1. **INTRODUCTION**

**Java Essential Dynamics** (JED) is a java library (a package of programs) for analyzing protein trajectories. The trajectories may be derived from MD, FIRST/FRODA, or any other dynamic simulations that output a trajectory as a set of PDB files. The program can handle single chain PDB files with no chain identifier as well as multi chain PDB files that use chain IDs. The user may specify the set of residues to be considered for the analysis, and this set need not be contiguous. A variety of utility tools are provided that use **Principal Component Analysis** (PCA) that are not found in MD-simulation packages or other stand alone PCA software, especially in regards to comparative analysis of multiple trajectories. JED is capable of running on any platform with a suitable Java Runtime Environment (JRE).

**Expected Input to JED:**

Ideally, each PDB structure must follow standard PDB-format. Note that some deviations from standard will often work fine, but JED expects standard format. Moreover, it is required that the structures have been prepared in such a way that there are no gaps in residue labeling. If residues or consecutive regions of residues are missing, these need to be fixed, or the residue labeling has to be altered in order to remove gaps in residue labels. The first residue label must start at 1 or higher. No 0 or negative numbers are allowed for residue labels. All preprocessing of the PDB files must be done with external software before using JED. It is convenient to label PDB files using leading zeros in the name of the files to simplify tracking time progression. For example, if a simulation generates 100,000 frames in the trajectory, it is best to name the PDB files like <file\_name\_000000>, <file\_name\_000001>, … <file\_name\_100001>, which specifies that relative to the starting structure 100,000 frames are generated in successive order. Although this naming scheme is not required, it is highly recommended because it allows the user to track time order easily on operating systems that sort order by literal alphabetic-characters, rather than interpreting 34 is less than 100, for example.

**JED Preprocessing Output:**

As a pre-processing step, JED reads in all PDB files in a specified directory and aligns all the structures in the trajectory to a specified reference structure using a quaternion alignment algorithm. A matrix of the **read** **PDB coordinates,** obtained from all the residues in the input PDB files, is created so that it can be used for all subsequent JED runs. A list of all the residues (**residue list**) found in the PDB files (along with the chain IDs when appropriate) is generated. The original and transformed **conformation rmsd** are determined for each member structure in the trajectory relative to the specified reference structure. The **residue rmsd** (also commonly referred to as **RMSF**)is determined from the entire trajectory. An **edited PDB file** is also generated where the B-factors are replaced with the **residue rmsd** values for visualization purposes. This output automatically happens and is non-optional.

**Carbon Alpha Atoms:**

The current implementation of JED only considers Cα atoms. As such, we speak about residues because the information is tied to the Cα atoms, which represents the dynamics of the residue at a coarse grained level of description. For example, the distance between two residues is modeled in JED as the distance between the two Cα atoms associated with the two residues. This choice of working only with Cα atoms allows the labeling of the Cα atoms to be associated with residue labels. For a single chain protein, this is a simple 1 to 1 mapping. For multiple chain proteins, JED also tracks the chain ID.

**Different Types of PCA:**

The core element of essential dynamics is to perform PCA. JED implements two variations of PCA. The first and most common method is based on Cartesian coordinates (**cPCA**). Note that cPCA using ***n*** **residues** will yield eigenvectors having ***3n*** components, each corresponding to one Cartesian coordinate. The second method is based on internal coordinates using either all-to-all residue distances (**dPCA**) and/or residue-pair distances (**dpPCA**). The dPCA is much more computationally expensive and the interpretation can be difficult when the number of residues in the subset exceeds ten. Note that dPCA using ***n*** **residues** will yield eigenvectors having ***n\*(n-1)/2*** components, each corresponding to one set of inter-residue distances. Thus, for five residues, one obtains ten pairs of interactions, and this becomes difficult to interpret. In contrast, dpPCA using ***n*** **residue-pairs** will yield eigenvectors having ***n*** components, each corresponding to one of inter-residue distance pairs.

All of the PCA methods can be performed using a **covariance matrix** (**Q**) and/or **correlation matrix** (**R**). The correlation matrix is a normalized version of the covariance matrix. The results obtained from **Q** and **R** generally differ somewhat due to the inherent statistical biases in each approach. The current implementation does both.

**Conditioning of the Q and R Matrices:**

JED handles the removal of outliers prior to the PCA analyses with two approaches. First, the user can specify the **percent** (a decimal [0,1]) of the structures to be removed based on the conformation rmsd. The most deviant structures are tagged as outliers and subsequently removed prior to the PCA analysis. In this first method, frames that are identified as an outlier are thrown out from the sample. Second, the user can specify a **z-score cutoff** (a decimal ≥ 0) such that when the value of a PCA variable (either a Cartesian or internal distance coordinate) has a |deviation| from the variable mean that exceeds the z-score cutoff, it is identified as an outlier. For each PCA-entry that is identified as an outlier, it is replaced with its mean. This process is done per variable over all frames, and each PCA-entry is treated independently. In this second method, a frame is never thrown away, but some entries within a frame may be modified. Both methods are intended to reduce the condition numbers of **Q** and **R**. While the first method of conditioning is most commonly employed in the protein field (if at all), the second method of conditioning is most commonly used in the field of statistics, and is the preferred method due to its superior effectiveness. It should be noted here that without applying the conditioning, the results of a PCA can be highly skewed due to the presence of outliers as such analyses are highly dependent on the quality of the sampling. It is strongly recommended to use the z-score cutoff conditioning method in all applications to avoid misinterpreting the PCA results.

**Visualization of cPCA modes:**

JED computes the **PCA modes** (RMSD and MSD, with and without weight by the corresponding eigenvalue) from the Cartesian eigenvectors so that the cPCA results can be mapped to the residue set. As noted above, sets of structures can be generated to visually inspect the cPCA modes. Eigenvectors from dPCA or dpPCA cannot be mapped to the residue set in any simple way, so no mapping or visualization is attempted. The user can specify the number of Cartesian modes to visualize. Mode visualization is done by creating a set of 20 PDB files that capture the displacement of the alpha carbons for the given mode. A scale-factor parameter is adjustable to control the amount of displacement in the modes. A Pymol© script is generated to animate the frames.

**Dimension Reduction Level:**

The primary purpose of applying PCA to capture the essential dynamics of a protein is to reduce the large dimension of variables to a much smaller number of variables that captures the greatest variance in protein motion. The **Q** and **R** matrices, once diagonalized, provide a set of eigenvalues and eigenvectors. The eigenvalues for proteins typically fall off fast for the first several modes, out of possibly thousands of modes. The number of dimensions needed to provide a fair assessment of the essential dynamics in a protein is system-dependent. The user can specify any number (say 20, which typically is more than needed) to obtain results for all possible selections ranging from 1 up to the value selected. In this way, the user can see how the added dimensions help glean more information, albeit making it harder to interpret the greater number of dimensions. Eventually, the user must decide, based on their purpose/goals, the optimal number of dimensions to use for representing the essential dynamics.

**Displacement Vectors:**

A set of **displacement vectors** (**DV**s) based on the full conformational space is calculated using a specified reference structure. Those **DV**s are then projected onto a set of eigenvector directions to create delta vector projections (**DVP**s), which are similar to principle components (**PC**s). The **PC**s are delta vector projections, but according to the standard definition used in statistics, they are always relative to the mean conformation position as defined in the construction of the **Q** or **R** matrix. In studying the essential dynamics of a protein, it is common to use a reference structure that has a particular physical or biochemical meaning, which is why we call these displacements **DVP**s, and not **PC**s. The DVPs are very useful to have for visualizing protein motions. For example, if the first two eigenvector directions are selected (those eigenvectors associated with the highest and second highest eigenvalues, or variance) the **DVP**s can be plotted for each frame to construct the trajectory in conformational space projected onto a two dimensional cross-section. Other eigenvector directions can be specified, allowing the user to investigate how the trajectory projects into the space defined by each eigenvector. The **DVP**s are given using un-normalized and normalized inner products, as well as weighted by the corresponding eigenvalue.

**Post PCA Comparative Subspace Analysis:**

JED performs a subspace analysis (**SSA**) on the two equidimensional sets of eigenvectors generated from the **Q** and **R** variants of PCA. The results provide comparisons for all subspace dimensions up to the dimension chosen by the user when selecting the number of Cartesian or Distance modes to process in an iterative fashion. This allows one to quantitatively determine how different the PCA results are due only to the choice of PCA model, while also assessing the size of the essential subspace. Additional analysis can be done using the driver programs for the Subspace Analysis class. To perform these kinds of tests, it is a best practice to first generate equidimensional sets of eigenvectors from each trajectory of interest, as well as from a pooled trajectory to use as a reference set, while ensuring that the subsets of residues analyzed are identical. Subspace analysis is done by comparing the sets of eigenvectors, directly or iteratively, and determining the root mean square inner products (**RMSIP**s), Principal Angles (**PA**s), cumulative overlap (**CO**s), cosine products (**CP**s), vectorial angular sum (**VAS**), and the maximum angle between subspaces of the given vector space.

(In this tutorial, code, file paths, and text file content are shown in dark blue 9 point Consolas)

1. **Understanding JED**

**JED Install Instructions:**

Java is **platform independent** and JREs exist for all common architectures. The machine on which JED is to be run should have **JRE version 1.6 or higher** installed. The programs can be run from compiled source or from the provided executable jar files. While JED can be installed in any directory that is part of your Java classpath, the sources must be compiled on the local machine to insure runtime integrity. When compiling from source, be sure to compile the JAMA MATRIX package as JED uses that library. Alternatively, no source code or compilation is needed to run the executable jar files. These can be placed in any directory that is on the Java classpath. For most applications, a **64bit OS is required** to address the amount of memory needed for the analyses. It is critical that the environment variable Java **CLASSPATH** be correctly set to run Java programs at the command prompt. Alternatively, you can always add the **-cp** option to the **java** command, which allows you to specify the path that contains your Java classes.

