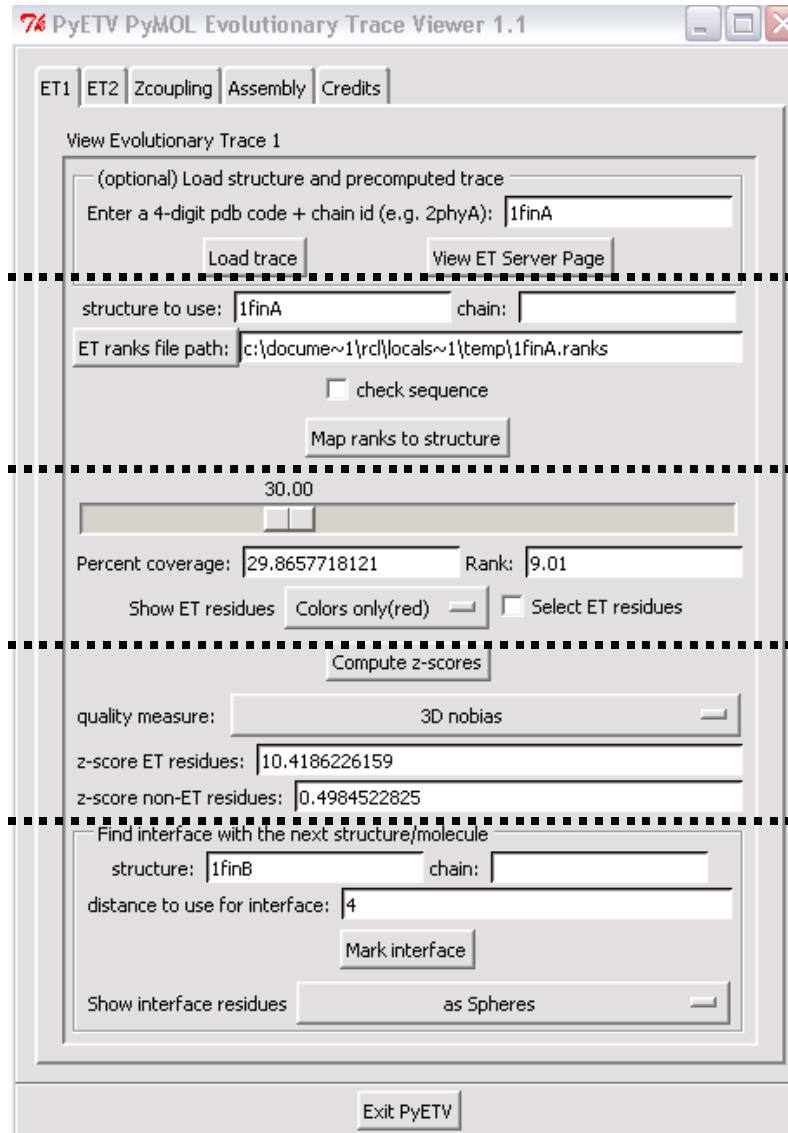


PyMOL Evolutionary Trace Viewer 1.1

Lichtarge Lab
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I. Basic sequence-evolution-structure analysis (ET1 or ET2)



Load trace with structure from the ET Server (Ref. 2)

Map ET rank data to a PyMOL structure

Vary the selection of ET residues

Assess clustering of ET residues

Mark interface of the structure with another PyMOL structure or ligand

I.1 Load trace with structure from the ET server (Ref. 2)

This will download a PDB chain into PyMOL and an ET ranks file. The boxes below automatically get filled with the correct information.

PyETV PyMOL Evolutionary Trace Viewer 1.1

ET1 ET2 Zcoupling Assembly Credits

View Evolutionary Trace 1

(optional) Load structure and precomputed trace

Enter a 4-digit pdb code + chain id (e.g. 2phyA): 1finA

Load trace View ET Server Page

structure to use: 1finA chain:

ET ranks file path: c:\docume~1\rcl\locals~1\temp\1finA.ranks

check sequence

Map ranks to structure

30.00

Percent coverage: 29.8657718121 Rank: 9.01

Show ET residues Colors only(red) Select ET residues

Compute z-scores

quality measure: 3D nobias

z-score ET residues: 10.4186226159

z-score non-ET residues: 0.4984522825

Find interface with the next structure/molecule

structure: 1finB chain:

distance to use for interface: 4

Mark interface

Show interface residues as Spheres

Exit PyETV

Enter a single PDB code with chain indicator.

