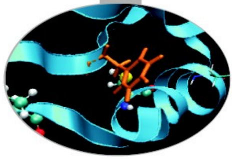
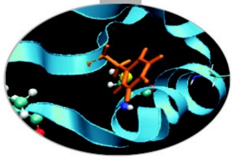


Analysis of MD trajectories (Essential Dynamics of Proteins)

Neva Bešker



Molecular dynamics MD computer simulation method for studying the physical movements of atoms and molecules.

The atoms and molecules are allowed to interact giving a view of the dynamical evolution of the system. The trajectories of atoms and molecules are determined by numerically solving Newton's equations of motion for a system of interacting particles, where forces between the particles and their potential energies are calculated using interatomic potentials or molecular mechanics force fields.

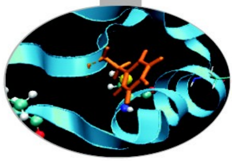
What if MD were
- perfectly accurate
- infinitely fast

Could easily perform arbitrary computational experiments
- understand dynamics by watching the system move
- transform messy et-stuff into nice dry data mining

But … MD is not infinitely fast

We know that biological phenomena occurs between 10 microsecond – some ms
- structural changes
- interactions with other proteins/nucleic acids/ drugs
- folding

Do you remember how many ns/day you did in Tutorial 1?



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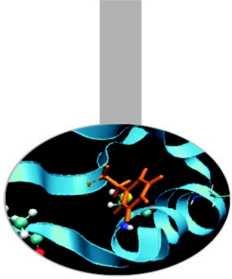
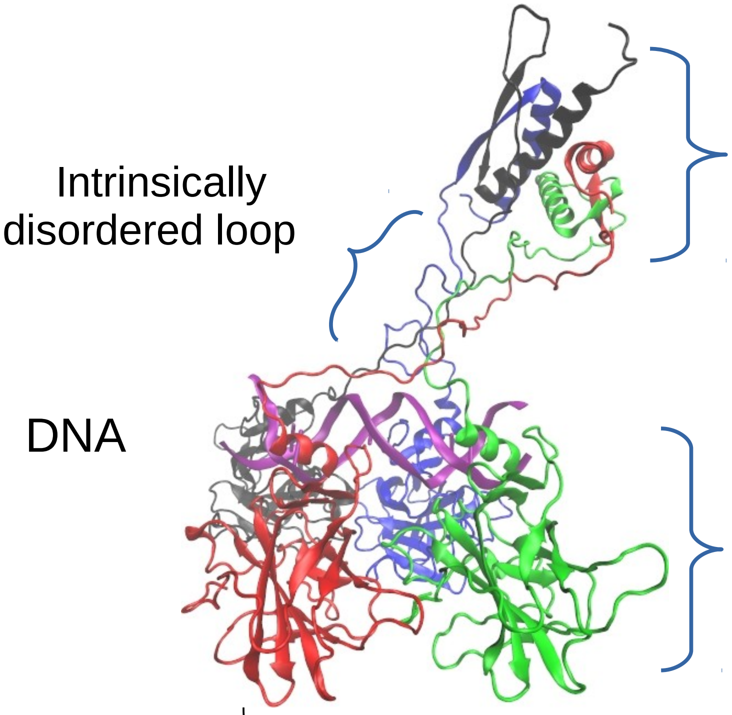
Do you remember how many ns/day you did in Tutorial 1?

What do long trajectories look like?

p53 example





Intrinsically disordered loop

DNA

C-terminal Domain

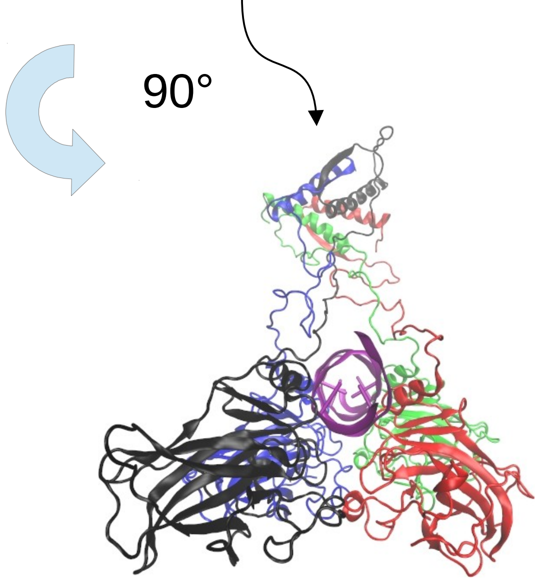
TEtramerization Domain

DNA Binding Domain

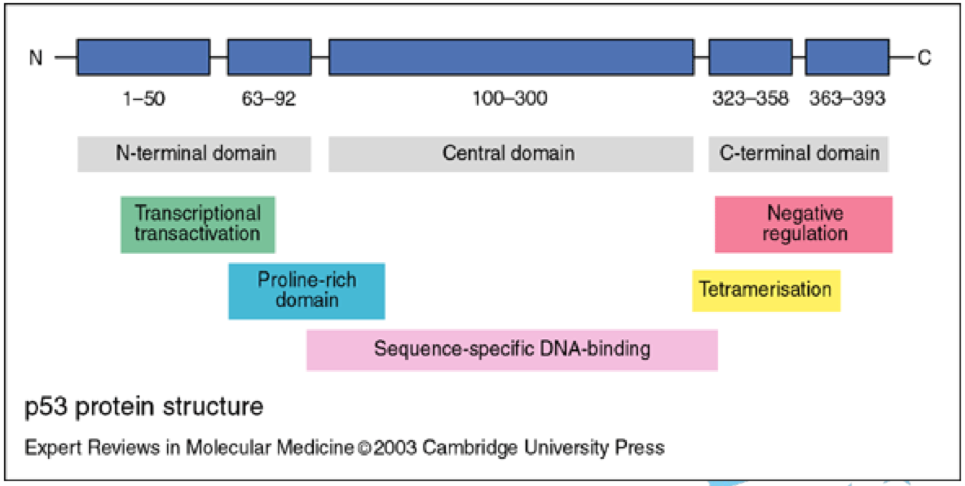
Central domain

4 MD of 25 ns

~ 347000 atoms



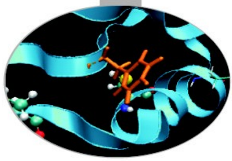
90°



p53 protein structure

Expert Reviews in Molecular Medicine ©2003 Cambridge University Press

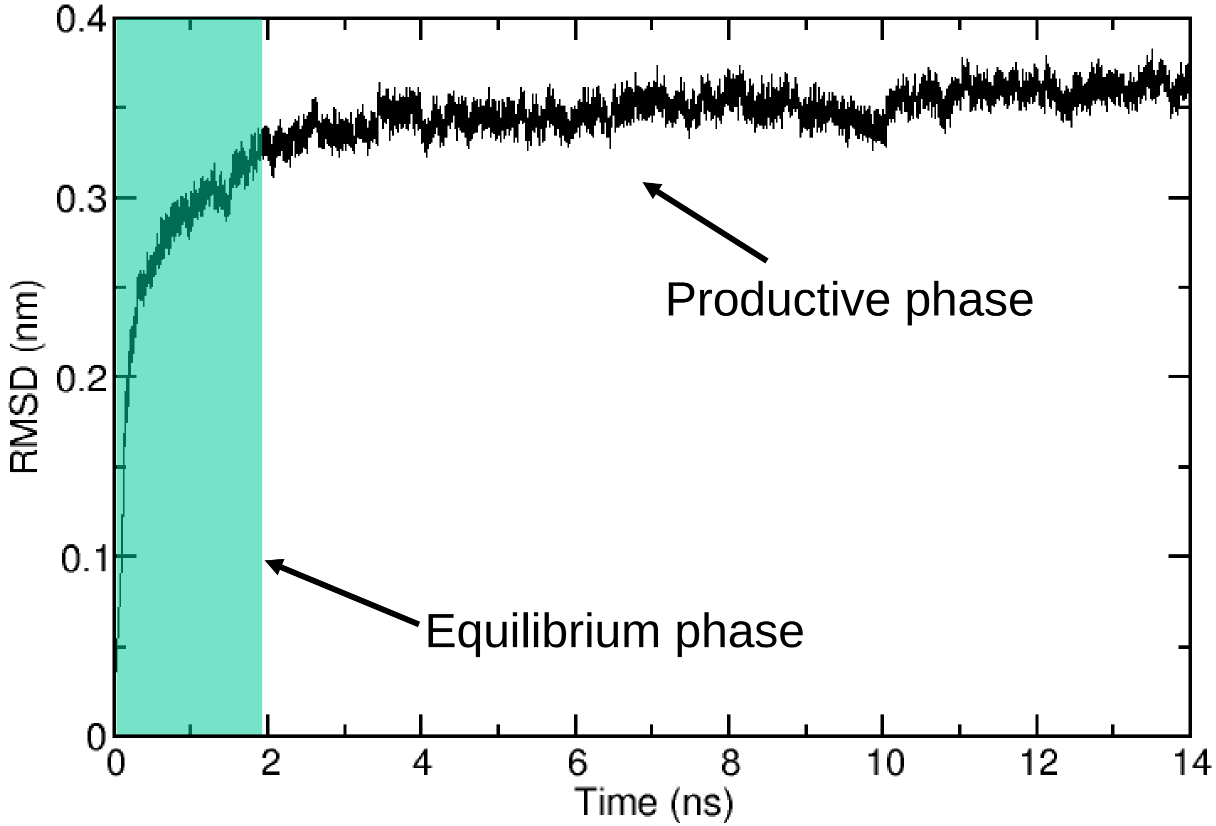
Did we reach equilibrium…?