**Expected Memory Requirements:**

On high performance computer clusters make sure the 64 bit JRE is installed. Memory use is demanding because JED loads the complete covariance matrix (among other data structures) and performs a full matrix diagonalization, which scales as O(N3). Typically 8 to 32 GB of RAM will be needed depending on the size of the protein. For very large proteins consisting of thousands of residues and/or many tens of thousands of frames, make available as much memory per node as possible (~ 1TB). On most platforms, Java can be optimized by specifying parameters at runtime for heap space, perm space, etc.

**Two Kinds of JED Drivers:**

There are two driver programs for JED: One (**JED\_Driver**) runs a single job using parameters specified in the input file, and the other (**JED\_Batch\_Driver**) runs a batch of jobs sequentially. The first is suited for running a single job at the command line or when using submit scripts on computer cluster resources. This can be implemented using job arrays so that your jobs run in parallel rather than sequentially. The second is suited for running multiple jobs on a single computer so that a user can submit a batch of jobs, and come back a few hours later with many different jobs finished without having to launch each one separately. Note: The input file formats for the two driver programs are NOT equivalent.

**Input File and Data for JED Driver:**

JED requires an input file for job parameters. The format of this file will be described below. The run command takes only one argument, which is the name of the input file that includes the absolute path to the file. If no argument is specified, then JED assumes that the default input file name is used and the file is located in the same directory from which the Java Virtual Machine (JVM) was called. The default input file names are:

**JED\_Driver.txt** for JED\_Driver.java (or .jar file)

**JED\_Batch\_Driver.txt** for JED\_Batch\_Driver.java (or .jar file)

Each job should be assigned to its own directory, which must contain either the PDB files to read or the coordinate file to process, along with the reference PDB file and residue lists for specifying either the Cartesian and/or Distance subsets.

**JED Command Line format:**

To run **JED\_Driver** at the command prompt or within a PBS script, you can use one of the following commands:

java -d64 JED\_Driver “/path/to/your/input/file.txt” (runs the compiled java program)

java -jar -d64 JED\_Driver.jar “/path/to/your/input/file.txt” (runs the executable jar file)

To run **JED\_Batch\_Driver** at a command prompt or in a PBS script, you can use one of the following commands:

java -d64 JED\_Batch\_Driver “/path/to/your/input/file.txt”)

java -jar -d64 JED\_Batch\_Driver.jar “/path/to/your/input/file.txt”

*Remember to include command line switches to optimize the Java runtime environment for your jobs.*

**Organization of Output Files:**

Output files from JED are written to subdirectories within the working directory, structured to organize the multitude of files produced in a meaningful manner. The top level of this directory tree is named "JED\_RESULTS\_**$description**", where **$description** is a user set parameter that succinctly describes the job. Limbs of the tree separate Cartesian PCA (**cPCA**), Distance PCA (**dPCA**), Distance-Pair PCA (**dpPCA**), and Mode Visualization Analysis (**VIZ**), when present. Each of these in turn contains limbs for **Q** (**COV**) and **R** (**CORR**) compartmentalization, which also contains a subdirectory for the subspace analysis (**SSA**). Most of the output file names include the **number of residues** in the selected subset for reference plus a description of the file contents.

**Current Limitations:**

Initial input of the protein trajectory must be done using PDB files that are expected to conform to the standard format, or a matrix of PDB coordinates containing the alpha carbon atomic positions only (see below for a description of this file). Only carbon-alpha atomic positions are used to create a **Q** or **R** matrix for essential dynamic analysis.

Each PDB file must have the exact same number of residues. The matrix of alpha carbon coordinates is determined from the first PDB file read. If other files in the working directory do not match exactly, then the array sizes will not match and the program will crash. *IF JED crashes during the reading of PDBs, this is probably the reason*.

While JED can process a PDB file with missing residues, it can NOT interpret files that have alternate conformations within a given frame based on fractional **occupancy** values. Only a single conformation per frame is allowed. Note that the original residue coordinates in the PDB files are mapped to the rows of the coordinates matrix and thus care must be exercised when specifying residue subsets.

1. **Step by Step Instructions to Run JED**

**IN ALL CASES:**

A preliminary run with NO PCA must be performed to generate the JED formatted coordinate matrix file for all the alpha carbons in the PDB files. This makes subsequent subset analyses much faster to perform. It also serves to guarantee that the specified residues for subset selection are correctly chosen. After this initialization step, the PDB files can be deleted or archived, with the exception of the reference PDB file. Once the coordinate matrix is created, it should be used for all subsequent analyses using different residue subsets and different job parameters.

The name of the coordinate file matrix produced from the PDB files is: "original\_PDB\_coordinates.txt"

The matrix packing is as follows:

**Rows are coordinate variables and columns are frames.**

For N residues, there are 3N rows: N x-coordinates, N y-coordinates, and N-z coordinates, stacked in that order.

* **The file to use in all subsequent JED analyses is the original\_PDB\_coordinates matrix.**

*This matrix contains all the residues in the PDB files and thus can be used for any subset of those residues. When a subset is chosen, a new correspondence set is generated and a new transformation is done to optimize the alignment of the structures. This removes overall translation and rotation.*

In subsequent analyses, it is critical that no residues are requested that do not actually exist in the PDB files!

JED maps the specified residue list to an internal list that is aligned to the rows of the coordinates matrix.

Note: The most critical step in preparing to run JED is in the creation of the input file. The input file must have the correct format (shown in examples below) and the entries must be accurate. If either of these conditions is violated, the program will crash, or worse, the results will be corrupt.

**Common Causes for JED to Crash**

If **any** **directory** cannot be found or if **any** **file** cannot be found, JED will crash.

If unexpected format is found in **any** of the input files, JED will crash.

Note that the JED driver programs employ many checks during the reading of the input files and the execution of the program. There are checks to validate the number formats of numeric data. There are checks to ensure that enough parameters were specified for the particular job. There are checks to ensure that the number of modes requested does not exceed the actual number of modes available. JED also verifies that directories and files exist before performing any analysis. The developers have gone to great lengths to provide meaningful information when the program crashes to facilitate making the necessary corrections. The specified input file is echoed to standard out as are the assignment of parameters, while any detected problems or errors have their messages directed to standard error. In many cases, a stack trace will also be sent to standard error.

1. **JED DRIVER:**
2. **The Preliminary Run**
3. **Run Command:**

java -jar -d 64 JED\_Driver.jar “/path/JED\_Driver.txt”

***The PDB files (including the PDB reference file) must be in the working directory.***

***JED input file may be in the working directory.***

This pre-processing step will read all PDB files in the working directory, but will perform **no PCA.**

The purpose of **no PCA** this is to generate the matrix of coordinates for performing subset analyses efficiently.

1. **Standard Preliminary Run Output Files:**

These are written to the **root** of the JED Results directory tree:

/working/directory/JED\_Results\_Description/

**JED LOG** providing a summary of the job parameters and results:

JED\_Log.txt

**PDB READ LOG** listing all the PDB files read, in the order they were read:

PDB\_READ\_Log.txt

**coordinates matrix** from all the alpha carbon coordinates in the PDB files:

original\_PDB\_coordinates.txt

**transformed coordinates matrix**, which aligns all the frames to the reference frame :

ss**\_$num**\_res\_transformed\_PDB\_coordinates.txt

**list of all residues** found in the PDB files for subsequent editing and use:

All\_PDB\_Residues\_JED.txt

All\_PDB\_Residues\_Multi\_JED.txt (for Multi runs)

**original and transformed conformation RMSDs**:

ss\_**$num**\_res\_original\_conformation\_rmsds.txt

ss\_**$num**\_res\_conformation\_rmsds.txt

**residue RMSDs (RMSF)**:

ss\_**$num**\_res\_residue\_rmsd.txt

**edited PDB file** containing all the residues with the RMSF replacing B-factors:

ss\_**$num**\_res\_edidited.PDB

**coordinates z-score matrix** from all the alpha carbon coordinates in the PDB files:

ss\_**$num**\_res\_coordinate\_Z\_scores.txt

**percent** of the frames to remove based on conformation RMSD (OPTIONAL)

**z-cutoff** for adjusting coordinate outliers to their mean values (OPTIONAL)

If **percent** or **z-cutoff** are set to a value other than **zero**, additional output files are generated with the results. However, the appropriate time to handle outliers is during runs that actually perform PCA and it is recommended that for pre-processing runs, these be set to zero.

1. **Single Chain PDB files: The Preliminary Run**

-----------------------------------------------------------------------------------------------------

0.00

0.00

1 0

0 0 0 0

/working/directory/job/

Description reference\_PDB\_file.pdb

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Notes: This is a **whitespace** separated file with **6 lines**.

Line 1 Field 1: specifies the **percent** (double) of frames to remove from the data: 0 🡪NONE

Line 2 Field 1: specifies the **z-cutoff** (double) for adjusting outliers: 0 🡪NO ADJUSTMENT

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪1 = yes

Field 2: specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 0 = no

Line 4 Field 1: specifies the **number of Cartesian** (integer) modes to process 🡪0 = none

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪0 = none

Line 5 Field 1: specifies the **working directory** (String)

Line 6 Field 1: specifies the **description** (String) for the requested job

Field 2: specifies the **reference PDB** (String) for the requested job

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**Key Points**:

The **z-cutoff** can be set to check the number of outliers per variable and examine the variable z-scores.

The **Read** flag must be set to **1**.

* When the **Read** flag is set to **1**, all other PCA analyses are turned off.

The **Multi** flag must be set to **0** for Single Chain PDBs with no Chain IDs.

* **The file to use in all subsequent JED analyses is the original\_PDB\_coordinates matrix.**

1. **Multi-Chain PDB files: The Preliminary Run**

**Differences from Single-Chain analysis shown highlighted in amber**

-----------------------------------------------------------------------------------------------------

0.00

0.00

1 **1 2 A B 795 151 0 0**

0 0 0 0

/working/directory/job/

Description reference\_PDB\_file.pdb

-----------------------------------------------------------------------------------------------------

Notes: This is a **whitespace** delineated file with **6 lines**.