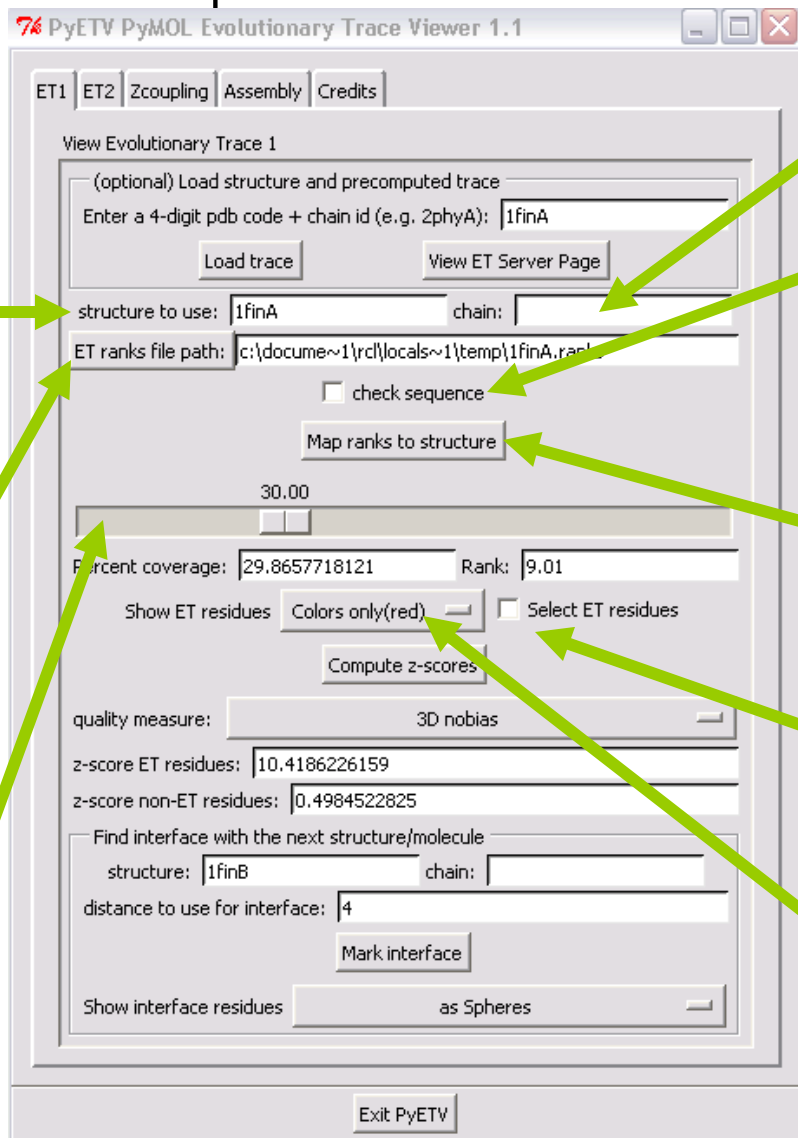
This will open an internet browser and take the user to the ET Server search results.

1.2 Map ET rank data to a PyMOL structure and vary the selection of top ET-ranked residues

Enter the PyMOL name of the structure. This structure must be present in the PyMOL graphics window.

Select an ET ranks file. The button opens a file selection window.

Vary selection of ET residues (Left mouse click, drag, then release). The slider value corresponds to a percent coverage.



