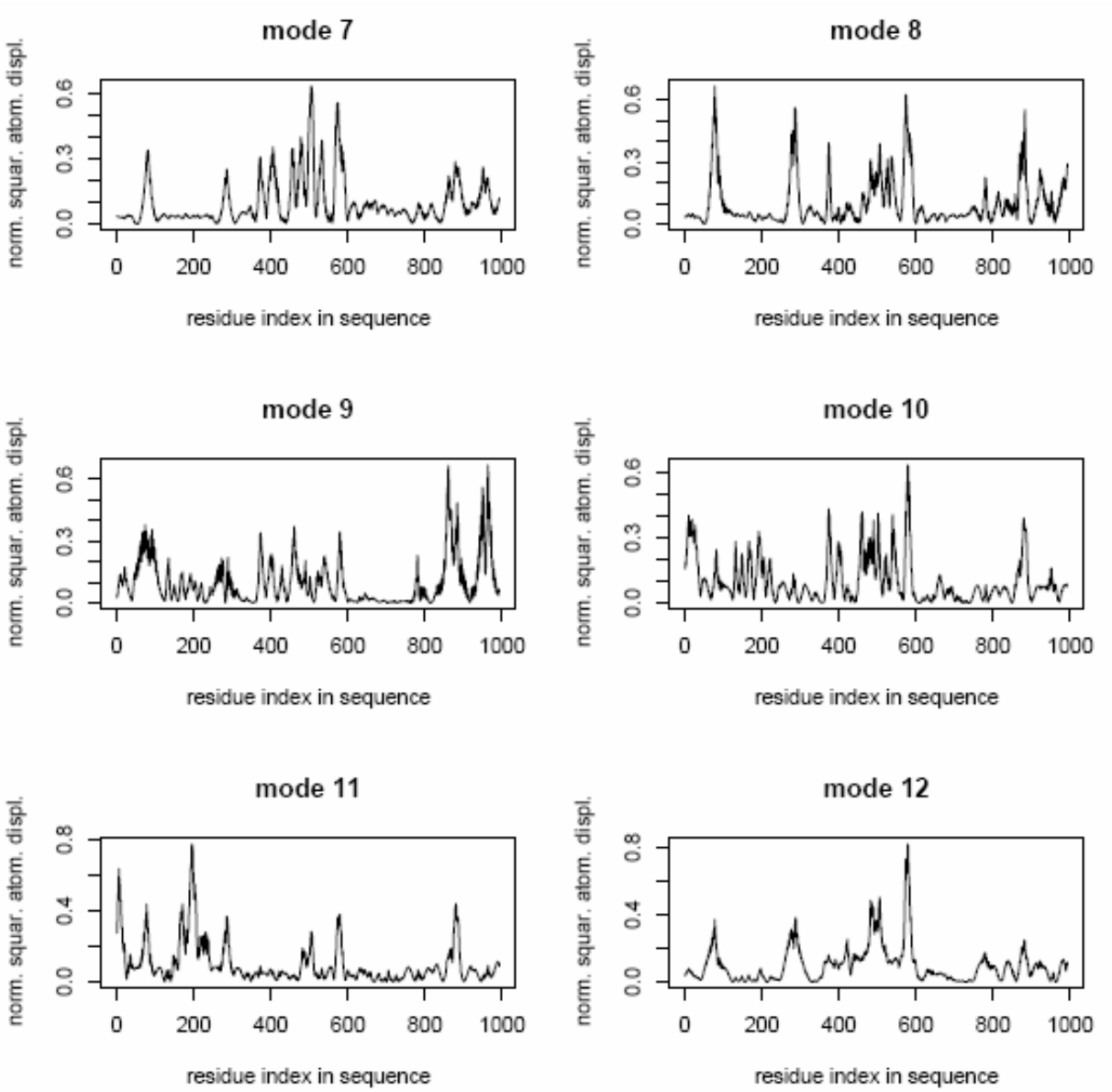
Vector field:

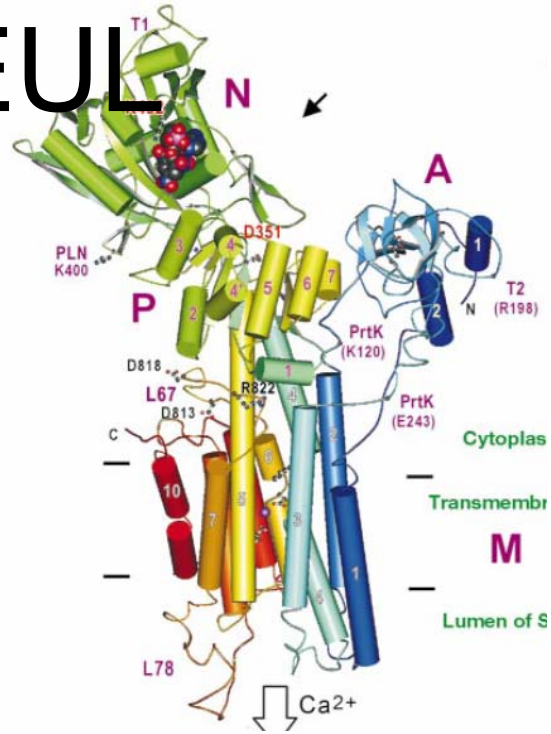
The vector field representation is calculated as described by Thomas et al. (Proteins, 1999). The vector field is calculated over cubic regions with an edge length of 3 Angstroms, containing on average 1.3 Calpha atoms. The vector field defined on a regular lattice at the center of each cube is the mass-weighted average of the displacements of the atoms in the cube.