Gromacs tool: gmx rms

$$= \left[\frac{1}{M}\sum_{i=1}^{N} m_i \|\mathbf{r}_i(t_1)-\mathbf{r}_i(t_2)\|^2\right]^{\frac{1}{2}}$$

We need to make sure that all the chemical and physical properties of the system have reached an equilibrium, where their averages do not longer change as a function of time. A simple way to test this is by measuring the RMSD (root mean square deviation) of Cα carbon atoms position with respect to start.

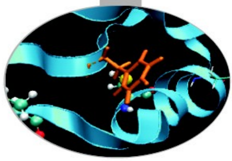


Productive phase

Equilibrium phase

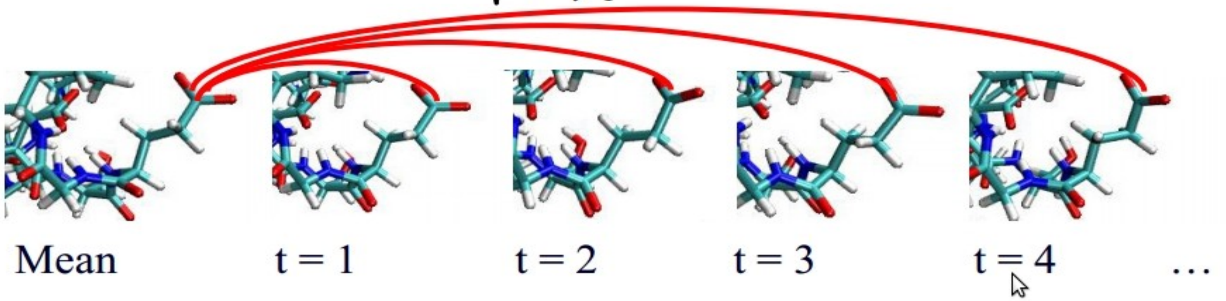
Measuring chain flexibility

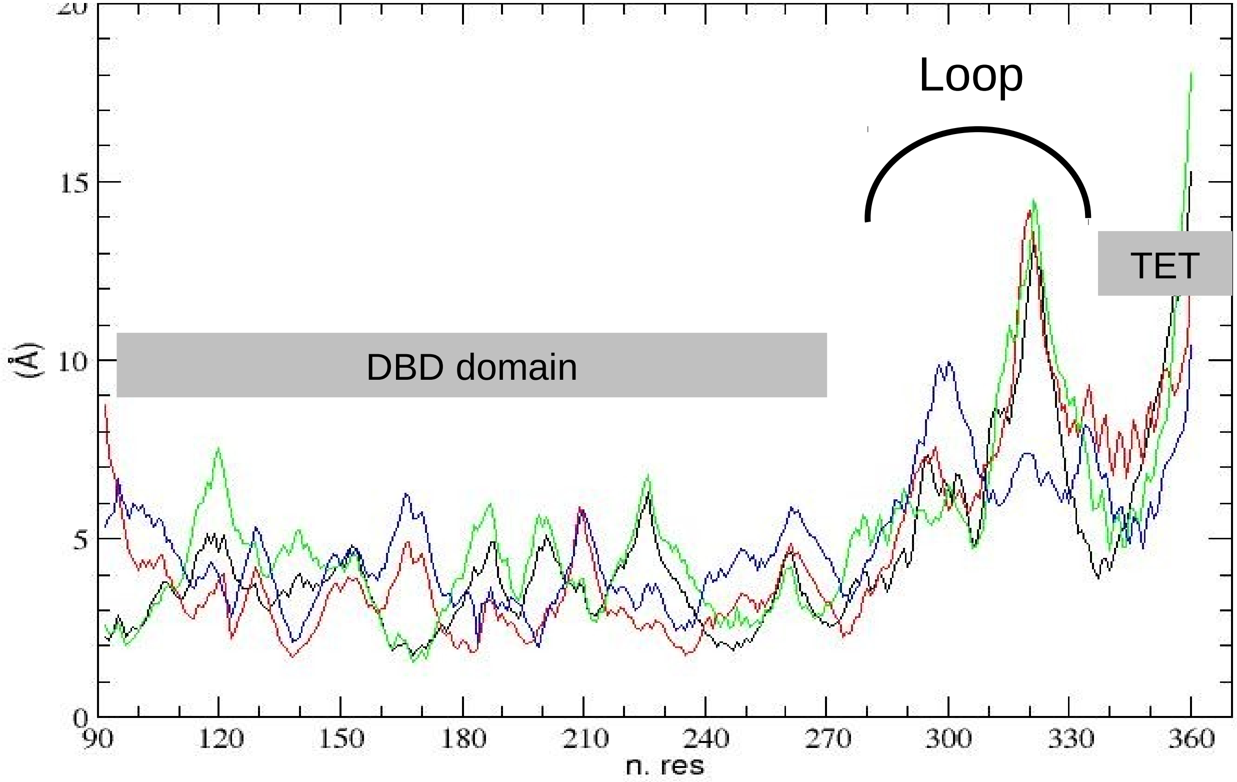
p53 in water



$$RMSF_{(v)} = \sqrt{\frac{1}{T}\sum_{t=1}^{T}(v_t - \bar{v})^2}$$

T = #(Frames)

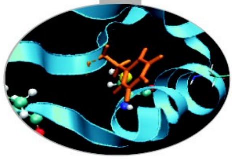




RMSF is an analysis to measure the flexibility of the polypeptide chain. It calculates the fluctuation of C-alpha atoms coordinates from their average position.

D'Abramo et al. Oncogene, October 2015;doi: 10.1038/onc.2015.388

Essential Dynamics analysis



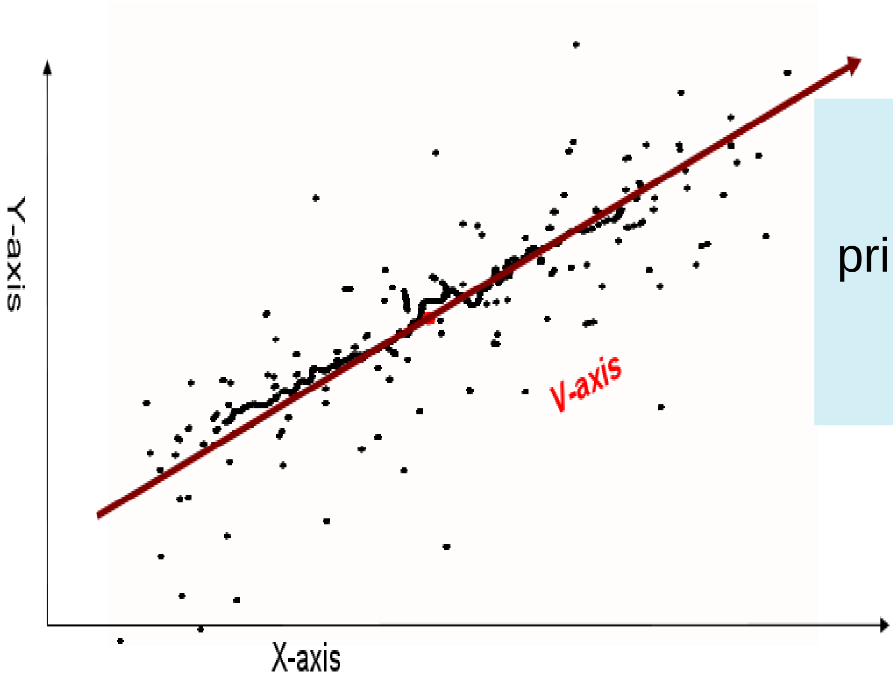
Essential dynamics analysis

How represent the "principal motion directions" of large systems?

The essential dynamics (ED)* is a technique able to represent the principal motion directions by a set of eigenvectors

(look at eigenvectors as important motion directions!)