Optional chain indicator

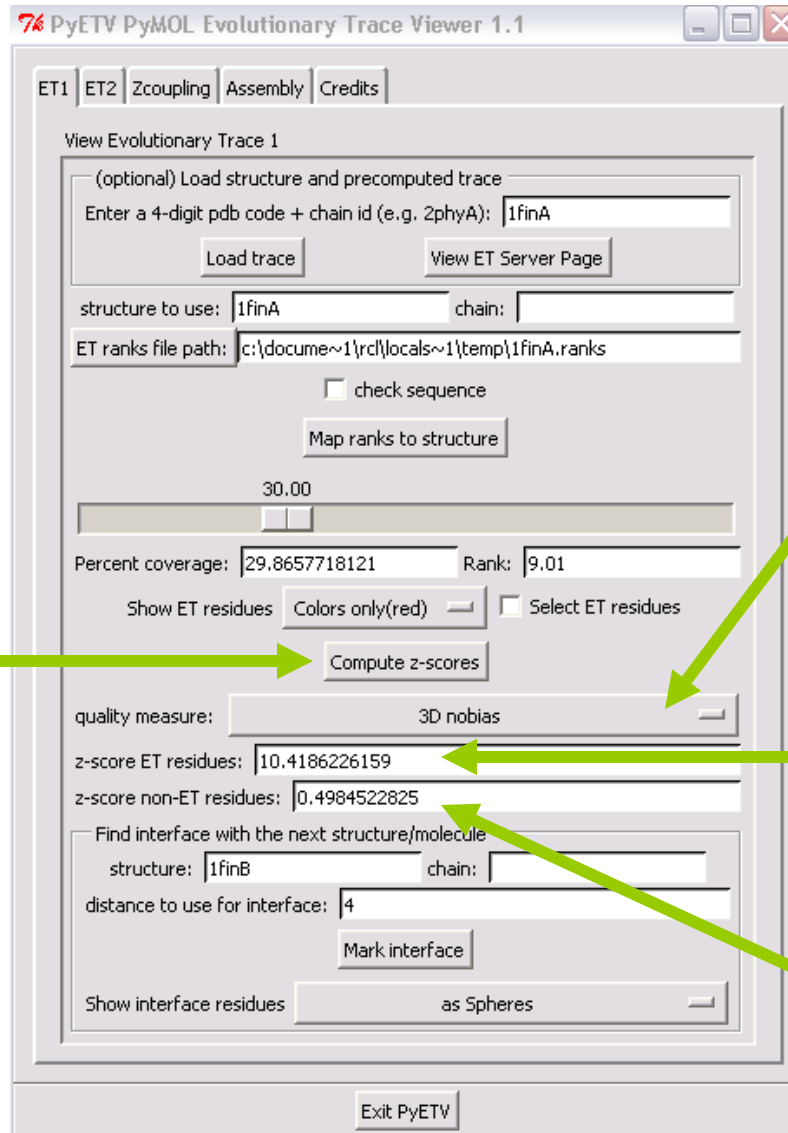
Check the box to compare the sequences between the structure and ET ranks file.

Map ET rank data to the residues in the structure.

Check the box to create a PyMOL selection of the ET residues.

Select display options to distinguish ET residues from the rest of the structure.

I.3 Assess the statistical significance of the spatial clustering of top ET-ranked residues



Start computation of the ET clustering z-scores

Select clustering measure (nobias, or with bias for residues more distant in sequence (Ref. 3))

Clustering z-score of the top-ranked residues at the current ET rank threshold.

Clustering z-score of the rest of the residues in the structure

1.4 Find interface residues on main ET1 structure

Enter the PyMOL name of the partner structure. A structure with this name must be present in the PyMOL graphics window.

Start finding the interface residues between the main **ET1** structure (e.g. 1finA) and the partner structure (e.g. 1finB)

PyETV PyMOL Evolutionary Trace Viewer 1.1

ET1 ET2 Zcoupling Assembly Credits

View Evolutionary Trace 1

(optional) Load structure and precomputed trace

Enter a 4-digit pdb code + chain id (e.g. 2phyA): 1finA

Load trace View ET Server Page

structure to use: 1finA chain:

ET ranks file path: c:\docume~1\rcl\locals~1\temp\1finA.ranks

check sequence

Map ranks to structure

30.00

Percent coverage: 29.8657718121 Rank: 9.01

Show ET residues Colors only(red) Select ET residues

Compute z-scores

quality measure: 3D nobias

z-score ET residues: 10.4186226159

z-score non-ET residues: 0.4984522825

Find interface with the next structure/molecule

structure: 1finB chain:

distance to use for interface: 4

Mark interface

Show interface residues as Spheres

Exit PyETV

Optional chain indicator

This input distance refers to the atom-atom distance (Å) threshold between the interface residues of the partners.