Line 1 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 🡪NONE

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 0.00 🡪NO ADJUSTMENT

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪1 = yes

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 1 = yes

Field 3 specifies the number of chains 🡪 (2)

Field 4 specifies the first chain ID 🡪 (A)

Field 5 specifies the second chain ID 🡪 (B)

Field 6 specifies the number of residues in chain A🡪 (795)

Field 7 specifies the number of residues in chain B🡪 (151)

Field 8 specifies the offset of Chain A 🡪 (0)

Field 9 specifies the offset of Chain B 🡪 (0)

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪0 = none

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪0 = none

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String) for job one

Field 2 specifies the **reference PDB** (String) for job one

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**Key Points**:

The **z-cutoff** can be set to check the number of outliers per variable and examine the variable z-scores.

The **Read** flag must be set to **1**.

* When the **Read** flag is set to **1**, all other PCA analyses are turned off.

The **Multi** flag must be set to **1** for Multi Chain PDBs.

**Multi Chain PDBs must have unique chain identifiers for every chain.**

* **A missing chain identifier will cause JED to crash.**
* **The file to use in all subsequent JED analyses is the original\_PDB\_coordinates matrix.**

1. **Debugging Crashes Part I:**

Things that will generally make your life miserable…

1. **Simple mistakes:**

Does the path to the input file exist?

Does the input file exist in the proper location?

Does the input-file start on the first line?

Is the **number format** correct? (20.0 will NOT parse as an integer)

Are the **Read** and **Multi** flags set correctly?

Did you forget a parameter declaration?

Does the working directory string end in “**/**” or “**\\**”?

Does the working directory exist?

Does the working directory contain PDB files of different sizes?

Does the working directory contain the reference PDB file?

1. **More subtle mistakes:**

The directory contains PDB files with 2 chains, but no chain IDs.

If the PDB file names are sorted in a different order than how they were generated, then the conformation RMSD results will not reflect what actually occurred in the simulation. Naming the PDB files appropriately by padding the numbers with leading zeros will ensure proper sorting to prevent this problem caused by the operating system.

If the conformation RMSD is very different from what you expect, then you may be using PDB files that contain occupancy information. JED does not use that information. Your results will not be accurate.

If you have pooled data, make sure the combined matrix is constructed the way you think it is and the reference column is the frame you think it is.

1. **Performing Cartesian PCA**
2. **Run command:**

java -jar -d64 JED\_Driver.jar “/path/JED\_Driver.txt”

***The working directory must contain: The coordinates matrix, the PDB reference file, and the residue list.***

***JED input file may be in the working directory.***

The purpose of this type of run is to perform Essential Dynamics using cPCA based on **Q** and **R**. The user specifies the subset of interest for the analysis, which may be the entire protein or a sub-region, which can be non-contiguous, by providing a residue list file. This task is simplified since JED has already created a list of all the residues in the protein. The user can simply edit this file. The cPCA results are written to the sub-directory "cPCA" and the Visualizations of the top modes (when selected) are written to the subdirectory "VIZ". The directory cPCA has sub directories for the **Q** and **R** analysis, as does the VIZ directory.

1. **Standard Output Files:**

These are written to the **root** of the JED Results directory tree:

/working/directory/JED\_Results\_Description/

**JED LOG** providing a summary of the job parameters and results:

JED\_Log.txt

**subset transformed coordinates matrix**:

ss\_**$num**\_res\_transformed\_PDB\_coordinates.txt

**original and transformed conformation RMSDs**:

ss\_**$num**\_res\_original\_conformation\_rmsds.txt

ss\_**$num**\_res\_conformation\_rmsds.txt

**residue RMSDs (RMSF)**:

ss\_$num\_res\_residue\_rmsd.txt

**subset edited PDB file** with the RMSF replacing B-factors:

ss\_**$num**\_res\_edidited.PDB

**subset coordinates Z-Score matrix**:

ss\_**$num**\_res\_coordinate\_Z\_scores.txt

**list of frames removed**, based on the **percent** parameter:

ss\_**$num**\_res\_ Removed\_Conformation\_Outliers.txt

**trimmed transformed coordinate matrix**:

ss\_**$num**\_res\_ trimmed\_$percent\_percent\_PDB\_coordinates\_COLS.txt

**list of coordinate variables adjusted**, based on the **z-cutoff** parameter:

ss\_**$num**\_res\_ adjustments\_per\_variable.txt

**adjusted transformed coordinate matrix**:

ss\_**$Z**\_threshold\_**$z**-cutoff\_adjusted\_PDB\_coordinates\_ROWS.txt

1. **Standard cPCA Output Files:**

These are written to the **/cPCA subdirectory**  of the JED Results directory tree:

/working/directory/JED\_Results\_Description/cPCA/

**centroids (means) of the variables:**

ss\_**$num**\_res\_centroids\_of\_variables.txt

**standard deviations of the variables:**

ss\_**$num**\_res\_std\_devs\_of\_centered\_variables.txt

**displacement vectors:**

ss\_**$num**\_res\_delta\_vectors.txt

* **The Q output files are written to the /COV subdirectory of /cPCA**

/working/directory/JED\_Results\_Description/cPCA/COV/

* **The R output files are written to the /CORR subdirectory of /cPCA**

/working/directory/JED\_Results\_Description/cPCA/CORR/

**covariance matrix:**

ss\_**$num**\_res\_COV\_matrix.txt

**eigenvalues:**

ss\_**$num**\_res\_eigenvalues\_COV.txt

**top eigenvalues:**

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_eigenvalues\_COV.txt

**top eigenvectors:**

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_eigenvectors\_COV.txt

**top pca modes and top weighted pca modes:**

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_pca\_modes\_COV.txt

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_weighted\_pca\_modes\_COV.txt

**top square pca modes and top weighted square pca modes:**

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_square\_pca\_modes\_COV.txt

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_weighted\_square\_pca\_modes\_COV.txt

**top DVPs and top weighted DVPs:**

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_DVPs\_COV.txt

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_weighted\_DVPs\_COV.txt

**top DVPs and top weighted DVPs:**

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_normed\_DVPs\_COV.txt

ss\_**$num**\_res\_top\_**$num**\_of\_cart\_modes\_weighted\_normed\_DVPs\_COV.txt

1. **SSA Output Files:**

These are written to the **/SSA subdirectory**  of /cPCA:

/working/directory/JED\_Results\_Description/cPCA/SSA/

**The Fast SSA Iterated Log:**

JED\_FSSA\_Iterated\_log.txt

**The SSA Log:**

JED\_SSA\_dim\_$top\_num\_cart\_modes\_log.txt

**The Random SSA Log**

JED\_Random\_SSA\_log.txt

**There are many more files in the /SSA directory that are flat files of the results reported in the log files:**

**RMSIPs**

**PAs**

**COs**

**Cosine Products**

**Vectorial Sum of Angles**

1. **Single-Chain PDB files: cPCA**

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0.01

3.00

0 0

20 0 0 2 1.0

/working/directory/

Description reference\_PDB\_file.pdb 0

residues.txt

original\_PDB\_Coordinates.txt 0

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Notes: This is a **whitespace** delineated file with **8 lines**.

Line 1 Field specifies the **percent** (double) of frames to remove from the data: 0.01 🡪 1%

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 0 = no

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 20 = top 20 modes

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪0 = none

Field 5 specifies the **scale factor** for the visualizations (double) 🡪 1.0 = default value

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String)

Field 2 specifies the **reference PDB** (String)

Field 3 specifies the **offset** for the residue numbering (integer)

Line 7 Field 1 specifies the **Cartesian** **residue list** (String)

Line 8 Field1 specifies the **coordinate matrix** (String)

Field 2 specifies the **reference column** (integer)

------------------------------------------------------------------------------------------

**Key Points**:

Both the **Read** flag and the **Multi** flag must be set to **0** for Single chain PDBs.

The number of cPCA modes must not be 0.

There must be a Cartesian residue list specified.

The number of dPCA modes and dpPCA modes must be zero.

There must not be a Distance residue list specified.

If the subset you have chosen contains N residues, then you must NOT request more than 3N modes.

If you request N cPCA modes then you can only visualize up to N top modes.

* **If number of cPCA modes > 0, then there must be a Cartesian residue list file!**

1. **Multi-Chain PDB files: cPCA**

**Differences from Single-Chain analysis shown highlighted in amber**

------------------------------------------------------------------------------------------

0.01

3.00

0 1 **2 A B 795 151 0 0**

20 0 0 2 1.0

/working/directory/

Description reference\_PDB\_file.pdb (no offset needed here)

residues.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes: This is a **whitespace** delineated file with **8 lines**.

Line 1 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.01 🡪 1%

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 1 = yes

Field 3 specifies the number of chains 🡪 (2)

Field 4 specifies the first chain ID 🡪 (A)

Field 5 specifies the second chain ID 🡪 (B)

Field 6 specifies the number of residues in chain A🡪 (795)

Field 7 specifies the number of residues in chain B🡪 (151)

Field 8 specifies the offset of Chain A 🡪 (0)

Field 9 specifies the offset of Chain B 🡪 (0)

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 20 = top twenty modes

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪0 = none

Field 5 specifies the **scale factor** for the visualizations (double) 🡪 1.0 = default value

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String)

Field 2 specifies the **reference PDB** (String)

Line 7 Field 1 specifies the **residue list** (String)

Line 8 Field 1 specifies the **coordinate matrix** (String)

Field 2 specifies the **reference column** (integer)

------------------------------------------------------------------------------------------

**Key Points**:

The **Read** flag must be set to **0**. The **Multi** flag must be set to **1**

* Chain Offsets are specified on line 3, not after the reference pdb file on line 6.

The number of cPCA modes must be **>** 0. There must be a Cartesian residue list specified.

The number of dPCA modes must be 0. There must not be a distance residue list specified.

If the subset you have chosen contains N residues, then you must not request more than 3N modes.

If you request ***N*** cPCA modes then you can only visualize up to ***N*** top modes.