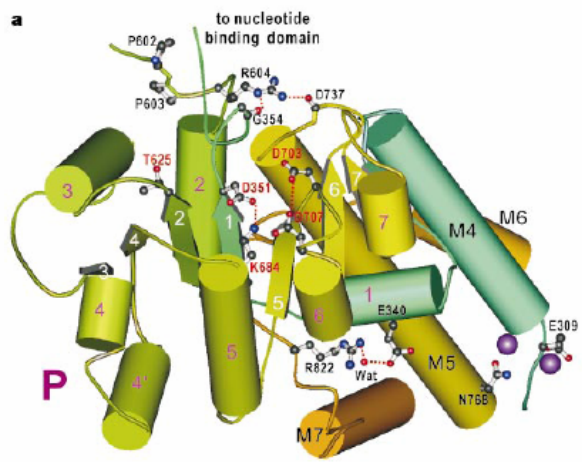
VMD files: the vector field can be visualized using the VMD program. (1) Download the vmd file corresponding to the mode you want to visualize.(3) Launch VMD on your computer and load the pdb file you submitted to our server. (3) Use 'load state' to load the vmd file.

Plot Modes – 1EUL

elapsed time (normal modes calculations) 236.86 seconds

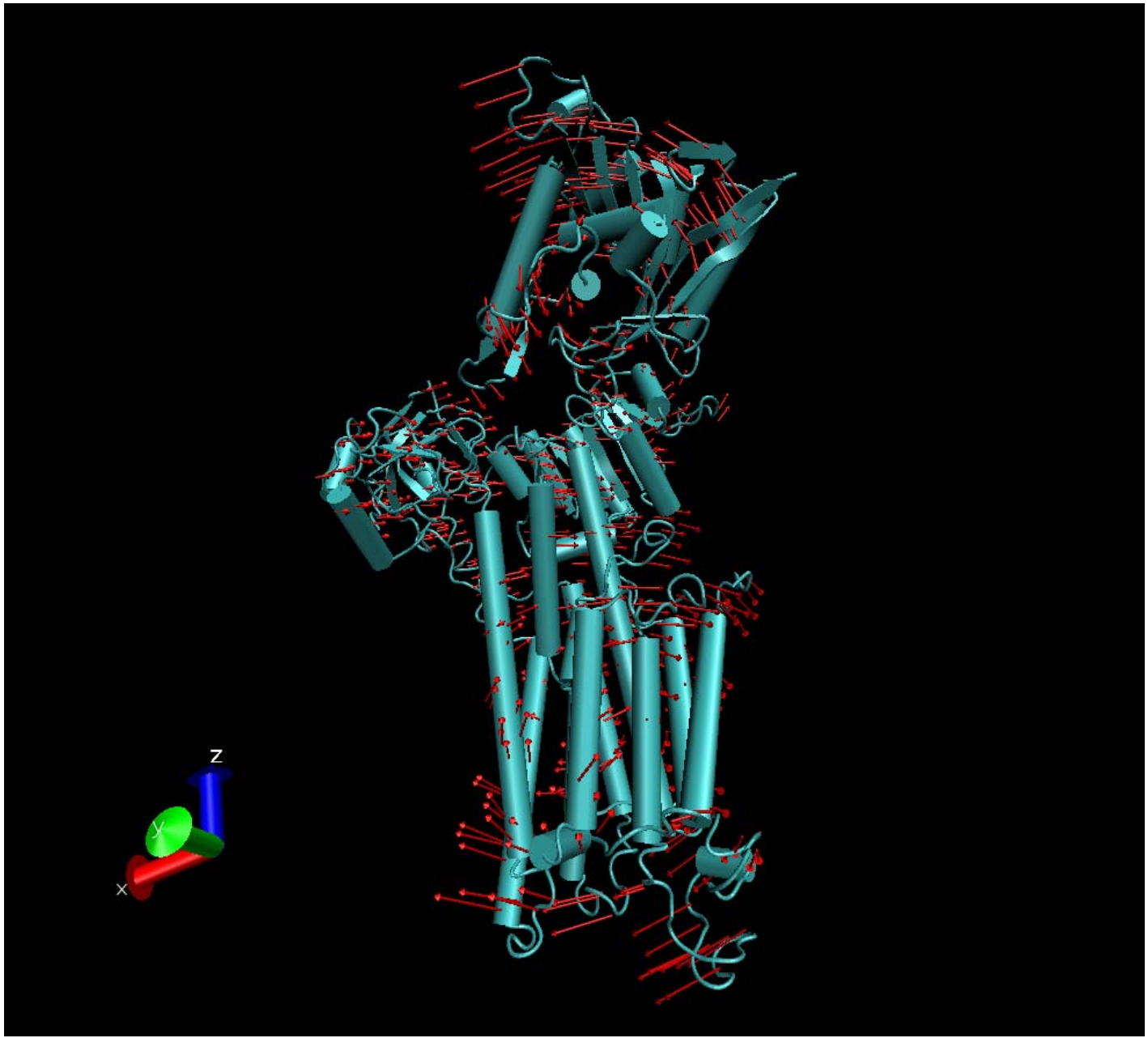






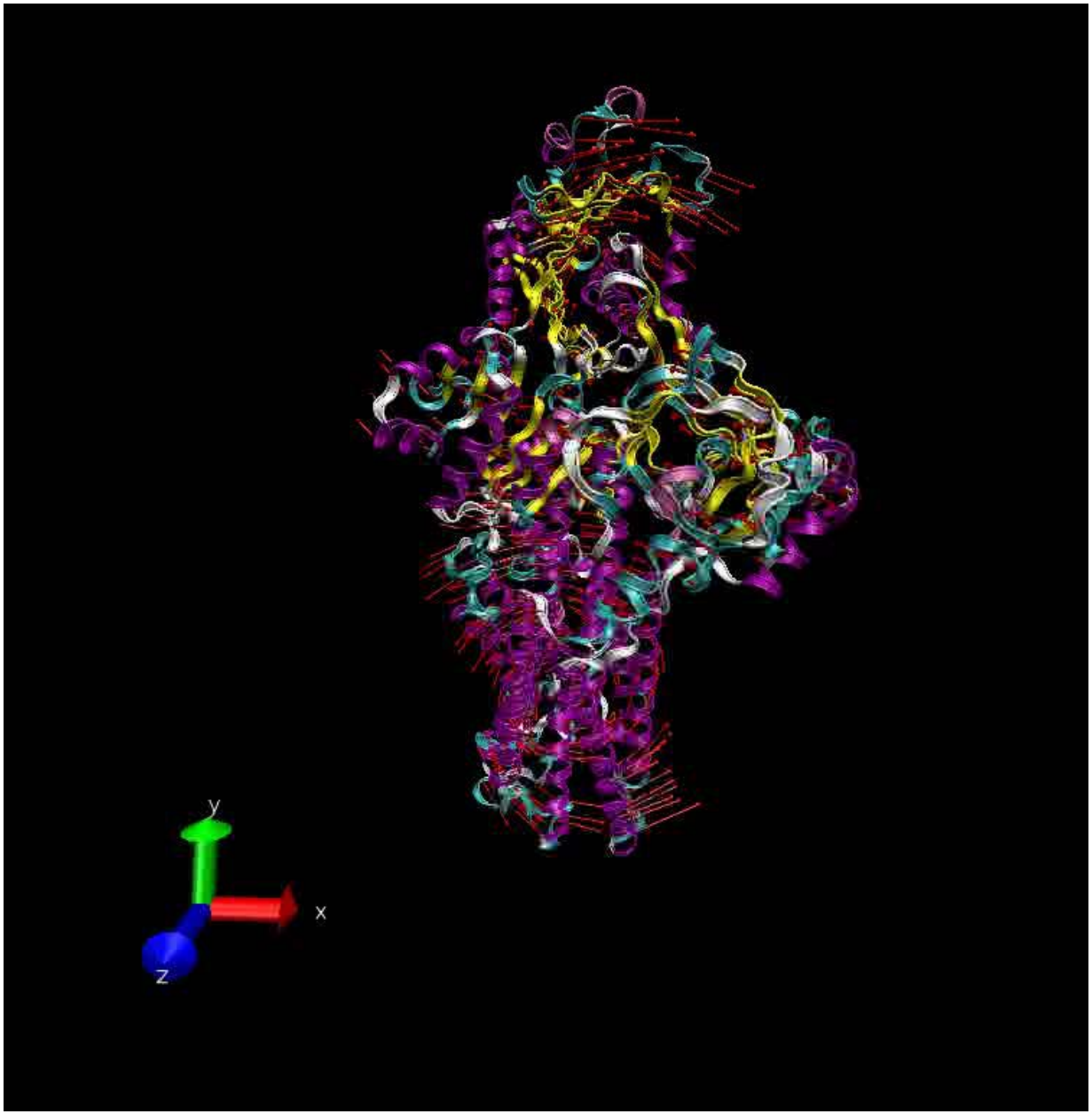
Architecture of Phosphorylation domain (D351 is phos. site)