Select display option for interface residues (Color(red), Spheres, Sticks, PyMOL selection) 6

II. Zcoupling – ET coupling z-score computation (dependent on ET1 and ET2 pages) and interface selection (bottom)

The screenshot shows the PyETV PyMOL Evolutionary Trace Viewer 1.1 interface. The 'Zcoupling' tab is active. The top section, 'Compute ET coupling z-score between Trace 1 and Trace 2', contains a 'Compute coupling z-score' button and a 'Currently disabled' label. Below this, the z-score is 2.35193207684, the number of ET couples is 14, the <C> average is 6.45100671141, and the c stdev is 3.20969868259. The bottom section, 'Mark interface between structures 1 and 2', contains fields for structure 1 (1fin, chain: A), structure 2 (1fin, chain: B), and distance to use for interface (4). There are 'Mark interface' and 'Exit PyETV' buttons. Two dropdown menus for 'Show interface residues' are set to 'Colors only(red)'. Annotations with arrows point to various parts of the interface: 'Start computation' points to the 'Compute coupling z-score' button; 'Computes the ET coupling z-score of the structures and current ET selections made with ET1 and ET2.' points to the z-score and related statistics; 'Specify PyMOL structures. Must be present in the PyMOL graphics window.' points to the structure 1 and 2 fields; 'Start interface computation' points to the 'Mark interface' button; 'chain indicators (optional)' points to the chain: A and B fields; and 'Select display option for interface residues (Color(red), Spheres, Sticks, PyMOL selection)' points to the dropdown menus.

Currently disabled

The z-score and its components;
 $Z = (c - \langle c \rangle) / \text{stdev}$
(Ref. 5)

Start computation

Computes the ET coupling z-score of the structures and current ET selections made with **ET1** and **ET2**.

Specify PyMOL structures. Must be present in the PyMOL graphics window.

Start interface computation

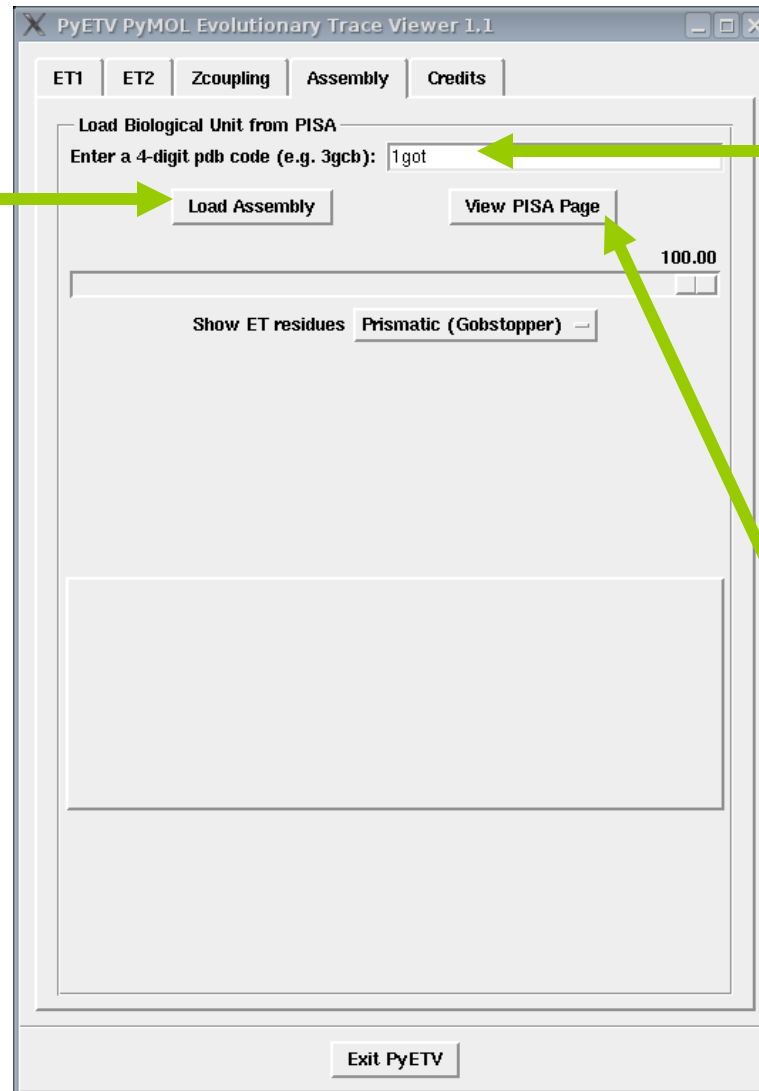
chain indicators (optional)

