gRINN Documentation

Release 1.0.1

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gRINN is a software for residue interaction enery-based analysis of protein MD simulation trajectories.

Current version is v1.1.0.

Start with the Tutorial.

CHAPTER 1

Introduction

Welcome to gRINN (get Residue Interaction Energies and Networks).

gRINN is a software tool for residue interaction-energy based investigation of protein Molecular Dynamics simulations.

Main functionality includes:

- Calculation of pairwise amino acid non-bonded interaction energies from NAMD or GROMACS generated Molecular Dynamics (MD) simulation trajectories by interoperating with NAMD/GMX simulation engines.
- Equal-time linear correlations between interaction energy time series.
- Custom residue selections & multiple processor usage.
- A visualization interface for:
  - Viewing pairwise interaction energies and their correlations alongside an embedded PyMol molecular viewer.
  - Protein Energy Network construction and visualization of simple residue-based local network metrics (Degrees, Betweenness Centrality and Closeness Centrality)
  - Shortest path analysis.

CHAPTER 2

Obtaining gRINN

gRINN is free and open to all users.

2.1 Dependencies

gRINN is designed to work with NAMD or GROMACS-generated MD simulation trajectories, hence *topology*, *structure* and *trajectory* files are required.

Moreover, the tool interoperates with NAMD or GROMACS, so you will need to provide the *location of the NAMD or GROMACS(gmx) executable* you've used for your MD simulation before using gRINN.

Since you're interested in using this tool, you're probably already a user of either NAMD or GROMACS; however, if the executable is for some reason not installed/located on your system, gRINN would not work. You should obtain and install them from the respective developers as we can't distribute them ourselves.

2.1.1 Obtaining NAMD

Please use a non-CUDA multicore version.

Please note that downloading NAMD requires registration.

gRINN was tested against and confirmed to work fine with the following NAMD versions in Linux:

- NAMD 2.12b1, NAMD 2.12, NAMD 2.11, NAMD 2.10 and NAMD 2.9.

gRINN was tested against and confirmed to work fine with the following NAMD versions in Mac OSX High Sierra:

- NAMD 2.11 and 2.10.

WARNING: NAMD 2.12 and NAMD 2.12b1 MacOSX versions lead to hang ups during gRINN operation. Please don't use these versions.

Download NAMD here.

2.1.2 Obtaining GROMACS

gRINN was tested against and confirmed to work fine with the following GROMACS versions in Linux:

- gromacs 2016.1, 2016.2, 2016.3, 2016.4, 5.1.2, 5.1.4.

gRINN will surely not work with gromacs versions below 5.x and it may not be compatible with versions other than those listed above.

Download GROMACS here.

2.2 Download gRINN

Please read the EULA first. By downloading and using gRINN, you agree to the terms and conditions contained therein.

gRINN is available for both Linux (x64) and Mac OSX operating systems.

Download gRINN v.1.1.0 for Linux x64

Download gRINN v.1.1.0 for Mac OSX

Windows support is currently a work-in-progress. Stay tuned and visit back for a Windows compatible executable.

2.3 Starting the application

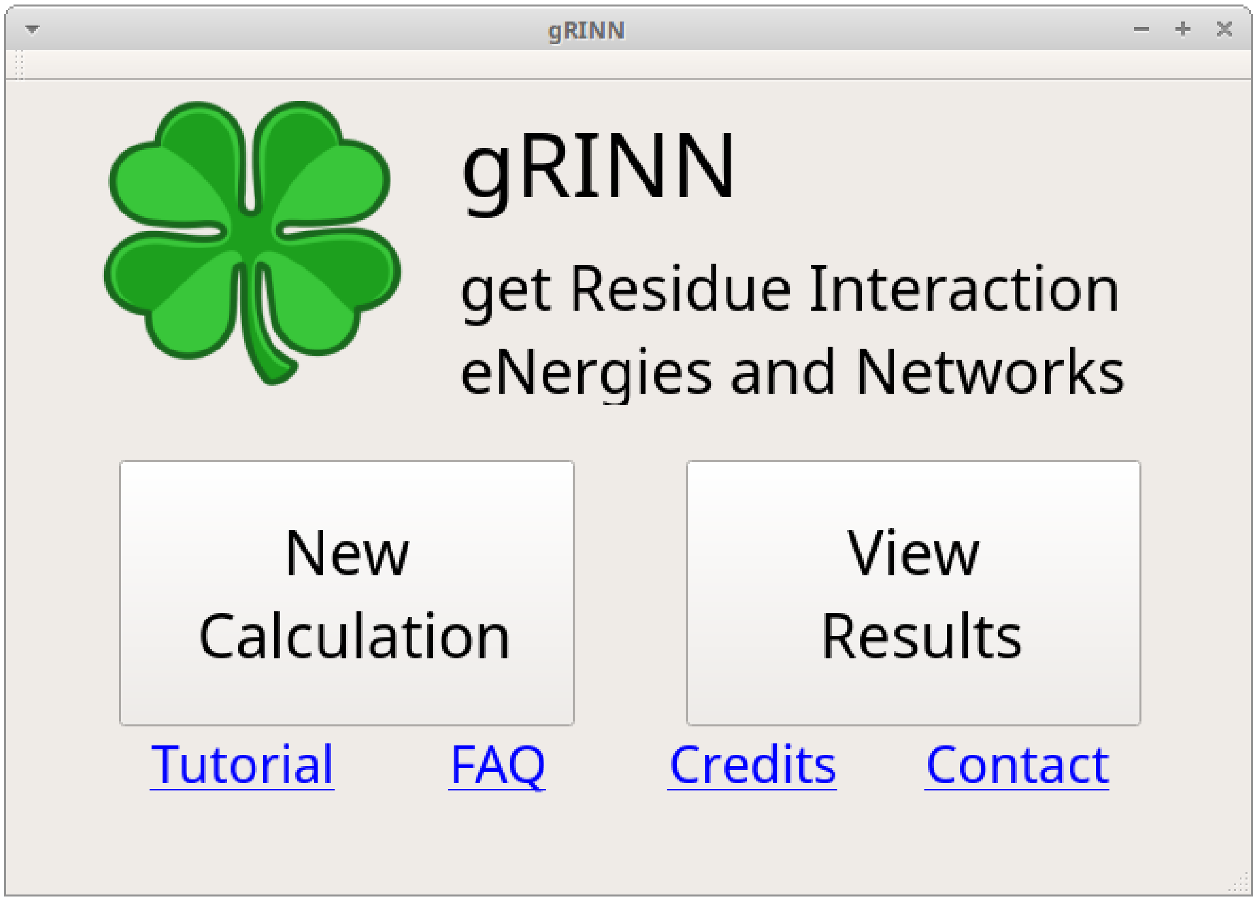
No installation is required.

Extract the archive you downloaded to a folder of your choice. Start a terminal in this folder and start the executable by typing:

```
$ ./grinn
```

Please be patient for the application to start for the first time.

If you see the gRINN Main Window as shown below, you can continue with the Tutorial.


gRINN
gRINN
get Residue Interaction eNergies and Networks
New Calculation
View Results
Tutorial
FAQ
Credits
Contact

CHAPTER 3

Tutorial

If you're here for the first time, obtain gRINN first.

Sample data used in this tutorial is already bundled with gRINN, however the contents are extracted to a temporary system folder which can be difficult to find. If you want to download it separately, click here.

3.1 Preparing input data

In this tutorial, we will use sample NAMD data from a short MD simulation of the trypsin enzyme which is already prepared for gRINN. Therefore, preparation of any input data is not required for completing this tutorial. The steps below describe the preparation steps you need to follow for your own data.

3.1.1 For NAMD/CHARMM-generated trajectories

When using your own data, you are advised to delete solvent molecules from your input Protein Data Bank (PDB), Protein Structure File (PSF) and DCD files before using gRINN. If you don't, a very high amount of RAM will be required by gRINN for processing the trajectory file, particularly if you choose to use multiple CPU cores. The completion time will increase significantly as well.

gRINN does not offer a function for removing the solvent molecules from input files, however this can be done using standart software such as VMD.

If you have used psfgen or VMD's Autopsf plug-in, usually the first PSF/PDB pair generated during system preparation for MD simulation **prior to solvation step** is what you need. To remove the solvent from the DCD, you can load the trajectory into VMD and save the coordinates of "protein" atoms into a new DCD file.

More information on removing non-protein atoms from input PSF/PDB/DCD files can be found here.

If you're observing molecules/atoms breaking & jumping out of the simulation box during the simulation, you need to "unwrap" them and save the resulting trajectory to a DCD file, then use that trajectory as input to gRINN. **gRINN does not handle such cases.**

3.1.2 For GROMACS-generated trajectories

If you're submitting a GROMACS trajectory, there is no need for additional data preparation. You can just give your topology (TOP), run input (TPR) and trajectory (XTC or TRR) files as input to gRINN. However, a common practice with protein MD simulations is to keep only protein atoms and remove solvent/ions from trajectory files to save disk space unless solvent-protein interactions are the focus of a study. In this case, TPR and TOP files WITHOUT non-protein atoms should be prepared for gRINN. More information on how to do that can be found here.

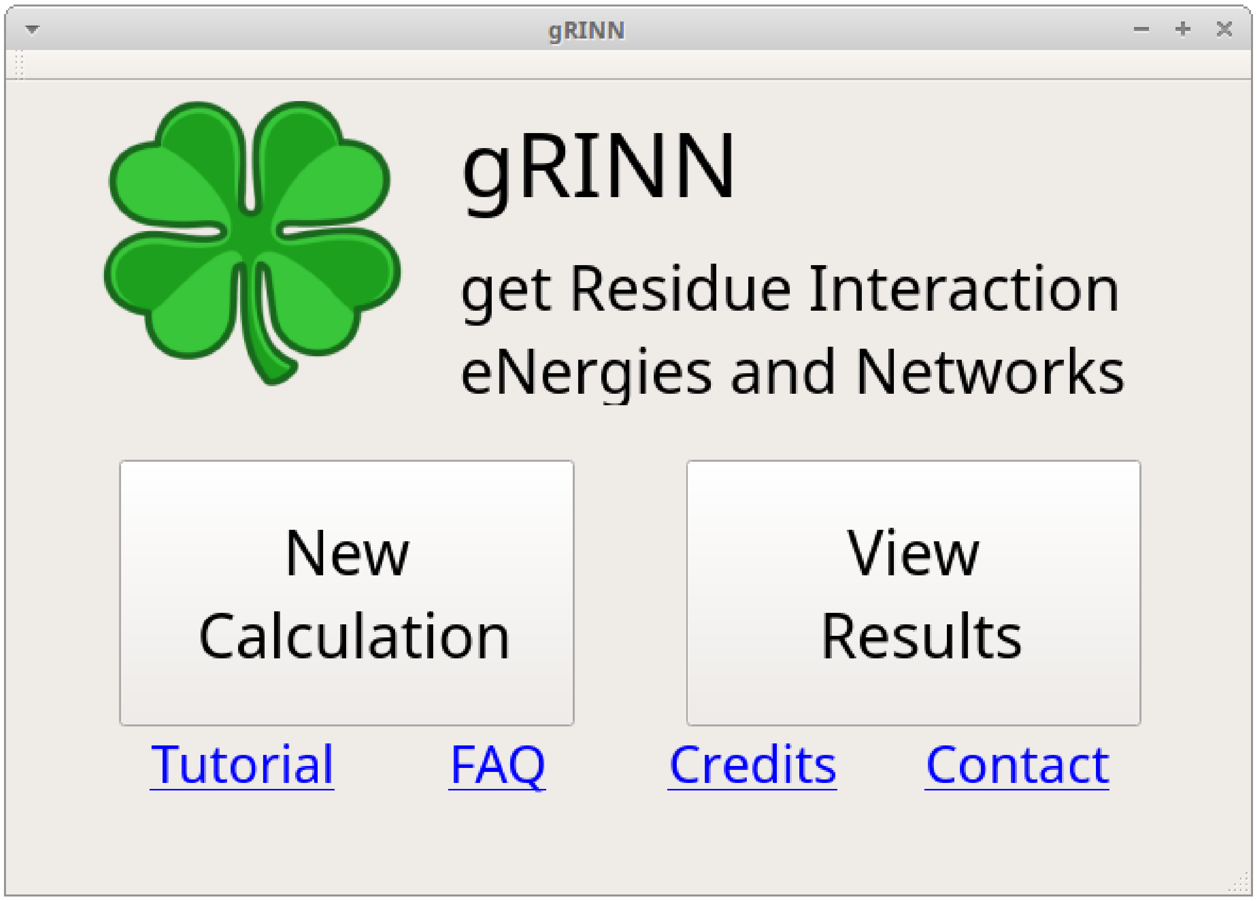
If you're observing molecules/atoms breaking & jumping out of the simulation box during the simulation, you need to remove PBC conditions with gmx trjconv and save the resulting trajectory to a new XTC/TRR file, and then use that trajectory as input to gRINN.

