S. Jonic - Tutorial 1

Hybrid Electron Microscopy Normal Mode Analysis (HEMNMA) within Scipion

This tutorial is on use of HEMNMA [1,2] in Scipion to first compute normal modes of an atomic structure or an EM density map of a molecular complex and, then, use these normal modes to analyze EM images of isolated copies of the complex to detect conformational changes of the complex in the images [3]. For this purpose, we use the same synthetic data set that we used to show the HEMNMA protocol in Xmipp [2].

A set of 200 synthetic images was obtained by projecting the atomic structure of adenylate kinase (PDB:4AKE) being displaced (elastically deformed) using a combination of normal modes. The same displacement amplitude was used along normal modes 7 and 8, which was uniformly distributed in the ranges [-100, 0], while the displacement amplitude along all other normal modes was 0. Image orientations and positions were also random. The two out-of-plane angles were distributed uniformly in the [0°, 360°] and [0°, 180°] ranges. The in-plane angle was 0°. The two in-plane translations were distributed uniformly in the range [0,5] pixels. The images were computed with the size of 128 ×128 pixels and the pixel size of 1 Å × 1 Å.

These images were analyzed based on Normal Mode Analysis of the 4AKE structure as well as based on Normal Mode Analysis of the density map that was computed using the 4AKE structure (density-map size: 128 ×128 ×128 voxels; voxel size: 1 Å × 1 Å × 1 Å). We show the computation of 20 normal modes and the image analysis using only the first three “elastic” normal modes i.e. modes 7-9 (the first six normal modes are not used as they describe rigid-body motions). Including more modes is possible but it would just require a longer processing time.

REFERENCES


1. In Scipion, create a new project and switch to HEMNMA protocol (in “View” menu at left):

![Screenshot of Scipion interface](image)

2. Import an atomic structure or an EM density map (double click on “Import PDB” or “Import volume” in the menu at left).

2.1 Dialog to import an atomic structure (after a double click on “Import PDB”):

![Protocol: scipion - import pdb volumes](image)

After filling the dialog and clicking on “Execute” (red button in the dialog):

![Screenshot of Scipion interface](image)
2.2 Dialog to import an EM density map (after a double click on “Import volume”):

After filling the dialog and clicking on “Execute” button:

The imported data can be checked (visualized) by first clicking on the corresponding “scipion – import … finished” box and then on “Analyze Results” (red button) at right. The density map is visualized by showing its slices and the atomic structure is shown using VMD.

3. If an EM density map was imported, convert it into pseudoatoms (double click on “Convert volume to PDB” in the menu at left):
Dialog to convert an EM map into pseudoatoms (after a double click on “Convert volume to PDB”):

After filling the dialog and executing (“Execute” button):

The results of the conversion can be visualized by first clicking on the “xmipp3 – convert to pseudoatoms finished” box and then on the “Analyze Results” button at right. The input density map and pseudoatom representation are superposed in Chimera. Slices of the input density map and its approximation are also visualized. Here is an example:
4. Perform Normal Mode Analysis of the atomic or pseudoatomic structure (double click on “Modes analysis & selection” in the menu at left):

The dialog for Normal Mode Analysis that opens after double-clicking on “Modes analysis & selection” is the same for atomic and pseudoatomic (obtained from EM density map) structures but some fields are read only if an atomic structure is entered in the “Input structure” field and some other fields are read only if a pseudoatomic structure is entered in the “Input structure” field. These structure-related fields are visible in “Advanced” Expert Level mode (fields shown in grey). The user does not have to bother about them because their default values work well in general. The dialog consists of the following two tabs: “Normal Mode Analysis” and “Animation”.

4.1 Normal Mode Analysis of the input atomic structure:

“Normal Mode Analysis” tab:
“Animation” tab:

After executing (click on “Execute” button):

The Normal Mode Analysis results can be visualized by first clicking on the “xmipp3 – nma analysis finished” and then on “Analyze Results” button at right. This opens the following dialog in which normal modes can be viewed:
“Display output Normal Modes” viewer:

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“Plot max distance profile” viewer:

“Display mode animation with VMD” viewer:  

“Plot mode distance profile” viewer:
4.2 Normal Mode Analysis of the input pseudoatomic structure:

“Normal Mode Analysis” tab:

The default parameter values in the “Animation” tab can be kept unchanged (the same values as in the case of atomic structure).