Example - reducing bi-dimensional to monodimensional

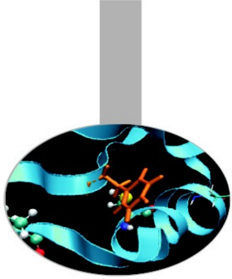


used for biological systems

principal modes ⟷ biological function

Based on PCA

Essential Dynamics: workflow in GROMACS



Least square fit of protein coordinates with respect to a reference structure to remove roto-translation in the simulation box.

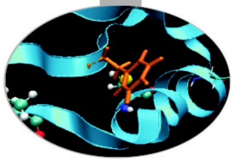
Calculate elements of the positional fluctuations covariance matrix of the Cα protein carbon atoms.

$$C_{ij} = \left\langle M_{ii}^{\frac{1}{2}}(x_i - \langle x_i \rangle) M_{jj}^{\frac{1}{2}}(x_j - \langle x_j \rangle) \right\rangle$$

Diagonalisation of the covariance matrix and output of the corresponding eigenvectors and eigenvalues.

Sort eigenvector in descending eigenvalue index and determine principal components

Eigenvalue equation



$$Cv=\lambda v$$

Eigenvectors

Eigenvalues

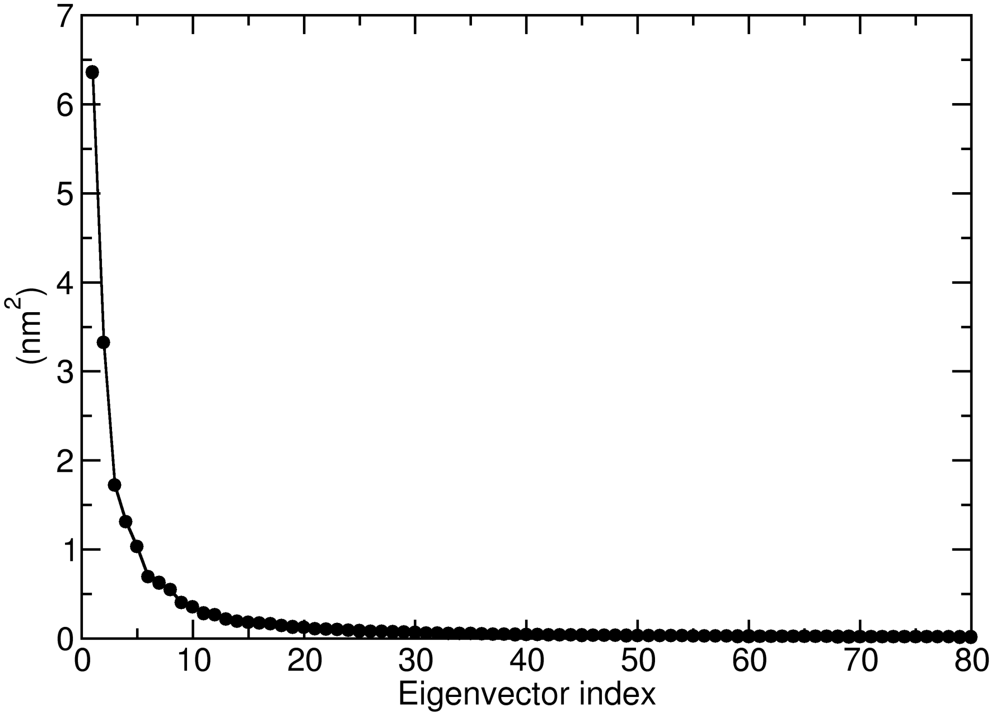
Eigenvectors represent directions where the σ^2 returns its maximum value.

Moreover, it can be shown that σ^2 are numerically equivalent to calculated eigenvalues

Eigenvectors, also called principal or essential modes.

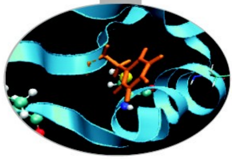
R defines a transformation to a new coordinate system. The trajectory can be projected on the principal modes to give the principal components

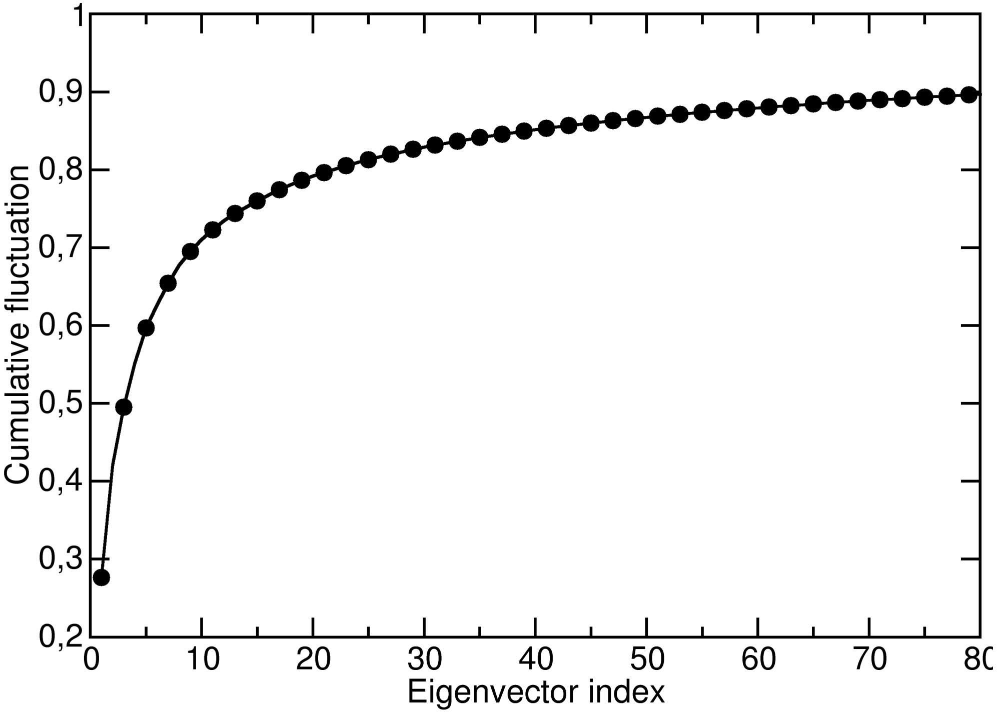
Essential Dynamics of Proteins



Eigenvalues are sorted in descending order: the first one corresponds to the maximum variance of the projected points. The corresponding eigenvectors are the best principal components of associated eigenvalues.

Essential Dynamics of Proteins

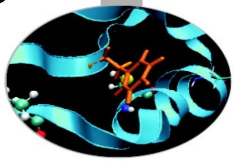




$$CF = \frac{\lambda_1 + \lambda_2 + \cdots + \lambda_n}{\sum_{i=1}^{N} \lambda_i}$$

It can be shown that about 70-75 % of all cumulative protein fluctuation is spanned by the first 10 principal components (eigenvectors)

Essential Dynamics: general procedure



Essential Dynamics Analysis is based on the computation of the elements of positional fluctuations covariance matrix of protein Cα carbon atoms as follows:

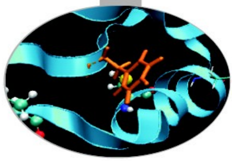
$$\Gamma_{ij} = \frac{1}{n}\sum_{h=1}^{n}\left(x_{hi}-\bar{x}_{i}\right)\times\left(x_{hj}-\bar{x}_{j}\right)$$

```
gmx covar –f traj.xtc –s reference.tpr –b start –e end –ascii
```

Output files:

Eigenvec.trr	→ eigenvectors file
Eigenval.xvg	→ eigenvalues file
Covar.dat	→ covariance matrix in ascii

Principal components analysis



```
gmx anaeig –f trajectory.xtc –v eigenvec.trr
         –s reference.tpr –b start –e end -first eig-first -last eig-last
```

gmx anaeig reads a set of eigenvectors and eigenvalues as input files and returns a set of output files that can be selected using appropriate flags:
Here are some examples:

-proj to project an MD trajectory along a selected eigenvector

-2d to project an MD trajectory in two dimensions of selected eigenvectors (essential subspace)

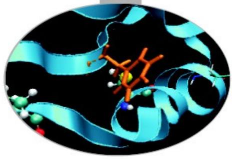
-rmsf to plot the RMS fluctuation per atom of selected eigenvectors