3.2 Starting the Application

If you have not done already, start gRINN by following the steps here.

3.3 gRINN Main Window

Upon execution of `grinn`, the following window appears:



This is the main window of gRINN. The links at the bottom direct the user to respective sections of this website.

gRINN offers two interfaces: *New Calculation* and *View Results*.

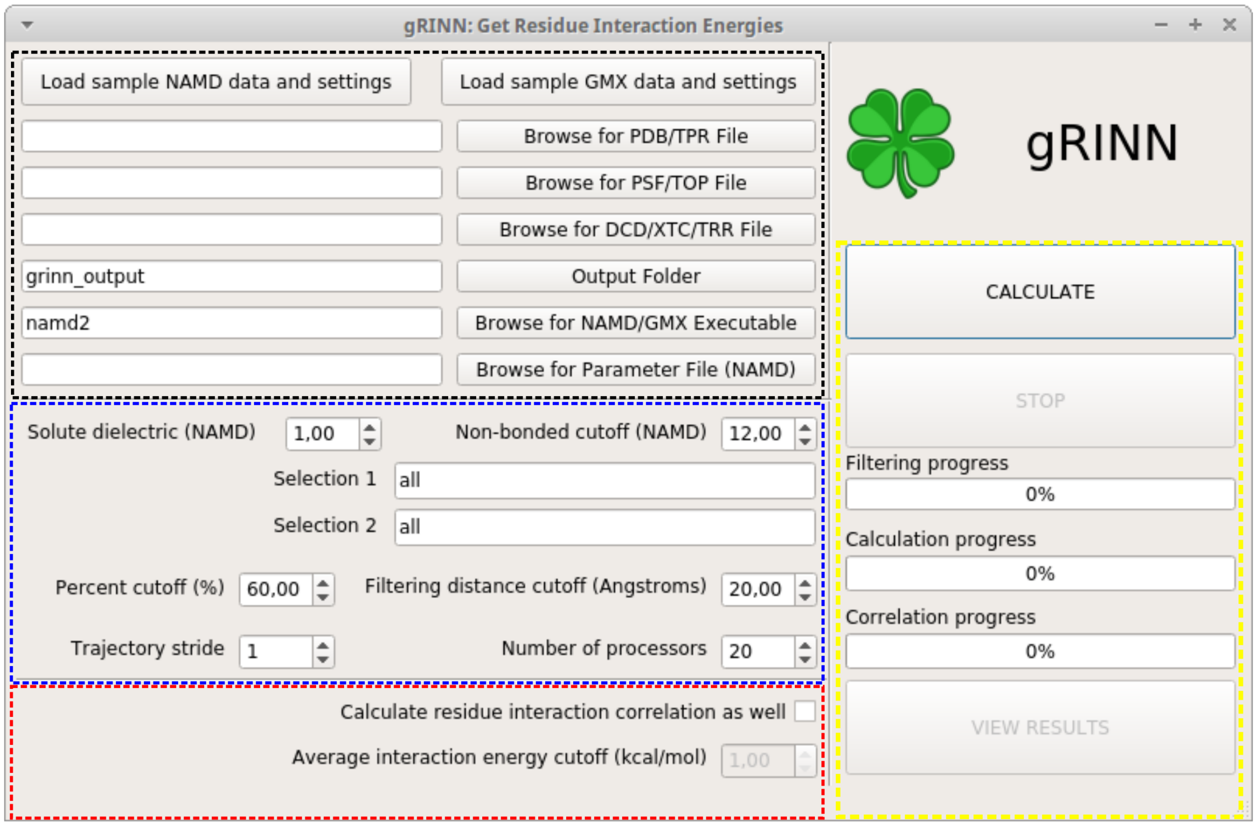
New Calculation: This interface is used for pairwise residue interaction energy and (optional) interaction energy correlation calculations.

View Results: This interface is used to visualize results from *New Calculation* and construct Protein Energy Networks using these data.

Go ahead and click on *New Calculation* now.

3.4 gRINN New Calculation

You should now see a window like the following one:


gRINN: Get Residue Interaction Energies
Load sample NAMD data and settings
Load sample GMX data and settings
Browse for PDB/TPR File
Browse for PSF/TOP File
Browse for DCD/XTC/TRR File
Output Folder
Browse for NAMD/GMX Executable
Browse for Parameter File (NAMD)
gRINN
CALCULATE
STOP
VIEW RESULTS

grinn_output
namd2

Solute dielectric (NAMD) 1,00
Non-bonded cutoff (NAMD) 12,00
Selection 1 all
Selection 2 all
Percent cutoff (%) 60,00
Filtering distance cutoff (Angstroms) 20,00
Trajectory stride 1
Number of processors 20
Calculate residue interaction correlation as well
Average interaction energy cutoff (kcal/mol) 1,00

Filtering progress 0%
Calculation progress 0%
Correlation progress 0%

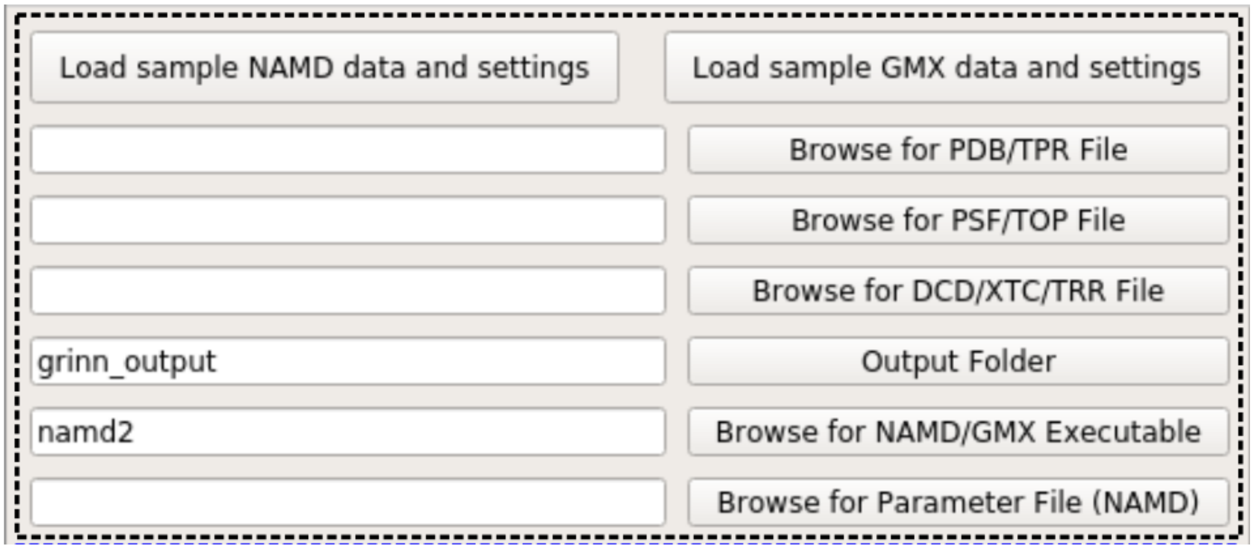
This is the *New Calculation* interface. gRINN New Calculation is used to:

- specify paths to input files, NAMD/GMX executables and other custom calculation settings
- start interaction energy and/or correlation calculations
- monitor the progress of computation
- start "View Results" interface once the calculation is completed.

The *New Calculation* UI elements can be grouped into four main parts based on their functionality. These are shown in the snapshot above in various color frames. We shall first describe the interface, then loading of sample NAMD data and starting a calculation.

3.4.1 Input files, output folder and NAMD/GMX executable paths

The black frame includes UI elements for specifying the input files and the output folder.


Load sample NAMD data and settings
Load sample GMX data and settings
Browse for PDB/TPR File
Browse for PSF/TOP File
Browse for DCD/XTC/TRR File
Output Folder
Browse for NAMD/GMX Executable
Browse for Parameter File (NAMD)

grinn_output
namd2

Full paths to PDB/PSF/DCD files (in case of NAMD data) or TPR/TOP and XTC or TRR files (in case of GROMACS data) can be specified either by typing/pasting the full file path to corresponding text edit boxes or via browsing by clicking the *"Browse for… "* buttons.

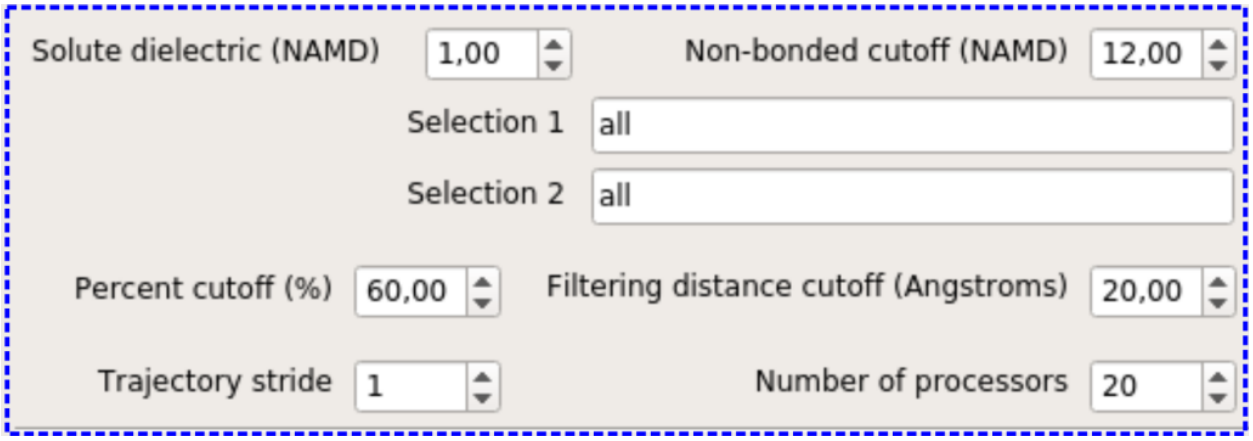
Output folder specifies the folder in which gRINN results are stored. **Note that the output folder you specify should NOT exist prior to calculation. "Output Folder" button should be used as a convenience for selecting a parent folder for your output folder.** gRINN will create the output folder inside this parent folder.

