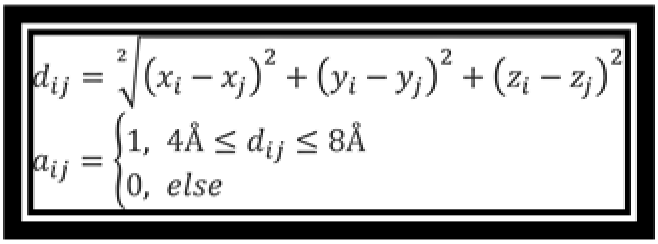
# Protein Contact Networks Miner: A tool for the Analysis of Protein Contact Networks

## 1 Introduction and motivation

Protein Contact Networks Miner is a command line tool (and now a Graphic User Interface) designed to annotate allosteric domains of a protein based on its representation through an unweighted graph. This graph is also known as Protein Contact Network (PCN).

A PCN is an unweighted graph where: (i) nodes are the amino acids of the protein and (ii) there exists an edge that connects two nodes *i* and *j* only if the euclidean distance between them has a value included in the interval: 4 Angstrom (threshold for only non covalent interactions) and 8 Angstrom (threshold for only significant interactions). The distance between two aminoacids i and j is approximated with the distance between the amino acids alpha carbons.

Given the distance between two nodes *i* and *j,* user can only modify covalent (min) and significant (max) threshold distance for PCN construction.



$$d_{ij} = \sqrt[2]{\left(x_i - x_j\right)^2 + \left(y_i - y_j\right)^2 + \left(z_i - z_j\right)^2}$$

$$a_{ij} = \begin{cases} 1, & 4\text{Å} \leq d_{ij} \leq 8\text{Å} \\ 0, & else \end{cases}$$

PCN global descriptors (like graph diameter) or local descriptors (like node centrality measures) are useful to model and analyze protein functions.

## 2 PCN Miner tool short description

The here available tool named PCN Miner allows the user to identify modules (also called communities or clusters) in protein molecules using three different approaches:

1. spectral clustering: it extracts clusters from a graph with a clustering approach based on the Laplacian matrix eigenvectors (see tutorial on spectral clustering [1]);
2. embedding+clustering: it uses one of the embedding algorithm in the GEM library [2] and then apply clustering;
3. community detection: it uses one of the community detection algorithms in the cdlib library [3].

PCN Miner allows to:

(i) identify the putative allosteric paths and regions in protein structures (thus to help the design of allosteric drugs);

(ii) define hypothesis and make tests about the functional effects of mutations (example variants of SARS CoV-2 Spike Protein);

(iii) recognise functional domains (communities or clusters) in proteins.

PCN-Miner includes the use of the following methods:

1. Spectral Clustering: Both Hard (K-Means) and Soft (Fuzzy C-Means) clustering approach used on the eigenvectors of the Laplacian matrix (both normalized or unnormalized form). The Shi Malik spectral clustering approach is also supported, to resolve the generalized eigenvalues problem;
2. Embedding + Clustering: Node2vec, HOPE, Laplacianeigenmaps embedding followed by a supported spectral clustering algorithm;
3. Community Detection: Louvain, Leiden, Walktrap, Infomap, Asyn FluidC, Greedy Modularity, Spinglass;
4. Centrality Measures: Closeness Centrality, Betweenness Centrality, Eigenvector Centrality, Degree Centrality.

The results obtained by using these algorithms, outputs (node centrality, clusters or communities) of the supported algorithms are then plotted on the 3D structure of the protein using PyMol scripts.

## 3 PCN Miner installation guide

It can be installed and used by using the setup files on github and using a command line in the operating system.

*-Microsoft Windows O.S. :*

```
git clone https://github.com/hguzzi/ProteinContactNetworks.git

setupWindows.bat
```

*-Linux-MACOSX O.S. :*

```
git clone https://github.com/hguzzi/ProteinContactNetworks.git

source setupLinux-MacOSX.sh
```

It is possible to install the tool by using Python Package Index (the pip), the installation tool of Python.

Moreover, to install PCN Miner please consider that this project includes and uses PyMOL and GraphEmbeddingMethods libraries.  Since both libraries are not available on PyPI, in case they are not available, pymol can be installed by using anaconda.

This can be performed by using the anaconda prompt and typing the following command:

```
conda create -n PCN python=3.8.3

conda activate PCN

conda install -c schrodinger pymol-bundle
```

GEM library can be installed by using pip+git and this library using TESTPYPI as:

```
pip install git+https://github.com/palash1992/GEM.git
pip install --extra-index-url https://pypi.org/simple -i
https://test.pypi.org/simple/ PCN-Miner
```

The PCN Miner tool can be similarly installed by using the two commands *pip* and *git* as in the following example:

```
conda create -n PCN python=3.8.3

conda activate PCN

conda install -c schrodinger pymol-bundle

pip install git+https://github.com/hguzzi/ProteinContactNetworks.git
```

## 4 Examples, use case and howto use of PCN Miner

The command line tool version can be used as follows:

```
conda activate PCN

cd pcn

cd tools

python pcn_main.py
```

The GUI tool version can be used by running is as:

```
conda activate PCN

cd pcn

cd tools

python pcn_gui_main.py
```

GUI interface instance is reported in the following example

### Case Study 1.

Description: Calculation of PCN from PDB file

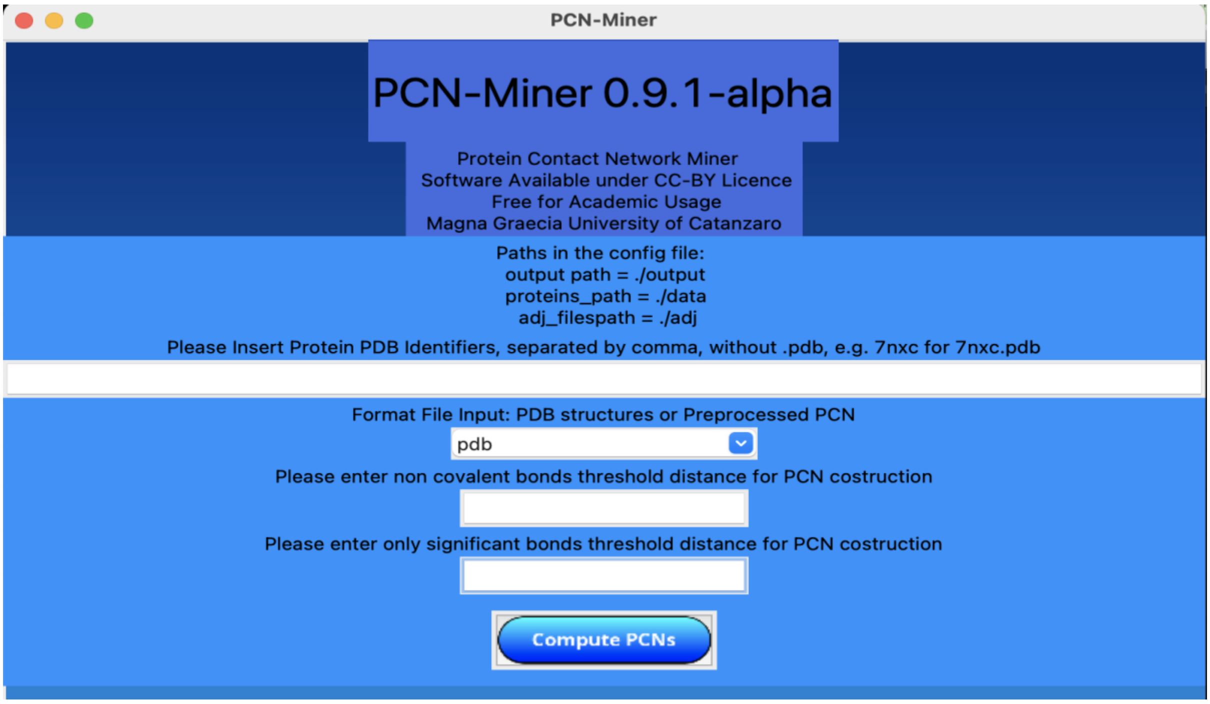
Use the entry PDB code: *6nxc*

Analysis of PCN: *Method: Centrality Analysis*

Algorithm: *Betweenness Centrality Analysis*

Visualization: *Pymol Embedded*

Step 1: Loading the PDB File into PCN-Miner



PCN-Miner

PCN-Miner 0.9.1-alpha

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Paths in the config file:
output path = ./output
proteins_path = ./data
adj_filespath = ./adj