Select display option for interface residues (Color(red), Spheres, Sticks, PyMOL selection)

Interface selection is independent of the z-score computation.

III. Assembly tool – loading and viewing a PISA assembly (Ref. 6)

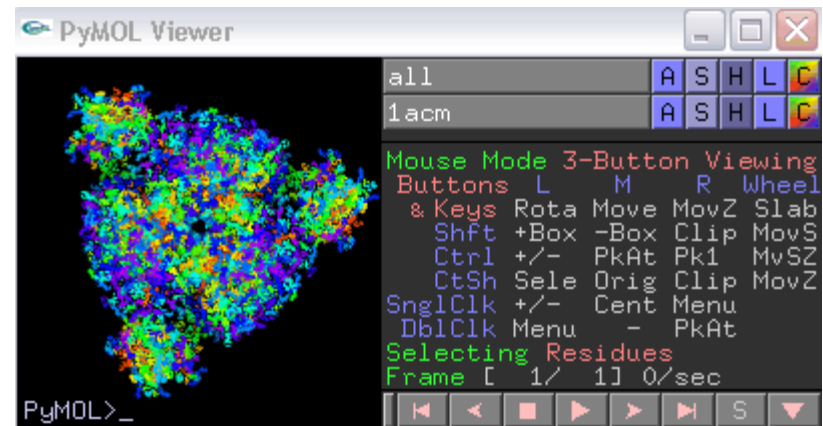
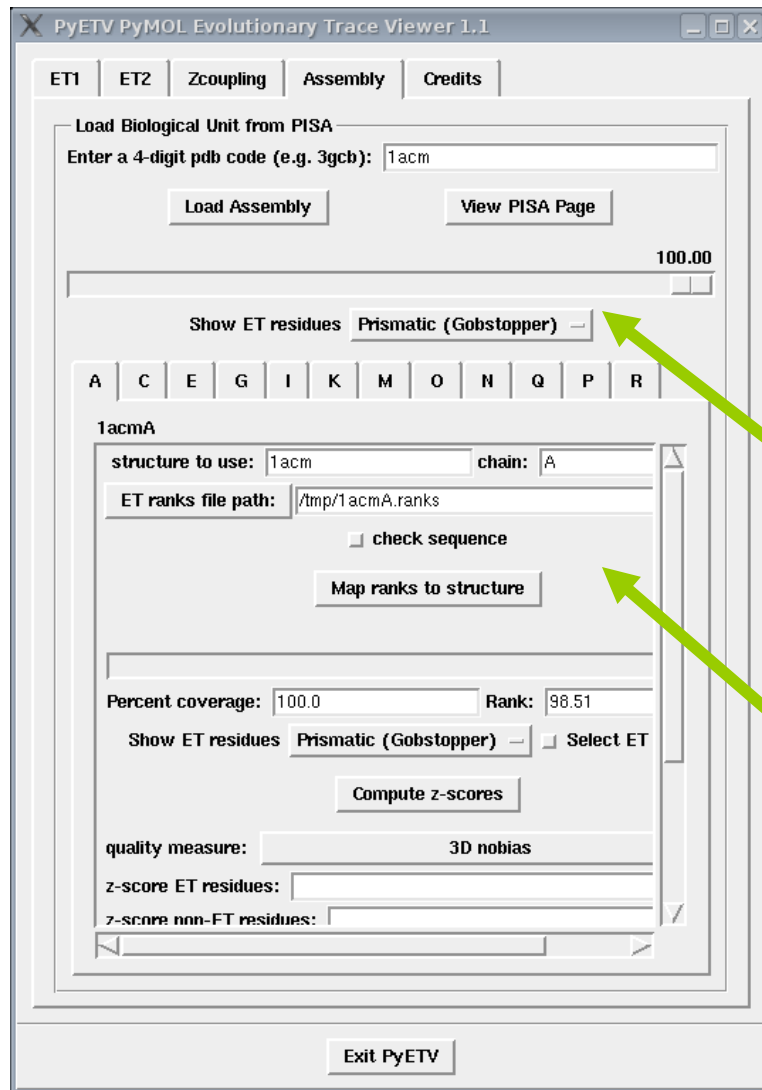
This will start the download of the multimer into PyMOL. PyETV will also attempt to match ET rank data from the ET server to each chain in the assembly. A page for each chain will be created below the **Assembly** page, organized into a tabbed folder.