Normal Modes Illustrated



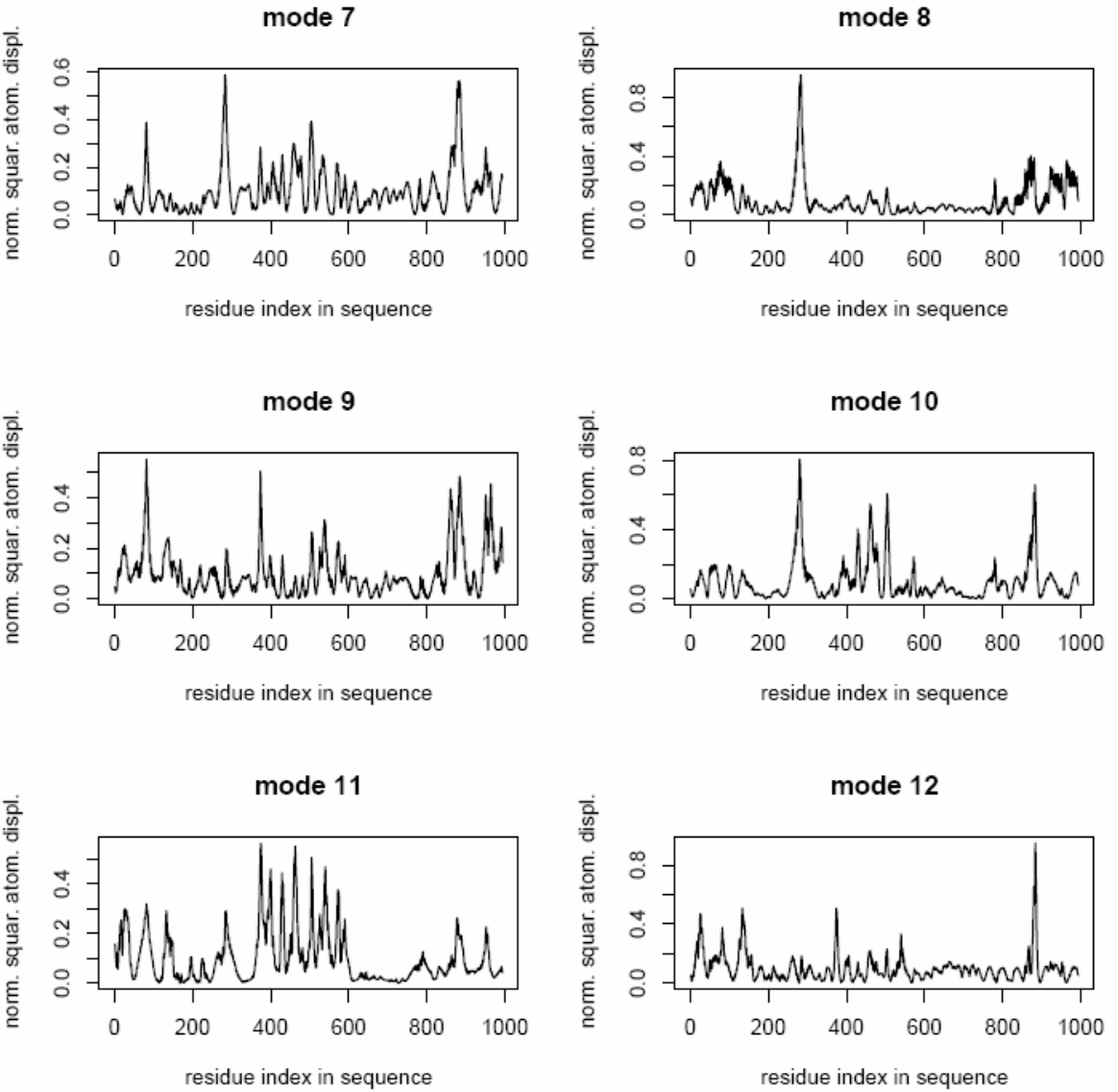
1) “Load State…” 1EUL-mode7.vmd
2) “New Mol...” 1EUL.pdb