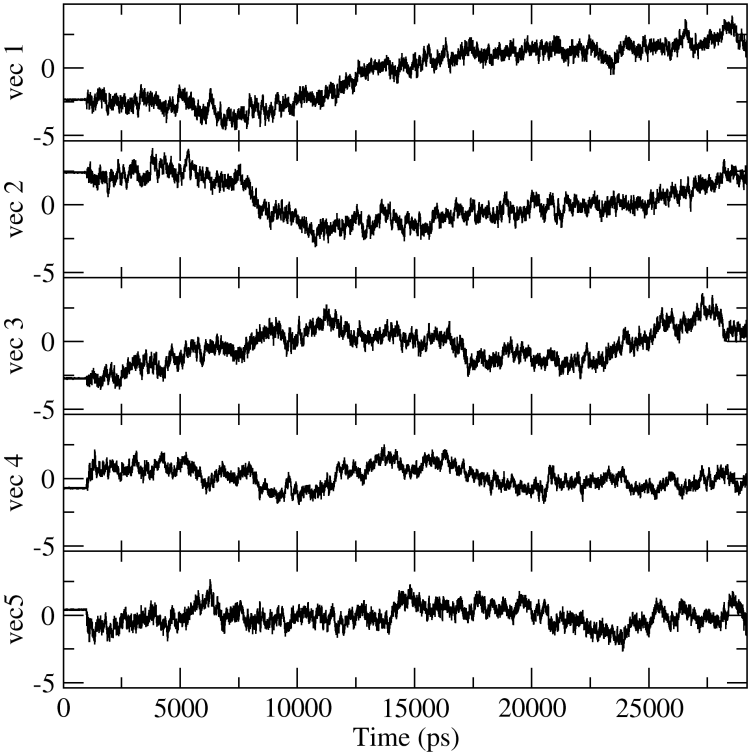
-comp to extract the eigenvector (atomic) components

-extr to extract structures projecting on the extreme of the selected eigenvectors

-filt to filter trajectory along selected eigenvectors

gmx anaeig: output of flag –proj

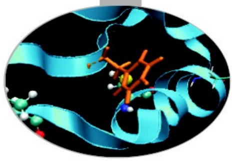


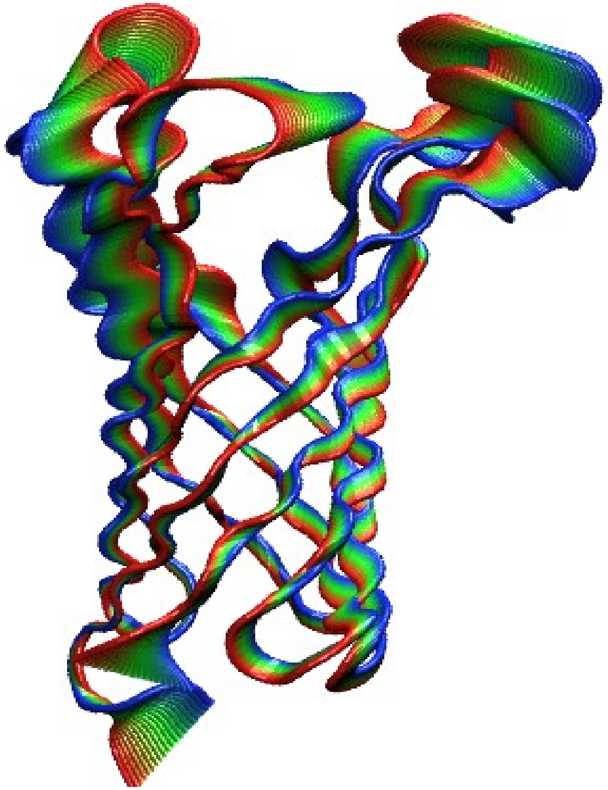


8 eigenvectors are considered for output using gmx anaeig. This option can be set by using the flags `–first` and `–last`

```
gmx anaeig -f trajectory.xtc -v eigenvec.trr -eig eigenval.xvg -s reference.tpr
                    -proj proj.xvg -first 1 -last 5
```

gmx anaeig: flag -extr

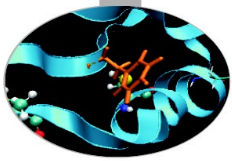


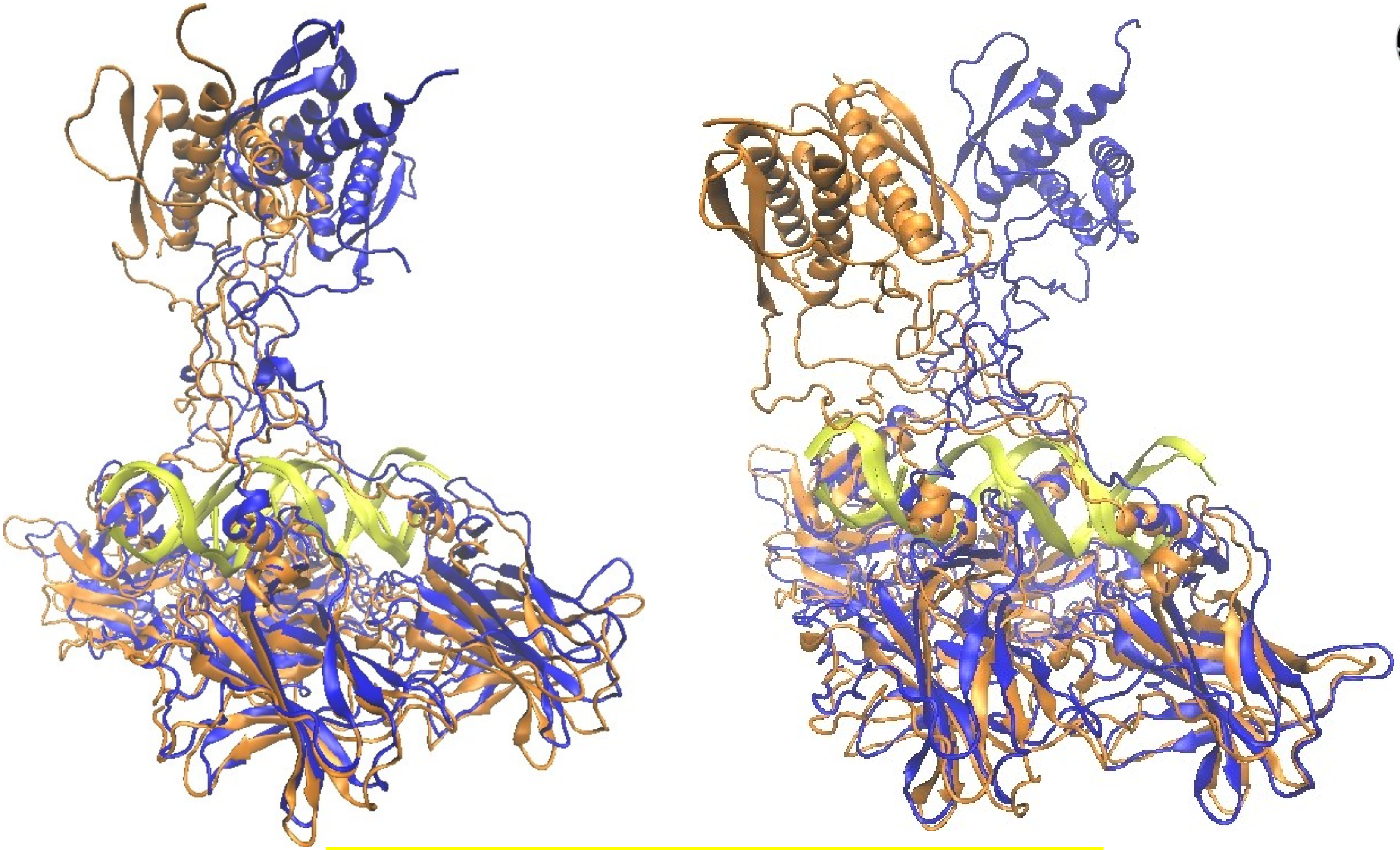


The concerted movements along the first eigenvectors of the extracellular loops of the OmpA porine are shown

```
gmx anaeig -f trajectory.xtc -v eigenvec.trr -eig eigenval.xvg -s reference.gro
                    -extr extreme.pdb -first 1 -last 3 -nframes 50
```