Please Insert Protein PDB Identifiers, separated by comma, without .pdb, e.g. 7nxc for 7nxc.pdb

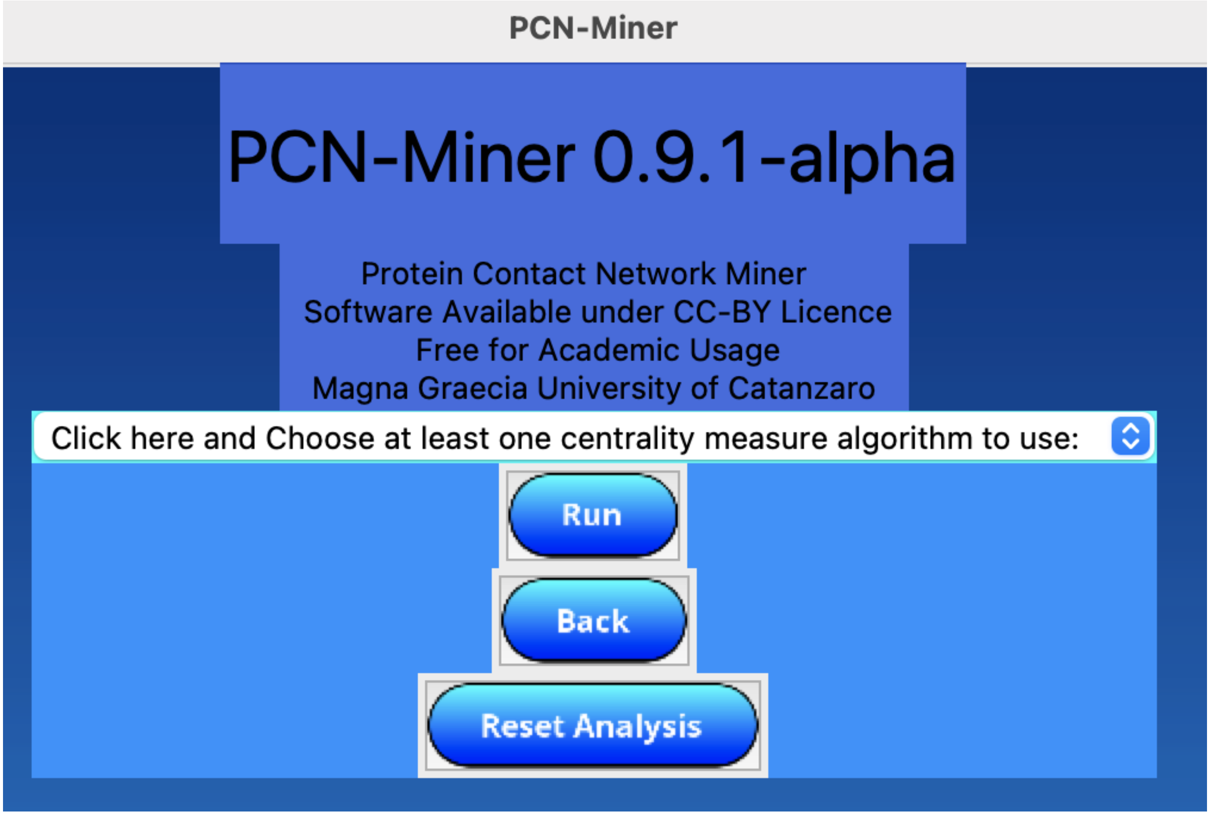
Format File Input: PDB structures or Preprocessed PCN

pdb

Please enter non covalent bonds threshold distance for PCN costruction

Please enter only significant bonds threshold distance for PCN costruction

Step 2 Choice of the desired centrality measure



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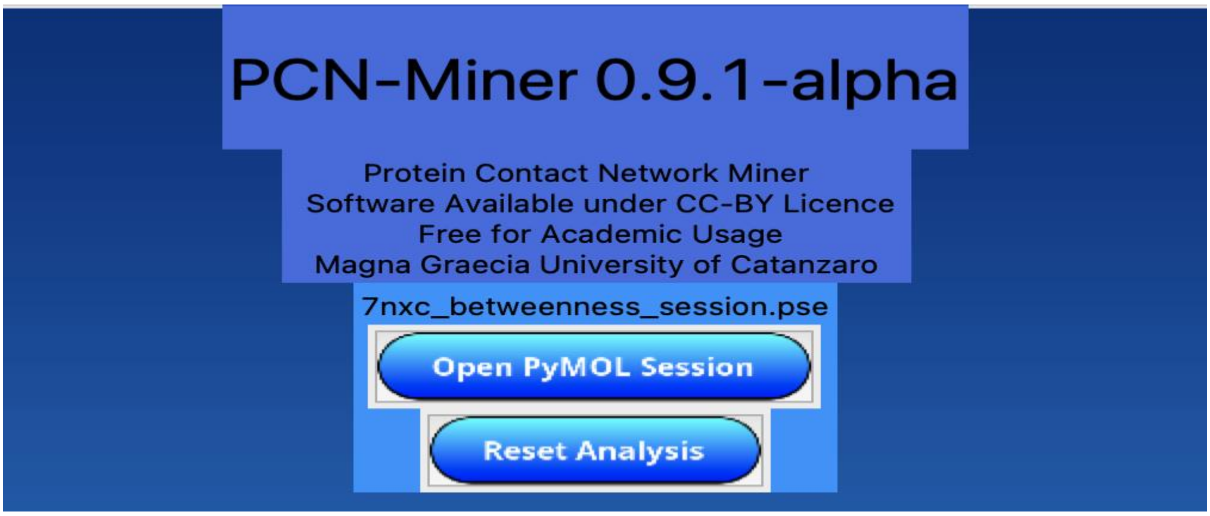
Click here and Choose at least one centrality measure algorithm to use:

Step 3 Loading and Visualization of the results through Pymol.