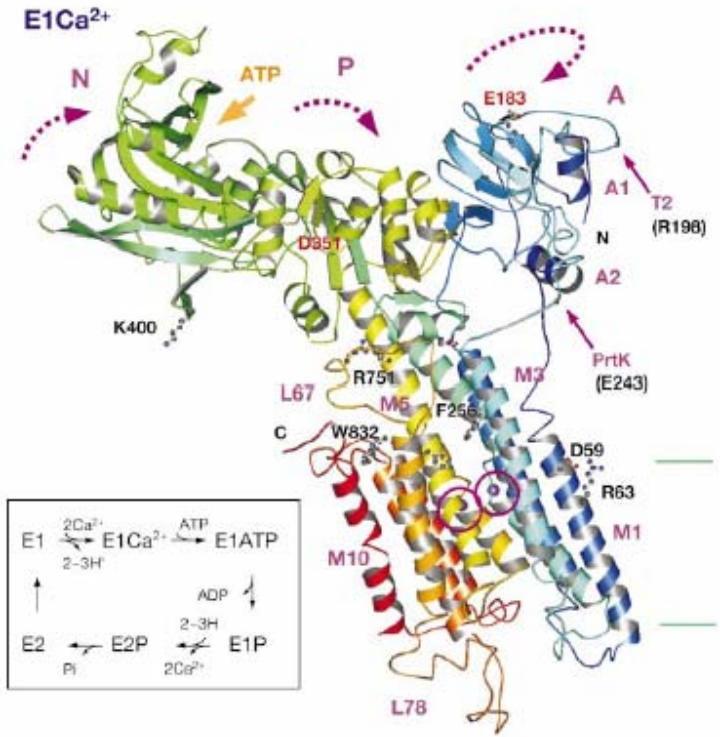
Normal Mode Vectors Animated



Plot Modes – 1IWO – A chain

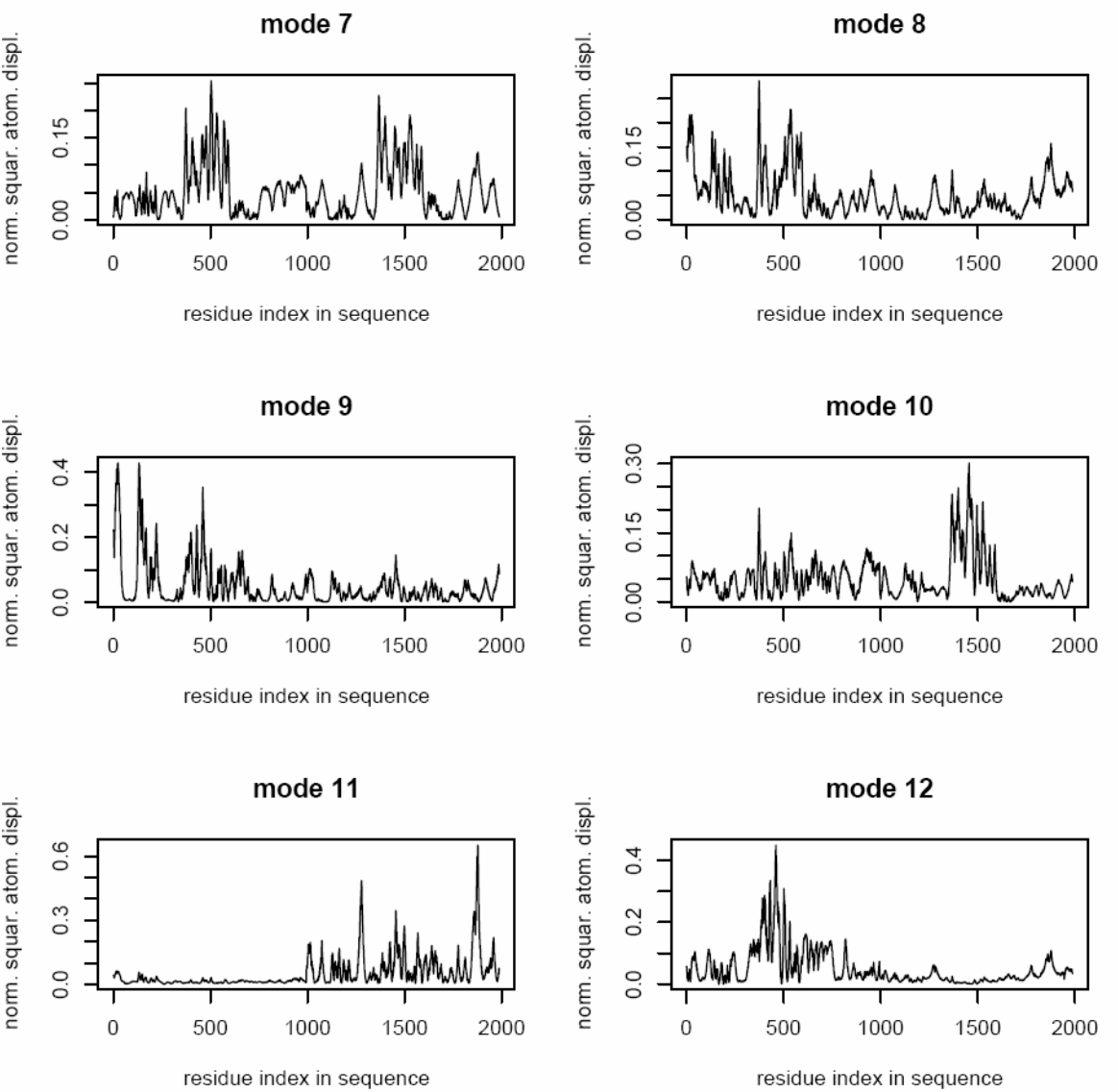
elapsed time (normal modes calculations) 246.61 seconds

