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# **Practical 10: Protein dynamics and visualization Documentation**

*Release 1.0*

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For background on using [VMD](#), look at [\[Hsin2008\]](#) and the [VMD tutorial](#).

Basics of protein structure are illustrated in [\[BrandenTooze\]](#).

**Contents:**



# BASICS OF PROTEIN STRUCTURE

See also illustrations in slides and [BrandenTooze].

Introduction to protein structure

- **polypeptides, amino acids**
  - chemical structure of the 20 aa
  - natural aa: L-form (note: look down H-Ca and read CORN == L, otherwise D)
  - peptide bond
  - repeating unit: residue, same backbone, differing sidechain R
  - sequence, *primary structure*
- hydrogen bonds: donor-H...acceptor
- **secondary structure**
  - **alpha-helix (n..n+4 H-bonds of main chain, ~3.6 res per turn)**
    - \* peptide units: phi, psi angles: flexible, omega (peptide bond) fixed (cis/trans)
    - \* must be right handed (otherwise clashes; only very short helices are left-handed)
  - beta sheet (extended), parallel/anti-parallel
  - (coiled coil)
  - 3-10 helix (n..n+3, 10 atoms between donor and acceptor, 3 res per turn)
  - pi helix (n..n+5)
- tertiary structure: - helices - sheets - hairpins, loops - coiled-coil
- quaternary structure





# ANALYZING PROTEIN STRUCTURE AND TOPOLOGY

Use [VMD](#) to learn more about a protein. The example we use is adenylate kinase (“AdK”).

For background on using [VMD](#), look at [Hsin2008] and the [VMD tutorial](#).

## 2.1 Topology and 2ndary structure

- get *adk\_open.pdb* from <http://becksteinlab.physics.asu.edu/pages/courses/2013/SimBioNano/09/>
- settings in VMD:
  - Display -> Orthographic
  - Display -> Rendermode: GLSL
  - Graphics -> Color: Display: Background: White
- select protein
- *new cartoon* representation, color by secondary structure

## 2.2 Exercises

### 2.2.1 Identify secondary structure and topology

Sketch a topology diagram on paper (use open structure for analysis):

- number helices H1 - H8 (ignore short < 4 res, count all helices as helix, alpha, pi, 3-10)
- sheets A, B, C, number strands SA1-SA5, SB1-3, SC1-2
- start with central b-sheet
- locate N-terminus (resid 1 and name CA, color blue, VDW) and C terminus

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**Note:** You can do ranges: `resid 1 to 5` or multiple ranges: `resid 1 to 5 20 to 50`.

You can use boolean operators `and`, `or`, `not`. See [selections](#) in the user manual for details.

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- draw connections between secondary elements in your topology diagram and label secondary structure elements.