1. **Residue List Format cPCA**
   1. **Single Chain PDBs**

**ONE column of integers: residue numbers**

* 1. **Multi Chin PDBs**

**TWO columns: first Strings, then integers tab separated: Chain IDs, residue numbers**

**Performing All-to-All Distance PCA: dPCA**

* 1. **Run command:**

java -jar -d64 JED\_Driver.jar “/path/JED\_Driver.txt”

***The working directory must contain: The coordinates matrix, the PDB reference file, and the residue list.***

***JED input file may be in the working directory.***

The purpose of this type of run is to perform Essential Dynamics using dPCA based on **Q** and **R**. The user specifies the subset of interest for the analysis, which is typically less than 10 residues, by providing a residue list file. This task is simplified since JED has already created a list of all the residues in the protein. The user must simply edit this file. PCA is performed on the set of all to all distances calculated from the entered residues. The dPCA results are written to the sub-directory "dPCA". Note that for dPCA no transform is needed since internal distances are used for coordinates and no visualization can be done in JED for the distance modes. The directory dPCA has sub directories for the **Q** and **R** analysis as well as for the subspace analysis (SSA). Choosing more than ten residues for the dPCA analysis makes the interpretation of the results challenging as each component of the distance eigenvectors corresponds to an inter-residue distance pair. Often subsets with less than ten can be used to investigate experimental findings in critical areas like binding pockets or clefts.

* 1. **Standard Output Files:**

These are written to the **root** of the JED Results directory tree:

/working/directory/JED\_Results\_Description/

**JED LOG** providing a summary of the job parameters and results:

JED\_Log.txt

**subset transformed coordinates matrix**:

ss\_**$num**\_res\_transformed\_PDB\_coordinates.txt

**original and transformed conformation RMSDs**:

ss\_**$num**\_res\_original\_conformation\_rmsds.txt

ss\_**$num**\_res\_conformation\_rmsds.txt

**residue RMSDs (RMSF)**:

ss\_**$num**\_res\_residue\_rmsd.txt

**subset edited PDB file** with the RMSF replacing B-factors:

ss\_**$num**\_res\_edidited.PDB

**subset coordinates Z-Score matrix**:

ss\_**$num**\_res\_coordinate\_Z\_scores.txt

**list of frames removed**, based on the **percent** parameter:

ss\_**$num**\_res\_ Removed\_Conformation\_Outliers.txt

**trimmed transformed coordinate matrix**:

ss\_**$num**\_res\_ trimmed\_$percent\_percent\_PDB\_coordinates\_COLS.txt

**list of coordinate variables adjusted**, based on the **z-cutoff** parameter:

ss\_**$num**\_res\_ adjustments\_per\_variable.txt

**adjusted transformed coordinate matrix**:

ss\_**$Z**\_threshold\_**$z**-cutoff\_adjusted\_PDB\_coordinates\_ROWS.txt

* 1. **Standard dPCA Output Files:**

These are written to the **/dPCA subdirectory**  of the JED Results directory tree:

/working/directory/JED\_Results\_Description/dPCA/

**subset distance residue stats** from all the alpha carbon coordinates in the **subset**:

ss\_**$num**\_res\_distance\_residue\_stats.txt

**subset distance Z-Score matrix** from all the alpha carbon coordinates in the **subset**:

ss\_**$num**\_res\_distance\_Z\_scores.txt

**subset all-to-all** **distances matrix** from all the alpha carbon coordinates in the **subset**:

ss\_**$num**\_res\_all\_to\_all\_distances.txt

**subset distance variables** adjusted:

ss\_**$num**\_res\_ outliers\_per\_variable.txt

**subset centroids (means) of the variables:**

ss\_**$num**\_res\_centroids\_of\_variables.txt

**subset standard deviations of the variables:**

ss\_**$num**\_res\_std\_devs\_of\_centered\_variables.txt

**subset displacement vectors:**

ss\_**$num**\_res\_delta\_vectors.txt

* **The Q output files are written to the /COV subdirectory of /dPCA** (Shown below)

/working/directory/JED\_Results\_Description/cPCA/COV/

* **The R output files are written to the /CORR subdirectory of /dPCA**

**covariance matrix:**

ss\_**$num**\_res\_distance\_COV\_matrix.txt

**eigenvalues:**

ss\_**$num**\_res\_distance\_eigenvalues\_COV.txt

**top eigenvalues:**

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_distance\_eigenvalues\_COV.txt

**top eigenvectors:**

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_distance\_eigenvectors\_COV.txt

**top pca modes and top weighted pca modes:**

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_distance\_pca\_modes\_COV.txt

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_weighted\_distance\_pca\_modes\_COV.txt

**top square pca modes and top weighted square pca modes:**

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_square\_distance\_pca\_modes\_COV.txt

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_weighted\_square\_distance\_pca\_modes\_COV.txt

**top DVPs and top weighted DVPs:**

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_DVPs\_COV.txt

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_weighted\_DVPs\_COV.txt

**top normed DVPs and top weighted normed DVPs:**

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_normed\_DVPs\_COV.txt

ss\_**$num**\_res\_top\_**$num**\_of\_dist\_modes\_weighted\_normed\_DVPs\_COV.txt

* 1. **Standard SSA Output Files:**

These are written to the **/SSA subdirectory**  of /cPCA:

/working/directory/JED\_Results\_Description/cPCA/SSA/

**The Fast SSA Iterated Log:**

JED\_FSSA\_Iterated\_log.txt

**The SSA Log:**

JED\_SSA\_dim\_$top\_num\_cart\_modes\_log.txt

**The Random SSA Log**

JED\_Random\_SSA\_log.txt

**There are many more files in the /SSA directory that are flat files of the results reported in the log files:**

**RMSIPs**

**PAs**

**COs**

**Cosine Products**

**Vectorial Sum of Angles**

1. **Single-Chain PDB files: dPCA**

------------------------------------------------------------------------------------------

0.00

3.00

0 0

0 3 0 0

/working/directory/

Description reference\_PDB\_file.pdb 0

residues\_dist.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 = none

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 0 = no

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 0 = none

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪3 = top 3 modes

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪0 = none

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String)

Field 2 specifies the **reference PDB** (String)

Field 3 specifies the **offset** (integer)

Line 7 Field 1specifies the **residue list** (String)

Line 8 Field 1 specifies the **coordinate matrix** (String)

Field 2 specifies the **reference column** (integer)

------------------------------------------------------------------------------------------

**Key Points**:

Both the **Read** PDB file flag and the **Multi** flag MUST be set to **ZERO**.

This tells JED to perform the analysis on Single Chain PDBs.

The number of cPCA modes MUST be zero.

There must be no Cartesian residue list specified.

The number of dPCA modes MUST NOT be zero.

The number of dpPCA modes MUST be zero.

There must be a Distance residue list specified.

If the subset you have chosen contains ***N*** residues, then you must NOT request more than ***N(N-1)/2*** modes.

**If number of dPCA modes > 0, then there must be a Distance residue list file!**

1. **Multi-Chain PDB files: dPCA**

**Differences from Single-Chain analysis shown highlighted in amber**

------------------------------------------------------------------------------------------

0.00

3.00

0 1 **2 A B 795 151 0 0**

0 3 0

/working/directory/

Description reference\_PDB\_file.pdb (no offset here)

residues\_dist.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 🡪 none

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 1 = yes

Field 3 specifies the number of chains 🡪 (2);

Field 4 specifies the first chain ID 🡪 (A);

Field 5 specifies the second chain ID 🡪 (B);

Field 6 specifies the number of residues in chain A🡪 (795);

Field 7 specifies the number of residues in chain B🡪 (151);

Field 8 specifies the offset of Chain A 🡪 (0);

Field 9 specifies the offset of Chain B 🡪 (0);

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 0

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪3 = top 3 modes

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪0 = none

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String)

Field 2 specifies the **reference PDB** (String)

Line 7 Field 1 specifies the **residue list** (String)

Line 8 Field 1 specifies the **coordinate matrix** (String)

Field 2 specifies the **reference column** (integer)

------------------------------------------------------------------------------------------------------------------------------------------------------

**Key Points**:

The **Read** PDB file flag MUST be set to **ZERO**.

The **Multi** flag MUST be set to **ONE**;

This tells JED to perform the analysis on MULTI Chain PDBs.

The number of cPCA modes MUST be zero.

There must be no Cartesian residue list specified.

The number of dPCA modes MUST NOT be zero.

There must be a Distance residue list specified.

The number of dpPCA modes MUST be zero.

If the subset you have chosen contains ***N*** residues, then you must NOT request more than ***N(N-1)/2*** modes.

Remember that the format of the residue list file is TWO columns for Multi Chain Analysis: **Chain ID, residue** **number** while for Single Chain Analysis, the file has ONE column: **residue number**.

**If number of dPCA modes > 0, then there must be a Distance residue list file!**

1. **Performing Distance-Pair PCA: dpPCA** 
   1. **Run command:**

java -jar -d64 JED\_Driver.jar “/path/JED\_Driver.txt”

***The working directory must contain: The coordinates matrix, the PDB reference file, and the residue list.***

***JED input file may be in the working directory.***

The purpose of this type of run is to perform Essential Dynamics using dpPCA based on **Q** and **R**. The user specifies the set of residue pairs of interest for the analysis, by providing a residue pair list file. This file is a two column file for Single Chain PDBs in which the pairs of interest are listed. However, for Multi Chain PDBs, the file is four columns, the first two for the chain ID and residue number of residue one, and the third and fourth columns for the chain ID and residue number of the second residue. The dpPCA results are written to the sub-directory "dpPCA". Note that for dpPCA no transform is needed since internal distances are used for coordinates and no visualization can be done in JED for the distance modes. The residue pair method is much easier to interpret as the number of components in the eigenvectors is equal to the number of residue pairs specified. The directory dpPCA has sub directories for the **Q** and **R** analysis as well as for the subspace analysis (SSA). Very large subsets can be used to investigate experimental findings in critical areas like binding pockets or clefts. Unfortunately, like the dPCA results, the dpPCA results cannot be visualized as no simple mapping can be made to the residues.