After executing (click on “Execute” button):

By first clicking on the “xmipp3 – nma analysis 2 finished” and then on “Analyze Results” button at right, a dialog opens by which normal modes can be viewed (the same dialog as in the case of atomic structures, see 4.1). Here is an example of the output of the “Display mode animation with VMD” viewer for the pseudoatomic structure:
5. Stop here if Normal Mode Analysis of structures was your only goal. Go to 6 if your final goal is to analyze images using normal modes.

Note that “Stop here or continue” in the menu at left will not execute anything. It is just an information (reminder).

6. Import images (double click on “Import particles” in the menu at left, fill the dialog, and execute):

Note that the images will not be phase-flipped after executing “Import particles” even if the answer to the question “Have data been phase-flipped?” is “No”. This will just set a flag in the corresponding metadata file. Also, all other information about the microscope that is entered in this dialog box will only be stored in the metadata file but will not play any role in the image analysis in this protocol (this metadata file could be used with some other protocol in the future). Therefore, it is recommended that the images are phase-flipped before entering HEMNMA protocol.
After executing “Import particles” (click on “Execute” button):

The imported particles can be visualized by first clicking on “scipion – import particles finished” and then on “Analyse Results” button at right.

If you do not wish to crop or resize images, go to 8.

7. Crop or resize images if you wish (double click on “Resize particles (optional)” in the menu at left, fill out the dialog, and execute):

“Crop/resize particles” dialog:
8. Analyze images with selected modes (double click on “Image analysis with selected modes” in the menu at left):

This opens a dialog box with the following two tabs: “Input” and “Angular assignment”. They are filled out the same way whatever type of the input structure is (atomic or pseudoatomic). As an example, we here show full details of the image analysis using normal modes of an atomic structure and only a few details of the analysis using normal modes of a pseudoatomic structure.

8.1 Image analysis using normal modes of the input atomic structure:

“NMA alignment” dialog:

“Input” tab:

“Angular assignment” tab:
After executing (click on “Execute” button):

By clicking on the “xmipp3 – nma alignment finished” and then on “Analyze Results” button at right, a dialog opens by which the results of the image analysis can be viewed. This allows analyzing results in 1D (amplitudes of displacement along 1 mode), 2D (amplitudes of displacement along 2 modes), or 3D (amplitudes of displacement along 3 modes). For instance, we show here 1D, 2D and 3D analysis of the results obtained using the input atomic structure.

Results analysis in 1D (amplitudes of displacement along the selected mode):

Results analysis in 2D (amplitudes of displacement along 2 selected modes):
Results analysis in 3D (amplitudes of displacement along 3 selected modes):

8.2 Image analysis using normal modes of the input pseudoatomic structure:

For the part of the project that uses the input pseudoatomic structure, we only show filling out the “Input” tab of the “NMA alignment” dialog box, the project structure after executing this task, and the results analysis. The parameter values in the “Angular assignment” tab of the “NMA alignment” dialog box were the same as in 8.1.

“Input” tab of the “NMA alignment” dialog box:

After executing (click on “Execute” button):
Here, we show 2D and 3D analysis of the results obtained using the input pseudoatomic structure.

**Results analysis in 2D (amplitudes of displacement along 2 selected modes):**

![Image of 2D analysis](image1)

**Results analysis in 3D (amplitudes of displacement along 3 selected modes):**

![Image of 3D analysis](image2)

9. Reduce dimension and create clusters or trajectories (double click on “Clustering and trajectories” in the menu at left):

![Image of SCION software](image3)
9.1 Dimension reduction:

The obtained results (the displacement amplitudes along normal modes computed by image analysis) should ideally be visualized in the space whose dimension is given by the number of normal modes used in the image analysis. However, the visualization is only possible in a space of 1, 2, or 3 dimensions. When the number of used normal modes is larger than 3, one can employ dimension reduction methods to reduce the dimension to 1, 2, or 3.

In the example given here, the image analysis was done using only 3 normal modes, meaning that we may not need the dimension reduction. However, visualizing the results in a 2D space can be practical, for instance to create clusters or trajectories more easily. Therefore, we reduce the dimension to 2 in our example case.

The dimension reduction can be performed using several methods. For instance, we here show the use of Principal Component Analysis.

Dialog to reduce dimension (after a double click on “Clustering and trajectories” in the menu at left):

![Dialog to reduce dimension](image)

The “Conformational distribution” field should be filled in with the normal-mode displacement amplitudes computed by image analysis. Note that we here first show the analysis of the conformational distribution obtained using the input atomic structure.