gmx anaeig: flag -extr

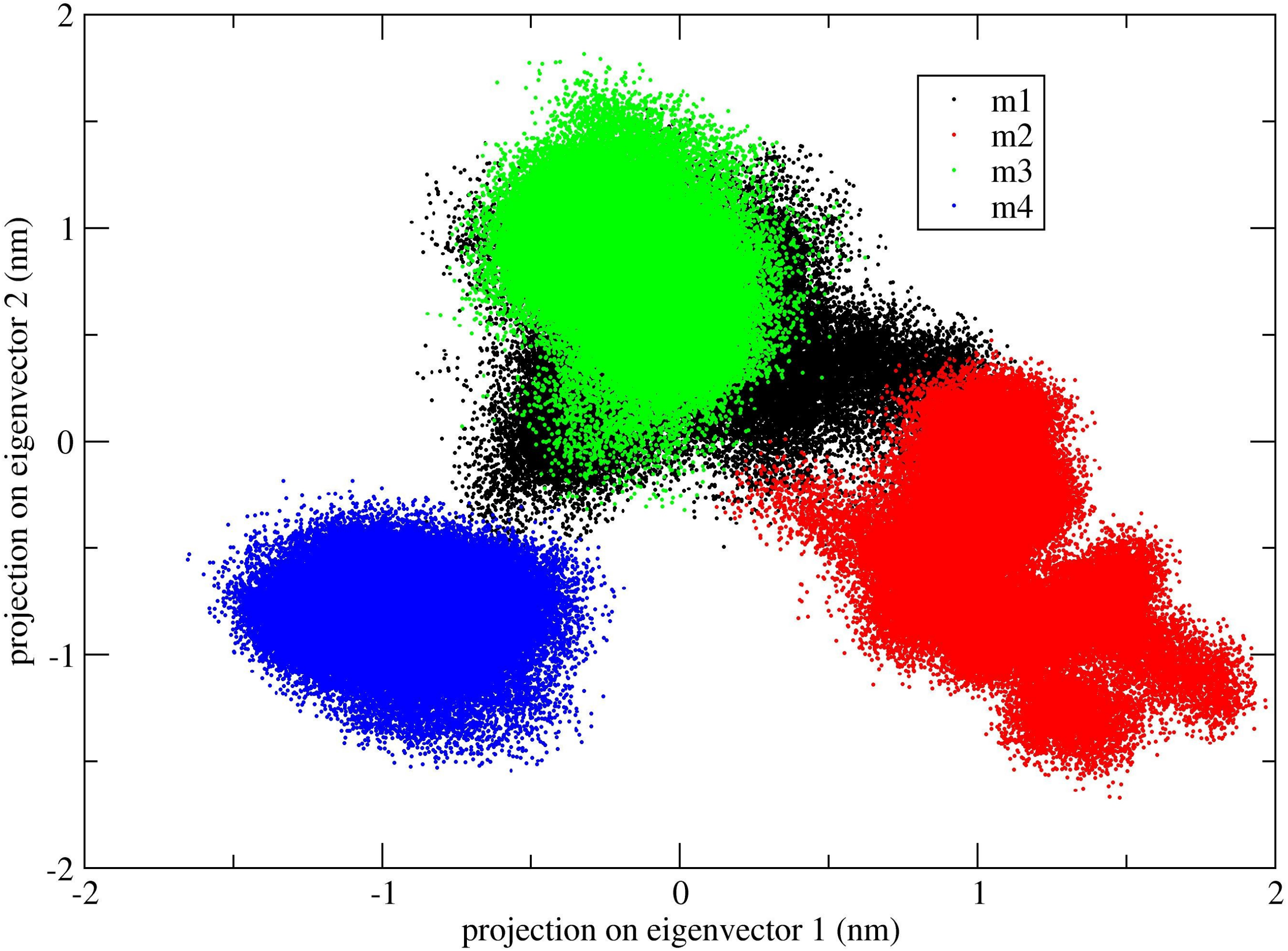


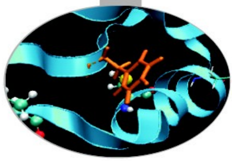


The concerted movements along the first (on the left) and the second (on the right) eigenvectors of DNA binding domain of p53