* 1. **Standard Output Files: Same as for dPCA**
  2. **v. Single-Chain PDB files: Performing dpPCA**

------------------------------------------------------------------------------------------

0.00

3.00

0 0

0 0 10 0

/working/directory/

Description reference\_PDB\_file.pdb 0

residues\_pairs.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 = none

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 0 = no

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 0 = none

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 10 = top 10 modes

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪0 = none

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String)

Field 2 specifies the **reference PDB** (String)

Field 3 specifies the **offset** (integer)

Line 7 Field 1specifies the **residue list** (String)

Line 8 Field 1 specifies the **coordinate matrix** (String)

Field 2 specifies the **reference column** (integer)

------------------------------------------------------------------------------------------

**Key Points**:

Both the **Read** PDB file flag and the **Multi** flag MUST be set to **ZERO**.

This tells JED to perform the analysis on Single Chain PDBs.

The number of cPCA modes MUST be zero.

There must be no Cartesian residue list specified.

The number of dPCA modes MUST be zero.

The number of dpPCA modes MUST NOT be zero.

There must be a Distance Residue Pair List specified.

**The file format is a two column list of integers: residue number-----residue number**

If the you have chosen ***N*** residue pairs, then you must NOT request more than ***N*** modes.

**If number of dpPCA modes > 0, then there must be a Distance Residue Pair List file!**

1. **Multi-Chain PDB files: Performing dpPCA**

**Differences from Single-Chain analysis shown highlighted in amber**

------------------------------------------------------------------------------------------

0.00

3.00

0 1 **2 A B 795 151 0 0**

0 0 10 0

/working/directory/

Description reference\_PDB\_file.pdb (no offset needed here)

residues\_dist.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 🡪 none

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 1 = yes

Field 3 specifies the number of chains 🡪 (2);

Field 4 specifies the first chain ID 🡪 (A);

Field 5 specifies the second chain ID 🡪 (B);

Field 6 specifies the number of residues in chain A🡪 (795);

Field 7 specifies the number of residues in chain B🡪 (151);

Field 8 specifies the offset of Chain A 🡪 (0);

Field 9 specifies the offset of Chain B 🡪 (0);

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 0

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 10 = top 10 modes

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪0 = none

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String)

Field 2 specifies the **reference PDB** (String)

Line 7 Field 1 specifies the **residue list** (String)

Line 8 Field 1 specifies the **coordinate matrix** (String)

Field 2 specifies the **reference column** (integer)

------------------------------------------------------------------------------------------------------------------------------------------------------

**Key Points**:

The **Read** PDB file flag MUST be set to **ZERO**.

The **Multi** flag MUST be set to **ONE**;

This tells JED to perform the analysis on MULTI Chain PDBs.

The number of cPCA modes MUST be zero.

There must be no Cartesian residue list specified.

The number of dPCA modes MUST be zero.

There must be a Distance Residue Pair List specified.

The number of dpPCA modes MUST NOT be zero.

If the subset you have chosen contains ***N*** Residue Pairs, then you must NOT request more than ***N*** modes.

Remember that the format of the Residue Pair List file is FOUR columns for Multi Chain Analysis:

**Chain ID, residue** **number-----Chain ID, residue number**

**If number of dpPCA modes > 0, then there must be a Distance Residue Pair List file!**

1. **Debugging Crashes Part II:**

Things that will generally make your life miserable…

1. **Simple mistakes:**

Did you set the Read and Multi flags correctly?

Did you request cPCA but not specify a Cartesian residue list?

Did you request dPCA but not specify a Distance residue list?

Did you request dpPCA but not specify a Distance Residue Pair List?

Did you set the number of modes appropriately?

Are you requesting to read PDBs when you should be specifying a coordinate matrix?

1. **Subtle mistakes:**

If the PDB file residue numbering starts at 5, then the chain offset is not 0, it is 4.

Did you request more modes than actually exist? For example, if your Cartesian subset contains 12 residues, but you asked for 50 modes, then you are going to crash JED because there are only 36 Cartesian modes in total.

Also, if your Distance subset contains 3 residues and you request 5 modes, then you are going to crash JED because there are only 3 distance modes in total.

If your trajectory has not equilibrated, then you must address the problem of outliers. If you do not, then the Q and R matrices will be highly ill-conditioned and may cause the eigenvalue decomposition to fail. You can check the variables in statistics packages that compute the Kaiser-Myer-Olkin (KMO) statistic as well as the Measure of Sampling Adequacy (MSA) for each coordinate variable to critically assess your data. If it is not well suited for PCA, you can condition the variables by setting the z-cutoff in JED between 2.0 and 3.0 when running your jobs. This type of conditioning is by far not very sophisticated, but it has the effect of lowering the condition numbers of Q and R as well as un-dilating the high and low regions of the eigenspectrum. In particular, it does not alter the ordinality of the eigenspectrum but does correct the distortion that arises from under sampling when trying to estimate the population covariance matrix from the sample covariance matrix.

1. **Performing Cartesian and Distance PCA with Mode Visualization**

***The working directory must contain: The coordinates matrix, the PDB reference file, and both residue lists.***

***It may also contain the JED input file***

JED is capable of doing both cPCA and dPCA, using both Q and R, and generating cPCA mode visualizations simultaneously. All outputs are delivered as discussed for the individual components.

The outputs to this type of job include the outputs for both the cPCA and dPCA analyses, as well as all the structures for the top modes chosen for visualization. JED will permute the reference structure for a given subset along the top eigenvectors selected for visualization and output structures (PDBs) that capture one cycle of this motion. The amplitude of the motion is determined by the value of the **$scale\_factor**, whose default value is 1.0, and can be adjusted as necessary. Setting the value too high will cause Visualization software like Pymol to break the ribbon diagrams of the structures. Ultimately, this is controlled by the magnitude of the eigenvector components for any given residue. Setting the scale factor between 1 and 3 is usually safe, but for proteins with highly mobile regions like loops, you may need to choose a smaller scale factor. This is done for both the top Q and R modes. Additionally, Pymol scripts are generated to animate those structures into a movie for better analysis of the physical meanings of the top modes.

These files will be located in the /VIZ subdirectory of the root of the JED results tree:

/working/directory/JED\_Results\_Description/VIZ/

The Q results will be in the subdirectory /COV and the R results will be in the subdirectory /CORR.

One huge advantage of JED is that it is highly configurable and can perform many types of Essential Dynamics analysis concurrently. Combined with the cluster resources or just using the batch feature allows a user to process a great deal of data efficiently.

**i. Single-Chain PDB files: Performing Cartesian and Distance PCA with Mode Visualization**

------------------------------------------------------------------------------------------

0.00

3.00

0 0

20 3 0 2 1.0

/working/directory/

Description reference\_PDB\_file.pdb 0

residues\_cartesian.txt

residues\_dist.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 = none

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 0 = no

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 20 = top twenty modes

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 3 = top 3 modes

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪 2 = top 2 modes

Field 5: specifies the **mode amplitude** (double) 🡪 1.0 = default

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String)

Field 2 specifies the **reference PDB** (String)

Field 3 specifies the **offset** (integer)

Line 7 Field 1 specifies the **Cartesian** **residue list** (String)

Line 8 Field 1 specifies the **Distance** **residue list** (String)

Line 9 Field 1 specifies the **coordinate matrix** (String)

Field 2 specifies the **reference column** (integer)

------------------------------------------------------------------------------------------

**Key Points**:

Both the Read PDB file flag and the Multi flag MUST be set to ZERO.

The number of cPCA modes MUST be > zero.

The number of dPCA modes MUST be > zero.

There must be a Cartesian residue list specified.

There must be a Distance residue list specified.

Residue Lists MUST be in the CORRECT ORDER: Cartesian first, then Distance

For Cartesian subsets containing ***N*** residues, you must NOT request more than **3*N*** modes.

For Distance subsets containing ***N*** residues, you must NOT request more than ***N(N-1)/2*** modes.

**ii. Multi-Chain PDB files: Performing Cartesian and Distance PCA with Mode Visualization**

**Differences from Single-Chain analysis shown highlighted in amber**

------------------------------------------------------------------------------------------

0.00

3.00

0 1 **2 A B 795 151 0 0**

20 3 0 2 1.0

/working/directory/

Description reference\_PDB\_file.pdb (no offset here)

residues\_cartesian.txt

residues\_dist.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 🡪 None

Line 2 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 3 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 1 = yes

Field 3 specifies the number of chains 🡪 (2);

Field 4 specifies the first chain ID 🡪 (A);

Field 5 specifies the second chain ID 🡪 (B);

Field 6 specifies the number of residues in chain A🡪 (795);

Field 7 specifies the number of residues in chain B🡪 (151);

Field 8 specifies the offset of Chain A 🡪 (0);

Field 9 specifies the offset of Chain B 🡪 (0);

Line 4 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 20 = top twenty modes

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 3 = top 3 modes

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪 2 = top 2 modes

Field 5: specifies the **mode amplitude** (double) 🡪 1.0 = default

Line 5 Field 1 specifies the **working directory** (String)

Line 6 Field 1 specifies the job **description** (String)

Field 2 specifies the **reference PDB** (String)

Line 7 Field 1 specifies the **Cartesian** **residue list** (String)

Line 8 Field 1 specifies the **Distance** **residue list** (String)

Line 9 Field 1 specifies the **coordinate matrix** (String)

Field 2 specifies the **reference column** (integer)

------------------------------------------------------------------------------------------

**Key Points**:

The read PDB file flag MUST be set to ZERO.

The Multi flag must be set to ONE; This tells JED to perform the analysis on MULTI Chain PDBs.

The number of cPCA modes MUST be > zero.

The number of dPCA modes MUST be > zero.

There must be a Cartesian residue list specified.

There must be a Distance residue list specified.

Residue Lists MUST be in the CORRECT ORDER: Cartesian first, then Distance

For cPCA subsets containing ***N*** residues, you must NOT request more than **3*N*** modes.

For dPCA subsets containing ***N*** residues, you must NOT request more than ***N(N-1)/2*** modes.

Remember that the format of the residue list file is TWO columns for Multi Chain Analysis: **Chain ID, residue** **number** while for Single Chain Analysis, the file has ONE column: **residue number**.