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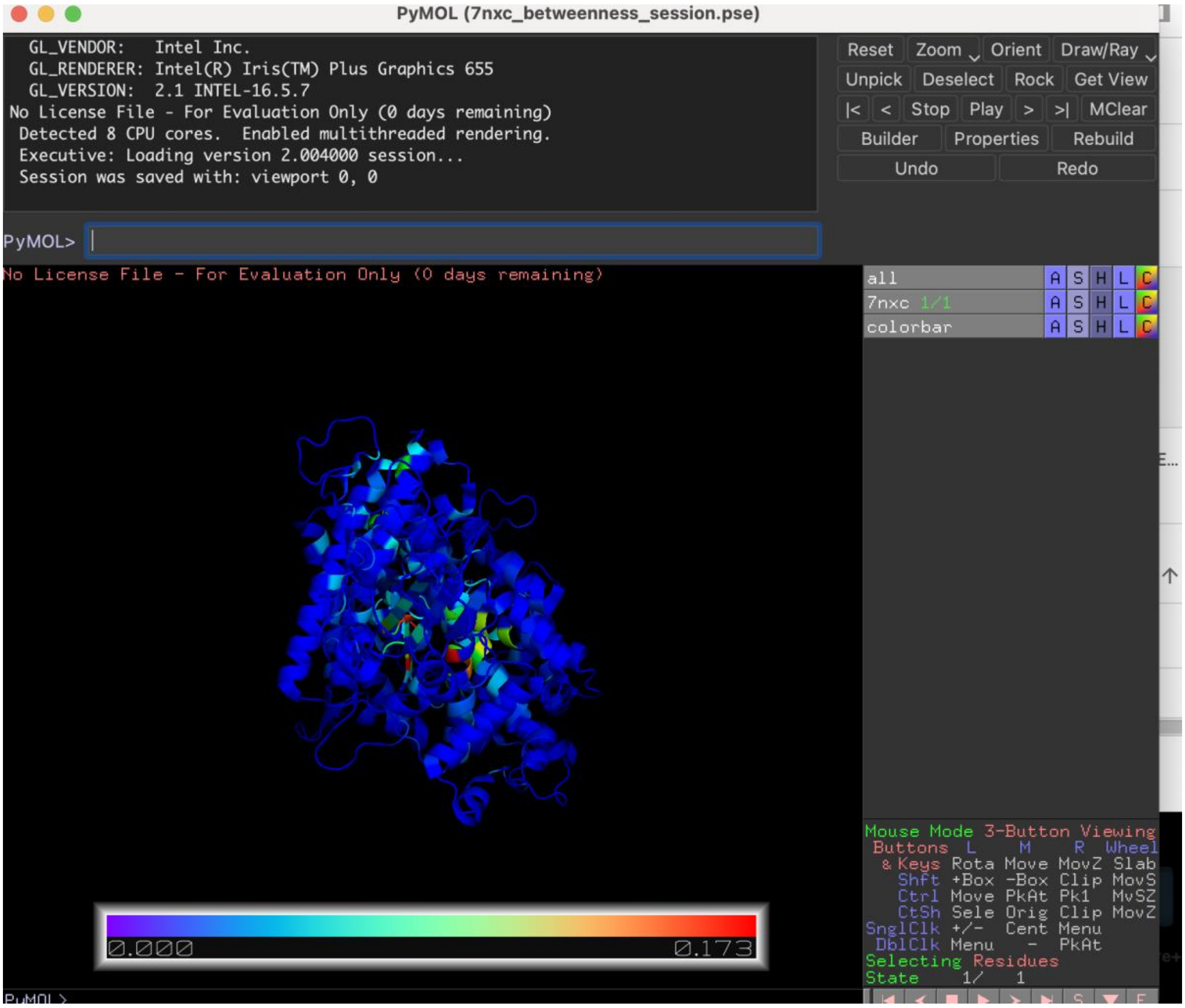
7nxc_betweenness_session.pse



PyMOL (7nxc_betweenness_session.pse)

```
  GL_VENDOR:   Intel Inc.
  GL_RENDERER: Intel(R) Iris(TM) Plus Graphics 655
  GL_VERSION:  2.1 INTEL-16.5.7
No License File - For Evaluation Only (0 days remaining)
 Detected 8 CPU cores.  Enabled multithreaded rendering.
 Executive: Loading version 2.004000 session...
 Session was saved with: viewport 0, 0
PyMOL>
No License File - For Evaluation Only (0 days remaining)
```

## Case Study 2: Spectral Clustering.

Description: Analysis of Modularity through Spectral Clustering

Use the entry PDB code: *6VXX (Coronavirus Spike Protein )*

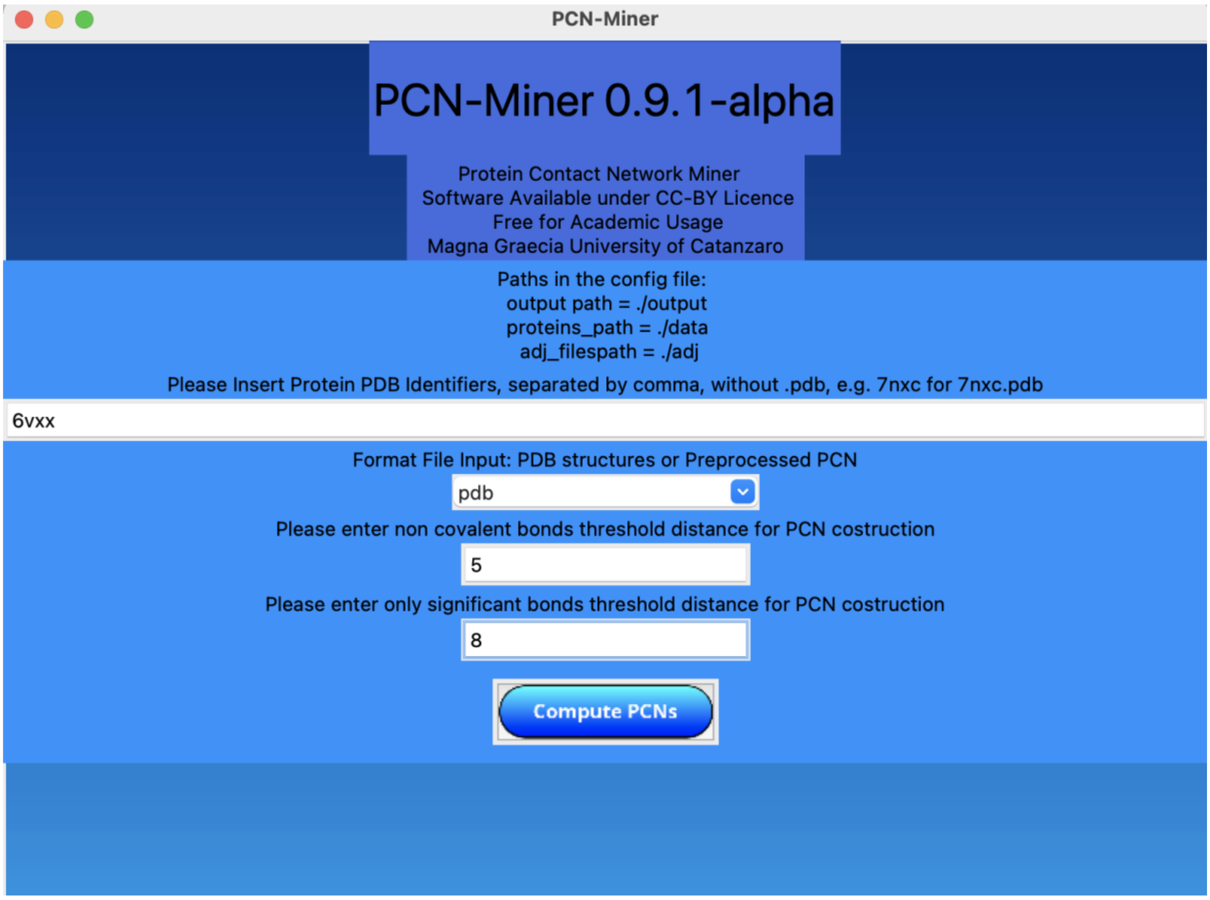
Analysis of PCN: *Method: Spectral Clustering*

*Parameters of Clustering: Number of Clusters 5*

Algorithm:

Visualization: *Pymol Embedded*

*Step 1: Loading of 6Vxx*



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Paths in the config file:
output path = ./output
proteins_path = ./data
adj_filespath = ./adj

Please Insert Protein PDB Identifiers, separated by comma, without .pdb, e.g. 7nxc for 7nxc.pdb