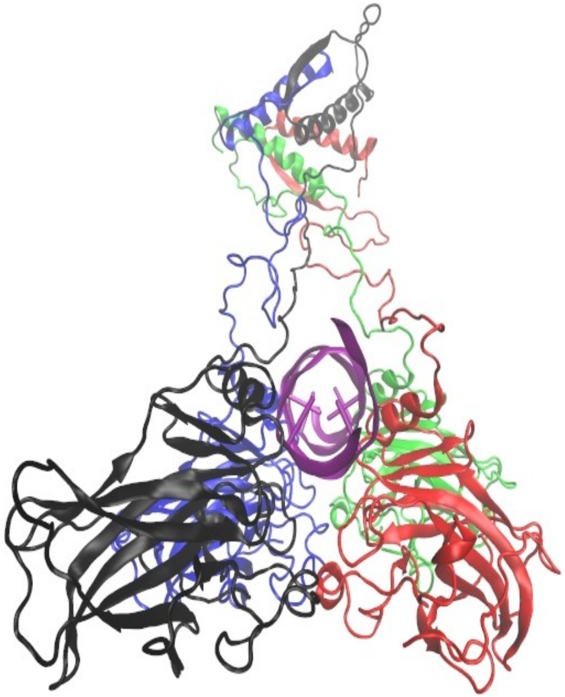
gmx anaeig: the -2d flag

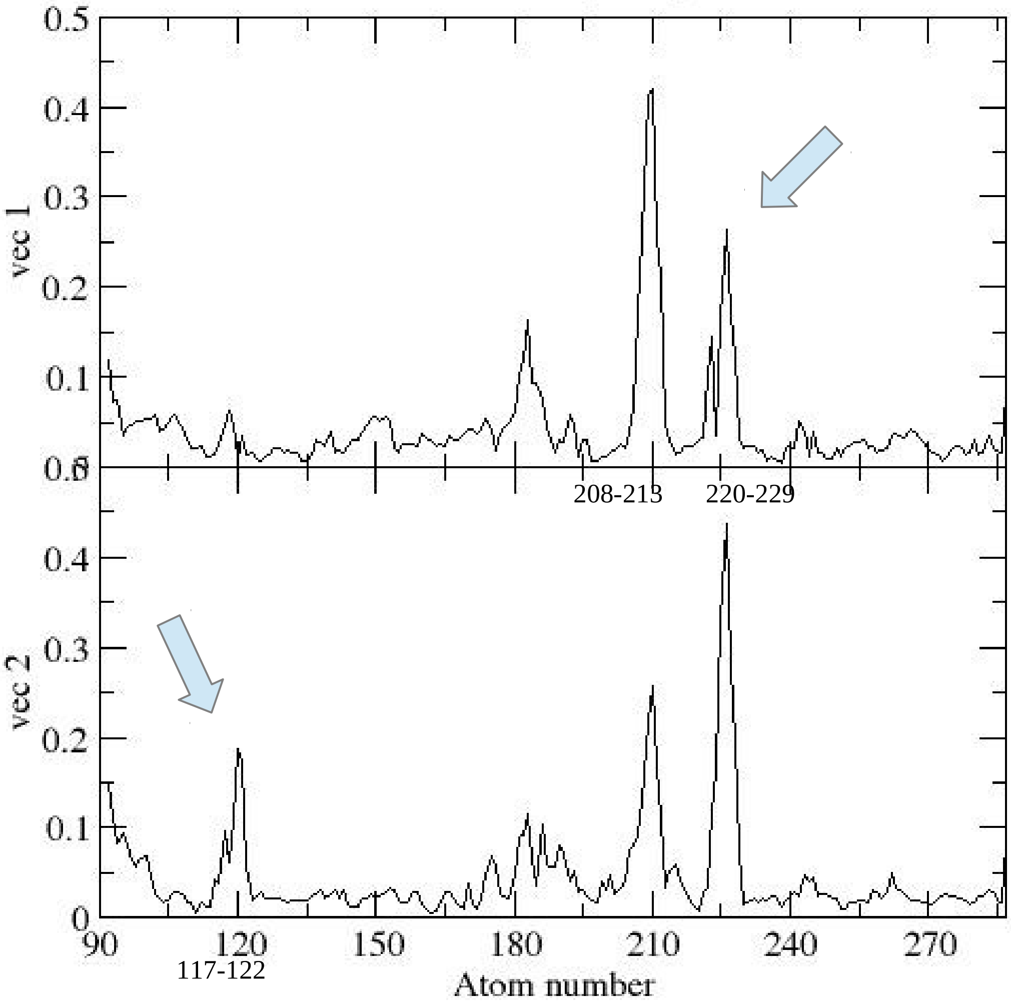
p53 example

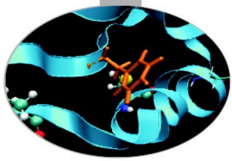




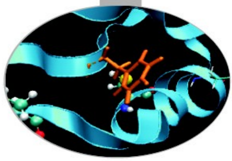
gmx anaeig: the –comp flag



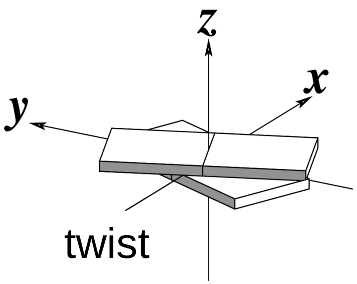


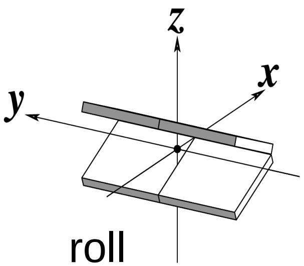


DNA structure

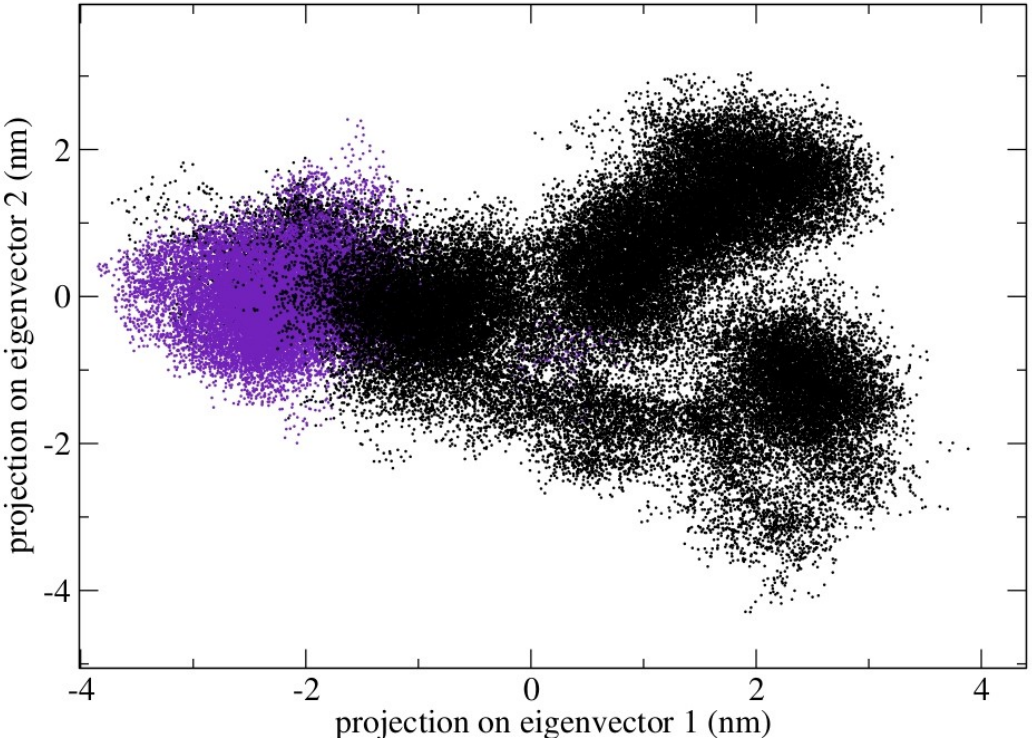


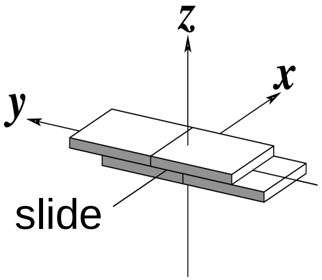
DNA is in the B form

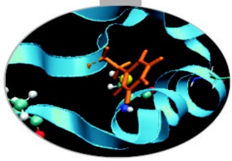




The conformations in which both the DNA roll and twist parameters for the central TA bases are significantly different from their corresponding values found in standard B-DNA are shown in purple. The essential subspace in which the DNA is distorted is separated along the first eigenvector from the great majority of the p53 tetramer conformations visited during the simulation.



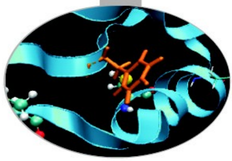




Tutorial T4

Just for exercise do on a trajectory of tutorial T1:

- rmsd
- rmsf
- covar (eigenval, eigenvec)
- anaeig (2dproj)



Tutorial T4

First of all:

```
module load profile/advanced
module load autoload gromacs/5.1.2
```

- rmsd

```
gmx rmsd -f traj.xtc -s topol.tpr
```

- rmsf

```
gmx rmsf -f traj.xtc -s topol.tpr
```

- covar (eigenval, eigenvec)

```
gmx covar -f traj.xtc -s topol.tpr -ascii
```

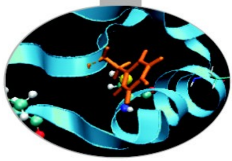
- anaeig (2dproj)

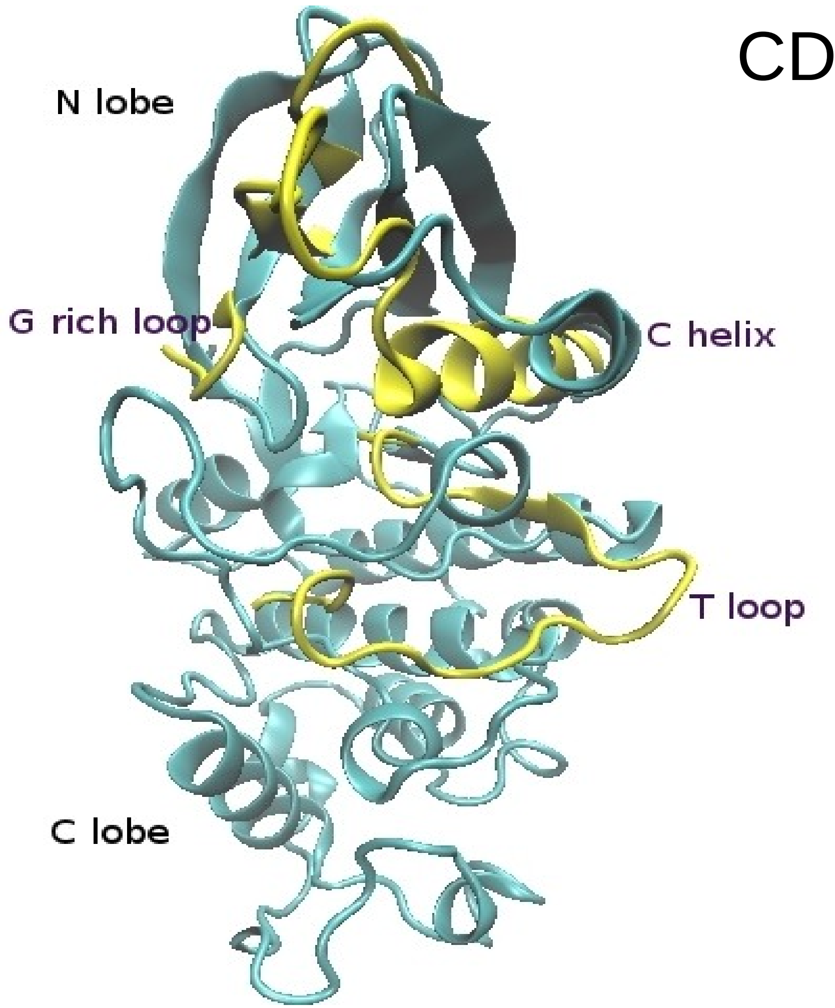
```
gmx anaeig -f traj.xtc -v eigenvec.trr -2dproj -first 1 – last 2
```

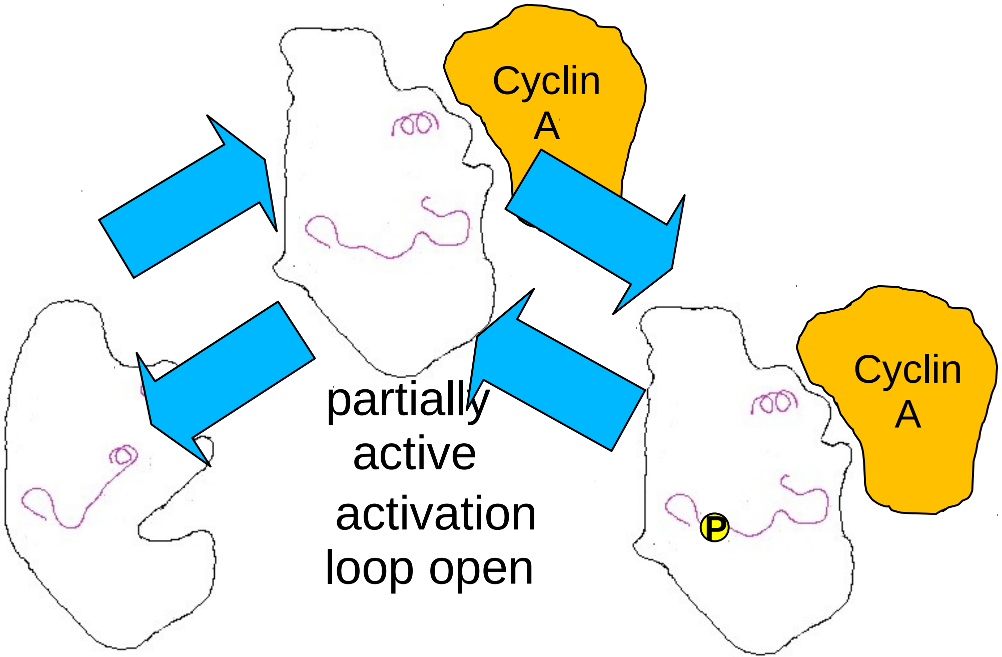
You can visualize files.xvg with xmgrace