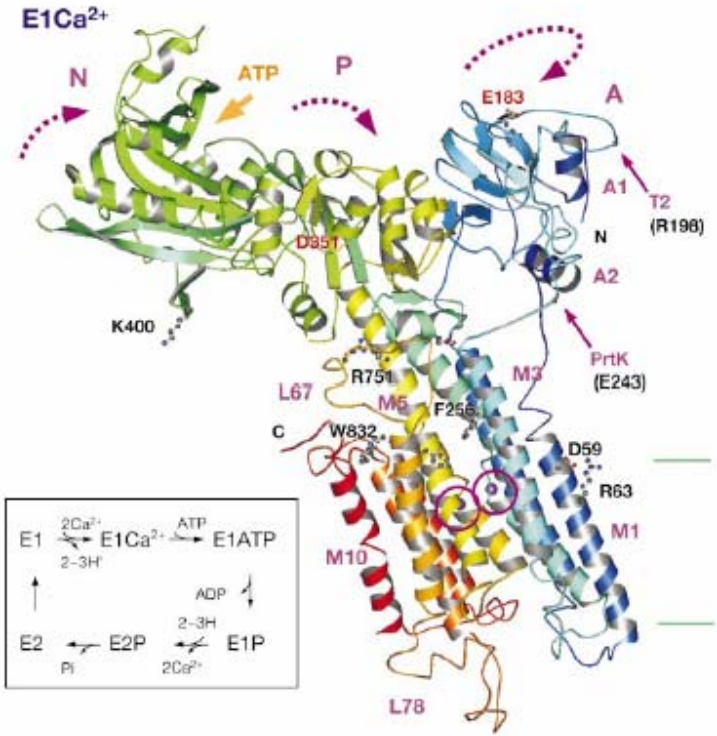




Plot Modes – 1IWO (2 prots)

elapsed time (normal modes calculations) 1359.03 seconds





http://molmovdb.mbb.yale.edu/molmovdb/nma/

molmovdb.org

Normal Mode Analysis of Protein Motions

with correlational study of fold flexibility

[Tutorial and Examples | Citation]

This tool allows the user to upload a query structure (or choose it from the motions database), calculate its lowest frequency Normal Mode, build the movie of this vibration and compare it with the pre-calculated flexibility regions based on either supplied B-factors or multiple structural alignment for the corresponding fold family (for single-domain queries).

PDB ID: Chain: (example: 4hhb)

or:

Upload PDB File: Browse...

☐ Use structural fold model for flexibility calculations (default: B-factors)

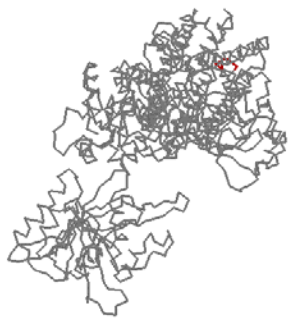
☐ Show five lowest normal modes (default: single lowest only)

Submit

Normal Mode Movies – Bound Ca^{2+}

- First five modes




Normal Mode Movies - Dissociated Ca^{2+}

- First five modes





Flex factors

Bound Ca^{2+}

Dissociated Ca^{2+}

Morphing between dissociated and bound structures

- Morphing is “adiabatic mapping”, but when applied in the CNS context means:
  1) Interpolate
  2) Minimize
  3) Repeat …

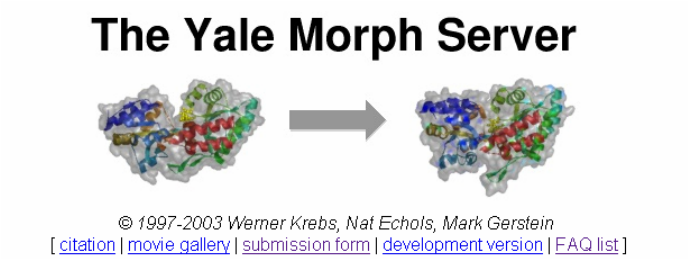
Make Movies in VMD!