Topology of AdK

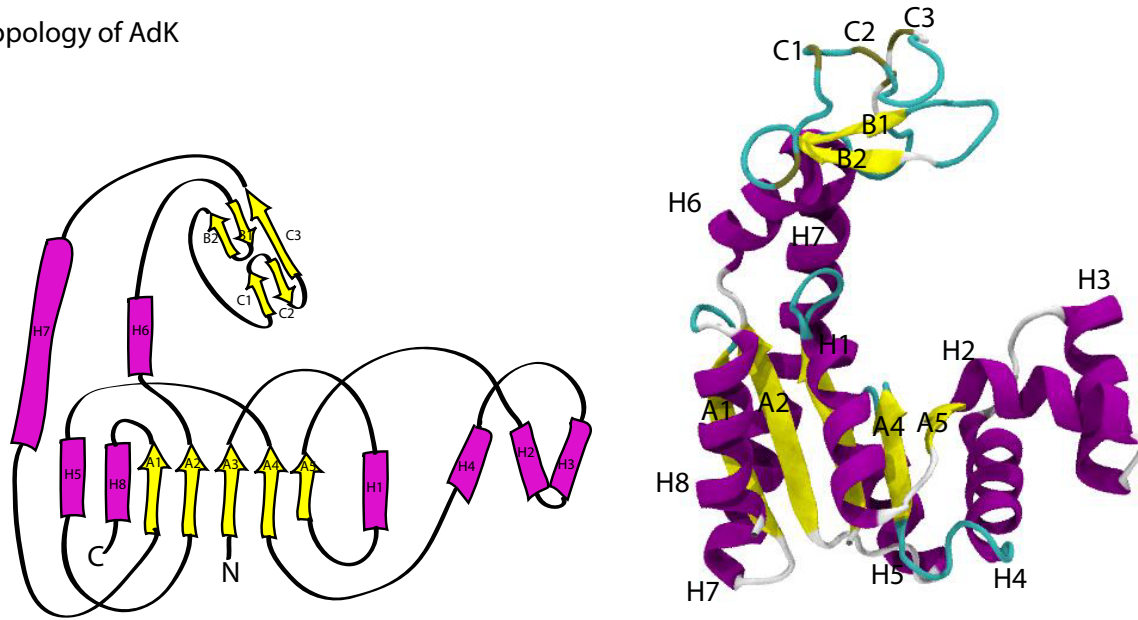


Figure 2.1: Topology diagram of AdK.

### 2.2.2 Identify domains (tertiary structure)

- compare to closed conformation
  - load *adk\_closed.pdb*
  - select protein
  - *new cartoon*, color differently from the open structure
  - superimpose: Extensions -> Analysis -> RMSD tool: protein

$$\text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_{a,i} - x_{b,i})^2}$$

What is the RMSD after superposition?

- Investigate how the conformation of the molecule changes.
  - \* Which regions (domains) “move”?
  - \* Identify two moving domains (called “NMP” and “LID”) and one constant region (“CORE”):
    - give residue ranges (tip: use Extensions -> Analysis -> Sequence Viewer)
    - color regions differently and also mark them in your topology diagram
- align on CORE domain only

What is the final RMSD when aligned on CORE only, i.e. how similar are the CORE regions in the two structures?

What is the overall protein RMSD, assuming that the two structures are superimposed on CORE?

### 2.2.3 Ligands

- Download [1AKE](#) from the PDB (or use `1ake.pdb` from the course directory)
- show protein chain A
- show the co-crystallized ligand Ap5 (`resname AP5"`)
  - `resname AP5, licorice, color: name`
- show charged residues within 5 Å of the ligand
  - `same residue as (charged and within 5 of resname AP5)`
  - use representation *licorice*, `color: name` for the residues, make ligand VDW.
  - Which side chains make contact with the ligand?
  - What have they got in common? (Tip: try out `color: restype`)
- water
  - show crystal water oxygens as VDW or licorice (small spheres)

### 2.2.4 Summary

#### AdK secondary and tertiary structure

AdK is a dynamic enzyme, which can undergo a large conformational change between an open and a closed conformation. The backbone RMSD between the two conformations is about 7.16 Å.

AdK contains a central beta sheet (with strands A1 to A5) between four helices (H1, H5, H7, H8), making up the CORE domain (residues 1-29, 60-121, 160-214). This CORE domain does not change much between the open and closed conformation (RMSD 1.97 Å of CORE).

The NMP domain, consisting of helices H2—H4 (residues 30-59), and the LID domain (H6 and two small beta sheets B1/B2 and C1/C2/C3, resids 122-159) are very mobile. On closing, the both fold towards CORE on hinges. When RMS-fitted on CORE, the overall RMSD is 8.0 Å.

#### AdK Ligand

The AP5 ligand mimics the transition state of the phospho-transfer reaction. Phosphates are negatively charged, hence only basic (positively charged, blue) residues are in contact.