Part B -EDS sampling

CDK2 example







Inactive form

activation loop closed

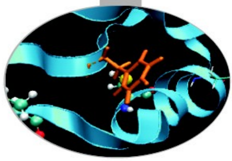
partially active

activation loop open

Different orientation of Cα helix

fully active

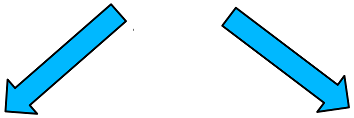
Essential Dynamics alghoritm to model conformational transition



Limit of the classic MD simulations: no transition in two MD simulations lasting 1 μs

ED sampling

- is based on previous essential dynamics analysis of native protein movements in its stable conformational states. The system is constrained to move along variables defined by the unbiased MD simulations i.e. in the space of the selected eigenvectors

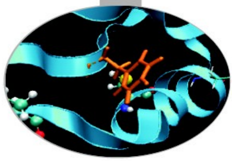


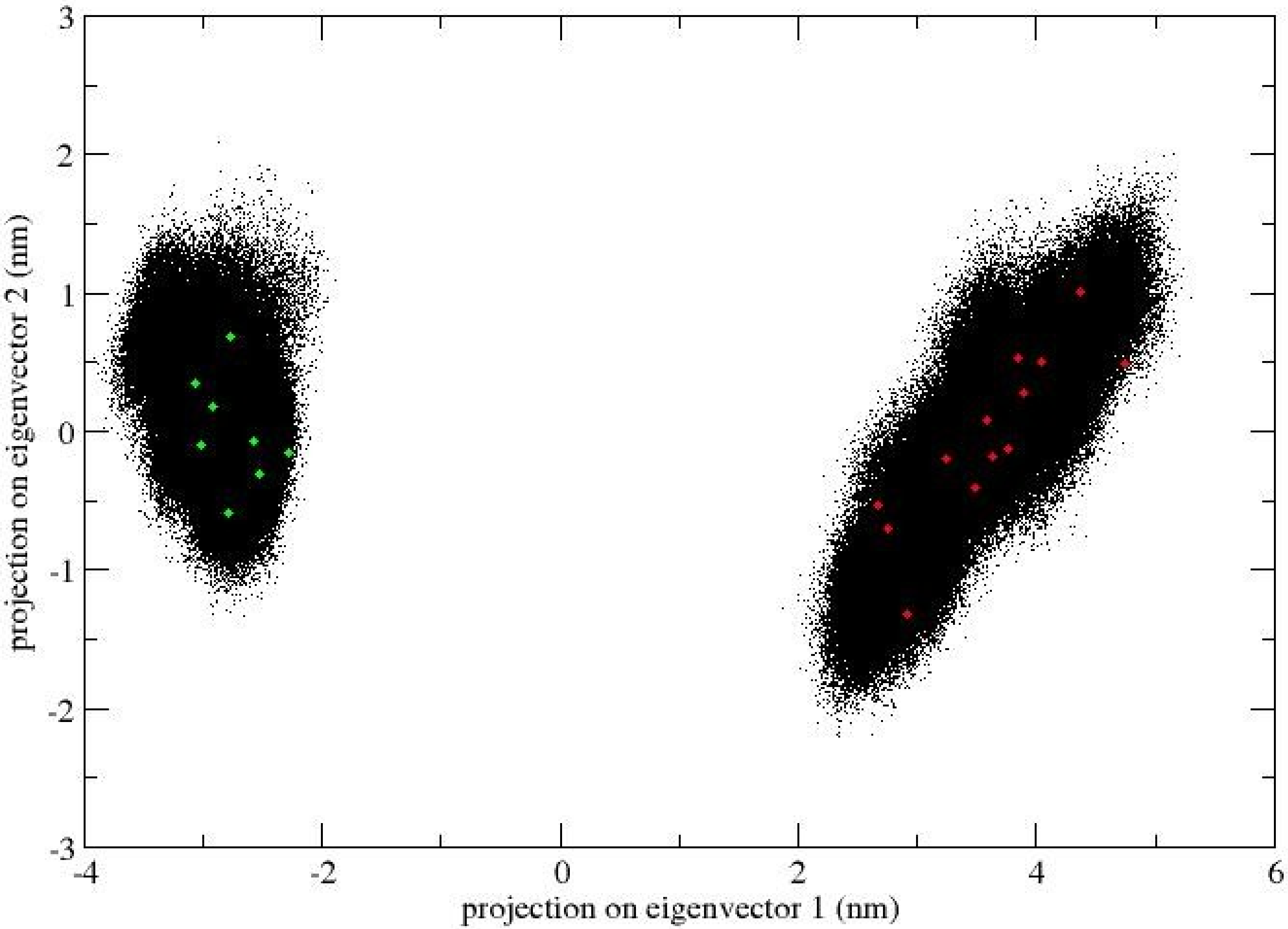
expansion procedure
increase the distance from a reference structure

contraction procedure
decrease the distance from a reference structure

$\Delta\xi = \Delta\xi_d$	per Δt	Per $r \le r_0$
$\Delta\xi = \Delta\xi_d + \Delta\xi_c$	per Δt	Per $r > r_0$

Amadei et al., J. Biomol. Struct&Dynamics, 13, 4, (1996), 615-625



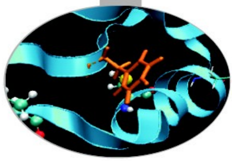


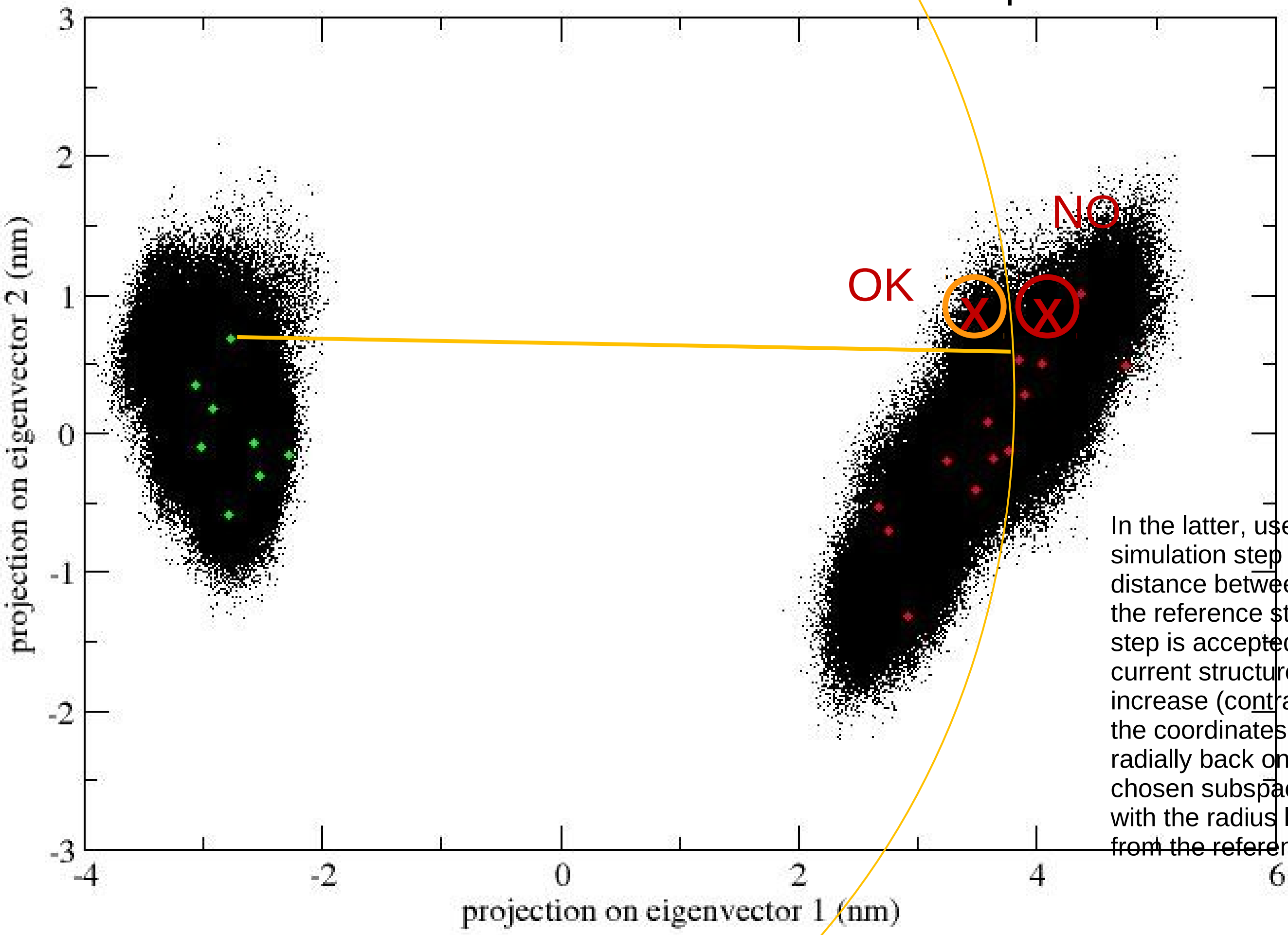
projection on eigenvector 2 (nm)
3
2
1
0
-1
-2
-3
-4
-2
0
2
4
6
projection on eigenvector 1 (nm)

the two trajectories were concatenated, covariance matrices of positional fluctuations (C-alpha only) were built and diagonalized.

which eigenvector – obtained by means of principal component analysis – well discriminate between the two protein conformational states ?