6vxx

Format File Input: PDB structures or Preprocessed PCN

pdb

Please enter non covalent bonds threshold distance for PCN costruction

5

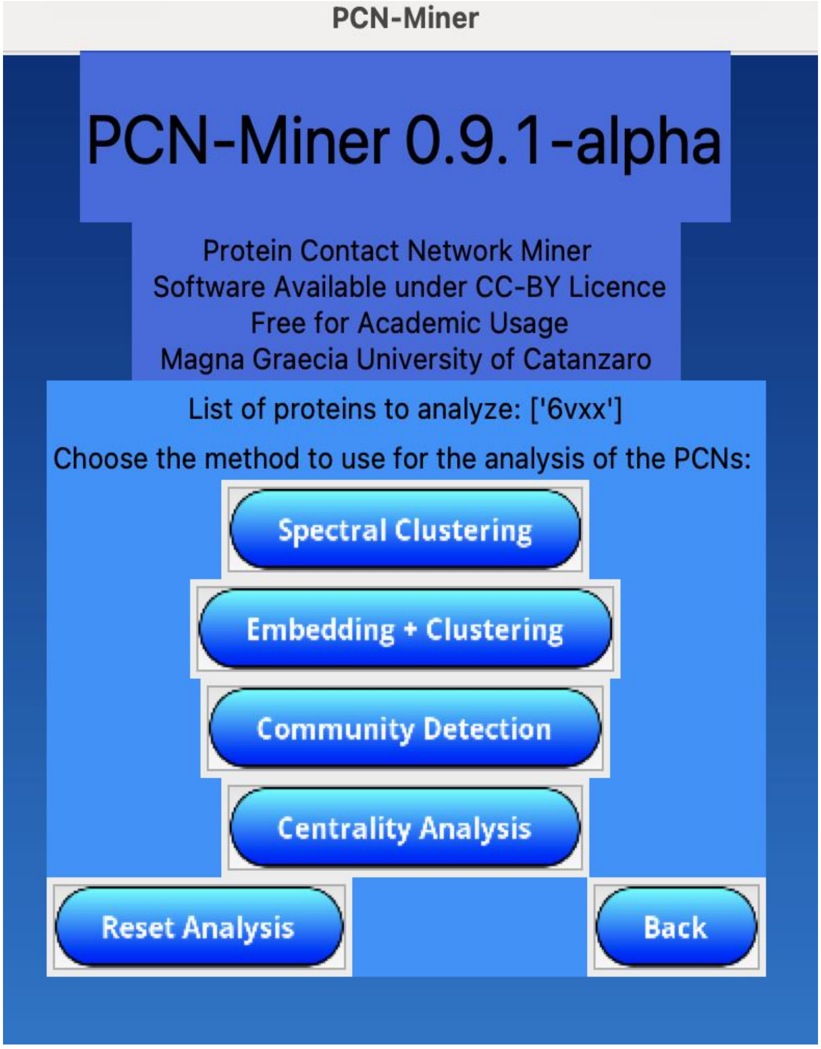
Please enter only significant bonds threshold distance for PCN costruction

8

*Step 2: Selection of Spectral Clustering Algorithm*

*Number of Clusters 5*

*Algorithm: Un-normalised Hard Spectral Clusteri*



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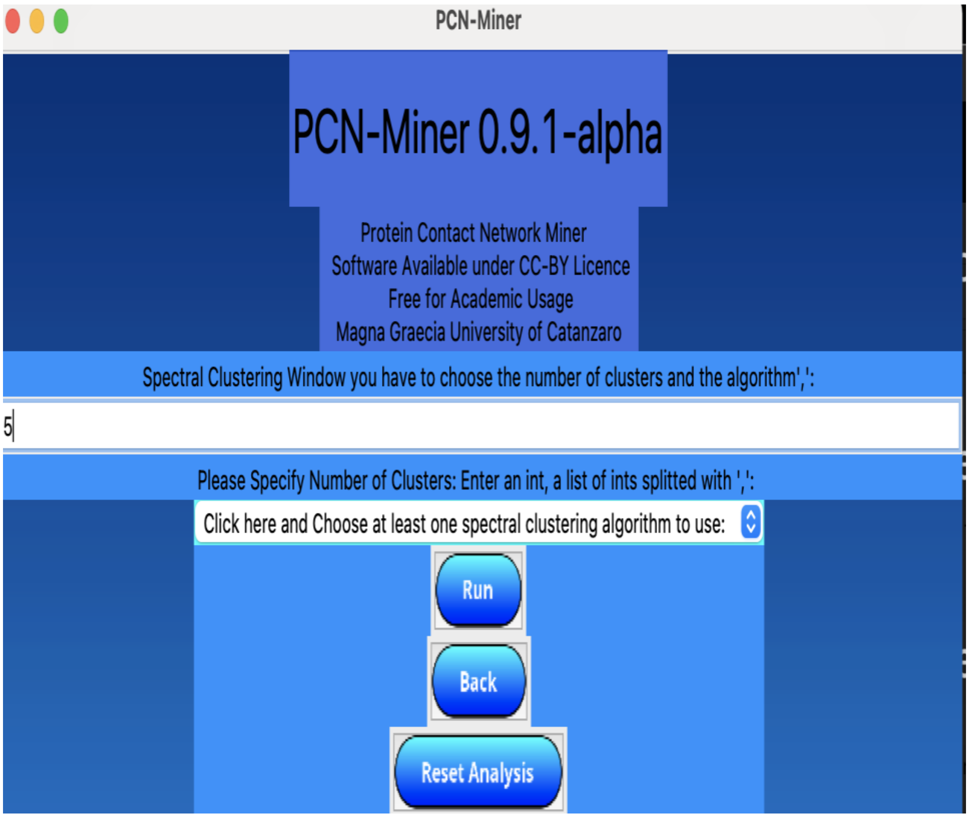
List of proteins to analyze: ['6vxx']

Choose the method to use for the analysis of the PCNs:



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Spectral Clustering Window you have to choose the number of clusters and the algorithm',':

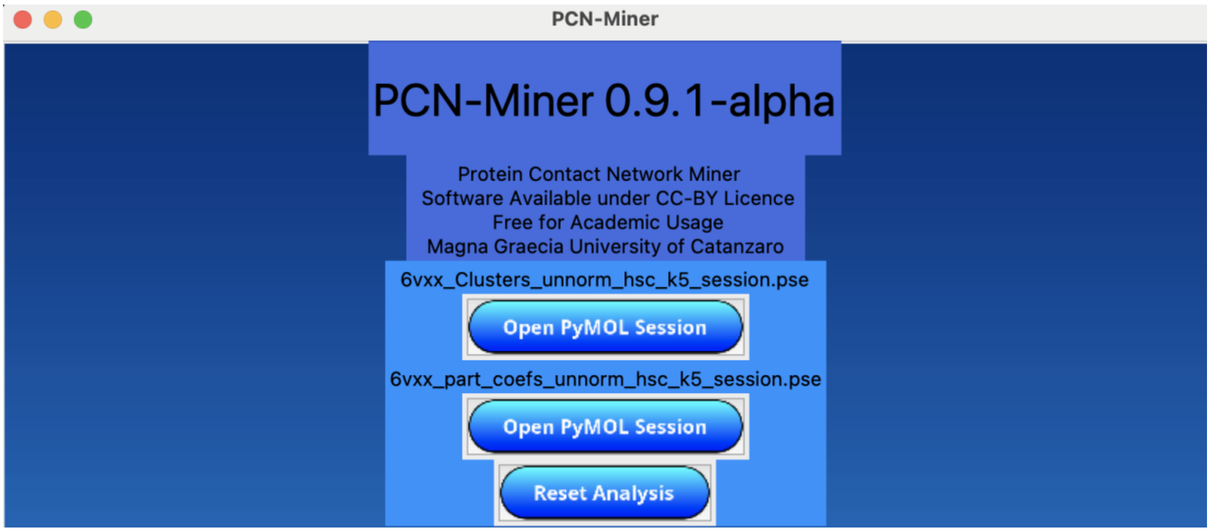
5

Please Specify Number of Clusters: Enter an int, a list of ints splitted with ',':

Click here and Choose at least one spectral clustering algorithm to use:

*Step 4: Selection of Visualization:*

*Clusters of Coefficient of Participation*



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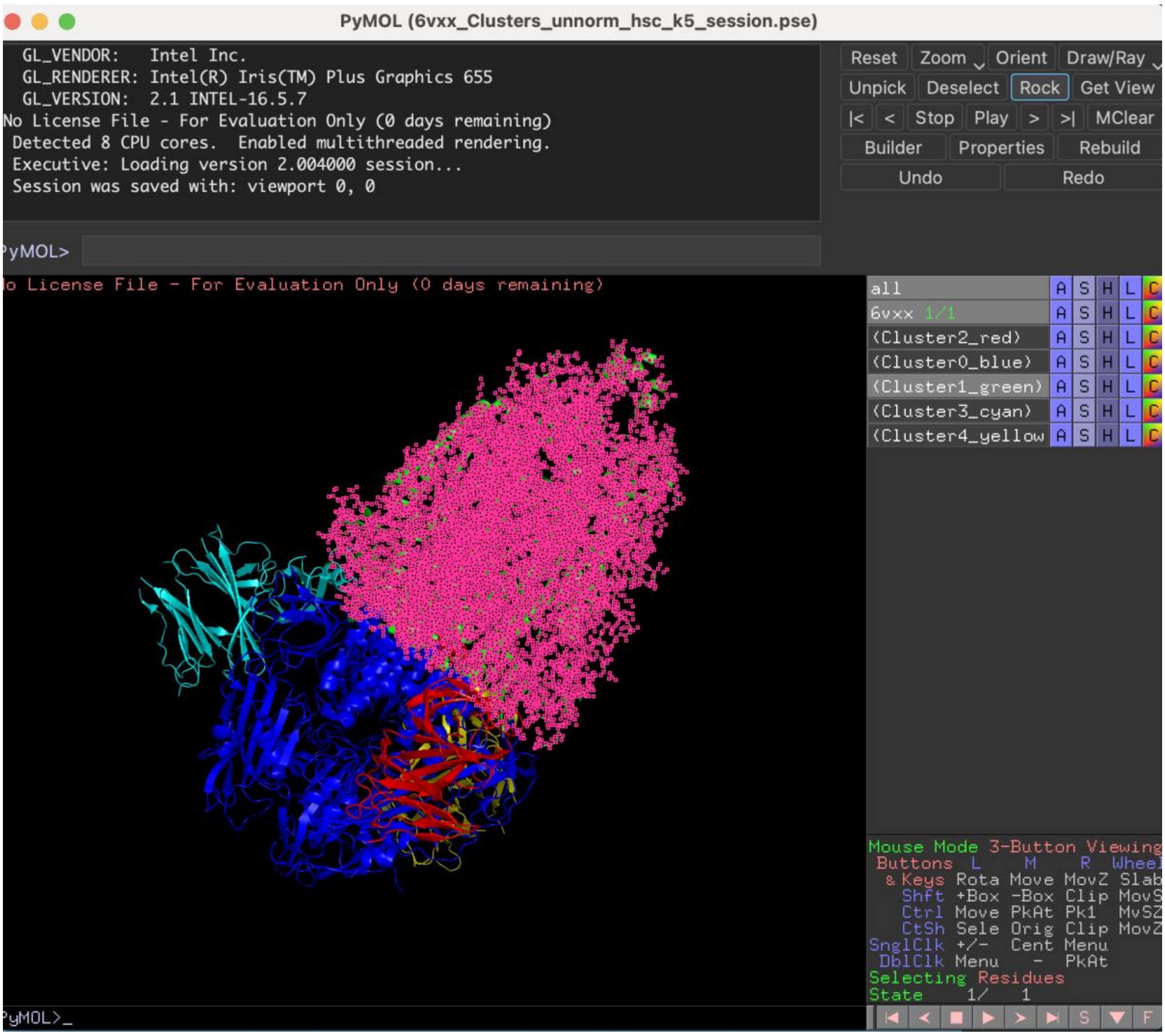
6vxx_Clusters_unnorm_hsc_k5_session.pse

6vxx_part_coefs_unnorm_hsc_k5_session.pse

*Step 5 Visualization*

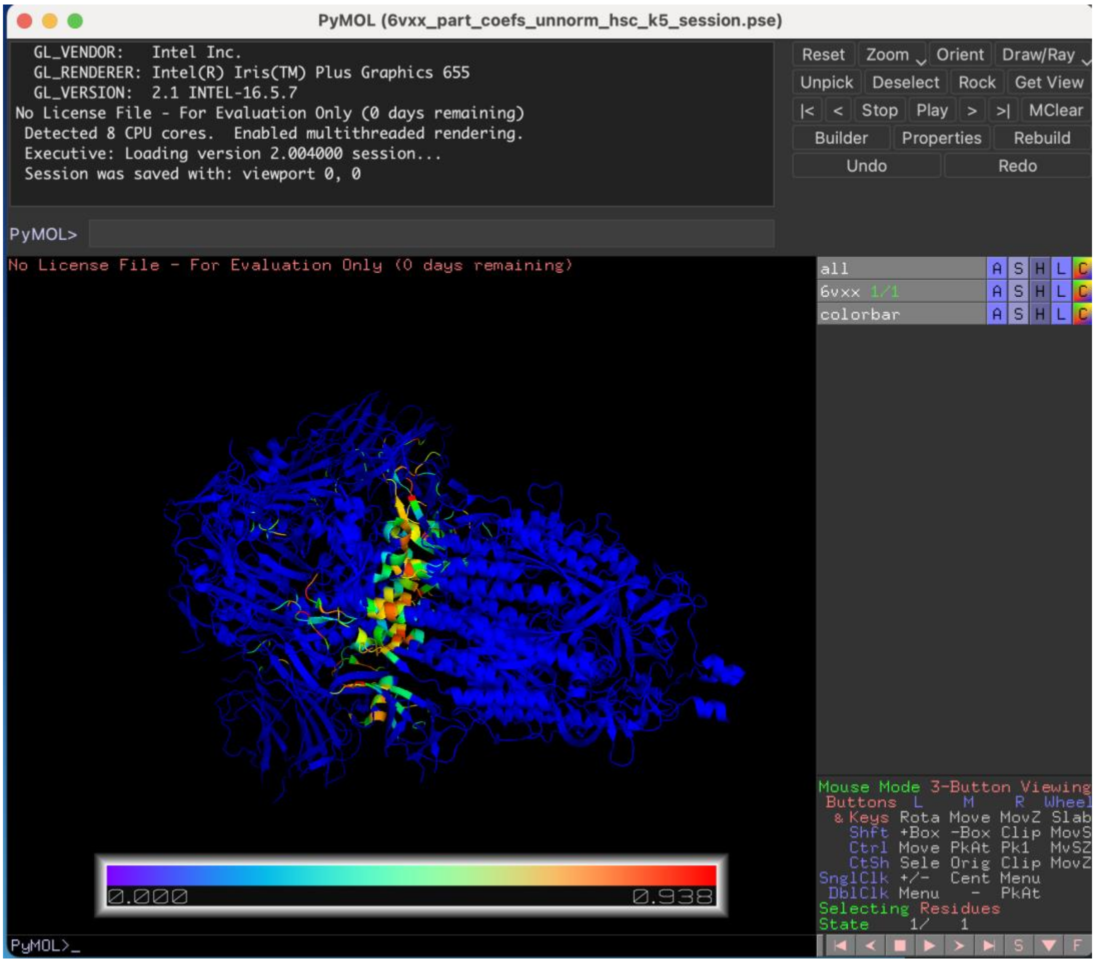
*Visualization of Clusters*



```
 GL_VENDOR:   Intel Inc.
 GL_RENDERER: Intel(R) Iris(TM) Plus Graphics 655
 GL_VERSION:  2.1 INTEL-16.5.7
No License File - For Evaluation Only (0 days remaining)
 Detected 8 CPU cores.  Enabled multithreaded rendering.
 Executive: Loading version 2.004000 session...
 Session was saved with: viewport 0, 0
PyMOL>
```

```
o License File - For Evaluation Only (0 days remaining)
PyMOL>_
```

*Visualization of Participation Coefficients*



PyMOL (6vxx_part_coefs_unnorm_hsc_k5_session.pse)

```
  GL_VENDOR:   Intel Inc.
  GL_RENDERER: Intel(R) Iris(TM) Plus Graphics 655
  GL_VERSION:  2.1 INTEL-16.5.7
No License File - For Evaluation Only (0 days remaining)
 Detected 8 CPU cores.  Enabled multithreaded rendering.
 Executive: Loading version 2.004000 session...
 Session was saved with: viewport 0, 0
PyMOL>
```