Topology of AdK

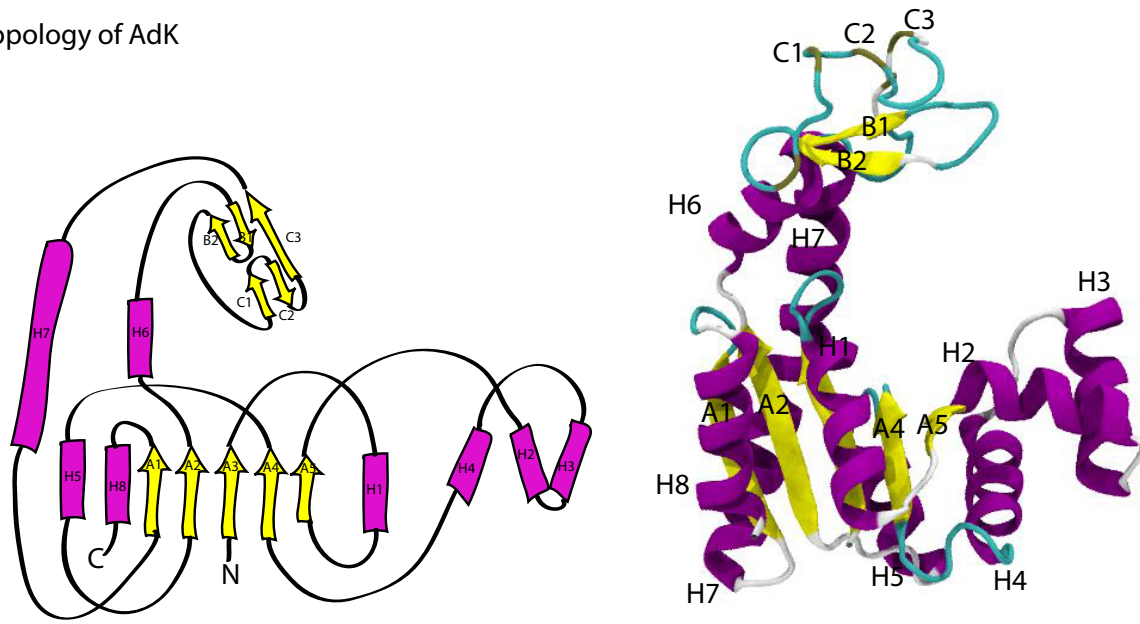