**If number of cPCA modes > 0, then there must be a Cartesian residue list file!**

**If number of dPCA modes > 0, then there must be a Distance residue list file!**

**F. Performing Cartesian and Distance PCA with Mode Visualization**

***The working directory must contain: The coordinates matrix, the PDB reference file, and three residue lists.***

***It may also contain the JED input file***

JED is capable of doing both cPCA, dPCA, and dpPCA, using both Q and R, and generating cPCA mode visualizations simultaneously. All outputs are delivered as discussed for the individual components.

Be sure that there is a corresponding input for each type of analysis that you turn on.

**i. Single-Chain PDB files: Performing cPCA, dPCA, and dpPCA with Mode Visualization**

------------------------------------------------------------------------------------------

0.00

3.00

0 0

20 5 10 2 1.0

/working/directory/

Description reference\_PDB\_file.pdb 0

residues\_cartesian.txt

residues\_dist.txt

residue\_pairs.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes:

There are 20 cPCA modes

There are 5 dPCA modes

There are 10 dpPCA modes

2 cPCA modes will be visualized

**There are three residue lists, in order, cPCA, dPCA, dpPCA.**

**If number of cPCA modes > 0, then there must be a Cartesian residue list file!**

**If number of dPCA modes > 0, then there must be a Distance residue list file!**

**If number of dpPCA modes > 0, then there must be a Distance Residue Pair List file!**

**ii. Multi-Chain PDB files: Performing cPCA, dPCA, and dpPCA with Mode Visualization**

**Differences from Single-Chain analysis shown highlighted in amber**

------------------------------------------------------------------------------------------

0.00

3.00

0 1 **2 A B 795 151 0 0**

20 5 10 2 1.0

/working/directory/

Description reference\_PDB\_file.pdb (no offset here)

residues\_cartesian.txt

residues\_dist.txt

residue\_pairs.txt

original\_PDB\_Coordinates.txt 0

------------------------------------------------------------------------------------------

Notes:

There are 20 cPCA modes

There are 5 dPCA modes

There are 10 dpPCA modes

2 cPCA modes will be visualized

**There are three residue lists, in order, cPCA, dPCA, dpPCA.**

**If number of cPCA modes > 0, then there must be a Cartesian residue list file!**

**If number of dPCA modes > 0, then there must be a Distance residue list file!**

**If number of dpPCA modes > 0, then there must be a Distance Residue Pair List file!**

**V. USING JED BATCH DRIVER**

**The batch version is identical to the non-batch version with the exception of the format of the input file.**

**i. Single Chain PDB files: The Preliminary Run**

-----------------------------------------------------------------------------------------------------

$num\_of\_jobs

0.00

0.00

1 0

0 0 0 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory/job1/

Description1 reference\_PDB\_file1.pdb

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory/job2/

Description2 reference\_PDB\_file2.pdb

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

-----------------------------------------------------------------------------------------------------

Notes: This is a whitespace delineated file.

Line 1 Field 1 specifies the **$num\_of\_jobs** (integer) for the batch.

Line 2 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 🡪NONE

Line 3 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 0.00 🡪NO ADJUSTMENT

Line 4 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪1 = yes

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 0 = no

Line 5 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪0 = none

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪 0 = none

Line 6 is a **separator line** between the batch parameters and the job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 7 Field 1 specifies the **working directory** (String) for job one

Line 8 Field 1 specifies the job **description** (String) for job one

Field 2 specifies the **reference PDB** (String) for job one

Line 9 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 10 Field 1 specifies the **working directory** (String) for job one

Line 11 Field 1 specifies the job **description** (String) for job two

Field 2 specifies the **reference PDB** (String) for job two

Line 12 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

-----------------------------------------------------------------------------------------------------

**Key Points**:

**For single jobs, it is preferred to use JED\_Driver.**

**Be sure that the number of jobs matches the number of job inputs.**

**Make sure to use separator lines after the batch parameters, between jobs, and after the last job.**

**ii. Multi-Chain PDB files: The Preliminary Run**

**Differences from Single-Chain analysis shown highlighted in amber**

-----------------------------------------------------------------------------------------------------

$num\_of\_jobs

0.00

0.00

1 **1 2 A B 795 151 0 0**

0 0 0 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory/job1/

Description1 reference\_PDB\_file1.pdb

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory/job2/

Description2 reference\_PDB\_file2.pdb

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

-----------------------------------------------------------------------------------------------------

Notes: This is a whitespace delineated file.

Line 1 Field 1 specifies the **$num\_of\_jobs** (integer) for the batch.

Line 2 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.00 🡪NONE

Line 3 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 0.00 🡪NO ADJUSTMENT

Line 4 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪1 = yes

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 1 = yes

Field 3 specifies the number of chains 🡪 (2);

Field 4 specifies the first chain ID 🡪 (A);

Field 5 specifies the second chain ID 🡪 (B);

Field 6 specifies the number of residues in chain A🡪 (795);

Field 7 specifies the number of residues in chain B🡪 (151);

Field 8 specifies the offset of Chain A 🡪 (0);

Field 9 specifies the offset of Chain B 🡪 (0);

Line 5 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪0 = none

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪 0 = none

Line 6 is a **separator line** between the batch parameters and the job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 7 Field 1 specifies the **working directory** (String) for job one

Line 8 Field 1 specifies the job **description** (String) for job one

Field 2 specifies the **reference PDB** (String) for job one

Line 9 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 10 Field 1 specifies the **working directory** (String) for job two

Line 11 Field 1 specifies the job **description** (String) for job two

Field 2 specifies the **reference PDB** (String) for job two

Line 12 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

-----------------------------------------------------------------------------------------------------

**Key Points**:

**For single jobs, it is preferred to use JED\_Driver.**

**Be sure that the number of jobs matches the number of job inputs.**

**Make sure to use separator lines after the batch parameters, between jobs, and after the last job.**

**iii. Single-Chain PDB files: Performing Cartesian PCA**

------------------------------------------------------------------------------------------

$num\_of\_jobs

0.01

3.00

0 0

20 0 0 2 1.0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory1/

Description1 reference\_PDB\_file1.pdb 0

residues1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory2/

Description2 reference\_PDB\_file2.pdb 0

residues2.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **$num\_of\_jobs** (integer) for the batch

Line 2 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.01 🡪 1%

Line 3 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 4 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 0 = no

Line 5 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 20 = top twenty modes

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪 2 = top 2 modes

Field 5 specifies the **scale factor** for the visualizations (double) 🡪 1.0 = default value

Line 6 is a **separator line** between the batch parameters and the job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 7 Field 1 specifies the **working directory** (String) for job one

Line 8 Field 1 specifies the job **description** (String) for job one

Field 2 specifies the **reference PDB** (String) for job one

Field 3 specifies the **offset** (integer) for job one

Line 9 Field 1 specifies the **residue list** (String) for job one

Line 10 Field 1 specifies the **coordinate matrix** (String) for job one

Field 2 specifies the **reference column** (integer) for job one

Line 11 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 12 Field 1 specifies the **working directory** (String) for job two

Line 13 Field 1 specifies the job **description** (String) for job two

Field 2 specifies the **reference PDB** (String) for job two

Field 3 specifies the **offset** (integer) for job two

Line 14 Field 1 specifies the **residue list** (String) for job two

Field 2 specifies the **offset** (integer) for job two

Line 15 Field 1 specifies the **coordinate matrix** (String) for job two

Field 2 specifies the **reference column** (integer) for job two

Line 16 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------------------------------------------------------------------

**iv. Multi-Chain PDB files: Performing Cartesian PCA**

**Differences from Single-Chain analysis shown highlighted in amber**

------------------------------------------------------------------------------------------

$num\_of\_jobs

0.01

3.00

0 1 **2 A B 795 151 0 0**

20 0 0 2 1.0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory1/

Description1 reference\_PDB\_file1.pdb (no offset here)

residues1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory2/

Description2 reference\_PDB\_file2.pdb (no offset here)

residues2.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **$num\_of\_jobs** (integer) for the batch

Line 2 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.01 🡪 1%

Line 3 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 4 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 1 = yes

Field 3 specifies the number of chains 🡪 (2);

Field 4 specifies the first chain ID 🡪 (A);

Field 5 specifies the second chain ID 🡪 (B);

Field 6 specifies the number of residues in chain A🡪 (795);

Field 7 specifies the number of residues in chain B🡪 (151);

Field 8 specifies the offset of Chain A 🡪 (0);

Field 9 specifies the offset of Chain B 🡪 (0);

Line 5 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 20 = top twenty modes

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 0 = none

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪 2 = top 2 modes

Field 5 specifies the **scale factor** for the visualizations (double) 🡪 1.0 = default value

Line 6 is a **separator line** between the batch parameters and the job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 7 Field 1 specifies the **working directory** (String) for job one

Line 8 Field 1 specifies the job **description** (String) for job one

Field 2 specifies the **reference PDB** (String) for job one

Line 9 Field 1 specifies the **residue list** (String) for job one

Line 10 Field 1 specifies the **coordinate matrix** (String) for job one

Field 2 specifies the **reference column** (String) for job one

Line 11 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 12 Field 1 specifies the **working directory** (String) for job two

Line 13 Field 1 specifies the job **description** (String) for job two

Field 2 specifies the **reference PDB** (String) for job two

Line 14 Field 1 specifies the **residue list** (String) for job two

Line 15 Field 1 specifies the **coordinate matrix** (String) for job two

Field 2 specifies the **reference column** (String) for job two

Line 16 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

---------------------------------------------------------------------------------------------------------------------------------------------------------------

**v. Single-Chain PDB files: Performing dPCA**

------------------------------------------------------------------------------------------

$num\_of\_jobs

0.01

3.00

0 0

0 3 0 0 (no scale factor)

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory1/

Description1 reference\_PDB\_file1.pdb 0

residues\_dist1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory2/

Description2 reference\_PDB\_file2.pdb 0

residues\_dist2.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **$num\_of\_jobs** (integer) for the batch

Line 2 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.01 🡪 1%

Line 3 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 4 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 0 = no

Line 5 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 0 = none

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 3 = top 3 modes

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪 0 = none

Line 6 is a **separator line** between the batch parameters and the job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 7 Field 1 specifies the **working directory** (String) for job one