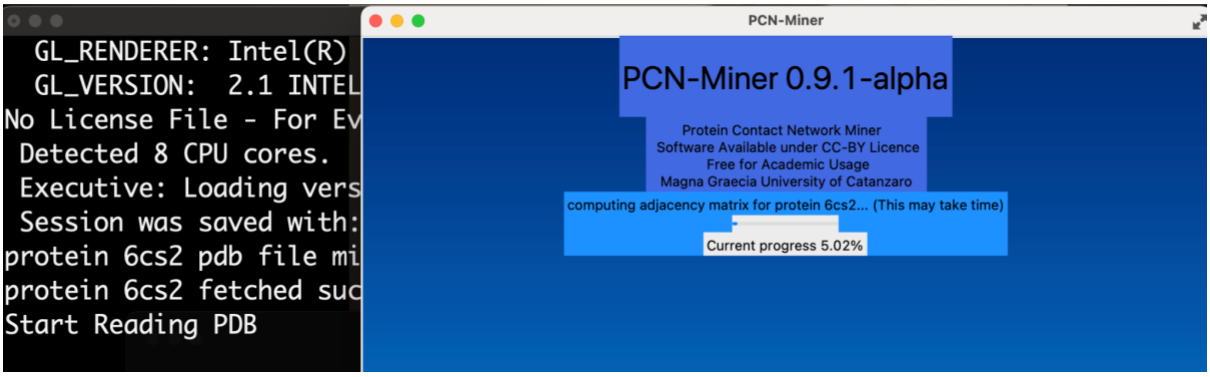
```
No License File - For Evaluation Only (0 days remaining)
```

```
Mouse Mode 3-Button Viewing
 Buttons  L    M    R   Wheel
  & Keys Rota Move MovZ Slab
    Shft +Box -Box Clip MovS
    Ctrl Move PkAt Pk1  MvSZ
    CtSh Sele Orig Clip MovZ
SnglClk  +/-  Cent Menu
 DblClk  Menu  -   PkAt
Selecting Residues
State      1/   1
```

```
PyMOL>_
```

# Discovery of a Putative Allosteric Site

## First Step: Loading the SARS-CoV S/ACE2 complex (PDB code 6cs2) Protein into PCN-Miner



```
  GL_RENDERER: Intel(R)
  GL_VERSION:  2.1 INTEL
No License File - For Ev
 Detected 8 CPU cores.
 Executive: Loading vers
 Session was saved with:
protein 6cs2 pdb file mi
protein 6cs2 fetched suc
Start Reading PDB
```

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computing adjacency matrix for protein 6cs2... (This may take time)

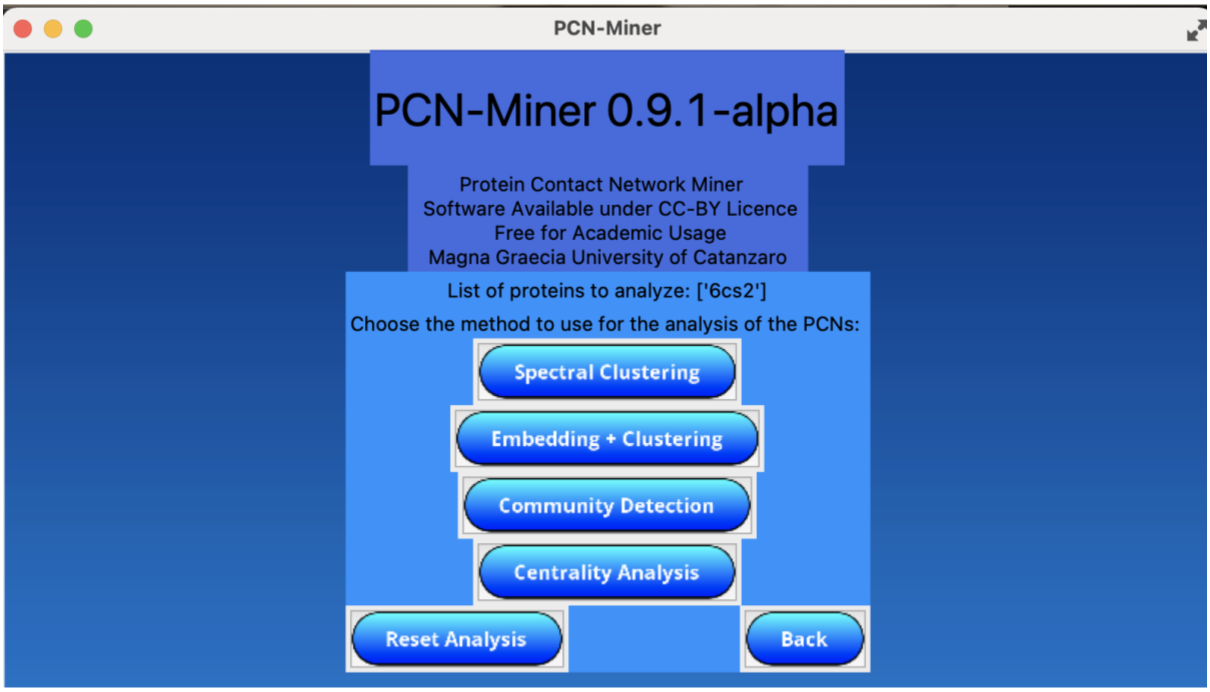
Current progress 5.02%

PCN-Miner is used to gather the structural information of the PDB files into a PCN. Network nodes are the amino acid residues represented by α-carbons, while . Links between nodes (residues) exist if the mutual distance of the residues (centered on α-carbons) were in the range between 5 and 8 Å.

To detect allosteric sites and functional regions activating upon binding we follow the approach described in Di Paola et al J Prot Res 2020 that demonstrated that network clusters (group of residues) correspond to functional regions in the protein. This methods is based on network spectral clustering of the PCN.

## STEP 2: Spectral Clustering of the PCN

Parameters: Cluster =2
Algorithm SKL Spectral Clustering



PCN-Miner

PCN-Miner 0.9.1-alpha

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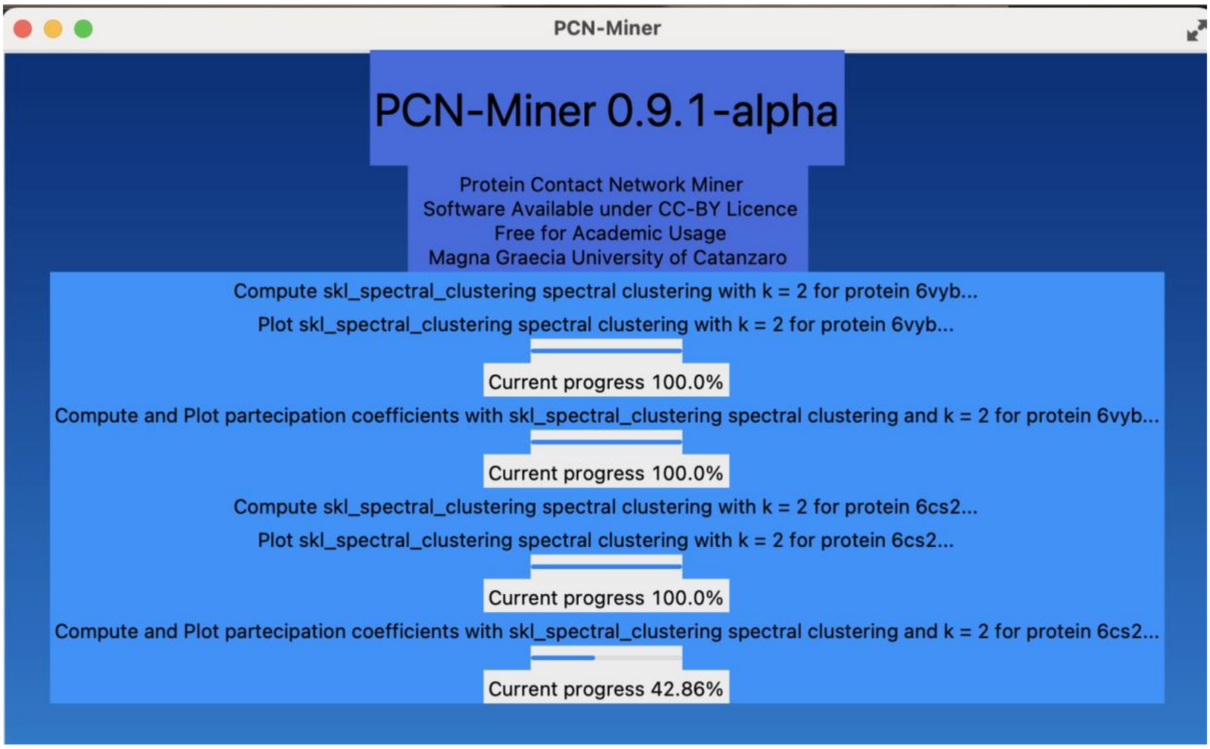
List of proteins to analyze: ['6cs2']