After executing (click on “Execute” button):

![Project HEMNMA_precomputed](image)
By clicking on the “xmipp3 – nma dimred finished” and then on “Analyze Results” button at right, a dialog opens by which the results of the dimension reduction can be viewed. Here, we show the projection of the conformational distribution onto the first two principal axes:

The principal axes to project the conformational distribution onto should be given in the “Display raw deformation” field of the “NMA dimred” viewer. Thus, note that “Mode 1” and “Mode 2” in the above figure (figure axes and title) mean “Principal axis 1” and “Principal axis 2”.

9.2 Clustering:

To open “Clustering Tool”, select “Open clustering tool” in the “NMA dimred” viewer, by clicking on the eye next to it. To select the points that will be assigned to a cluster, in the “Expression” field, enter a logical expression for the selected axes (in the “Axes” field, x1, x2, …) following the given example. Click on “Update Plot” to highlight the selected points (yellow points in the far right figure) To create a cluster with the selected points, enter the desired cluster name in the “Cluster name” field and click on “Create Cluster” button.

We here show an example of selecting points with “Clustering Tool” to create a cluster named “cluster1”:

In this example case, the points do not naturally group into clusters (separated groups of points) but rather follow a trajectory. However, we make two clusters from these points to show the use of the clustering tool.
After clicking on “Create Cluster” in the clustering tool right above, “cluster1” is created:

As explained above, we here create a second cluster as well. We create this cluster (“cluster2”) so that it does not overlap with “cluster1”:

After clicking on “Create Cluster” in the clustering tool right above, “cluster2” is created:
The clustering results, for instance those for “cluster1”, can be visualized by first clicking on “cluster1 finished” and then on “Analyze Results”. This allows visualizing images in “cluster1” as well as slices of the 3D reconstruction from these images.

Slices of the density map reconstructed from images in “cluster1”:

Images in “cluster1”:

One can also visualize isosurfaces of the density maps reconstructed from clusters by using Chimera on the command line. The density map reconstructed from a cluster of images (“reconstruction.vol”) can be found on the following path: “~/ScipionUserData/projects/PROJECTNAME/Runs/XXXXXX_BatchProtNMACluster/extra/”.

Here are superposed two density maps reconstructed from images in “cluster1” and “cluster2”: 
9.3 Trajectories:

To open “Trajectories Tool”, select “Open trajectories tool” in the “NMA dimred” viewer, by clicking on the eye next to it. Optionally, some points can be removed from the plot so that the trajectory can be created only from the remaining points. To select the points to be removed, in the “Expression” field, enter a logical expression for the selected axes (in the “Axes” field, x1, x2, …) following the given example. Click on “Update Plot” to remove the selected points. To create (animate) a trajectory from the remaining points, enter first the desired trajectory name in the “Name” field. Then, designate the starting and stopping trajectory points on the plot (one click for the starting point and double click for the stopping point). This will mark these 2 points and 8 points between them with red color. The red points will be taken to animate the trajectory. Finally, click on “Generate Animation” to animate the trajectory with VMD. This trajectory can also be found on the following path: “~/ScipionUserData/projects/PROJECTNAME/Runs/XXXXXX_XmippProtDimredNMA(extra/anima\_TRAJECTORYNAME/”.

Removing some points and creating a trajectory from the remaining points (“trajectory1”) in our example data case is shown here:
9.4 Dimension reduction, clusters, and trajectories when the conformational distribution is obtained using a pseudoatomic structure:

The dimension reduction, creation of clusters, and creation of trajectories can be performed in the same way for a conformational distribution obtained using a pseudoatomic structure as for the one obtained using an atomic structure (9.1-9.3)

We give below the results of the dimension reduction (from 3 to 2), cluster creation (2 clusters), and trajectory animation for our example data case with the pseudoatomic structure from the input density map.

Dialog to reduce the dimension (after a double click on “Clustering and trajectories” in the menu at left):

The “Conformational distribution” field is here filled with the conformational distribution obtained using the pseudoatomic structure from the input density map.

Creation of “cluster1”:

Creation of “cluster2”:
The project after creating the two new clusters:

Density maps reconstructed from images in “cluster1” and “cluster2” and superposed in Chimera:

Trajectory animated with VMD: