

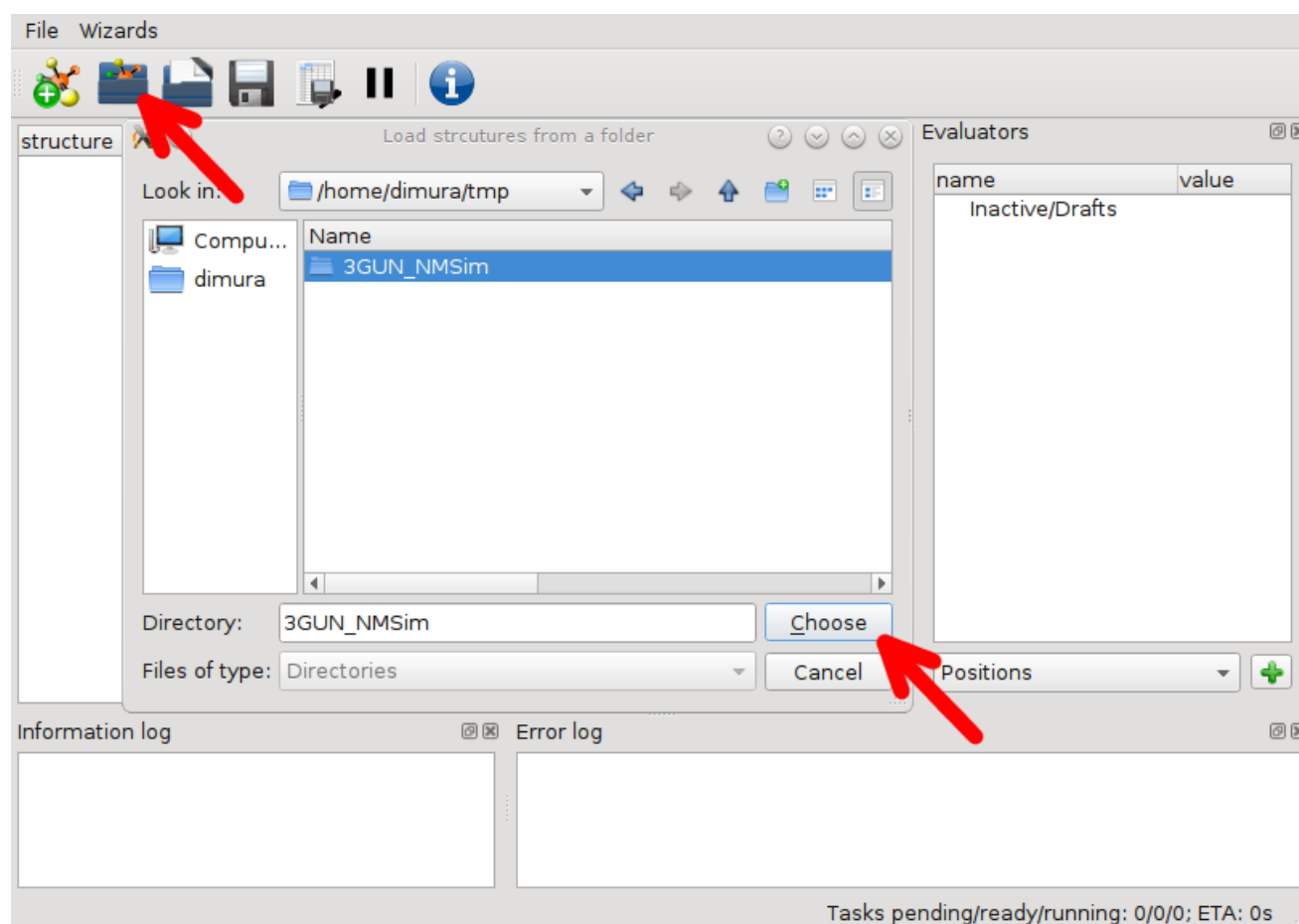
# Tutorial: FRET screening of structural models

## Summary

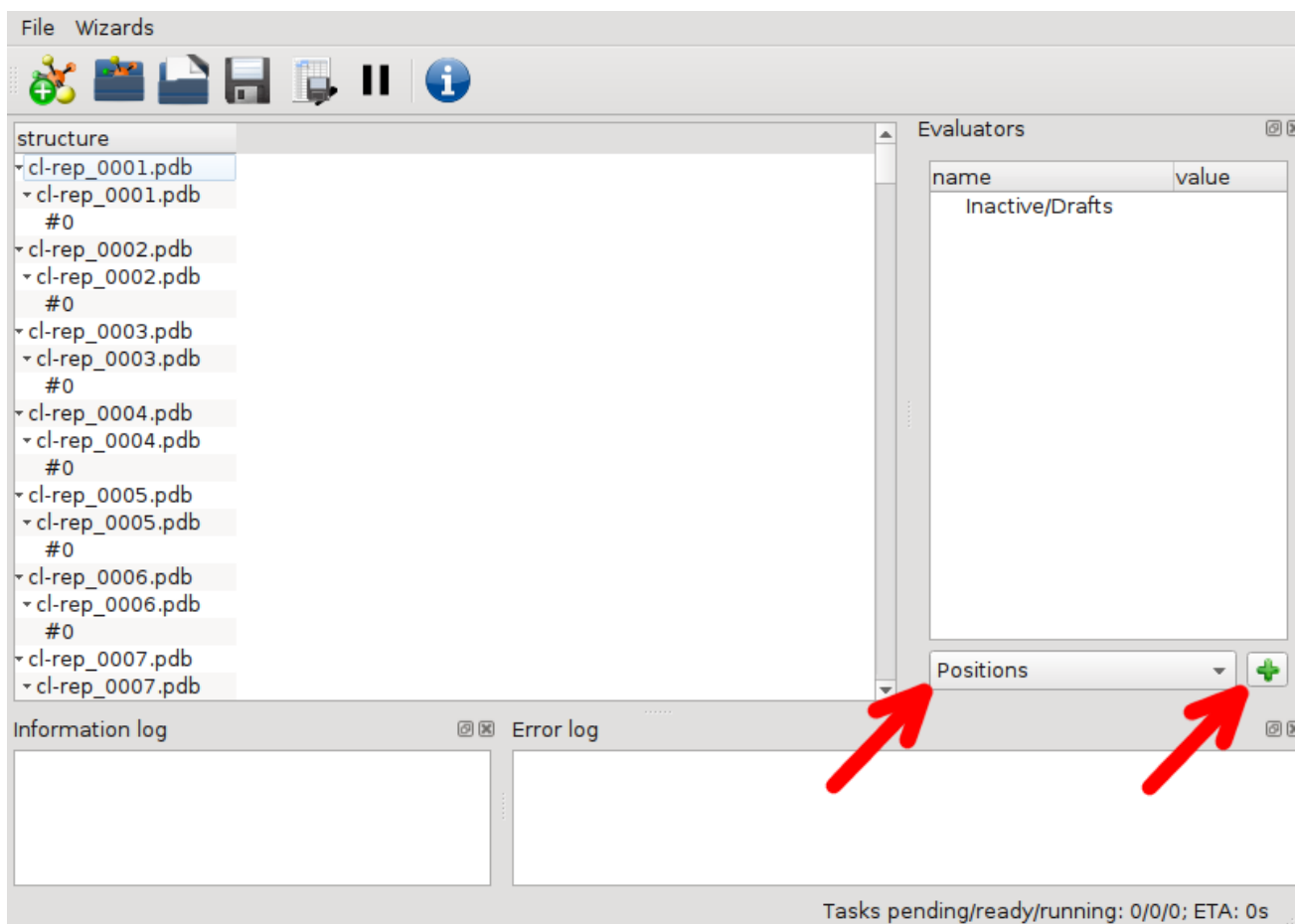
In this tutorial we walk through the calculation of FRET-average inter-dye distances for the case when organic dyes are used, i.e. Alexa 488 as a donor and Alexa 647 as an Acceptor. We use T4 lysozyme (T4L) protein as an example.

## Screening

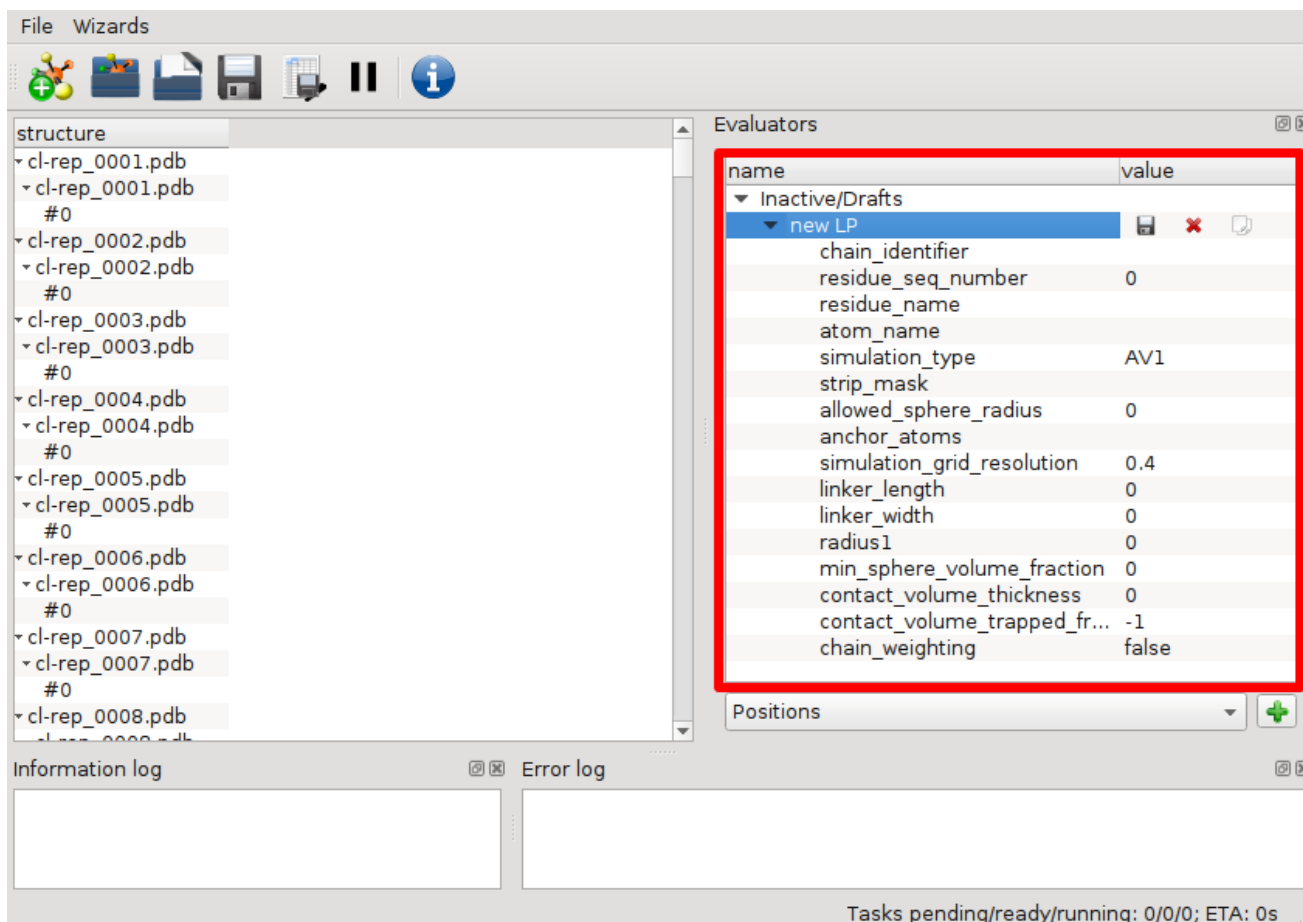
1. Start Olga software ( `olga.exe` executable).
2. Press "Import structures from directory" button to load a set of PDB files. Select the directory that contains files you need to screen. In this example we use a [trajectory](#), which contains structural models of T4L protein generated by [NMSim](#) software from the 3GUN crystal structure. The trajectory was clustered using RMSD as the distance metric and a threshold of 1.8 Angstrom. You will need to extract the .zip archive first.



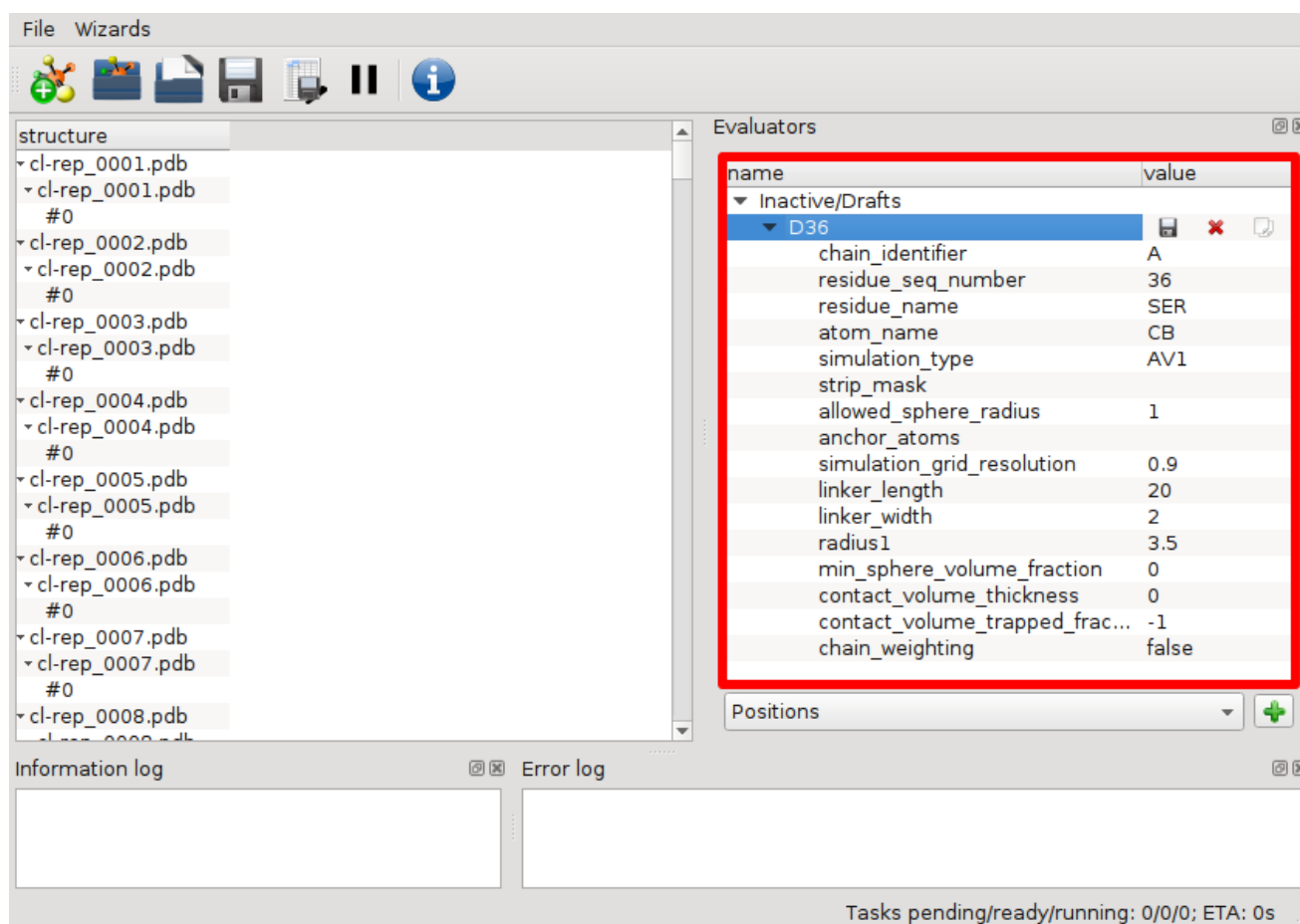
3. In this example we are calculating FRET-average distance between the dyes attached to residue #36 (SER, donor) and #132 (ASN, acceptor). First, we create a labelling position evaluator for the donor. Select "Position" option from the dropdown menu, then press the "+" button.



New labelling position will appear in the evaluators panel. Unfold it to see its settings.



4. Now Labelling Position properties can be filled out. First one has to choose the simulation type (here AV1 is selected). In this example Donor label is shown, Alexa 488 is used as a dye. Chromophore moiety approximated by a sphere with the radius of 3.5 Angstrom ( `radius1 = 3.5` ). It's linker has length of 20 Angstrom at maximum extension ( `linker_length = 20` ). We use linker width of 2 Angstrom; digitization step is set to 0.9 Angstrom ( `simulation_grid_resolution = 0.9` ). You will need to specify chain ID ( `chain_idenfifier = A` ), residue ID ( `residue_seq_number = 36` ), residue name ( `residue_name = SER` ) and name of the atom, to which fluorophore linker is attached. In experiment, typically, labelled residue is mutated to cysteine and then the dye is covalently bound to it by maleimide linker. To mimic that, we use C $\beta$  atom as the attachment point ( `atom_name = CB` ). User can specify `allowed_sphere_radius` option, which tells algorithm to ignore obstacles in the given radius in AV simulation. For example, side chain atoms would not exist in experiment, but they are present in the PDB files and can be disregarded for AV simulations. We can rename evaluator from default `new LP` to, e.g. `D36` by double-clicking on its name.



5. Copy the Donor labelling position and modify the copy to represent the Acceptor. We rename the copy to `A132` . We increase linker length to 22 Angstrom, since acceptor linker is longer in this case. We also adjust residue name ( `residue_name = ASN` ) and residue id ( `residue_seq_number = 132` ).

File Wizards

structure

- cl-rep\_0001.pdb
  - cl-rep\_0001.pdb
  - #0
- cl-rep\_0002.pdb
  - cl-rep\_0002.pdb
  - #0
- cl-rep\_0003.pdb
  - cl-rep\_0003.pdb
  - #0
- cl-rep\_0004.pdb
  - cl-rep\_0004.pdb
  - #0
- cl-rep\_0005.pdb
  - cl-rep\_0005.pdb
  - #0
- cl-rep\_0006.pdb
  - cl-rep\_0006.pdb
  - #0
- cl-rep\_0007.pdb
  - cl-rep\_0007.pdb
  - #0
- cl-rep\_0008.pdb
  - cl-rep\_0008.pdb
  - #0

Evaluators

name	value
Inactive/Drafts	
▶ D36	
▼ A132	
chain_identifier	A
residue_seq_number	132
residue_name	ASN
atom_name	CB
simulation_type	AV1
strip_mask	
allowed_sphere_radius	1
anchor_atoms	
simulation_grid_resolution	0.9
linker_length	22
linker_width	2
radius1	3.5
min_sphere_volume_fraction	0
contact_volume_thickness	0
contact_volume_trapped_frac...	-1
chain_weighting	false

Positions

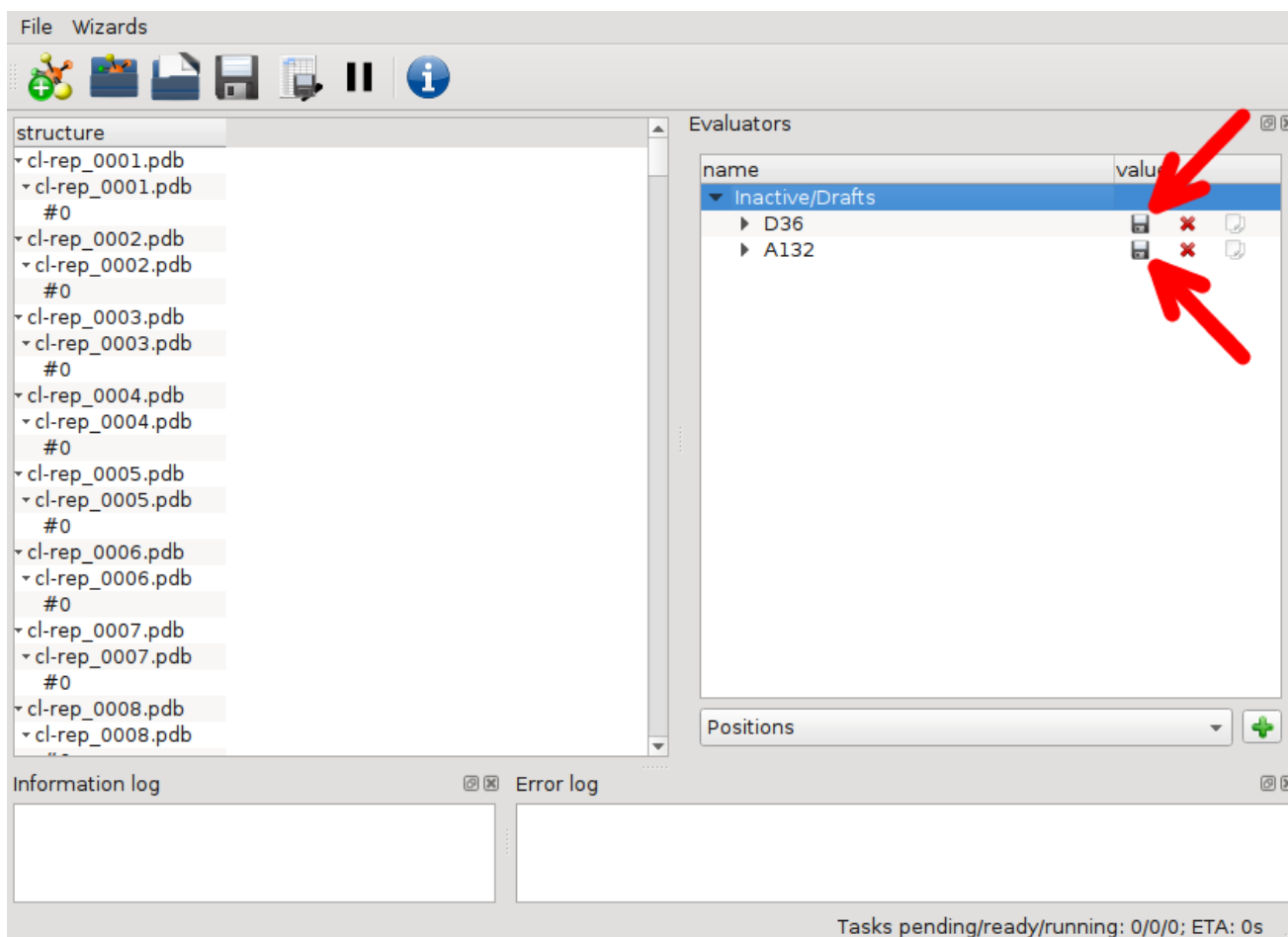
Information log

Error log

Tasks pending/ready/running: 0/0/0; ETA: 0s

**copy**

6. Activate Donor and Acceptor labelling position drafts by pressing "save" button.



7. Create a distance evaluator. To do this select "Distance" from dropdown menu and press "+", as in step 3.

8. First we select the distance type, possible options are:

- **RDAMean** - average iter-dye distance, typically obtained from TCSPC-like analysis.
- **RDAMeanE** - FRET-average inter-dye distance, typically obtained from single molecule / PDA analysis.
- **Rmp** - distance between mean positions of accessible volumes, usually can not be directly obtained from FRET experiments.

Then we pick donor and acceptor labelling position, specify the Förster radius. If available, measured distance and error can be specified, otherwise default values can stay. Save the distance evaluator to start calculation.

File Wizards

structure

- cl-rep\_0001.pdb
  - cl-rep\_0001.pdb
  - #0
- cl-rep\_0002.pdb
  - cl-rep\_0002.pdb
  - #0
- cl-rep\_0003.pdb
  - cl-rep\_0003.pdb
  - #0
- cl-rep\_0004.pdb
  - cl-rep\_0004.pdb
  - #0
- cl-rep\_0005.pdb
  - cl-rep\_0005.pdb
  - #0
- cl-rep\_0006.pdb
  - cl-rep\_0006.pdb
  - #0
- cl-rep\_0007.pdb
  - cl-rep\_0007.pdb
  - #0
- cl-rep\_0008.pdb
  - cl-rep\_0008.pdb
  - #0

Evaluators

name	value
Inactive/Drafts	
36_132	
distance_type	RDAMean
position1_name	D36
position2_name	A132
distance	37.6
error_neg	5.8
error_pos	5.8
Forster_radius	52
Positions	
D36	
A132	

Distances

Information log

Error log

Tasks pending/ready/running: 0/0/0; ETA: 0s

9. Once the distance evaluator is saved, corresponding column will appear next to the structure list. At first this column will only show `...`. Once the corresponding calculation is finished, calculated distance value will show up (or "nan" if the distance could not be calculated). Repeat steps 7-9 to calculate multiple FRET distances.

File Wizards

structure 36\_132 60\_132

structure	36_132	60_132
cl-rep_0001.pdb		
cl-rep_0001.pdb		
#0	36.222145	53.694662
cl-rep_0002.pdb		
cl-rep_0002.pdb		
#0	35.114470	54.797716
cl-rep_0003.pdb		
cl-rep_0003.pdb		
#0	36.675563	55.535447
cl-rep_0004.pdb		
cl-rep_0004.pdb		
#0	38.924891	54.801695
cl-rep_0005.pdb		
cl-rep_0005.pdb		
#0	41.704150	52.876858
cl-rep_0006.pdb		
cl-rep_0006.pdb		
#0	37.681152	52.020438
cl-rep_0007.pdb		
cl-rep_0007.pdb		
#0	40.189171	52.392372
cl-rep_0008.pdb		
cl-rep_0008.pdb		

Evaluators

name	value
Inactive/Drafts	
Positions	
D36	✗
A132	✗
D60	✗
Distances	
36_132	✗
60_132	✗
AV File	

AV File

Information log

Error log

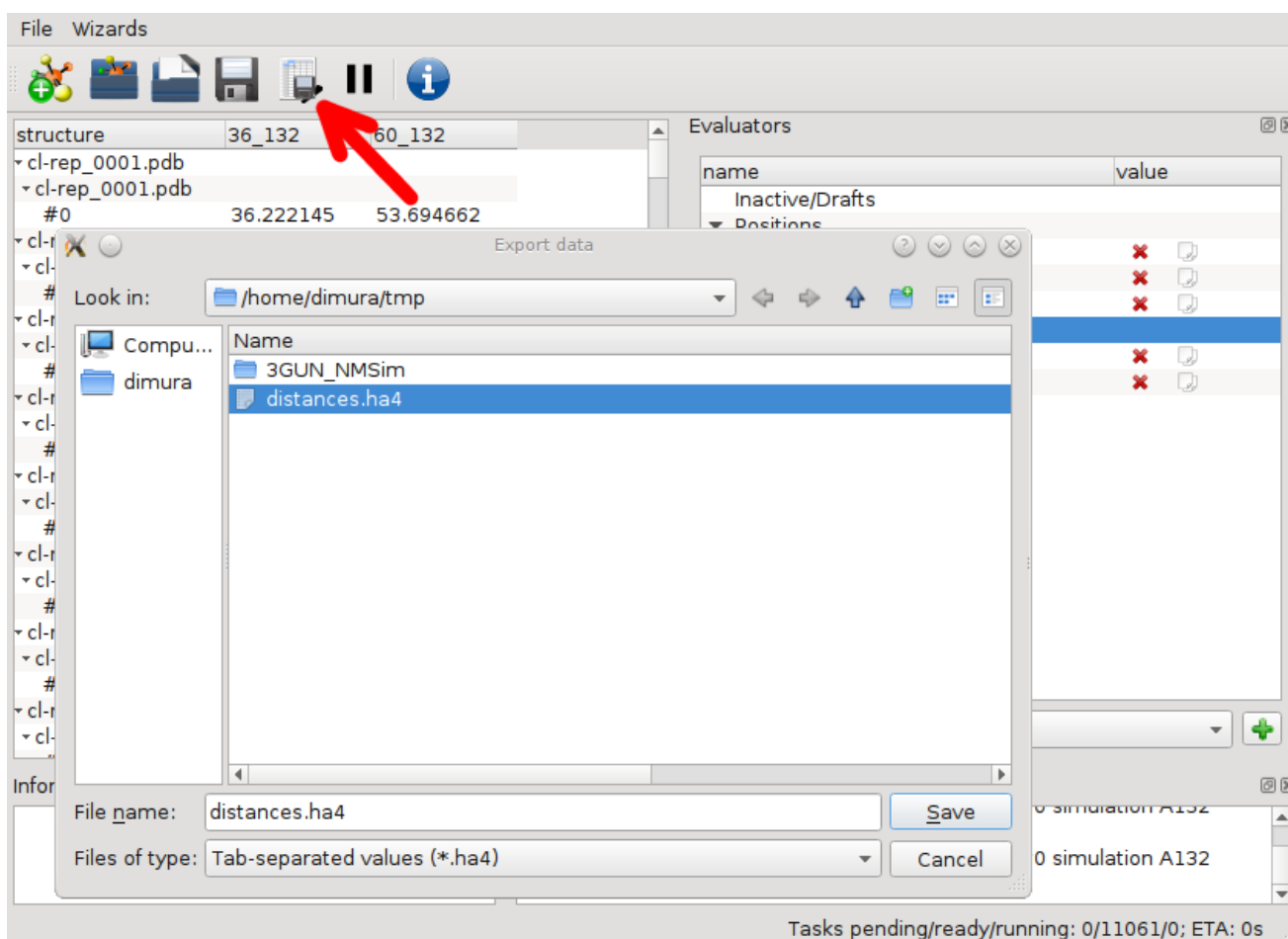
```

/home/dimura/tmp/3GUN_NMSim/cl-rep_3657.pdb#0 simulation A132
failed: empty AV
/home/dimura/tmp/3GUN_NMSim/cl-rep_3672.pdb#0 simulation A132
failed: empty AV

```

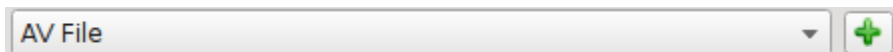
Tasks pending/ready/running: 0/11061/0; ETA: 0s

10. Results table can be saved by pressing "Export calculated values" button.



## Saving the Accessible Volume (AV) files

1. Create the "AV file" evaluator.



2. Select the labelling position, format (i.e. .xyz ) and directory to save the files ( write\_dir ). If write\_dir is empty, directory of the PDB file is used. Once the AV File evaluator is saved, program will automatically simulate AVs and save AV clouds for each of the loaded PDBs.



File Wizards

structure	36_132	60_132	D36_AV
cl-rep_0001.pdb			
cl-rep_0001.pdb #0	36.222145	53.694662	1
cl-rep_0002.pdb			
cl-rep_0002.pdb #0	35.114470	54.797716	1
cl-rep_0003.pdb			
cl-rep_0003.pdb #0	36.675563	55.535447	1
cl-rep_0004.pdb			
cl-rep_0004.pdb #0	38.924891	54.801695	1
cl-rep_0005.pdb			
cl-rep_0005.pdb #0	41.704150	52.876858	1
cl-rep_0006.pdb			
cl-rep_0006.pdb #0	37.681152	52.020438	1
cl-rep_0007.pdb			
cl-rep_0007.pdb #0	40.189171	52.392372	1
cl-rep_0008.pdb			
cl-rep_0008.pdb #0			

Evaluators

name	value
Inactive/Drafts	
Positions	
D36	✗
A132	✗
D60	✗
Distances	
36_132	✗
60_132	✗
AV File	
D36_AV	✗
position_name	D36
write_dir	
only_shell	false
openDX	false

AV File

Information log

Error log

```

/home/dimura/tmp/3GUN_NMSim/cl-rep_3657.pdb#0 simulation A132
failed: empty AV
/home/dimura/tmp/3GUN_NMSim/cl-rep_3672.pdb#0 simulation A132
failed: empty AV

```

Tasks pending/ready/running: 567/10494/254; ETA: 4s

3. AV cloud files can be now found in the `write_dir`. Here Pymol software is used to view the resulting cloud.