Enter a single PDB code (e.g. *1got*) to get the most probable PISA solution, or a PDB code with PISA assembly numbers (e.g. *1got:1,1* for the top solution).

This will open an internet browser and take the user to the PISA search results.

III.1 Assembly tool – completion of Load Assembly

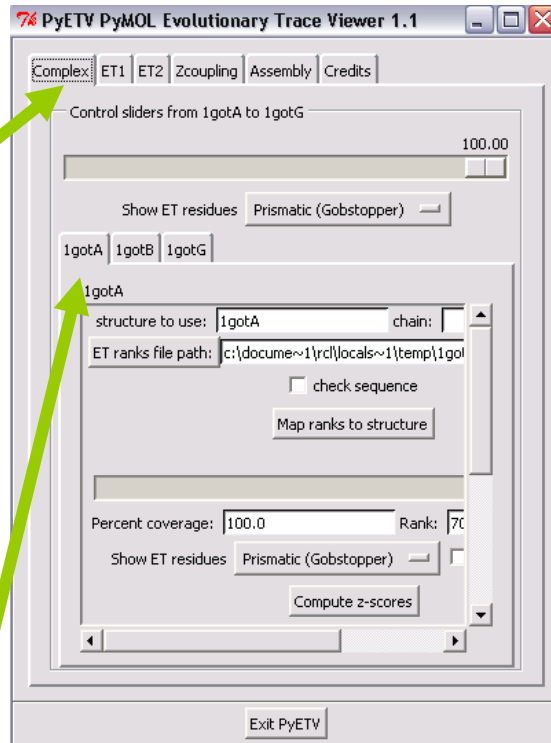


Use the slider and select options to modify the ET residue selections for all chains in the assembly

The page for each chain (**A,C,E,...**) operates like **ET1** or **ET2** (without the **Load Trace** feature)

IV. New tab created when traces are loaded via PyMOL scripts

Case 1: More than one trace



Complex tab created after running a PyMOL script and opening the plugin. This tab is similar to **Assembly**.