Choose the method to use for the analysis of the PCNs:



PCN-Miner

PCN-Miner 0.9.1-alpha

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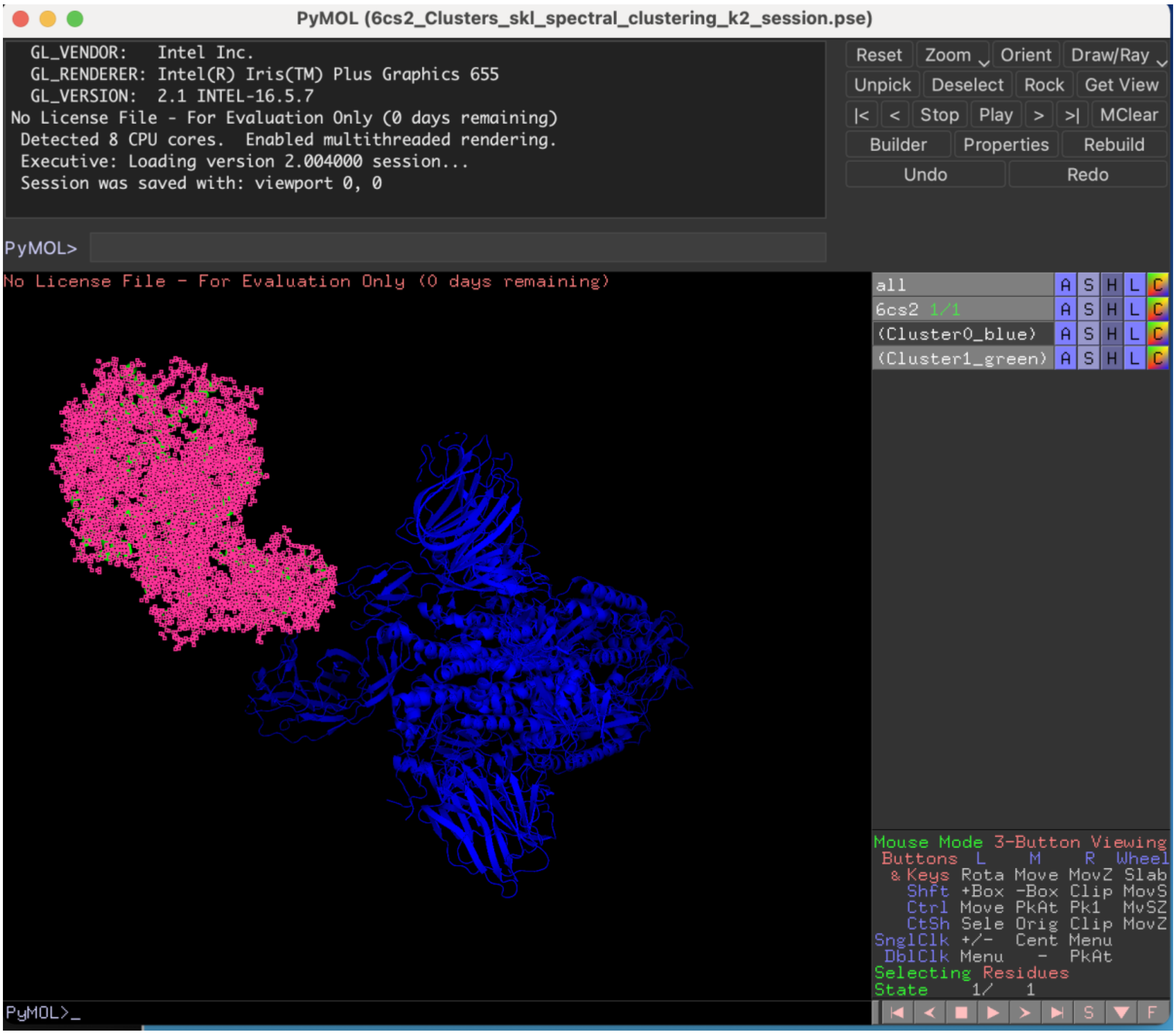
```
Compute skl_spectral_clustering spectral clustering with k = 2 for protein 6vyb...
Plot skl_spectral_clustering spectral clustering with k = 2 for protein 6vyb...
Current progress 100.0%
Compute and Plot partecipation coefficients with skl_spectral_clustering spectral clustering and k = 2 for protein 6vyb...
Current progress 100.0%
Compute skl_spectral_clustering spectral clustering with k = 2 for protein 6cs2...
Plot skl_spectral_clustering spectral clustering with k = 2 for protein 6cs2...
Current progress 100.0%
Compute and Plot partecipation coefficients with skl_spectral_clustering spectral clustering and k = 2 for protein 6cs2...
Current progress 42.86%
```

We plot both the detected clusters and and the map of the participation coefficient projected onto the ribbon structure of the SARS-CoV/ACE2 complex.

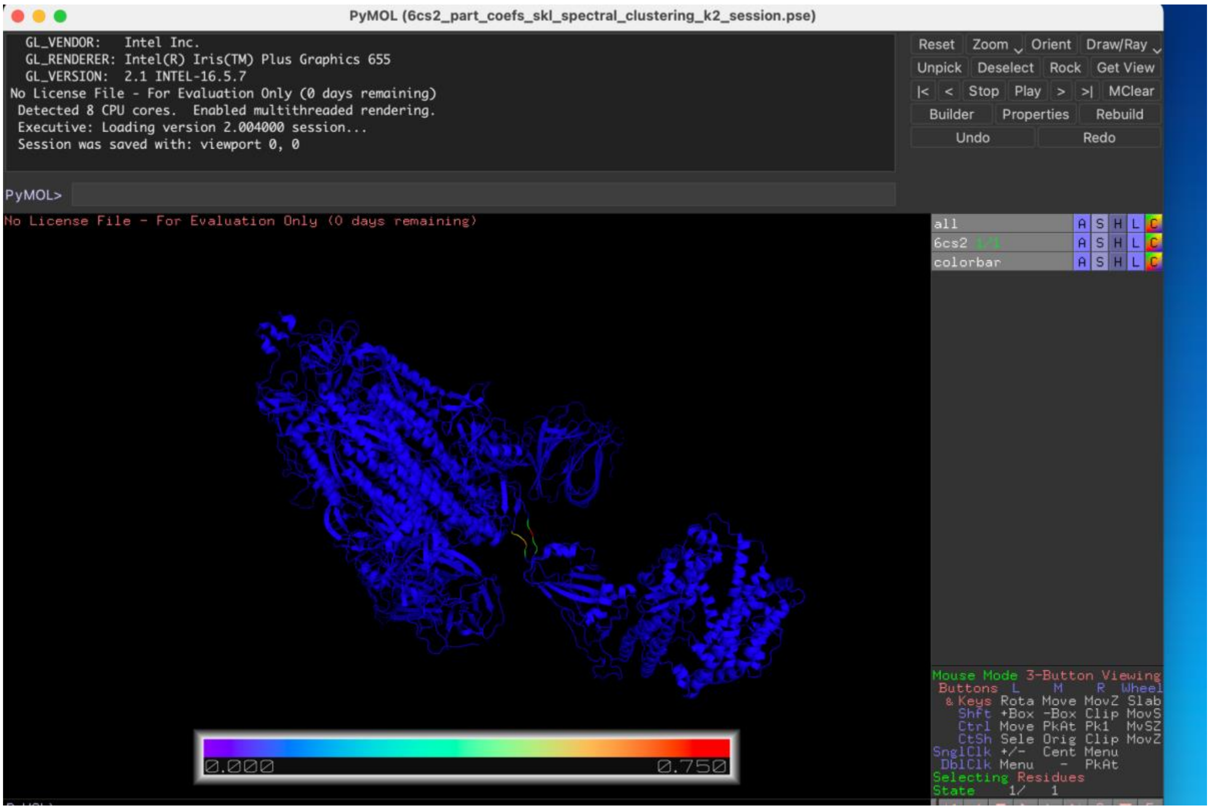
The following figure shows that the complex does not have a modular structure,