Line 8 Field 1 specifies the job **description** (String) for job one

Field 2 specifies the **reference PDB** (String) for job one

Field 3 specifies the **offset** (integer) for job one

Line 9 Field 1 specifies the **distance** **residue list** (String) for job one

Line 10 Field 1 specifies the **coordinate matrix** (String) for job one

Field 2 specifies the **reference column** (integer) for job one

Line 11 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 12 Field 1 specifies the **working directory** (String) for job two

Line 13 Field 1 specifies the job **description** (String) for job two

Field 2 specifies the **reference PDB** (String) for job two

Field 3 specifies the **offset** (integer) for job two

Line 14 Field 1 specifies the **distance** **residue list** (String) for job two

Field 2 specifies the **offset** (integer) for job two

Line 15 Field 1 specifies the **coordinate matrix** (String) for job two

Field 2 specifies the **reference column** (integer) for job two

Line 16 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------------------------------------------------------------------

**vi. Multi-Chain PDB files: Performing dPCA**

**Differences from Single-Chain analysis shown highlighted in amber**

------------------------------------------------------------------------------------------

$num\_of\_jobs

0.01

3.00

0 1 **2 A B 795 151 0 0**

0 3 0 0 (no scale factor)

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory1/

Description1 reference\_PDB\_file1.pdb (no offset here)

residues\_dist1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory2/

Description2 reference\_PDB\_file2.pdb (no offset here)

residues\_dist2.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

Notes:

Line 1 Field 1 specifies the **$num\_of\_jobs** (integer) for the batch

Line 2 Field 1 specifies the **percent** (double) of frames to remove from the data: 0.01 🡪 1%

Line 3 Field 1 specifies the **z-cutoff** (double) for adjusting outliers: 3.00 🡪 values beyond |3.0|

Line 4 Field 1 specifies whether to **read PDB files** (0 or 1) 🡪 0 = no

Field 2 specifies if the PDB files are **Multi** Chain (0 or 1) 🡪 1 = yes

Field 3 specifies the number of chains 🡪 (2);

Field 4 specifies the first chain ID 🡪 (A);

Field 5 specifies the second chain ID 🡪 (B);

Field 6 specifies the number of residues in chain A🡪 (795);

Field 7 specifies the number of residues in chain B🡪 (151);

Field 8 specifies the offset of Chain A 🡪 (0);

Field 9 specifies the offset of Chain B 🡪 (0);

Line 5 Field 1 specifies the **number of Cartesian** (integer) modes to process 🡪 0 = none

Field 2: specifies the **number of all-to-all Distance modes** (integer) to process 🡪 3 = top 3 modes

Field 3: specifies the **number of residue-pair Distance modes** (integer) to process 🡪 0 = none

Field 4: specifies the **number of Cartesian modes to Visualize**(integer) 🡪 0 = none

Line 6 is a **separator line** between the batch parameters and the job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 7 Field 1 specifies the **working directory** (String) for job one

Line 8 Field 1 specifies the job **description** (String) for job one

Field 2 specifies the **reference PDB** (String) for job one

Line 9 Field 1 specifies the **distance** **residue list** (String) for job one

Line 10 Field 1 specifies the **coordinate matrix** (String) for job one

Field 2 specifies the **reference column** (String) for job one

Line 11 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

Line 12 Field 1 specifies the **working directory** (String) for job two

Line 13 Field 1 specifies the job **description** (String) for job two

Field 2 specifies the **reference PDB** (String) for job two

Line 14 Field 1 specifies the **distance** **residue list** (String) for job two

Line 15 Field 1 specifies the **coordinate matrix** (String) for job two

Field 2 specifies the **reference column** (String) for job two

Line 16 is a **separator line** between sets of job parameters \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

---------------------------------------------------------------------------------------------------------------------------------------------------------------

**vii. Single-Chain PDB files: Performing cPCA, dPCA, and Visualization**

------------------------------------------------------------------------------------------

$num\_of\_jobs

0.01

3.00

0 0

20 3 0 2 1.0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory1/

Description1 reference\_PDB\_file1.pdb 0

residues1.txt

residues\_dist1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory2/

Description2 reference\_PDB\_file2.pdb 0

residues2.txt

residues\_dist2.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

**viii. Multi-Chain PDB files: Performing cPCA, dPCA, and Visualization**

------------------------------------------------------------------------------------------

$num\_of\_jobs

0.01

3.00

0 1 **2 A B 795 151 0 0**

20 3 2 1.0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory1/

Description1 reference\_PDB\_file1.pdb (no offset here)

residues1.txt

residues\_dist1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory2/

Description2 reference\_PDB\_file2.pdb (no offset here)

residues2.txt

residues\_dist2.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

**vii. Single-Chain PDB files: Performing cPCA, dPCA, dpPCA, and Visualization**

------------------------------------------------------------------------------------------

$num\_of\_jobs

0.00

2.00

0 0

20 3 10 2 1.0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory1/

Description1 reference\_PDB\_file1.pdb 0

residues1.txt

residues\_dist1.txt

residue\_pairs1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory2/

Description2 reference\_PDB\_file2.pdb 0

residues2.txt

residues\_dist2.txt

residue\_pairs2.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

**viii. Multi-Chain PDB files: Performing cPCA, dPCA, dpPCA, and Visualization**

------------------------------------------------------------------------------------------

$num\_of\_jobs

0.00

2.00

0 1 **2 A B 795 151 0 0**

20 3 10 2 1.0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory1/

Description1 reference\_PDB\_file1.pdb (no offset here)

residues1.txt

residues\_dist1.txt

residue\_pairs1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

/working/directory2/

Description2 reference\_PDB\_file2.pdb (no offset here)

residues2.txt

residues\_dist2.txt

residue\_pairs1.txt

original\_PDB\_Coordinates.txt 0

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

**VI. Additional Types of Analysis**

**A. Pooling Data:**

It is often useful to pool trajectory statistics. This can be done in JED by combining coordinate files and then performing the usual analysis. To combine the coordinate files, there is a utility program called **JED\_Pool\_Data.java** that will combine multiple matrices into one. Each matrix is appended to the last column of the preceding matrix. Of courses, the number of rows in the coordinate files must match. The matrices to combine are specified by an input file called **pool.txt** that the user must construct correctly.

**i. Run Command:**

java -jar -d 64 JED\_Pool\_Data.jar “/path/to/pool.txt”

**ii. Input File format:**

Line #1 specifies the number of jobs (integer)

**Then for each job you must specify the following:**

The number of matrices to combine (integer)

The output directory (string)

The path to the first matrix (String)

**Below is a sample pool.txt file:**

------------------------------------------------------------------------------------------

2

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

2

/output/directory/

/path/to/first/matrix/matrix\_1.txt

/path/to/first/matrix/matrix\_2.txt

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

2

/output/directory/

/path/to/first/matrix/matrix\_1.txt

/path/to/first/matrix/matrix\_2.txt

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

------------------------------------------------------------------------------------------

Notes:

Line #1 specifies the number of jobs (integer)

Line #2 Separator Line (Required)

Line #3 specifies the number of matrices to combine for Job 1 (integer)

Line #4 specifies the output directory for Job 1 (string)

Line #5 specifies the path to the first matrix for Job 1 (String)

Line #6 specifies the path to the second matrix for Job 1 (String)

Line #7 Separator Line (Required)

etc...

------------------------------------------------------------------------------------------

**iii. Output File format:**

The output is a single, augmented matrix with the same number of rows as the composite matrices and columns equal to the sum of all columns in the composite matrices.

The output file name is: **"Pooled\_Coordinates\_Matrix\_$*number\_of\_input\_matrices*.txt"**

**B. Subspace Analysis:**

Once JED Driver has been run on multiple trajectories as well as pooled trajectories, an analysis can be done to compare how similar the essential subspaces derived from those trajectories are to each other. JED contains a program called **Subspace\_Analysis.java** along with 3 driver programs that perform those functions. The core program takes as input two matrices of eigenvectors derived from PCA (or NMA, ANM, etc.). The matrices must have the same number of rows and columns, meaning the vectors being compared come from the same vector space and that the subspaces have the same dimensions. For example, in an analysis of lysozyme you might choose to process 20 cPCA modes while examining 10 different experimental conditions plus pooled data. As long as all the subsets in the analysis are the same then all the 20 dimensional subspaces can be compared.

Like most of the JED programs, the subspace analysis program driver reads an input file called **SSA.txt** to obtain runtime information. This file must be constructed properly by the user to perform the analysis. The three driver programs are **SSA\_Driver.java**, **FSSA\_Driver.java**, and **FSSA\_Iterated\_Driver.java** and are different in how much analysis is requested. The SSA\_Driver gives full outputs for non-iterated subspace comparison including both log files and individual flat files. The FSSA\_Driver is a light-weight version with only RMSIP and PA output in the log files. The Iterated version performs a recursive variation of the above where all equidimensional subspaces are compared up to the size that was provided, for example, from 1 to 20 by step-size 1 for a 20 column input file.

**i. Run Commands:**

java -jar -d 64 SSA\_Driver.jar “/path/to/SSA.txt”

java -jar -d 64 FSSA\_Driver.jar “/path/to/SSA.txt”

java -jar -d 64 FSSA\_Iterated\_Driver.jar “/path/to/SSA.txt”

**ii. Input File format:**

ALL three drivers use the same input file, only the outputs are different.