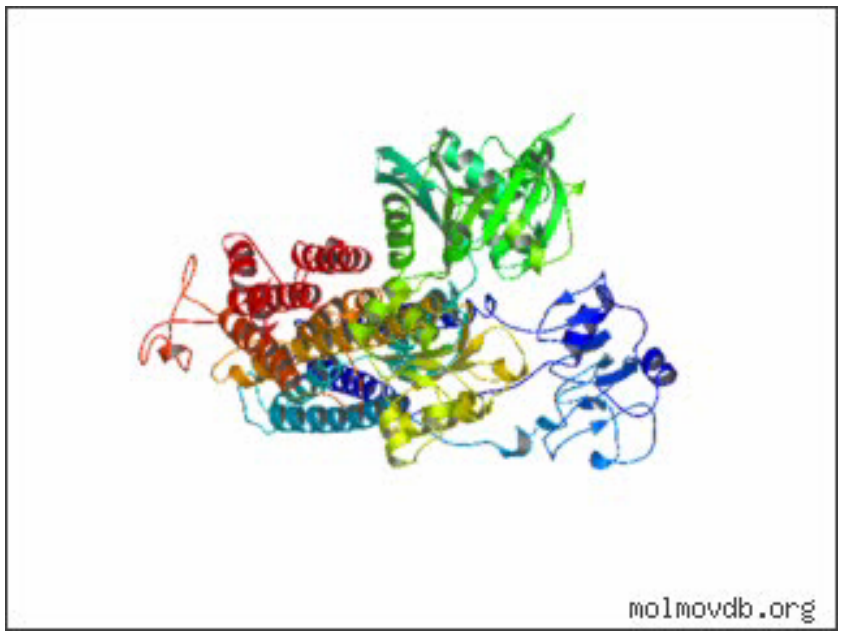
- Morphing (or another process)generates *n* PDB files
- $ source c:/animatepdbs.txt
- $ animatepdbs 0 32 "foo%d.pdb"
- Hit “Go” in the VMD frame editor
- $ vmdmovie

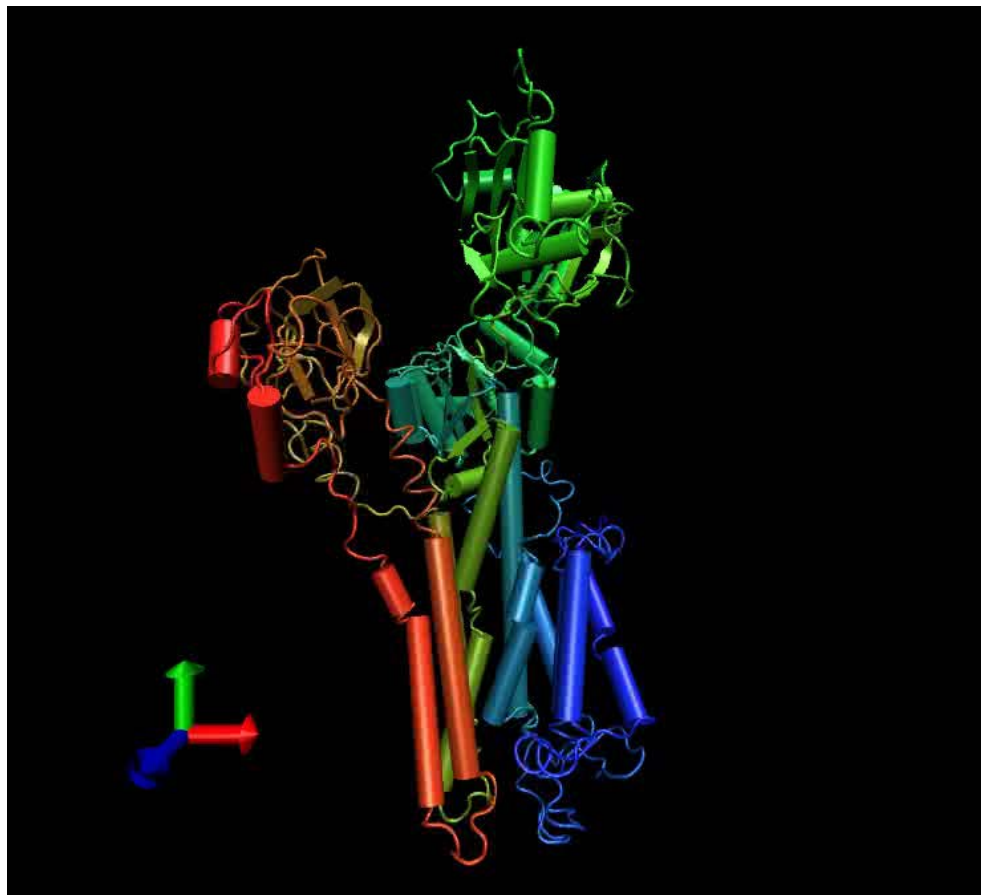
  (options, rendered, or bitmapped …)