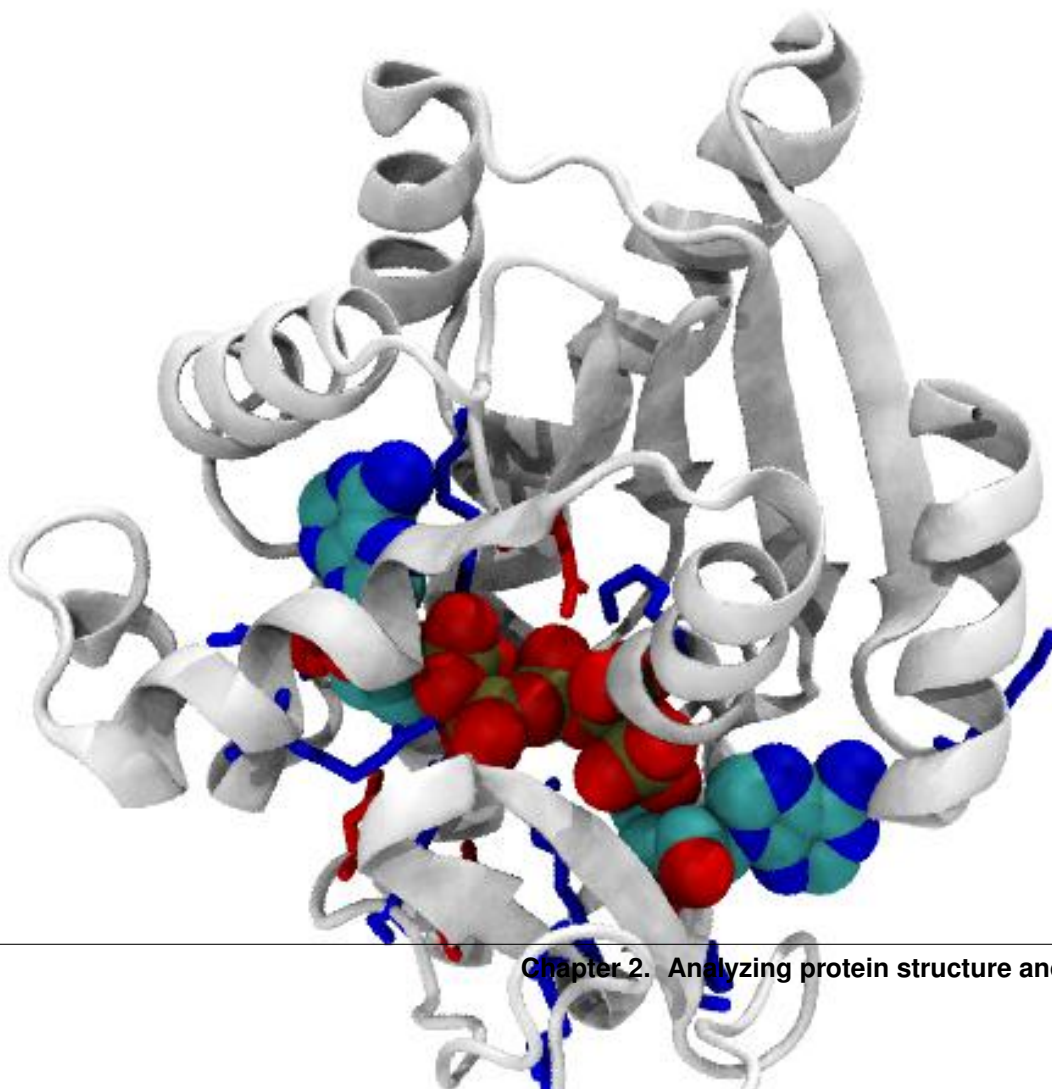
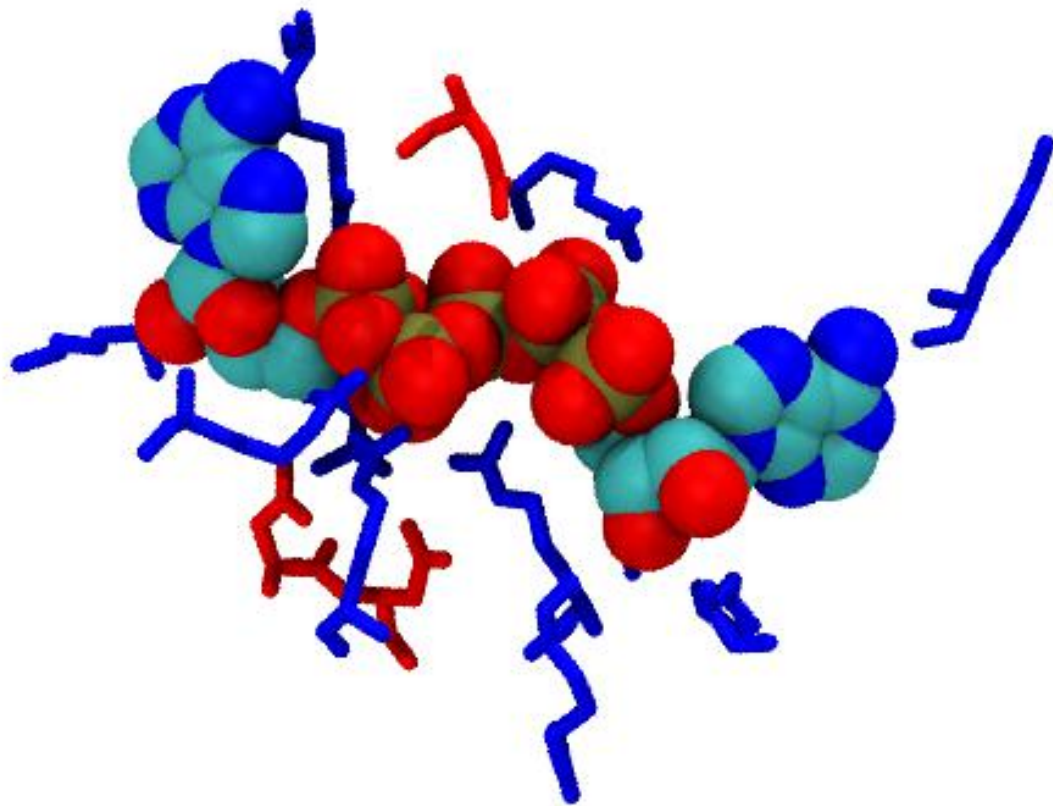