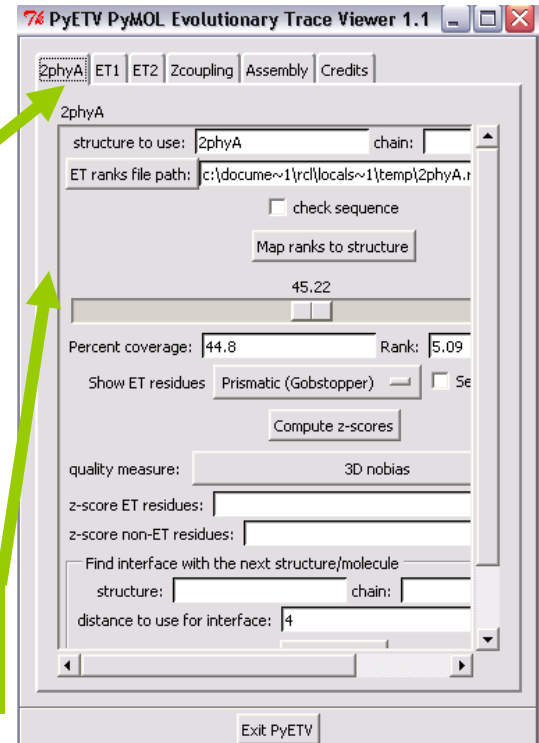
Three chains loaded

PyMOL script:
<http://mammoth.bcm.tmc.edu/ETserver2/pdbeasytrace/pmlFiles/1got.pml>

Case 2: One trace

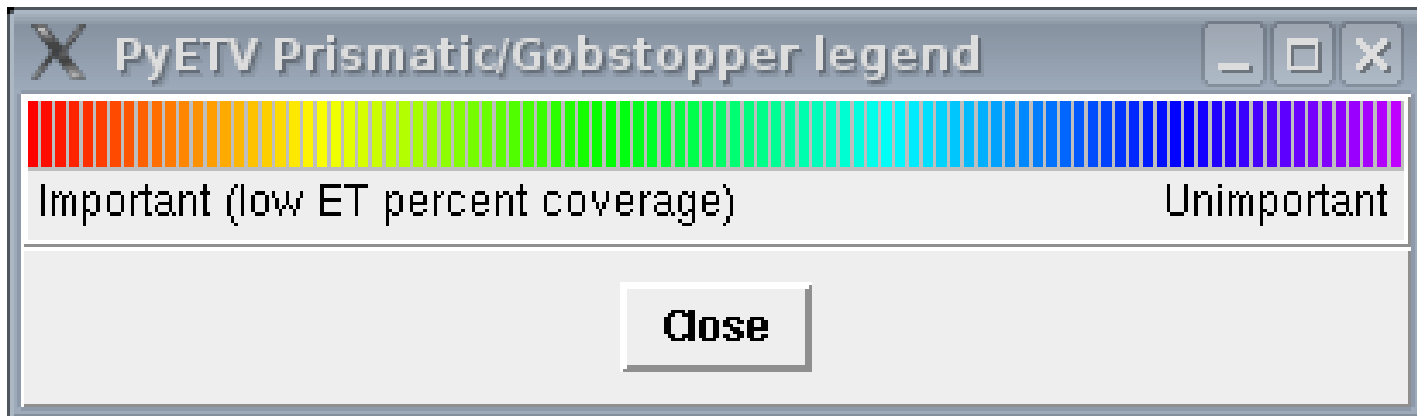
Tab created after running a PyMOL script and opening the plugin. This tab is similar to **ET1**.

One chain loaded



PyMOL script:
<http://mammoth.bcm.tmc.edu/ETserver2/pdbeasytrace/pmlFiles/2phy.pml>

V. Prismatic (Gobstopper) color ramp



This box appears whenever **Prismatic (Gobstopper)** is selected as the display option for the currently selected top ET-ranked residues.

References

1. Warren L. DeLano "The PyMOL Molecular Graphics System." DeLano Scientific LLC, San Carlos, CA, USA. <http://www.pymol.org>; The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC
2. <http://mammoth.bcm.tmc.edu/ETserver.html>; Mihalek, I., I. Res, et al. (2006). "Evolutionary trace report_maker: a new type of service for comparative analysis of proteins." Bioinformatics **22**(13): 1656-7.
3. Mihalek, I., I. Res, et al. (2003). "Combining inference from evolution and geometric probability in protein structure evaluation." J Mol Biol **331**(1): 263-79.
4. Wilkins, A. D., R. Lua, et al. (2010). "Sequence and Structure Continuity of Evolutionary Importance Improves Protein Functional Site Discovery and Annotation." Protein Science
5. <http://mammoth.bcm.tmc.edu/wiki/index.php/Zcoupling>
6. Krissinel, E. and K. Henrick (2007). "Inference of macromolecular assemblies from crystalline state." J Mol Biol **372**(3): 774-97.