PyMOL (6cs2_Clusters_skl_spectral_clustering_k2_session.pse)

```
  GL_VENDOR:   Intel Inc.
  GL_RENDERER: Intel(R) Iris(TM) Plus Graphics 655
  GL_VERSION:  2.1 INTEL-16.5.7
No License File - For Evaluation Only (0 days remaining)
 Detected 8 CPU cores.  Enabled multithreaded rendering.
 Executive: Loading version 2.004000 session...
 Session was saved with: viewport 0, 0
PyMOL>
No License File - For Evaluation Only (0 days remaining)
PyMOL>_
```

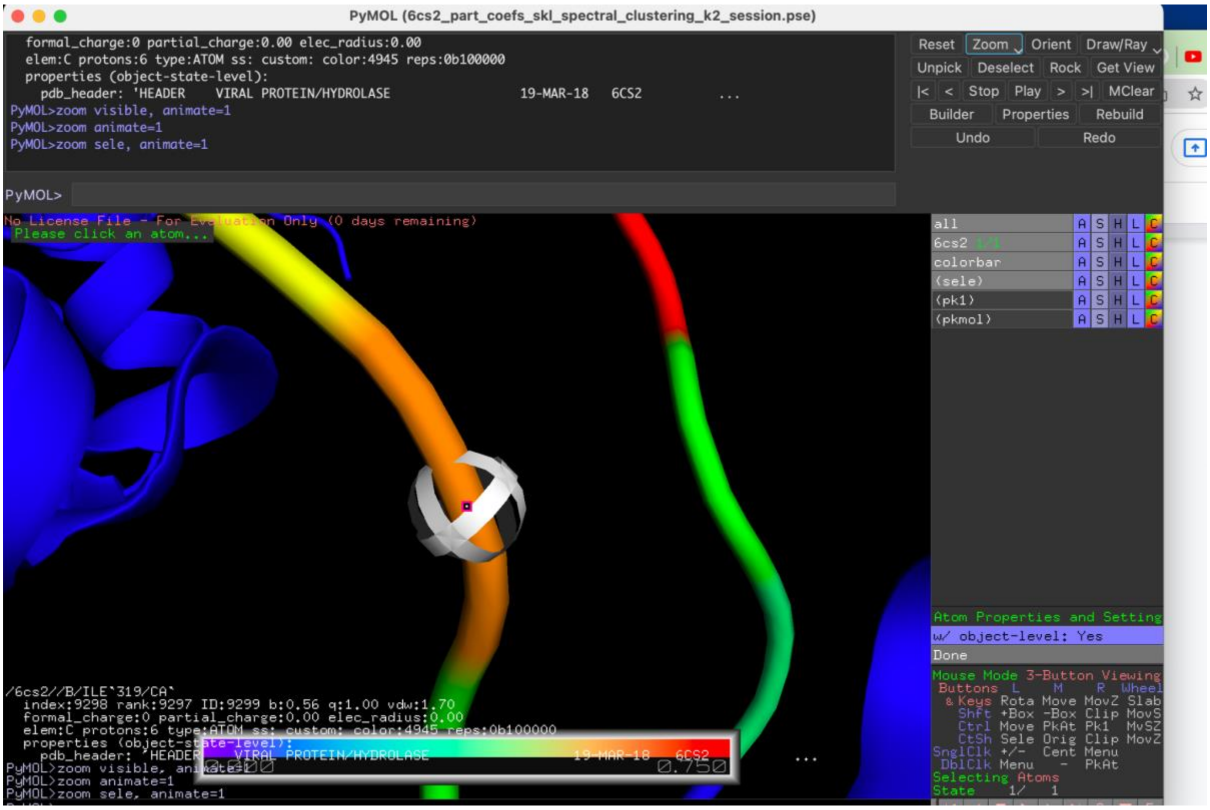
Conversely, followin figure  shows an active region ($P > 0$) in the junction between the fusion peptide and the trimeric bulk phase of the spike protein. This region is a good candidate to intervene in the allosteric regulation of complex formation.



PyMOL (6cs2_part_coefs_skl_spectral_clustering_k2_session.pse)
Reset Zoom Orient Draw/Ray
Unpick Deselect Rock Get View
|< < Stop Play > >| MClear
Builder Properties Rebuild
Undo Redo
all A S H L C
6cs2 A S H L C
colorbar A S H L C
0.000
0.750
Mouse Mode 3-Button Viewing
Buttons L M R Wheel
& Keys Rota Move MovZ Slab
Shft +Box -Box Clip MovS
Ctrl Move PkAt Pk1 MvSZ
CtSh Sele Orig Clip MovZ
SnglClk +/- Cent Menu
DblClk Menu - PkAt
Selecting Residues
State 1/ 1

```
  GL_VENDOR:   Intel Inc.
  GL_RENDERER: Intel(R) Iris(TM) Plus Graphics 655
  GL_VERSION:  2.1 INTEL-16.5.7
No License File - For Evaluation Only (0 days remaining)
 Detected 8 CPU cores.  Enabled multithreaded rendering.
 Executive: Loading version 2.004000 session...
 Session was saved with: viewport 0, 0
PyMOL>
No License File - For Evaluation Only (0 days remaining)
```

This is more clear when zooming on.



```
  formal_charge:0 partial_charge:0.00 elec_radius:0.00
  elem:C protons:6 type:ATOM ss: custom: color:4945 reps:0b100000
  properties (object-state-level):
    pdb_header: 'HEADER    VIRAL PROTEIN/HYDROLASE                 19-MAR-18   6CS2        ...
PyMOL>zoom visible, animate=1
PyMOL>zoom animate=1
PyMOL>zoom sele, animate=1
PyMOL>
No License File - For Evaluation Only (0 days remaining)
 Please click an atom...
/6cs2//B/ILE`319/CA`
  index:9298 rank:9297 ID:9299 b:0.56 q:1.00 vdw:1.70
  formal_charge:0 partial_charge:0.00 elec_radius:0.00
  elem:C protons:6 type:ATOM ss: custom: color:4945 reps:0b100000
  properties (object-state-level):
    pdb_header: 'HEADER    VIRAL PROTEIN/HYDROLASE                 19-MAR-18    6CS2        ...
PyMOL>zoom visible, animate=1
PyMOL>zoom animate=1
PyMOL>zoom sele, animate=1
```

## References:

Di Paola, L., Hadi-Alijanvand, H., Song, X., Hu, G., & Giuliani, A. (2020). The Discovery of a Putative Allosteric Site in the SARS-CoV-2 Spike Protein Using an Integrated Structural/Dynamic Approach. *Journal of proteome research*, *19*(11), 4576–4586. https://doi.org/10.1021/acs.jproteome.0c00273

[1] von Luxburg, U. A tutorial on spectral clustering. Stat Comput 17, 395–416 (2007). https://doi.org/10.1007/s11222-007-9033-z;

[2] https://github.com/palash1992/GEM;

[3] G. Rossetti, L. Milli, R. Cazabet. CDlib: a Python Library to Extract, Compare and Evaluate Communities from Complex Networks. Applied Network Science Journal. 2019. DOI:10.1007/s41109-019-0165-9 https://github.com/GiulioRossetti/cdlib