Figure 2.2: Topology diagram of AdK.







# PUBLICATION-QUALITY IMAGES

To make an image with AO (ambient occlusion lighting) and depth cueing:

- Settings
  - Display -> Rendermode -> GLSL
  - Display -> Display Settings:
    - \* Shadows On,
    - \* Amb Occl On
    - \* Cue Mode: Linear
    - \* Cue start: 1.75
    - \* Cue end: 3.0
  - white bg: Graphics -> Colors: Display : White
- rendering: File -> Render
  - *snapshot*
    - \* glossy material looks nice
  - *tachyon (internal)*: ray tracer (takes a while due to AO)
    - \* change materials to special AO materials (e.g. AOChalky)

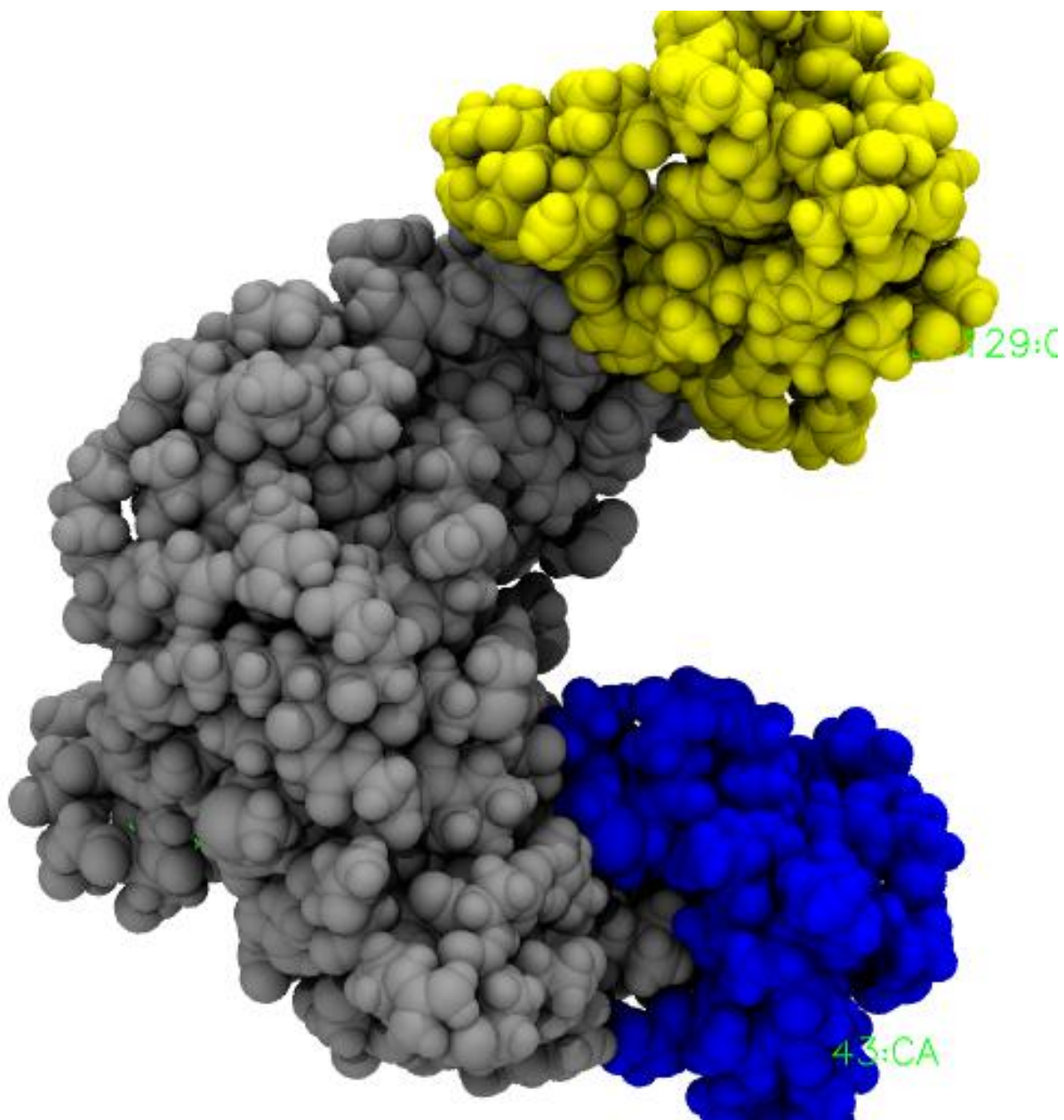


Figure 3.1: Example (AdK) was rendered with Tachyon (internal) with VDW representation, colors silver, yellow, and blue, no depth cueing, material AOChalky, and using Ambient Occlusion (AO) and Shadows (see Display → Displaysettings)



# ANALYZING PROTEIN DYNAMICS

Analyze a trajectory of the closed-to-open transition of AdK [Beckstein2009]:

## 4.1 Visualization

- load *adk.psf* with *adk\_dims.dcd* (use *load all at once*, this is faster in general)
- color domains and show as *new cartoon*
  - resid 30 to 59: blue
  - resid 122 to 159: yellow
  - not (resid 30 to 59 122 to 159) (or resid 1 to 29 60 to 121 160 to 214): gray
- play (loop: rock)

## 4.2 RMSD change

- RMSD change over the trajectory: Extensions/Analysis/RMSD Trajectory Tool
- Is the result consistent with the result of your *RMSD analysis of the static structures*?