The path of the NAMD/GMX executable is set to namd2 by default. This requires that a valid NAMD executable is present in the executable search path of your system (in linux, this is the PATH environment variable). If namd2 is not accessible via the executable search path, provide the full path here. The same logic applies to the gmx executable, i.e. typing just *gmx* in this text box will assume that the gmx executable is accessible via the executable search path.

For NAMD, at least one additional parameter file containing the parameters included in the force-field you've used for your simulation, is required. If you've used more than one parameter file, you can specify the full paths to these files in the text box by leaving one blank space between them. Alternatively, you can put all parameter files in the same directory and then select them via "Browse for Parameter File (NAMD)" button.

3.4.2 Calculation settings

The blue frame includes UI elements for specifying the settings used for non-bonded pairwise residue interaction energy calculations.



Solute dielectric (NAMD) 1,00
Non-bonded cutoff (NAMD) 12,00
Selection 1 all
Selection 2 all
Percent cutoff (%) 60,00
Filtering distance cutoff (Angstroms) 20,00
Trajectory stride 1
Number of processors 20

Solute dielectric (NAMD) specifies the dielectric constant that is used while computing the electrostatic component of the interaction energy (*dielectric* keyword in the NAMD configuration file). The default value is 1.0, which means that the electrostatic interactions will not be modified. Any value larger than this value lessens the electrostatic forces. More information can be found in NAMD User Guide.

Selection 1 and *Selection 2* define custom atom selections that include residue groups between which non-bonded interaction energies will be computed. ProDy atom selection syntax is used here. (e.g. for selecting only the residues of chain A that are within a certain cutoff distance (5 Angstroms) from residues of chain B and vice versa, you will type `chain A and within 5 of chain B` in *Selection 1* and `chain B and within 5 of chain A` in *Selection 2*)

Note: This setting is useful if you're only interested in pairwise residue interactions within a specific subset of your protein. If you want a full characterization, leave these selections at the default settings (all). Note that using a custom residue selection here will also lead to incorrect Protein Energy Network (PEN) construction while using the "View Results" interface.

Percent cutoff and *Filtering distance cutoff* settings specify criteria for selecting pairs of residues between which non-bonded interaction energies will be computed. Default values are 60% and 12 Angstroms, meaning that only pairs of residues whose centers-of-mass come closer than 12 Angstroms in at least 60 percent of trajectory frames will be included.

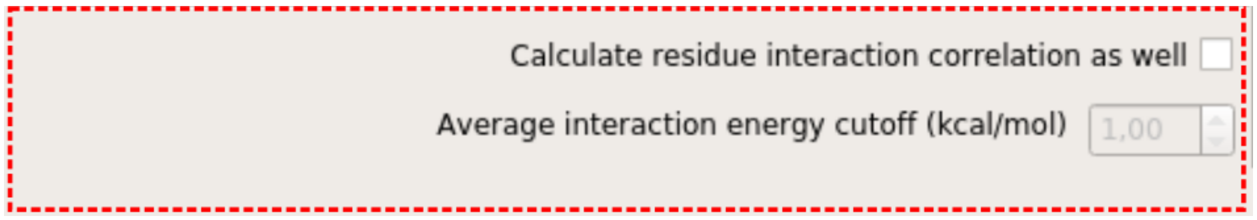
Non-bonded cutoff (NAMD) setting specific the cutoff distance for non-bonded energy calculations by NAMD (Angstroms). In other words, all non-bonded atom-atom interactions beyond this cutoff distance will be ignored. **Note that this settings does not apply to GROMACS trajectories (all calculation settings are as specified in the input TPR file)**

Trajectory stride specifies a stride value for the input trajectory. For example, if you have 1000 frames in your trajectory and set a value of 10 here, every 10th frame will be included in the calculation, yielding a total of 100 frames. Default stride value is 1 (all frames are included).

Number of processors specify the number of CPU cores to be used. The default value is the number of cores available in your system. This option is useful in cases where you have another CPU-intensive process running in the background and don't want to employ all available resources for gRINN.

3.4.3 Correlation settings

The red frame includes UI elements for activating residue interaction energy correlation (Pearson's product moment correlation).


Calculate residue interaction correlation as well ☐
Average interaction energy cutoff (kcal/mol) 1.00

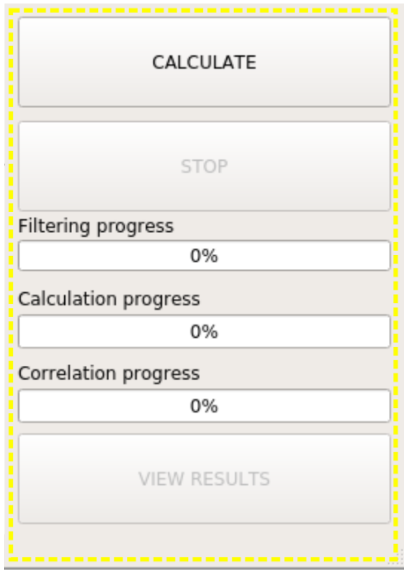
Calculate residue interaction correlation as well check box enables/disables correlation calculation between all pairwise residue interaction energy time series computed.

Average interaction energy cutoff specifies a cutoff for correlation calculations. Interactions with mean energy values below this cutoff value will be excluded from correlation calculations. Default value is set to 1 kcal/mol.

Note: Correlations are reported only if the value is significant (i.e. Pearson's r above 0.4) to reduce the noise in the reported data and maintain a manageable output file size.

3.4.4 Starting and monitoring the calculation

The yellow frame on the right side of the UI includes two buttons for starting and stopping the calculation and three progress bars to monitor the percentage of completion of the three steps involved in the calculation. Finally, at the bottom, a button to start *View Results* interface is included, which becomes activated once an interaction energy calculation task is successfully completed.



CALCULATE button, when pressed, will first check the input (files, settings, whether the output folder exists, etc.). If valid input is detected, computation starts with filtering the interacting pairs. Pairwise residue interaction energies are computed in the second step. Final step, correlation, is done only if the corresponding checkbox is selected.

STOP button is for stopping the operation of gRINN. This is useful if you notice that you need to change a setting after starting the operation or simply want to cancel it.

Note: Note that the *STOP* button **does not** pause the operation. You need to start all over again if you click on this button (A warning is spawned for confirmation to prevent accidental clicking here).

3.4.5 Start calculation with sample NAMD data

Let's load some sample data and start a calculation to see how gRINN works. Click on *load sample NAMD data and settings*.

You will see that the input file UI elements are now populated with some values. The top three text boxes include paths to PDB, PSF and DCD files from a 50 nanoseconds-long MD simulation of bovine cationic trypsin (structure extracted from PDB id 3OTJ).

Note: Note that the PSF and PDB files correspond to a step prior to solvation step during preparation of the system for MD simulation using VMD and psfgen. Solvent molecules in the DCD trajectory were removed by using VMD.

Note: The DCD file corresponds to an equilibrium stage of the simulation (between 25 and 50 nanoseconds). A stride value of 25 was applied in order to reduce the file size bundled with gRINN.

The path of the output folder is set to **grinn_output** in the current working directory. It is safe to change this path as long as it does not exist before starting the calculation.

The path of the NAMD executable is set to namd2 by default. This requires that a valid NAMD executable is present in the executable search path of your system (in linux, this is the PATH environment variable). If namd2 is not accessible via the executable search path, provide the full path here.

Parameter file text box is now filled with the path of the parameter file used for the sample MD simulation.

Click on *CALCULATE* now. After an initial input checking step, gRINN should start by filtering the residue pairs to be included in the interaction energy calculation. Once this is complete, interaction energies will be calculated, followed by equal-time correlation calculations. Depending on the capacity of your computer, the operation will take some time between a few minutes and an hour or two. You can follow the progress by keeping an eye on the progress bars which will show the estimated amount of remaining time as well.

Once gRINN finishes operation, you will be notified and *VIEW RESULTS* button will be enabled. You can now proceed to viewing the results either by clicking this button or *View Results* button on the gRINN Main Window.

3.5 gRINN View Results

Upon starting *View Results* interface, you will be immediately prompted to browse and select a folder containing results from the previous step in gRINN.

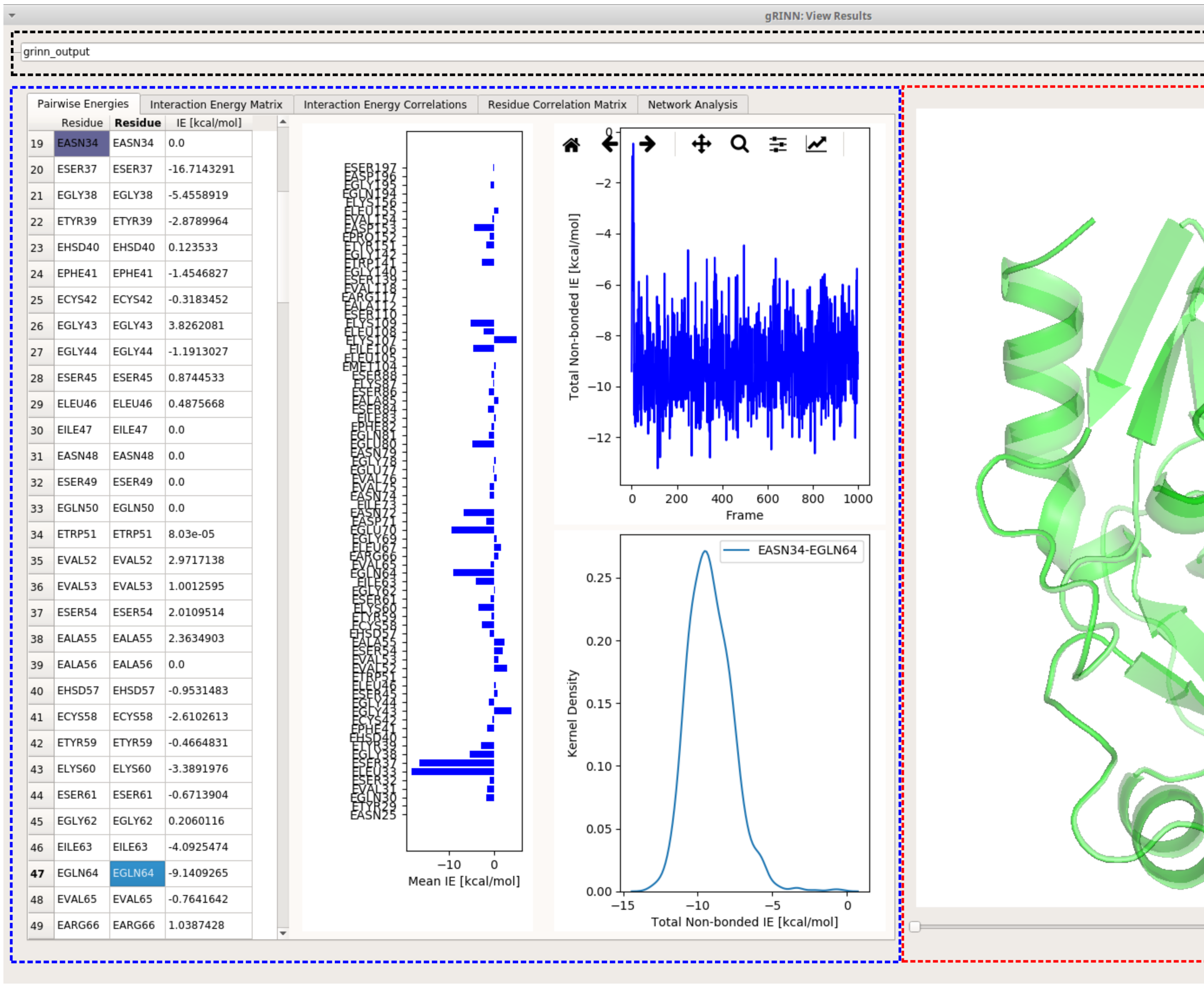
Go ahead and select this output folder now.

If the folder is valid, you will see the message *Please click OK to start loading your data…*. Just click OK to proceed.

After some time, another message will appear, saying *A trajectory exists in your output folder. Would you like to load it as well? Warning: This might slow down the display significantly if the trajectory file size is large.*. Click *Yes* to proceed.

Note: If you choose *No* here, the trajectory will not be loaded and the sliding bar to control the frame displayed in the molecular viewer embedded in the interface (see below) will be disabled.

Once all of the output is loaded into the UI, you will see the *View Results* interface looking like the following figure:



	Residue	Residue	IE [kcal/mol]
19	EASN34	EASN34	0.0
20	ESER37	ESER37	-16.7143291
21	EGLY38	EGLY38	-5.4558919
22	ETYR39	ETYR39	-2.8789964
23	EHSD40	EHSD40	0.123533
24	EPHE41	EPHE41	-1.4546827
25	ECYS42	ECYS42	-0.3183452
26	EGLY43	EGLY43	3.8262081
27	EGLY44	EGLY44	-1.1913027
28	ESER45	ESER45	0.8744533
29	ELEU46	ELEU46	0.4875668
30	EILE47	EILE47	0.0
31	EASN48	EASN48	0.0
32	ESER49	ESER49	0.0
33	EGLN50	EGLN50	0.0
34	ETRP51	ETRP51	8.03e-05
35	EVAL52	EVAL52	2.9717138
36	EVAL53	EVAL53	1.0012595
37	ESER54	ESER54	2.0109514
38	EALA55	EALA55	2.3634903
39	EALA56	EALA56	0.0
40	EHSD57	EHSD57	-0.9531483
41	ECYS58	ECYS58	-2.6102613
42	ETYR59	ETYR59	-0.4664831
43	ELYS60	ELYS60	-3.3891976
44	ESER61	ESER61	-0.6713904
45	EGLY62	EGLY62	0.2060116
46	EILE63	EILE63	-4.0925474
47	EGLN64	EGLN64	-9.1409265
48	EVAL65	EVAL65	-0.7641642
49	EARG66	EARG66	1.0387428

View Results interface can be separated into three main parts based on the functionality of UI elements.

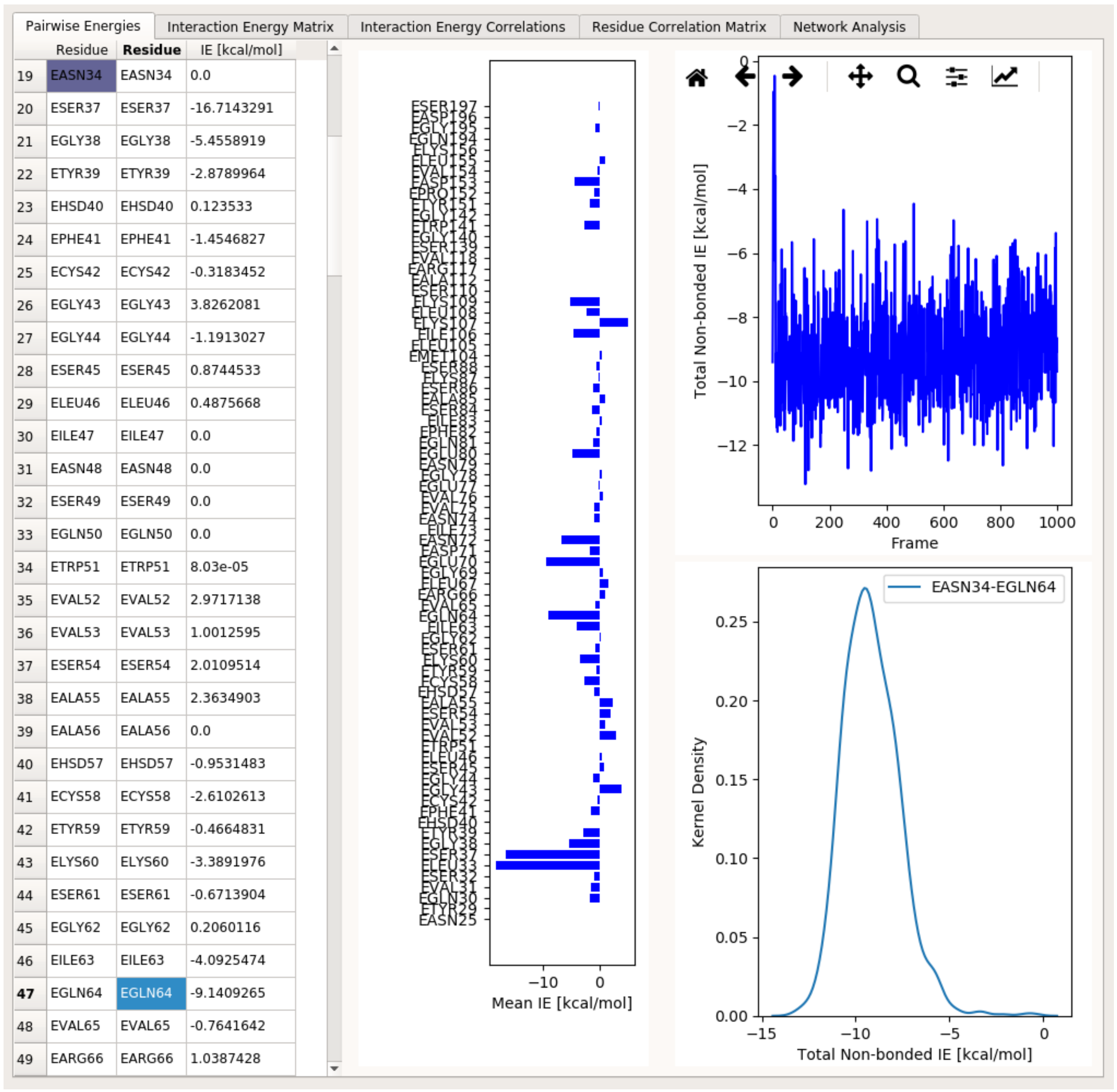
The black frame includes a text box and a button for selecting an output folder. Upon selecting an output folder by using the button, the contents of that folder (if it is a valid gRINN output folder) will be loaded, discarding the currently displayed output (if there's any).

The red frame includes an embedded molecular viewer (which is a PyMol instance) that is updated upon interaction with the blue frame UI elements. How this occurs is explained in the relevant sections of each tab below. The PyMol viewer allows zoom-in and out (rmb), rotation (lmb while on protein) and translation (middle mouse button) modes.

The blue frame includes UI elements, organized into several tab panels. The results displayed in this section are extracted from the files included in the output folder. The content of each tab is explained below.

3.5.1 Pairwise Energies

Pairwise Energies tab includes several UI elements that display individual pairwise residue interaction energies. The UI looks like the following:


Pairwise Energies
Interaction Energy Matrix
Interaction Energy Correlations
Residue Correlation Matrix
Network Analysis
Mean IE [kcal/mol]
Total Non-bonded IE [kcal/mol]
Frame
Kernel Density
EASN34-EGLN64

	Residue	Residue	IE [kcal/mol]
19	EASN34	EASN34	0.0
20	ESER37	ESER37	-16.7143291
21	EGLY38	EGLY38	-5.4558919
22	ETYR39	ETYR39	-2.8789964
23	EHSD40	EHSD40	0.123533
24	EPHE41	EPHE41	-1.4546827
25	ECYS42	ECYS42	-0.3183452
26	EGLY43	EGLY43	3.8262081
27	EGLY44	EGLY44	-1.1913027
28	ESER45	ESER45	0.8744533
29	ELEU46	ELEU46	0.4875668
30	EILE47	EILE47	0.0
31	EASN48	EASN48	0.0
32	ESER49	ESER49	0.0
33	EGLN50	EGLN50	0.0
34	ETRP51	ETRP51	8.03e-05
35	EVAL52	EVAL52	2.9717138
36	EVAL53	EVAL53	1.0012595
37	ESER54	ESER54	2.0109514
38	EALA55	EALA55	2.3634903
39	EALA56	EALA56	0.0
40	EHSD57	EHSD57	-0.9531483
41	ECYS58	ECYS58	-2.6102613
42	ETYR59	ETYR59	-0.4664831
43	ELYS60	ELYS60	-3.3891976
44	ESER61	ESER61	-0.6713904
45	EGLY62	EGLY62	0.2060116
46	EILE63	EILE63	-4.0925474
47	EGLN64	EGLN64	-9.1409265
48	EVAL65	EVAL65	-0.7641642
49	EARG66	EARG66	1.0387428

On the left, a table displays average interaction energies between selected pairs of residues. Due to the excessively high number of all possible pairwise interactions even in a small protein, not all pairs are displayed at once in this table. Instead, only one interaction pair is selected at one time via clicking relevant cells of the table. **The selected item** of the first column determines the first residue in an interaction pair. **The selected item** of the second column determines the second residue in an interaction pair.

So, for example, if you click on residue EGLN64 in the leftmost column, the average interaction energies of all other residues with this residue are displayed in the third column of the table. In addition to this, the vertical bar plot right next to the table is updated to reflect non-zero interaction energies of all other residues with EGLN64. If you then click on EASN34 in *the second column or on the bar plot*, the interaction pair is updated as EGLN64 and EASN34. This will cause the plots on the right hand side of this tab to reflect interaction energy time series and energy distribution belonging to these two residues over the trajectory frames.

Note: gRINN identifies residues with chain ID, amino acid type (three-letter code) and the residue number. For example, EASN34 means the residue 34 (ASN) of chain E in the protein structure.

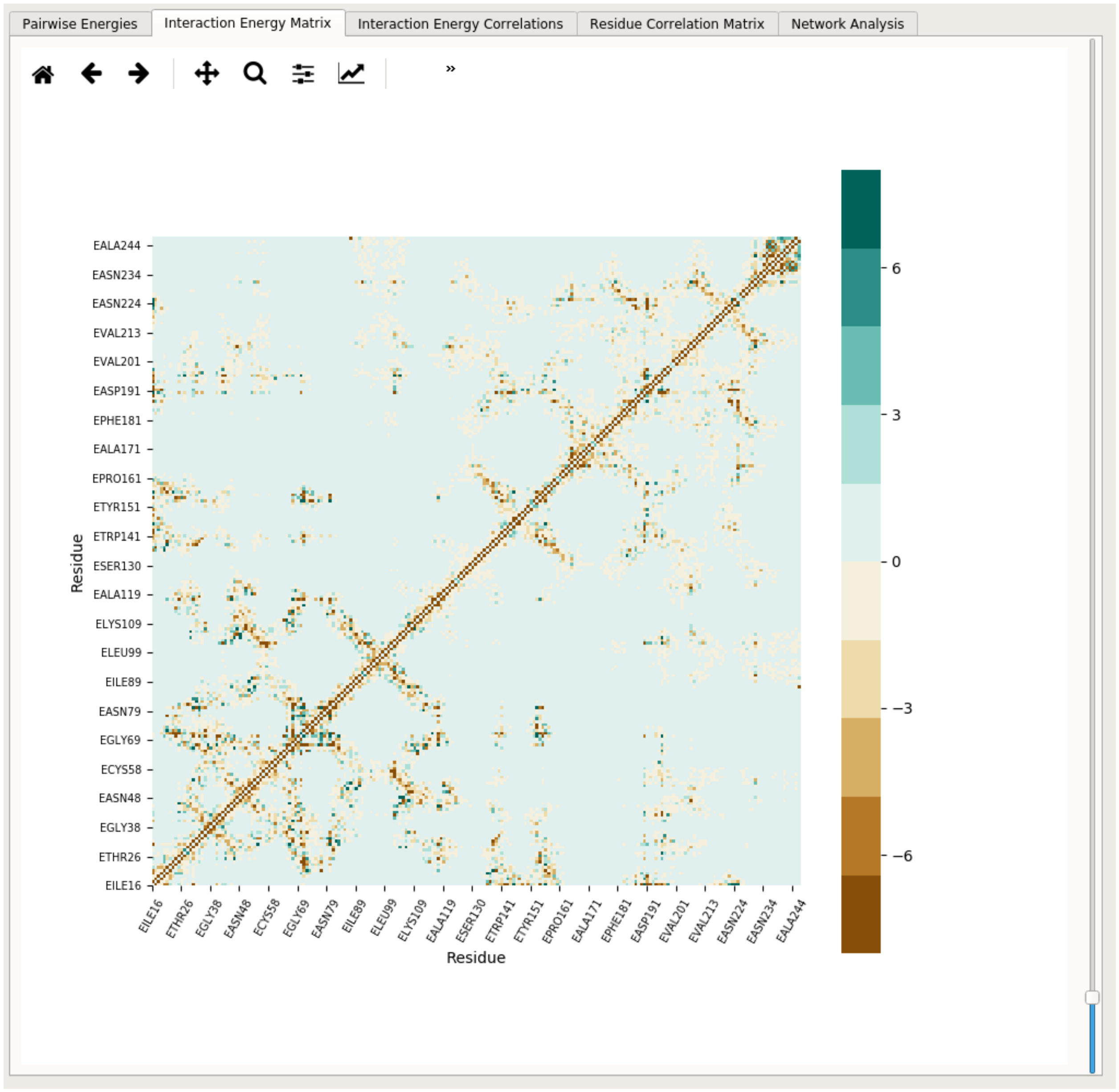
Once you select an interaction pair this way, the protein structure that is displayed in the molecule viewer embedded on the right will reflect this pair of residues as well.

Note: gRINN uses kcal/mol as the energy unit.

Note: Note that some interaction energies will be negative (attractive) and some will be positive (repulsive).

3.5.2 Interaction Energy Matrix

Interaction Energy Matrix tab includes a heat map that displays average interaction energies between all pairs of residues:

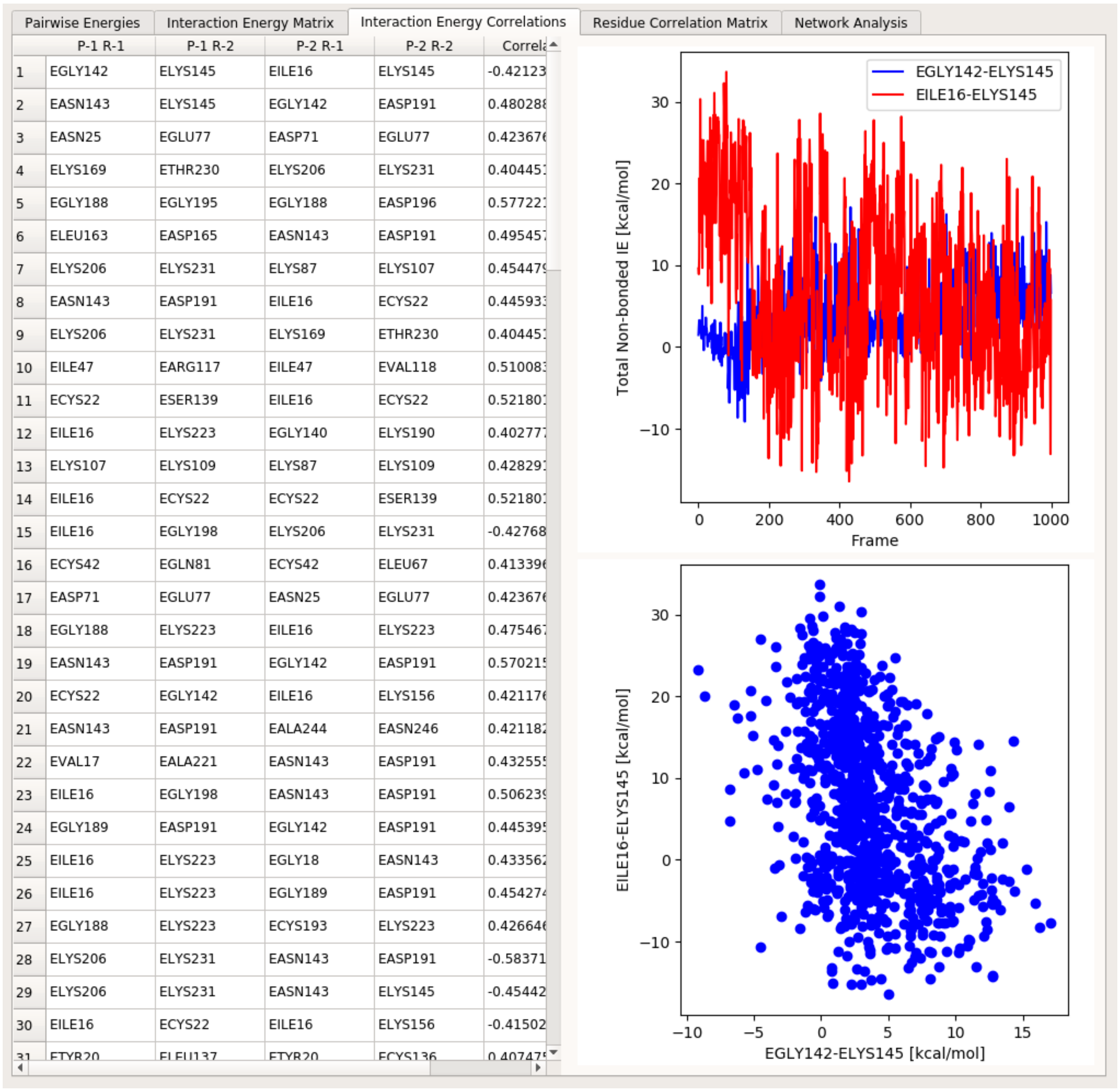


The heatmap shows so-called energetic "hot-spots" in the protein structure. Many of these spots correspond to secondary structure elements, disulfide bonds as well as residues that are not sequence neighbors but in close contact with each other in the folded protein structure. The heatmap can be zoomed in & out and saved into a file using the toolbar included above. The upper and lower boundaries of the heatmap can be adjusted by using the sliding bar located on the right-hand side of the heatmap. The boundary setting affects the total range from negative to positive interaction energies.

Double-clicking on a cell on this heatmap will update the right pane molecule viewer to reflect the selected residue pair on the protein structure.

3.5.3 Interaction Energy Correlations

Interaction Energies Correlations tab includes several UI elements that display data related to equal-time linear correlations between interaction energy time series:


Pairwise Energies
Interaction Energy Matrix
Interaction Energy Correlations
Residue Correlation Matrix
Network Analysis
EGLY142-ELYS145
EILE16-ELYS145
Total Non-bonded IE [kcal/mol]
Frame
EILE16-ELYS145 [kcal/mol]
EGLY142-ELYS145 [kcal/mol]