Morphing between two X-ray structures

http://molmovdb.mbb.yale.edu/molmovdb/morph/





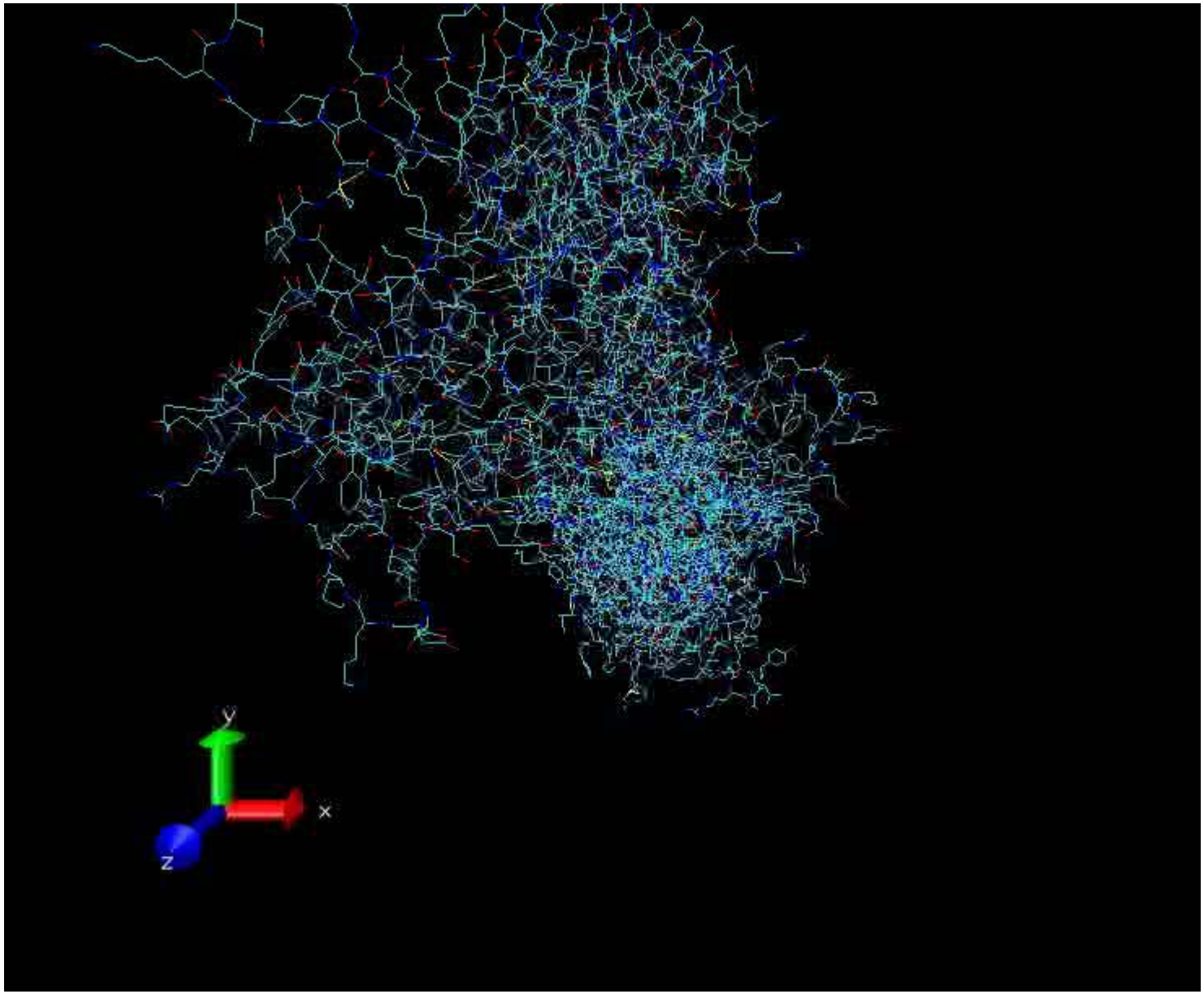


Future Directions

- MMTK uses deformation force field model
  (every residue approximated by virtual atom centered at C-α position)
- Examining certain subsets of lowest modes is often desirable
- Refine global dynamics
- Calculate cumulative square of overlap between the mode and vector difference, as function of mode number, for closed and open forms
- Compare to homology models
- Remove certain areas, like P domain in 1IWO coordinates movement of transmembrane and cytoplasmic domains
- Compare hinge region results to MD simulation
- Acts as ensemble of rigid bodies, is α-helix always rigid?
- MMTK's author, Hinsen, says,

> "If you do want to work with an all-atom model, but need only low-frequency modes, you could try subspace normal modes with the Fourier space. Finally, if you want the high-frequency modes, just cut your molecule into pieces and study them separately. The biggest protein complex I ever treated with MMTK had 8700 residues, I used a C-alpha model plus a Fourier subspace."

(1EUL has 994 AA)


y
x
z