The format for the file shown below is:

**LINE 1: Number\_of\_Jobs (integer)**

**LINE 2: Output\_Directory (string ending in "/" or "\\")**

**LINE 3: Batch\_Decription (string)**

**LINE 4: Separator Line (Required)**

**THEN FOR EACH JOB:**

**Description (string)**

**Directory1 (string ending in "/" or "\\") Name1 (string) (eigenvectors1)**

**Directory2 (string ending in "/" or "\\") Name2 (string) (eigenvectors2)**

**Separator Line (Required)**

**Below is a sample SSA.txt file:**

------------------------------------------------------------------------------------------

4

/output/directory/

Single\_Combo\_SSA

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

All-vs-A

/Users/physicslabs/ all\_combo\_SS\_75\_top\_20\_eigenvectors.txt

/Users/physicslabs/ 1a6n\_combo\_SS\_75\_top\_20\_eigenvectors.txt

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

All-vs-B

/Users/physicslabs/ all\_combo\_SS\_75\_top\_20\_eigenvectors.txt

/Users/physicslabs/ 1wit\_combo\_SS\_75\_top\_20\_eigenvectors.txt

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

All-vs-A+B

/Users/physicslabs/ all\_combo\_SS\_75\_top\_20\_eigenvectors.txt

/Users/physicslabs/ 1ubq\_combo\_SS\_75\_top\_20\_eigenvectors.txt

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

All-vs-A\_B

/Users/physicslabs/ all\_combo\_SS\_75\_top\_20\_eigenvectors.txt

/Users/physicslabs/ 1ypi\_combo\_SS\_75\_top\_20\_eigenvectors.txt

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

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**APPENDIX**

**A. Sample JED Log file:**

Java Essential Dynamics

Job Description: Single\_cPCA\_dPCA\_Viz

Working directory: C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Single\\

Output directory: C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Single\\JED\_Results\_Single\_cPCA\_dPCA\_Viz/

Performing cPCA: 20 modes.

Performing dPCA: 3 modes.

Performing mode visualization on top 2 cPCA modes

Alpha carbon coordinates were obtained from file: C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Single\\original\_PDB\_coordinates.txt

The number of residues read: 151

The number of conformations read: 2001

The dimension of the coordinates matrix is: 453 by 2001

The transformed PDB coordinates were obtained by quaternion least-squares alignment.

PDB reference file is 1A6N.pdb

Reference conformation (column) in the coordinate matrix: 0

Residue list for Cartesian subset: residues.txt

Number of residues in Cartesian subset: 81

The transformed data was trimmed by removing 10.0 percent of the samples based on conformation RMSD.

The coordinates were 'conditioned' by adjusting outliers with Z-scores beyond 3.0 to their mean value.

Condition number of the covariance matrix (Q): 52,101,318,291,300,056

LOG of the Q Condition Number: 17

Trace of Q: 69

Condition number of the correlation matrix (R): 35,505,888,101,013,780

LOG of the R Condition Number: 17

Trace of R: 243

PDB file with B-factors replaced by residue RMSDs: Single\_cPCA\_dPCA\_Viz\_SS\_81.pdb

Number of residues in distance subset: 12

The coordinates were 'conditioned' by adjusting outliers with Z-scores beyond |3.0| to their mean value.

Condition number of the distance Covariance matrix (Q\_dist): 80,427,675

LOG of Q\_dist: 8

Trace of Q\_dist: 2

Condition number of the distance Correlation matrix (R\_dist): 183,847

LOG of R\_dist: 5

Trace of R\_dist: 66

MEANs and STD\_DEVs for the residue distances:

Res1 Res2 Mean Std\_Dev

1 2 3.780 0.004

1 3 6.388 0.245

1 4 9.460 0.425

1 5 11.239 0.242

1 6 9.326 0.284

1 7 9.790 0.511

1 8 13.292 0.427

1 9 13.982 0.305

1 10 13.117 0.431

1 11 15.209 0.647

1 12 17.872 0.479

2 3 3.808 0.005

2 4 6.632 0.067

2 5 7.994 0.080

2 6 5.818 0.115

2 7 6.416 0.145

2 8 9.854 0.134

2 9 10.503 0.138

2 10 9.922 0.150

2 11 12.086 0.152

2 12 14.519 0.149

3 4 3.783 0.009

3 5 5.308 0.010

3 6 5.113 0.011

3 7 6.309 0.011

3 8 8.648 0.012

3 9 10.012 0.012

3 10 10.692 0.012

3 11 12.367 0.012

3 12 14.171 0.014

4 5 3.804 0.004

4 6 5.409 0.005

4 7 5.070 0.003

4 8 6.172 0.005

4 9 8.766 0.006

4 10 9.846 0.006

4 11 10.448 0.006

4 12 12.007 0.008

5 6 3.784 0.003

5 7 5.458 0.004

5 8 5.064 0.003

5 9 6.444 0.004

5 10 8.797 0.005

5 11 10.033 0.006

5 12 10.526 0.006

6 7 3.809 0.006

6 8 5.357 0.006

6 9 5.167 0.004

6 10 6.341 0.006

6 11 8.741 0.007

6 12 9.919 0.007

7 8 3.823 0.005

7 9 5.407 0.006

7 10 4.992 0.003

7 11 6.075 0.004

7 12 8.364 0.007

8 9 3.815 0.004

8 10 5.431 0.004

8 11 5.248 0.004

8 12 5.868 0.005

9 10 3.806 0.005

9 11 5.620 0.005

9 12 5.210 0.004

10 11 3.777 0.004

10 12 5.545 0.005

11 12 3.787 0.006

Sets of structures were generated to animate each of the top 2 cPCA modes using both Q and R PCA methods.

MODE AMPLITUDE: 1.000

Analysis completed: 2013-12-16 02:25:55

**B. Sample PDB READ Log file:**

1A6N.pdb

1A6N\_froda\_00000001.pdb

1A6N\_froda\_00000002.pdb

1A6N\_froda\_00000003.pdb

1A6N\_froda\_00000004.pdb

1A6N\_froda\_00000005.pdb

1A6N\_froda\_00000006.pdb

1A6N\_froda\_00000007.pdb

1A6N\_froda\_00000008.pdb

1A6N\_froda\_00000009.pdb

1A6N\_froda\_00000010.pdb

1A6N\_froda\_00000011.pdb

1A6N\_froda\_00000012.pdb

1A6N\_froda\_00000013.pdb

1A6N\_froda\_00000014.pdb

1A6N\_froda\_00000015.pdb

1A6N\_froda\_00000016.pdb

1A6N\_froda\_00000017.pdb

1A6N\_froda\_00000018.pdb

1A6N\_froda\_00000019.pdb

1A6N\_froda\_00000020.pdb

1A6N\_froda\_00000021.pdb

1A6N\_froda\_00000022.pdb

1A6N\_froda\_00000023.pdb

1A6N\_froda\_00000024.pdb

1A6N\_froda\_00000025.pdb

**C. Sample Single Chain PDB Residue List file:**

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

**D. Sample Multi Chain PDB Residue List file:**

A 1

A 2

A 3

A 4

A 5

A 6

A 7

A 8

A 9

A 10

A 11

A 12

A 13

A 14

A 15

A 16

A 17

A 18

A 19

A 20

A 21

A 22

A 23

A 24

A 25

B 1

B 2

B 3

B 4

B 5

B 6

B 7

B 8

B 9

B 10

B 11

B 12

B 13

B 14

B 15

B 16

B 17

B 18

B 19

B 20

**E. Sample Single Chain PDB Residue-Pair List file:**

1 3

2 5

3 8

4 9

5 12

6 22

7 28

8 45

9 48

10 90

**F. Sample Multi Chain PDB Residue-Pair List file:**

A 1 A 3

A 2 A 7

A 3 A 14

A 4 A 22

A 5 A 45

B 6 A 70

B 7 A 81

B 8 B 30

B 9 B 56

B 10 B 72

**G. Sample SSA Log File:**

Top\_COV\_Eigenvectors

Rows: 45

Cols: 3

Top\_CORR\_Eigenvectors

Rows: 45

Cols: 3

Projections file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_projections\_dim\_3.txt

Cumulative overlaps 1 --> 2 file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_CO\_1\_2\_dim\_3.txt

Cumulative overlaps 2 --> 1 file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_CO\_2\_1\_dim\_3.txt

Principle Angles file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_PA\_dim\_3.txt

Cosine Products file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_Cosine\_Products\_dim\_3.txt

Vectorial sums of angles file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_Vector\_sums\_of\_Angles\_dim\_3.txt

The absolute projections of each vector in subspace 1 with each vector in subspace 2 are:

0.76 0.52 0.07

0.31 0.69 0.47

0.06 0.37 0.70

The cumulative overlaps CO\_3 for each vector in subspace 1 with all the vectors in subspace 2 are:

Vector 1 0.919

Vector 2 0.890

Vector 3 0.797

The cumulative overlaps CO\_3 for each vector in subspace 2 with all the vectors in subspace 1 are:

Vector 1 0.820

Vector 2 0.936

Vector 3 0.850

The RMSIP score is 0.870

The principle angles (in degrees) are:

PA1 6

PA2 21

PA3 31

The cosine products (in degrees) are:

CP1 6

CP2 30

CP3 51

The vectorial sums of angles (in degrees) are:

VS1 6

VS2 22

VS3 38

Maximum possible angle between two subspaces of this dimension is 90 degrees

Analysis completed: 2013-12-16 02:15:05

**H. Sample FSSA Iterated Log File:**

Principle Angle Spectra file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_PA\_Spectra.txt

RMSIPs file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_RMSIPs.txt

RMSIPs:

Dim 1 0.758

Dim 2 0.839

Dim 3 0.870

The PA spectra for the range of subspaces are:

29 0 0

14 32 0

6 21 31

Analysis completed: 2013-12-16 02:15:05

**I. Sample Random SSA Log File:**

Average RMSIPs file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_avg\_random\_RMSIPs.txt

Average PAs file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_avg\_random\_PAs.txt

Average COs file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_avg\_random\_COs.txt

RMSIP Std Devs file written to:

C:\\Users\\Charles\\workspace\\JED\_1.0\\JED\_Test\\Multi\\JED\_results\_Multi\_cPCA\_dPCA\_Viz/dPCA/SSA/Multi\_cPCA\_dPCA\_Viz\_random\_RMSIP\_std\_devs.txt

The dimension of the vector space is 45

SS\_DIM avg\_RMSIP std\_dev

1 0.883 0.094

2 0.647 0.069

3 0.557 0.064

The avg random PA spectra for the range of subspaces are:

27 0 0

26 71 0

25 61 75

The avg random CO scores for the range of subspaces are:

0.794 0.000 0.000

0.803 0.176 0.000

0.810 0.242 0.156

Analysis completed: 2013-12-16 02:15:05