All the files for this tutorial will be available at the website:
http://cbp.cfn.kit.edu/downloads/MD-SS2017/materials-prak/ADD/

ADD1 – vibrational analysis vs principal component analysis

For PCA, perform an MD simulation of solvated K2 peptide first. Prior to that, an equilibration is necessary (minimization, 10ps of NVT heating, and 20ps of NPT density equilibration):

```
gmx grompp -f steep -o steep -c k2-ion
gmx mdrun -deffnm steep

gmx grompp -f nvt -o nvt -c steep
gmx mdrun -deffnm nvt

gmx grompp -f npt -o npt -c nvt
gmx mdrun -deffnm npt
```

Then the MD simulation itself. If you feel like, you may adjust the length of MD simulation (number of steps). I am performing a simulation over 100 ps, which may be a little too short:

```
gmx grompp -f md -o md -c npt
gmx mdrun -deffnm md
```

Then we may already perform the PCA; first we need to create a `.tpr` file that contains Protein only:

```
gmx convert-tpr -s md -o md-protein.tpr
```

- and select '1' for Protein

```
gmx covar -f md.xtc -s md-protein.tpr -mwa
```

- and select twice 'System' or 'Protein'

Check the eigenvalues. These describe the percentage of the total motion of the molecule that can be assigned to each respective eigenvector. Also, the eigenvalues may be converted to vibrational frequencies (wave numbers, in cm^-1). This can be done with a little script.

```
./eigenval-to-wavenum.awk eigenval.xvg > freq.xvg
```

(Note that the last six eigenvalues are close to zero and most likely correspond to six non-periodic modes of motion – which ones? Simply ignore the corresponding frequencies.)

For NMA, we need to minimize first, as close to a minimum as possible. To this end, we first extract the structure of the peptide from the steepest-descents minimization in water – this is already available in file `steep.gro`. We then minimize with L-BFGS (quasi-Newton method):

```
gmx_d grompp -f l-bfgs -o l-bfgs -c  steep
gmx_d mdrun -deffnm l-bfgs
```

Note that we need Gromacs compiled in double precision, in order to really come as close to the minimum as possible. The numerical inaccuracy of Gromacs compiled in single ('float') precision would be possibly too large.

Then, we calculate the Hessian. It is important to read in the full-precision trajectory with the '`-t`' option because the precision of the .gro file is too low (even lower than single precision, actually):

```
gmx_d grompp -f nma -o nma -t l-bfgs.trr -c l-bfgs.gro
gmx_d mdrun -deffnm nma
```

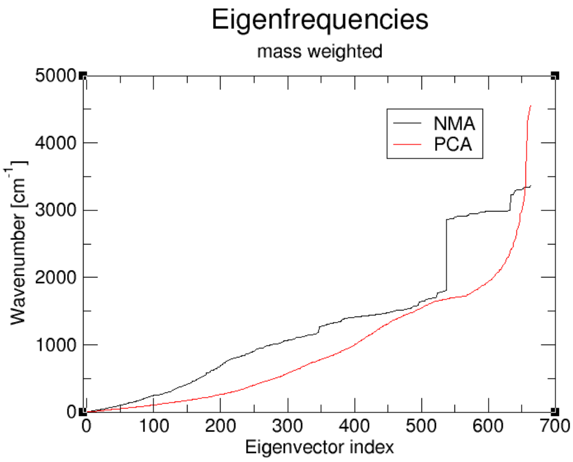
Then we diagonalize the Hessian to obtain vibrational frequencies and normal modes as the eigenvectors of the Hessian:

```
gmx_d nmeig -f nma -s nma -last 669
```

Check the frequencies in `eigenfreq.xvg` – do they look reasonable? Are the first six frequencies equal to zero? What modes of motion do they correspond to?

The eigenvectors may be visualized in the same way as described in the script to the large tutorial – please do so for a few eigenvectors! Beware the numbering of the eigenvectors (if the six trivial, non-interesting eigenvectors are the first or last ones).

Compare the frequencies computed with NMA and PCA, and see if the results are the same or at least similar! Why is the agreement in the high-frequency region particularly poor? Is that a problem? Which motions do these frequencies correspond to?



ADD2 – free energy of water pair / ion pair

Perform metadynamics simulations for two kinds of systems: (i) a box of water, (ii) Na+ and Cl- in a box of water. (Each student choose one system.) The topologies and coordinates of the systems are provided; the final coordinates from the equilibration are in file `npt.gro`.

For the water system, a pair of water-bridged water molecules are e.g. molecules 600 and 814, i.e. you can choose the reaction coordinate to be the distance of atoms 1798 and 2440.

For the ion-pair system, the atoms are simply numbers 1 and 2.

Perform metadynamics simulations with:

gmx grompp -f npt -p topol -o meta

gmx mdrun -deffnm meta -plumed plumed.dat -v

Analyze it with the command:

plumed sum_hills –hills HILLS

Look at the free energy in the file *fes.dat* with *xmgrace* and compare the two profiles.

The simulation will run for 5 ns and it will take a couple of hours.

Repeat the same simulations but with different metadynamics setup (height and width of the gaussians). To do that edit the file plumed.dat.

ADD3 – electron transfer to DNA

(i) simulation of neutral DNA

```
GMXLIB=amber99.ff gmx_d grompp -f neutral -c start.gro -p topol_qmmm.top -n
index_qmmm.ndx -o neutral
gmx_d mdrun -deffnm neutral
```

(ii) simulation of anionic DNA

```
GMXLIB=amber99.ff gmx_d grompp -f charged -c neutral -p topol_qmmm.top -n
index_qmmm.ndx -o charged
gmx_d mdrun -deffnm charged

gmx_d trjconv -n index_qmmm.ndx -f neutral.gro -o neutral-dna.gro -s neutral
vmd neutral-dna.gro charged.xtc
```

note 1: “`GMXLIB=amber99.ff`” gives Gromacs the force field in that directory
note 2: the atomic Mulliken charges are in the file `qm_dftb_charges.xvg`
- but pay attention to the atom ordering! both link atoms are together at the end, so thymine1 is $2 through $15 and additionally $30, while thymine 2 is $16 through $29 and additionally $31.

the charges on thymines can be summed from the individual atoms with the following script:

```
./calculate_charges_on_thymines.awk qm_dftb_charges.xvg > charges_thymines.xvg
xmgrace -nxy charges_thymines.xvg
```