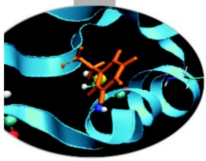
Hypersphere with r=the distance between two structures on the essential subspace

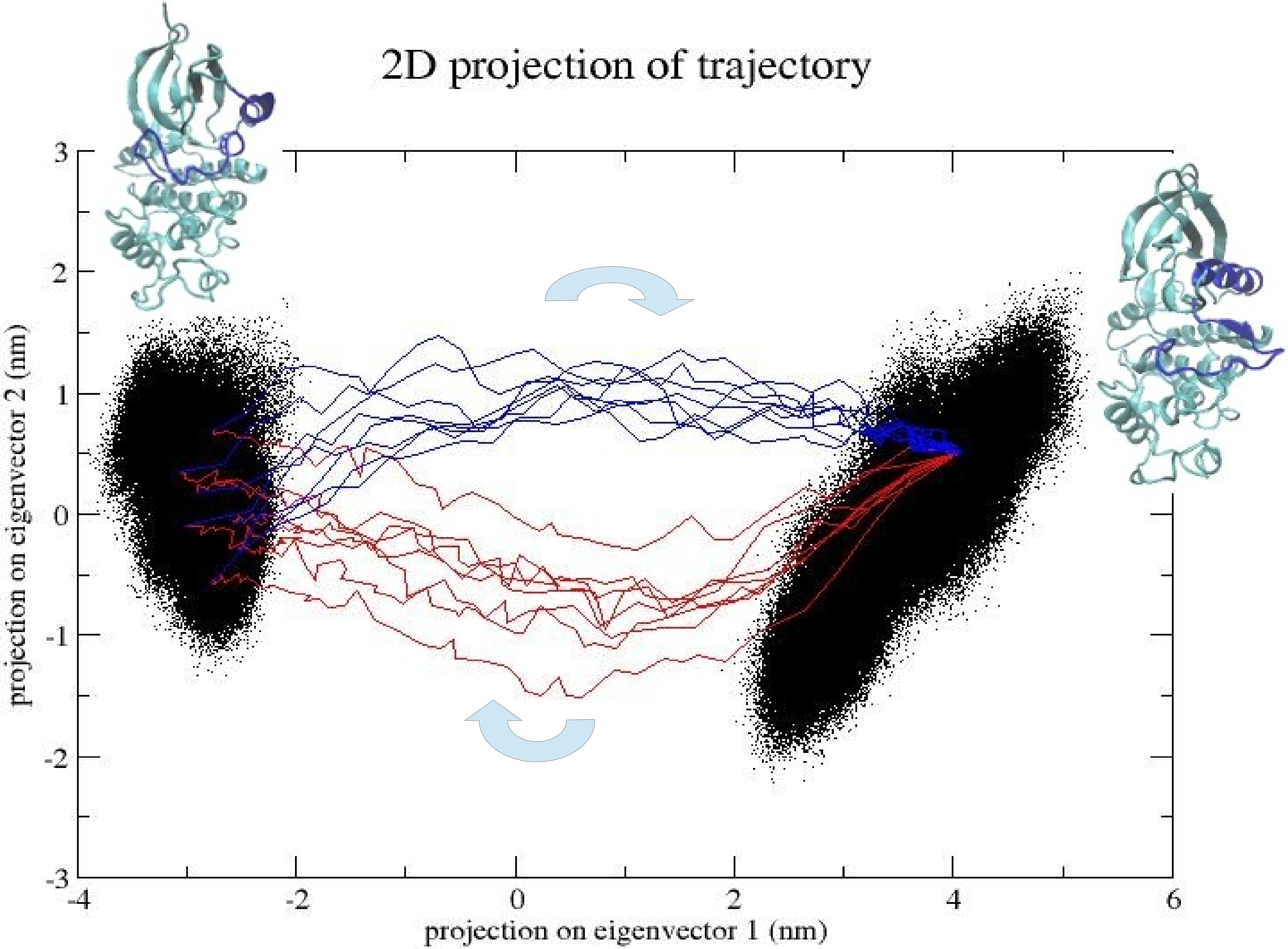



OK
NO
X
X
3
2
1
0
-1
-2
-3
-4
-2
0
2
4
6
projection on eigenvector 2 (nm)
projection on eigenvector 1 (nm)

In the latter, used in this work, a regular MD simulation step is performed and the distance between the current structure and the reference structure is calculated. The step is accepted if the distance between the current structure and the reference does not increase (contraction procedure); otherwise, the coordinates and velocities are corrected radially back onto the hypersphere (in the chosen subspace) centred in the reference, with the radius length given by the distance from the reference in the previous step.

THE SYSTEM IS CONSTRAINED TO MOVE TOWARDS A REFERENCE POINT (the origin of the hypersphere) IN THE ESSENTIAL SPACE

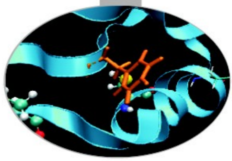



2D projection of trajectory
3
2
1
0
-1
-2
-3
-4
-2
0
2
4
6
projection on eigenvector 2 (nm)
projection on eigenvector 1 (nm)

RMSD from the refence structure; conservation of the secondary structure, conservation of the H bonds

Besker et al J. Biomol Struct Dyn. 2014 Dec;32(12):1929-35.

Essential Dynamics Tutorial: Essential Dynamics Analysis and ED sampling of open and closed CDK2



Input file on Eurora:

/gpfs/scratch/userinternal/nbesker1/MDcourse-aprile2016/Tutorial4.tar.gz

Copy Tutorial4.tar.gz file from Eurora:

- scp username@login.eurora.cineca.it
- Xmgrace /usr/local/grace/bin/xmgrace
- VMD /usr/local/bin/vmd

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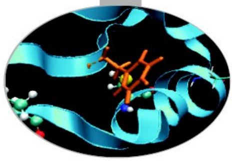

- Concatenate the trajectories: ✓

```
gmx trjcat -f ../cdk2-closed/dry.xtc ../cdk2-open/traj-cdk2open-mdcourse.xtc -o combined.xtc -settime
                          ---> combinetraj.xtc
```

- Calculate the covariance matrix ✓

```
gmx covar -f combined.xtc -s reference.gro
                          ---> eigenvec.trr, eigenval.xvg
```

- Find the essential plane ✓

```
gmx anaeig -f combinetraj.xtc -v eigenvec.trr -2d -s reference.gro -first -last
                          ---> 2dproj.xvg
```

- Extract the origin and a target structure ✓

```
gmx trjconv -f combinetraj.xtc -s reference.gro -b -e -o name.gro
                          ---> final-structure.gro (-s reference-open-cdk2.gro -b 39100)
                          ---> origin_sol.gro
```

- The control= projection of the extracted structures on the essential plane

```
gmx anaeig -f struct.gro -v eigenvec.trr -2d struct-2dproj.xvg -first 1 -last 2 -s reference.gro
```

- generates an essential dynamics (ED) sampling input file

```
gmx make_edi  -ori -radcon -tar -o sam.edi
```

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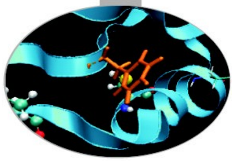

```
• gmx trjconv -f combinetraj.xtc -s reference-open-cdk2.gro -b 39100 -e 39100 -o fin.gro
• gmx make_edi -ori origin_solv.gro -radcon 298 -tar fin.gro -o sam.edi
• gmx grompp -f run.mdp -c origin_solv.gro -p topol-closed.top
• qsub eds.sh
• gmx anaeig -f traj_comp.xtc -v eigenvec.trr -2d traj-2dstruct2.xvg -first 1 -last 2 -s topol.tpr
```