## 4.3 Measuring distances

Measure distances interactively:

- ‘2’ (or Mouse -> Label -> Bonds)
- click tip of LID and tip of NMP
- play the trajectory: label changes dynamically
- Graphics -> Labels: Bonds - select - Graph (preview) - Graph...
- try different distances between residues (use the CA) and plot together: which change, which don't?
  - What would be good distances to report on conformational changes, e.g. for FRET?



# MAKING MOVIES IN VMD

## 5.1 Install the ffmpeg encoder

The iMacs are not well equipped for movie making so we need to install the `ffmpeg` encoder from <http://becksteinlab.physics.asu.edu/pages/courses/2013/SimBioNano/10/ffmpeg/>

```
cd ~/bin
curl -O http://becksteinlab.physics.asu.edu/pages/courses/2013/SimBioNano/10/ffmpeg/ffmpeg
chmod a+x ffmpeg
```

Then make sure that `ffmpeg` is found on your `PATH`:

```
echo "export PATH=$HOME/bin:$PATH" >> ~/.profile
```

(You might have to log out and log in for this to have full effect later on.)

## 5.2 AdK transition movie

We use the standard VMD plugin `MovieMaker`.

1. Load the AdK transition trajectory from *Analyzing protein dynamics*.
2. Use a nice representation, e.g. color the CORE, LID, and NMP domains. You can also find a number of salt bridges (pairs of basic/positively charged and acidic/negatively charge residues) that “zip up” AdK (see the [AdK zipping movie](#) from Beckstein et al).
3. Open *Extension -> Visualization -> Movie Maker*
4. Settings
  - *Renderer*: Snapshot (for “Hollywood”-style movies, use one of the ray tracers such as tachyon + AO)
  - *Movie Settings*: Trajectory
  - Set *Format* to MPEG-1 (ffmpeg). (MPEG-2 is better but the iMacs can’t display it).
  - Set your *working directory* to e.g. `~/USERNAME/p10/movies` (so that you can easily find the finished movie)
  - *Name* your movie, e.g. `adk_transition`.
5. *Make Movie*: should step through your trajectory, write out frames, and finally run `ffmpeg`.
6. In the shell, go to your movie directory and play it:

```
open adk_transition.mpg
```

## 5.3 Advanced movie making

In order to rotate and zoom a movie during a trajectory we can use the VMD [ViewChangeRender](#) plugin together with [MovieMaker](#).

Open [ViewChangeRender Extensions](#) -> [Visualization](#) -> [ViewChangeRender](#). Generate “viewpoints” of your movie:

1. Adjust position in trajectory, rotate, zoom.
2. Save Viewpoint

Repeat for as many viewpoints as you want to include.

*Add all viewpoints to movie:* The final movie will smoothly interpolate between these points. You can check with *Preview Movie*. (Note that this plugin has a few bugs so you might have to quit and restart when it stops working.)

Once you are ready to render the movie:

1. In [ViewChangeRender](#): *Enable*
2. In [MovieMaker](#):
  - (a) *Movie Settings: User defined procedure*
  - (b) Adjust other settings as needed...
  - (c) *Make movie*

# LEARNING OBJECTIVES

- basic understanding of protein structure
- familiarize with a molecular graphics program (VMD)
- loading of static structures and dynamics trajectories
- viewing a molecule (mouse, view modes)
- representations
- selections
- making images
- basic analysis
- use of RMSFitting tools



# REFERENCES





# INDICES AND TABLES

- *genindex*
- *modindex*
- *search*



# BIBLIOGRAPHY

- [BrandenTooze] C. Branden and J. Tooze. Introduction to Protein Structure. 2nd ed, 1999, Garland Publishing, New York.
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- [Beckstein2009] O Beckstein. EJ Denning, JR Perilla, and TB Woolf. Zipping and Unzipping of Adenylate Kinase: Atomistic Insights into the Ensemble of Open/Closed Transitions. *J Mol Biol* 394 (2009), 160–176. <http://dx.doi.org/10.1016/j.jmb.2009.09.009>



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