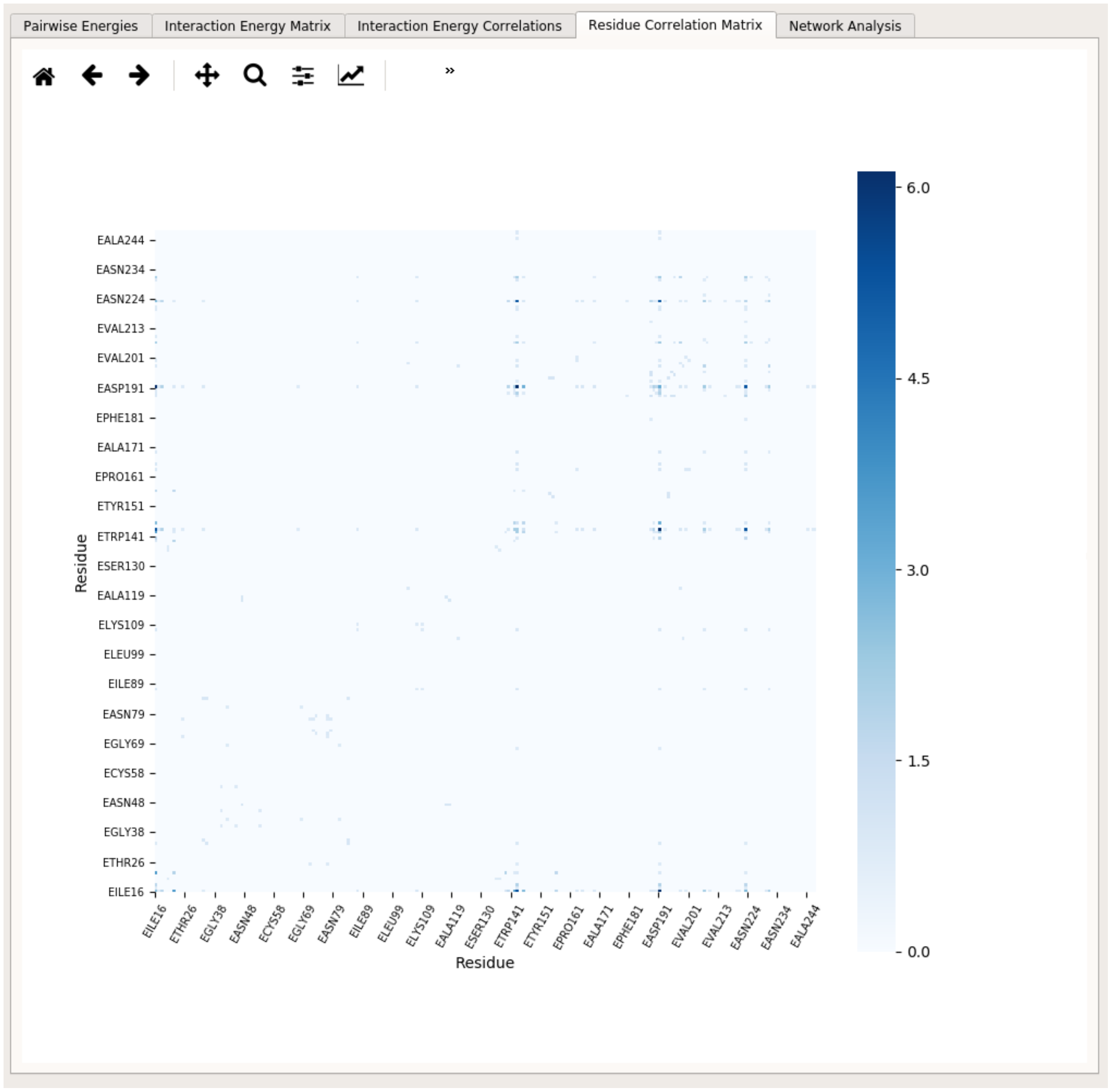
	P-1 R-1	P-1 R-2	P-2 R-1	P-2 R-2	Correl
1	EGLY142	ELYS145	EILE16	ELYS145	-0.42123
2	EASN143	ELYS145	EGLY142	EASP191	0.48028
3	EASN25	EGLU77	EASP71	EGLU77	0.42367
4	ELYS169	ETHR230	ELYS206	ELYS231	0.40445
5	EGLY188	EGLY195	EGLY188	EASP196	0.57722
6	ELEU163	EASP165	EASN143	EASP191	0.49545
7	ELYS206	ELYS231	ELYS87	ELYS107	0.45447
8	EASN143	EASP191	EILE16	ECYS22	0.44593
9	ELYS206	ELYS231	ELYS169	ETHR230	0.40445
10	EILE47	EARG117	EILE47	EVAL118	0.51008
11	ECYS22	ESER139	EILE16	ECYS22	0.52180
12	EILE16	ELYS223	EGLY140	ELYS190	0.40277
13	ELYS107	ELYS109	ELYS87	ELYS109	0.42829
14	EILE16	ECYS22	ECYS22	ESER139	0.52180
15	EILE16	EGLY198	ELYS206	ELYS231	-0.42768
16	ECYS42	EGLN81	ECYS42	ELEU67	0.41339
17	EASP71	EGLU77	EASN25	EGLU77	0.42367
18	EGLY188	ELYS223	EILE16	ELYS223	0.47546
19	EASN143	EASP191	EGLY142	EASP191	0.57021
20	ECYS22	EGLY142	EILE16	ELYS156	0.42117
21	EASN143	EASP191	EALA244	EASN246	0.42118
22	EVAL17	EALA221	EASN143	EASP191	0.43255
23	EILE16	EGLY198	EASN143	EASP191	0.50623
24	EGLY189	EASP191	EGLY142	EASP191	0.44539
25	EILE16	ELYS223	EGLY18	EASN143	0.43356
26	EILE16	ELYS223	EGLY189	EASP191	0.45427
27	EGLY188	ELYS223	ECYS193	ELYS223	0.42664
28	ELYS206	ELYS231	EASN143	EASP191	-0.58371
29	ELYS206	ELYS231	EASN143	ELYS145	-0.45442
30	EILE16	ECYS22	EILE16	ELYS156	-0.41502
31	ETYR20	ELEU137	ETYR20	ECYS136	0.40747

The table here displays a list of the pairs of residues involved in a specific correlation (with the first two columns indicating the two residues in the first interaction pair and the third and fourth columns indicating the two residues in the second interaction pair). The last column shows the correlation value.

Clicking on a row in this table will update the two plots next to the table. The top plot shows the interaction energy series involved in the correlation against trajectory frames. In the bottom, a plot of these two series against each other is displayed. The right pane molecular viewer will be updated to highlight the four residues involved in the correlation.

3.5.4 Residue Correlation Matrix

Residue Correlation Matrix tab includes a heatmap showing the "Residue Correlation Matrix" (RC Matrix) that is constructed using the interaction energy correlations. RC matrix is one way of extracting dynamical correlation information from interaction energies of residue pairs in the structure.



The matrix is constructed to be able to map the correlation values on the three dimensional structure *[KK2009]*. In other words, the correlation values are translated into a residue-residue type of information. The matrix is an NxN square matrix (N being the number of residues in the structure) and is constructed by considering the mutual occurence of a given pair of residues in both sides of a correlation. For example, if the correlation between the interaction EGLY142-ELYS145 and the interaction EILE16-ELYS145 is 0.6 and the correlation between the interaction EGLY16-ELYS223 and the interaction EGLY142-EASP191 is -0.5, the residue correlation between EILE16 and EGLY142 would be the sum of the absolute values of these two correlation coefficients (which is 1.1))*[0] This summation is performed for all calculated correlations for each residue pair in the structure.

Like the interaction energy matrix, the heatmap can be zoomed-in and out. Double-clicking a cell in the heatmap will highlight the corresponding residue pair in the right pane molecular viewer.

[0] Note that the values given here are exemplary and do not reflect the values you've just inspected in the previous tab.

3.5.5 Network Analysis

Protein Energy Network (PEN)

The term "Protein Energy Network" has been used for the first time by Vijayabaskar and Vishveshwara *[VV2010]* in a study where they constructed such networks of protein structures using pairwise residue interaction energies computed over ensembles of structures obtained from MD simulations.

In this method, a network is constructed by taking individual residues as nodes and average interaction energies between each residue pair as the "weight" for edges that are added between these residue nodes. Once the network is constructed, local (node-based) network metrics, such as degree, closeness and betweenness centralities can be obtained to assess the importance of each residue in terms of protein stability and dynamics.

gRINN constructs such a network once you load an output folder into the *View Results* interface. Each residue in the structure is taken as a node in the PEN. Edges are added if the absolute value of the interaction energy between any two residues is greater than a cutoff value. This cutoff can be set by changing the *Edge addition energy cutoff* value.

Adding an edge between two residues implies that they have a significant interaction. Yet, the way how the *strength* of this interaction is defined changes the results of global residue-based network metrics such as centrality values and shortest path analysis. Values of average interaction energies can be used to assign a relative strength (**weight**) value to an edge. Accordingly, an edge is added using the following **general** criteria:

In the above equation, ω_{ij} denotes the edge weight between residues i and j. χ_{ij} denotes the average interaction energy between residues i and j. Note that the addition of edges between covalently bound residues is optional (see below).

χ_{ij} is computed using the following formula:

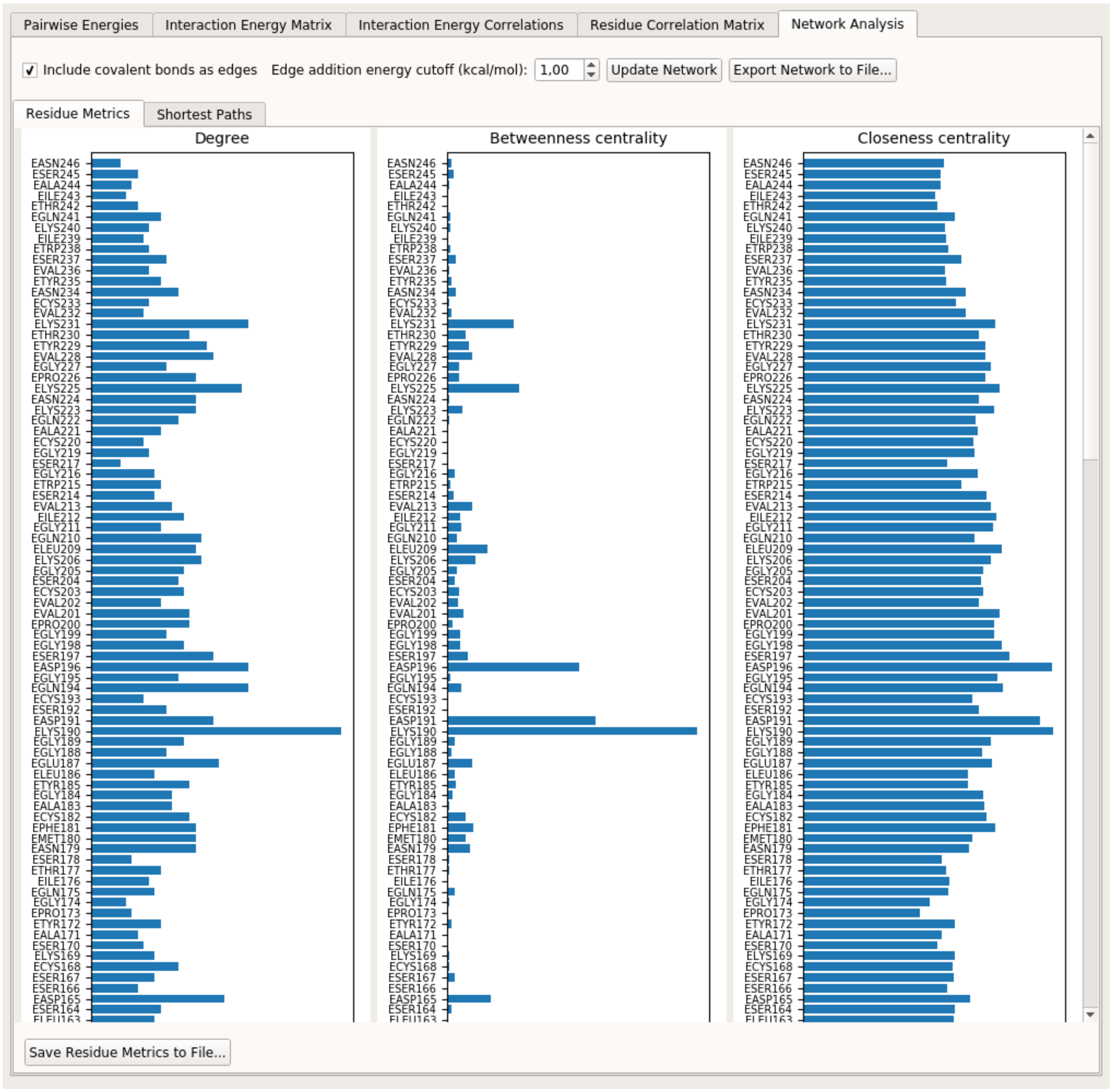
In this equation, ϵ_{ij} denotes the average interaction energy between residues i and j and ϵ_{att} denotes the array of attractive (negative) interaction energies. Note that this equation favors attractive interactions: the more attractive (negative) an interaction is, the higher weight will be assigned to the edge of that specific interaction. Repulsive interactions obtain zero weight.

Network Analysis Tab

Network Analysis tab includes UI elements for inspecting the node-level (residue-level) metrics and shortest paths in a Protein Energy Network (PEN) constructed by using the interaction energy matrix.

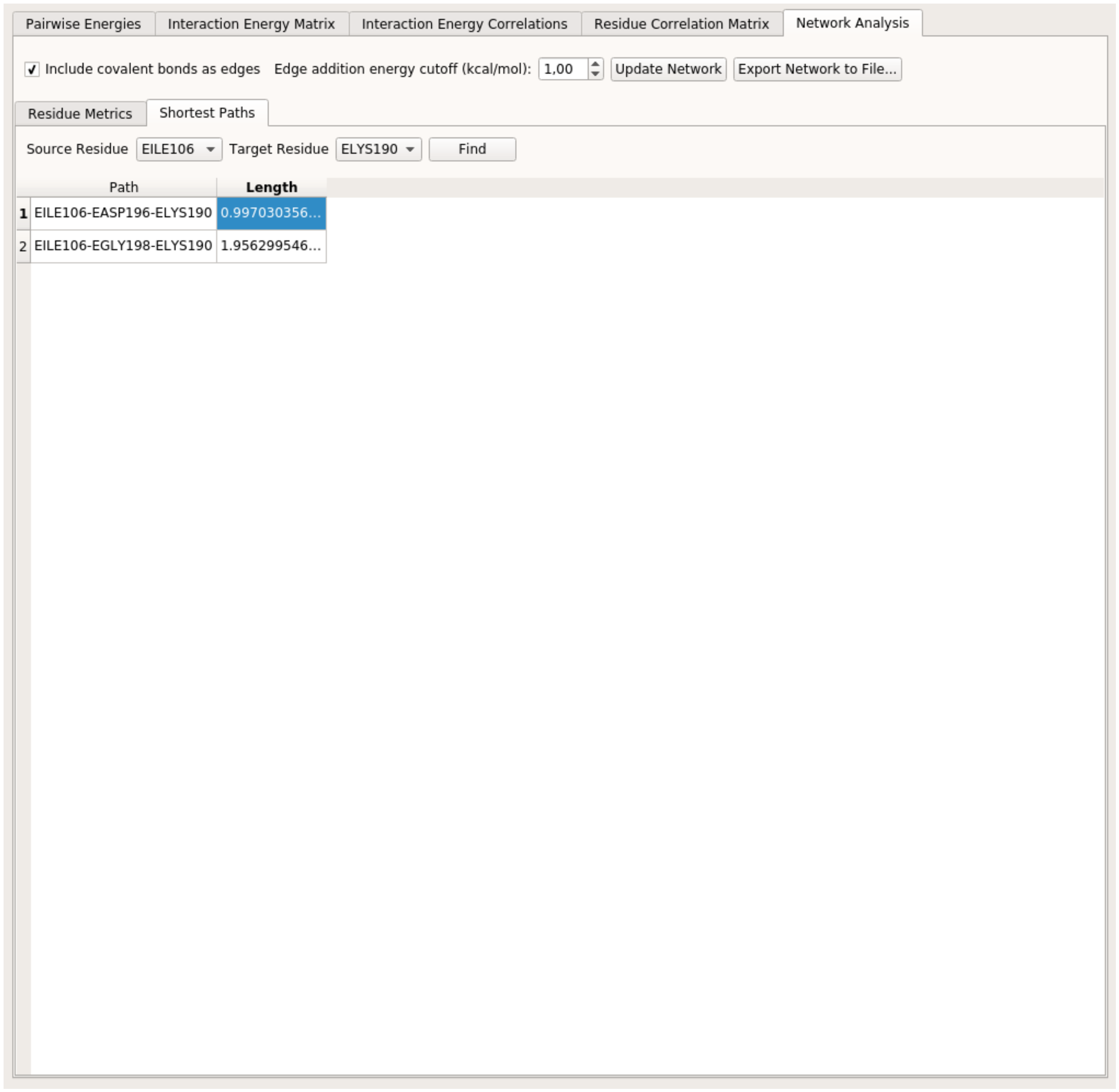
It is possible to include/exclude covalent bonds as edges and specify an interaction energy cutoff for edge addition using the respective checkbox and the spinbox at the top of this tab panel. It is necessary to update the network by clicking the *Update Network* button if these settings are changed.

Residue Metrics tab here shows three types of local metrics (node/residue-based): Degree, Betweenness Centrality and Closeness Centrality.


Pairwise Energies
Interaction Energy Matrix
Interaction Energy Correlations
Residue Correlation Matrix
Network Analysis
Update Network
Export Network to File...
Residue Metrics
Shortest Paths
Degree
Betweenness centrality
Closeness centrality
Save Residue Metrics to File...

✓ Include covalent bonds as edges Edge addition energy cutoff (kcal/mol): 1,00

Shortest Paths tab allows the user to select a source and a target residue and find all alternative short pathways between these two residues within the structure of the PEN. The shortest paths are found by using Dijkstra's algorithm *[DEW1959]*. Note that since this algorithm favors edges with lower weights and in reality we want stronger interactions to be preferred in a short path (which have higher edge weights), a new edge property (distance) is assigned to each edge by calculating $1 - \chi_{ij}$ and this distance value is considered as the edge weight when employing Dijkstra's algorithm.


Pairwise Energies
Interaction Energy Matrix
Interaction Energy Correlations
Residue Correlation Matrix
Network Analysis
Update Network
Export Network to File...
Residue Metrics
Shortest Paths
Find

✓ Include covalent bonds as edges Edge addition energy cutoff (kcal/mol): 1,00

Source Residue EILE106 Target Residue ELYS190

	Path	Length
1	EILE106-EASP196-ELYS190	0.997030356...
2	EILE106-EGLY198-ELYS190	1.956299546...

Upon clicking a path in the shortest path table, the right pane molecular viewer will be updated to reflect the path.

3.5.6 References

3.5.7 Output folder content

If gRINN completes the operation successfully, you should see the following files in the output folder:

- traj_dry.dcd
- system_dry.psf
- system_dry.pdb
- network.gml
- grinn.log

- energies_resIntCorr.csv (if you enabled interaction energy correlation)
- energies_resCorr.dat (if you enabled interaction energy correlation)
- energies_intEnVdW.csv
- energies_intEnTotal.csv
- energies_intEnMeanVdW.dat
- energies_intEnMeanTotalList.dat
- energies_intEnMeanTotal.dat
- energies_intEnMeanElec.dat
- energies_intEnElec.csv
- energies.pickle

traj_dry.dcd includes the frames of your input trajectory that were used by gRINN. *View Results* interface reads conformations of protein structure into the PyMol instance from this file.

system_dry.psf and **system_dry.pdb** contain your input protein structure topology and coordinates.

grinn.log is the log file produced by gRINN.

energies_resIntCorr.csv includes data displayed in *Interaction Energy Correlations* tab in *View Results* interface in comma-separated values (CSV) format.

energies_resCorr.dat includes the RC matrix that is displayed as heatmap in *Residue Correlations* tab.

energies_intEnVdW.csv includes non-bonded Van-der Waals interaction energies in CSV format.

energies_intEnTotal.csv includes non-bonded Total interaction energies (sum of Van der Waals and Electrostatic energies) in CSV format.

energies_intEnElec.csv includes non-bonded Electrostatic interaction energies in CSV format.

energies_intEnMeanVdW.dat includes average non-bonded Van der Waals interaction energy matrix between all pairs of amino-acids.

energies_intEnMeanElec.dat includes average non-bonded Electrostatic interaction energy matrix between all pairs of amino-acids.

energies_intEnMeanTotal.dat includes average non-bonded Total interaction energy matrix between all pairs of amino-acids. This data is displayed as heatmap in *Interaction Energy Matrix* tab.

energies.pickle is a pickled dictionary which contains all pairwise interaction energies between residues in protein structure. Once loaded into a Python working environment, the energy time series between specific residue pairs can be accessed as folows:

```python
import pickle
energies = pickle.load(open('energies.pickle','r'))
energies['EASN34-EGLN64']['Total']
```

3.6 gRINN with GROMACS data

gRINN can work with GROMACS-generated data and interoperate with the gmx executable.

Click on *load sample GMX data and settings*. The top three UI elements will contain a TPR, TOP and an XTC (gromacs trajectory). The data were obtained from the equilibrium section (25-50ns) of a 50ns-long MD simulation of a peptide-loade Major Histocompatibility Complex (pMHC, PDB ID: 1EEY).

Click on *CALCULATE*. Depending on the capacity of your computer, the operation will last between a few minutes to one-two hours. Once computation finishes, you can *VIEW RESULTS* as described above.

Note: GROMACS uses SI units of kJ/mol. This is converted to kcal/mol by gRINN.

3.7 gRINN Command-Line Interface

gRINN offers a command-line interface for interaction energy and correlation calculations as well. This is mostly useful if you decide to use gRINN for a batch of trajectories. You can include several gRINN commands in a e.g. shell script.

Command-line interface is activated by providing additional flags to `grinn`. Three modes are available here: `-calc`, `-corr` or `-results`. `-results` mode is used just for starting the *VIEW RESULTS* interface directly instead of starting the main window first.

You can get a full of list of flags and their explanations by typing `./grinn --help` in the terminal (or `grinn --help` if the executable is not in the current working directory) . Some examples are included below:

The following command will take 3otj_typsin_psfgen.pdb, 3otj_trypsin_psfgen.psf and 3otj_trypsin_25_50ns_dry_str25_aligned.dcd and par_all27_prot_lipid_na.inp as input files from the current working directory, assume that namd2 is present in the executable search path, apply a stride of 10 on the input trajectory and start an interaction energy calculation using 16 CPU cores by taking only residue pairs whose centers-of-mass come closer than 20 Angstroms in at least 75% of the trajectory frames with a 12 Angstroms cutoff for non-bonded energy computation. The results will be saved to folder `grinn_output`, which will be created in the current working directory.

```
$ grinn -calc --pdb 3otj_trypsin_psfgen.pdb --top 3otj_trypsin_psfgen.psf
--traj 3otj_trypsin_25_50ns_dry_str25_aligned.dcd --parameterfile par_all27.inp
--stride 10 --exe namd2 --pairfiltercutoff 20 --cutoff 12 --pairfilterpercentage 0.75
--outfolder grinn_output --numcores 16
```

The above command does not compute interaction energy correlations. If you want to include interaction energy correlations, you need to provide the `--calccorr` flag:

```
$ grinn -calc --pdb 3otj_trypsin_psfgen.pdb --top 3otj_trypsin_psfgen.psf
--traj 3otj_trypsin_25_50ns_dry_str25_aligned.dcd --parameterfile par_all27.inp
--stride 10 --exe namd2 --pairfiltercutoff 20 --cutoff 12 --pairfilterpercentage 0.75
--outfolder grinn_output --numcores 16 --calccorr --corrintencutoff 1
```

This will include correlation calculations for average interaction energies above 1 kcal/mol.

Alternatively, you can compute correlations separately after using `-calc` mode with the `--calccorr` flag. For this, use the `-corr` mode. This time, you need to provide the path to the CSV file that includes interaction energy time series. The results will be stored in the folder in which this CSV file is located.

```
$ grinn -corr --corrinfile grinn_output/energies_intEnTotal.csv
```

For GROMACS-generated data, just replace the `--exe` flag with the path to the gmx executable (or type `--exe gmx` if gmx is available in the executable search path), use `--tpr` flag instead of `--pdb` and provide your TOP and XTC/TRR files instead of PSF and DCD files as above.

CHAPTER 4

Miscellaneous

4.1 Deleting non-protein atoms from NAMD/CHARMM input data

gRINN requires "dry" NAMD/CHARMM MD simulation data in order to process them efficiently. It is fairly easy to remove non-protein atoms from input PDB/PSF/DCD files using VMD. Below we describe the steps in VMD (You need to have VMD installed on your computer).

4.1.1 Deleting non-protein atoms from PSF/PDB files

- Start vmd from the directory in which your psf and pdb files are located by typing `vmd` in a terminal.
- Save the following lines of Tcl code to a file named *make_dry_psf.tcl* into your current working directory.

```
mol load psf ionized.psf pdb ionized.pdb

set a [atomselect top "not protein"]
set l [lsort -unique [$a get segid]]

package require psfgen
readpsf ionized.psf
coordpdb  ionized.pdb

foreach s $l {
delatom $s
}

writepsf ionized_dry.psf
writepdb ionized_dry.pdb
```

- Replace the *ionized.psf, ionized.pdb, ionized_dry.psf and ionized.pdb* with the file names of your psf/pdb and your desired output file names.
- Run the tcl script by starting the TkConsole from VMD Main Window (Extensions>TkConsole) and typing `source make_dry_psf.tcl`.

- You should have obtained a psf/pdb file pair WITHOUT non-protein atoms now. Use them as input to gRINN.

4.1.2 Deleting non-protein atoms from DCD trajectory files

- Start vmd from the directory in which your psf and pdb files are located by typing `vmd` in a terminal.
- Load your psf file (e.g. ionized.psf) INCLUDING all atoms included in your simulation by choosing (File -> New Molecule) from VMD Main Window.
- Load your trajectory (dcd) onto this psf file by choosing (File -> New Molecule) and selecting "Load Files for…" option.
- Save a new trajectory file by choosing the molecule in Main Window, right-clicking on it and selection "Save Coordinates" option. Then, type *"protein"* to the "Selected Atoms" field and select "DCD" as the file-type. Click on "Save".
- Use this new DCD file as input to gRINN.

4.2 Deleting non-protein atoms from GROMACS input data

If you're submitting a GROMACS trajectory, there is no need for additional data preparation. You can just give your topology (TOP), run input (TPR) and trajectory (XTC or TRR) files as input to gRINN. However, a common practice with protein MD simulations is to keep only protein atoms and remove solvent/ions from trajectory files to save disk space unless solvent-protein interactions are the focus of a study. In this case, TPR and TOP files WITHOUT non-protein atoms should be prepared for gRINN. We describe here a step-by-step procedure to make TPR,TOP and TRR/XTC files that don't include any non-protein atoms.

4.2.1 Deleting non-protein atoms from TOP files

The only required action here is to delete lines containing non-protein atoms in the topology (TOP) file. For example, if your top file contains a certain number of water molecules, the end of the file might look like the following:

```
[ molecules ]
; Compound        #mols
Protein_chain_A     1
Protein_chain_B     1
Protein_chain_C     1
SOL         24933
NA               8
```

Simply delete the lines beginning with SOL and NA:

```
[ molecules ]
; Compound        #mols
Protein_chain_A     1
Protein_chain_B     1
Protein_chain_C     1
```

Save this file with a new file name (e.g. mytopol_dry.top) and use it as input to gRINN.

4.2.2 Deleting non-protein atoms from TPR files

The TPR file contains all information that is necessary to start an MD simulation with GROMACS. Making a new TPR file without any non-protein atoms is a bit more complicated than generating a "dry" topology file. The steps are described below:

- First, extract a PDB file from your TPR by calling **gmx editconf** in a terminal window:

```
gmx editconf -f mytpr.tpr -o mypdb.pdb
```

- Then, delete all non-protein atoms from this PDB file (using a suitable text edit, e.g. Sublime Text) and save it as e.g. "mypdb_dry.pdb".
- Next, modify your simulation input MDP file such that it does not contain any solvent/ion related parameters. In other words, prepare it as if you're going to run a simulation *in vacuo*. For example, if your MDP file contains such lines:

```
; Temperature coupling
tcoupl          = V-rescale
tc-grps         = Protein Non-Protein
ref_t           = 310     310
```

simply delete them. Then, save the file as e.g. "mymdp_dry.mdp".

- Next, create a new TPR file using your dry PDB and MDP files by calling **gmx grompp**:

```
gmx grompp -f mymdp_dry.mdp -c mypdb_dry.pdb -p mytopol_dry.top -o mymd_dry.tpr
```

- Use the resulting TPR as input to gRINN.

CHAPTER 5

History/Change Log

### v1.1.0 (2018/04/06)

This version introduces a major internal code rehaul, leaving major features of gRINN unaffected. There are additional new features as well as major/minor bug fixes:

New Features:

- A new calculation setting for non-bonded interaction cutoff for NAMD simulation input is introduced. In the previous version, filtering cutoff distance parameter specified both the filtering cutoff distance itself and the non-bonded interaction energy cutoff for NAMD simulation input.
- gRINN now supports Charmm simulation input as well.

Major/minor bug fixes:

- A bug which caused faulty reading of more than one parameter file for NAMD simulation input is fixed.
- A minor bug which caused incorrect protein structure display upon start of View Results interface in Mac OS version is fixed.

### v1.0.1 (2017/12/27)

Initial release of gRINN.

CHAPTER 6

FAQ

gRINN is fresh out-of-box software! We are still preparing a list of "Frequently Asked Questions" list to serve as clarifications regarding the use of the tool. Still, real FAQs will come up as you use the software and direct questions/comments at us. Please contact either Onur or Pemra directly:

onursercin AT gmail DOT com

pemra DOT ozbek AT marmara DOT edu DOT tr

CHAPTER 7

Credits

The core functionality of gRINN is provided by NAMD and GROMACS MD simulation software.

gRINN was written in Python programming language (version 2.7). In addition to the Python core library, several open source Python packages are used to provide the following functionality:

PyQt5 (a python wrapper around the Qt desktop application development environment, v5.6.0) is used for all GUI elements.

matplotlib (v2.0.2) and seaborn (v0.8.1) are used to display two-dimensional line and scatter plots as well as heatmaps included in the "View Results" interface.

ProDy (v1.9.3) is used for PDB and DCD trajectory manipulations, atom selections and all other general geometric tasks related to protein structures.

Mdtraj (v1.9.0) is used to convert GROMACS trajectory file formats to DCD for further processing using ProDy.

PyMol by Schrödinger (open source version v1.9.0.0) is used as the embedded molecule viewer in the "View Results" interface of gRINN.

pexpect (v4.3.1) is used to interact with gmx executable from within Python environment.

Numpy (v1.13.3) is used for all operations related to matrices, which occur throughout the computation workflow of gRINN.

pandas (v0.20.3) is used to store, process and save data throughout the computational workflow of gRINN.

networkx (v2.0) is used to construct Protein Energy Networks and calculation of local network metrics and shortest paths.

gRINN's logo, Shamrock lucky Icon was designed by www.iconka.com

CHAPTER 8

Contact

gRINN is developed and maintained primarily by Onur Serçinoğlu. For all technical issues/questions/requests/problems, please contact him directly at onursercin AT gmail DOT com.

For all scientific questions/discussions, contact the Principal Investigator (Dr. Pemra Ozbek) directly at pemra DOT ozbek AT marmara DOT edu DOT tr.

gRINN was part of Onur's work in Pemra Ozbek's lab at Marmara University Department of Bioengineering Computational Biology and Bioinformatics Research Group

CHAPTER 9

License

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gRINN Developers: Onur Serçinoğlu (onursercin@gmail.com) Pemra Ozbek (pemra.ozbek@marmara.edu.tr)

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9.2 Third-party tools included in gRINN:

gRINN is based on Python 2.7. Python is available under a Python license:

```
Python 2.7 license
This is the official license for the Python 2.7 release:

A. HISTORY OF THE SOFTWARE
==========================

Python was created in the early 1990s by Guido van Rossum at Stichting
Mathematisch Centrum (CWI, see http://www.cwi.nl) in the Netherlands
as a successor of a language called ABC.  Guido remains Python's
principal author, although it includes many contributions from others.

In 1995, Guido continued his work on Python at the Corporation for
National Research Initiatives (CNRI, see http://www.cnri.reston.va.us)
in Reston, Virginia where he released several versions of the
software.

In May 2000, Guido and the Python core development team moved to
BeOpen.com to form the BeOpen PythonLabs team.  In October of the same
year, the PythonLabs team moved to Digital Creations (now Zope
Corporation, see http://www.zope.com).  In 2001, the Python Software
Foundation (PSF, see http://www.python.org/psf/) was formed, a
non-profit organization created specifically to own Python-related
Intellectual Property.  Zope Corporation is a sponsoring member of
the PSF.

All Python releases are Open Source (see http://www.opensource.org for
the Open Source Definition).  Historically, most, but not all, Python
releases have also been GPL-compatible; the table below summarizes
the various releases.

    Release         Derived     Year        Owner       GPL-
                    from                                compatible? (1)
```

gRINN makes use of and includes several open-source software:

PyQt5 is distributed with gRINN. PyQt5 is dual licensed on all platforms under the Riverbank Commercial License and the GPL v3.

NumPy is distributed with gRINN. NumPy is available under NumPy license:

pandas is distributed with gRINN. pandas is available under BSD 3-Clause License.

networkx is distributed with gRINN. networkx is available under a BSD license:

ProDy is distributed with gRINN. ProDy is available under MIT license:

MDTraj is distributed with gRINN. MDtraj is available under the LPGL.

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CHAPTER 10

Indices and tables

- genindex
- modindex
- search

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