Tutorial: selection of the most informative FRET pairs

Summary

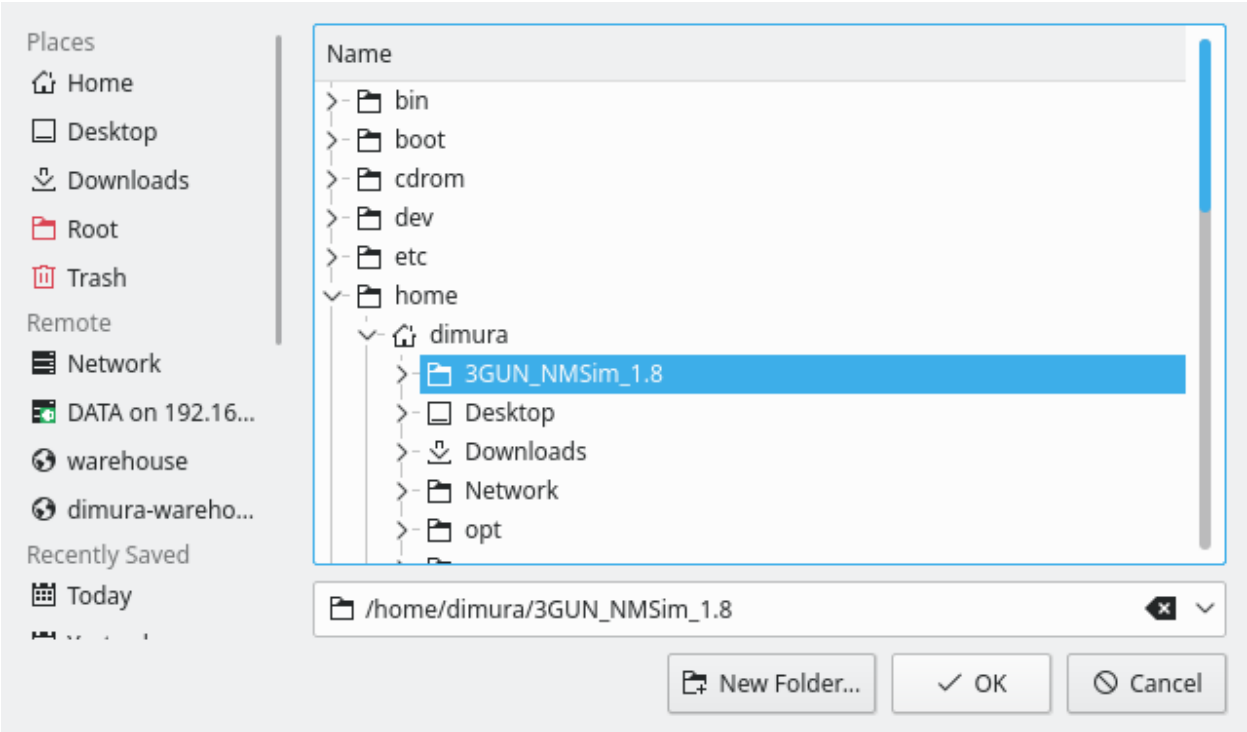
In this tutorial we walk through the procedure of selecting an informative set of FRET-pairs. In this context we define set as informative if it allows to distinguish any single conformation from a set of hypothetical structures (prior) using the FRET measurements. The software looks for such a set of FRET pairs, that expected structural precision is maximised with as little FRET pairs as possible. More information is available in the "Quantitative FRET" article DOI 10.1016/j.sbi.2016.11.012 :

> Dimura, M., Peulen, T.O., Hanke, C.A., Prakash, A., Gohlke, H. and Seidel, C.A., 2016. Quantitative FRET studies and integrative modeling unravel the structure and dynamics of biomolecular systems. Current opinion in structural biology, 40, pp.163-185.

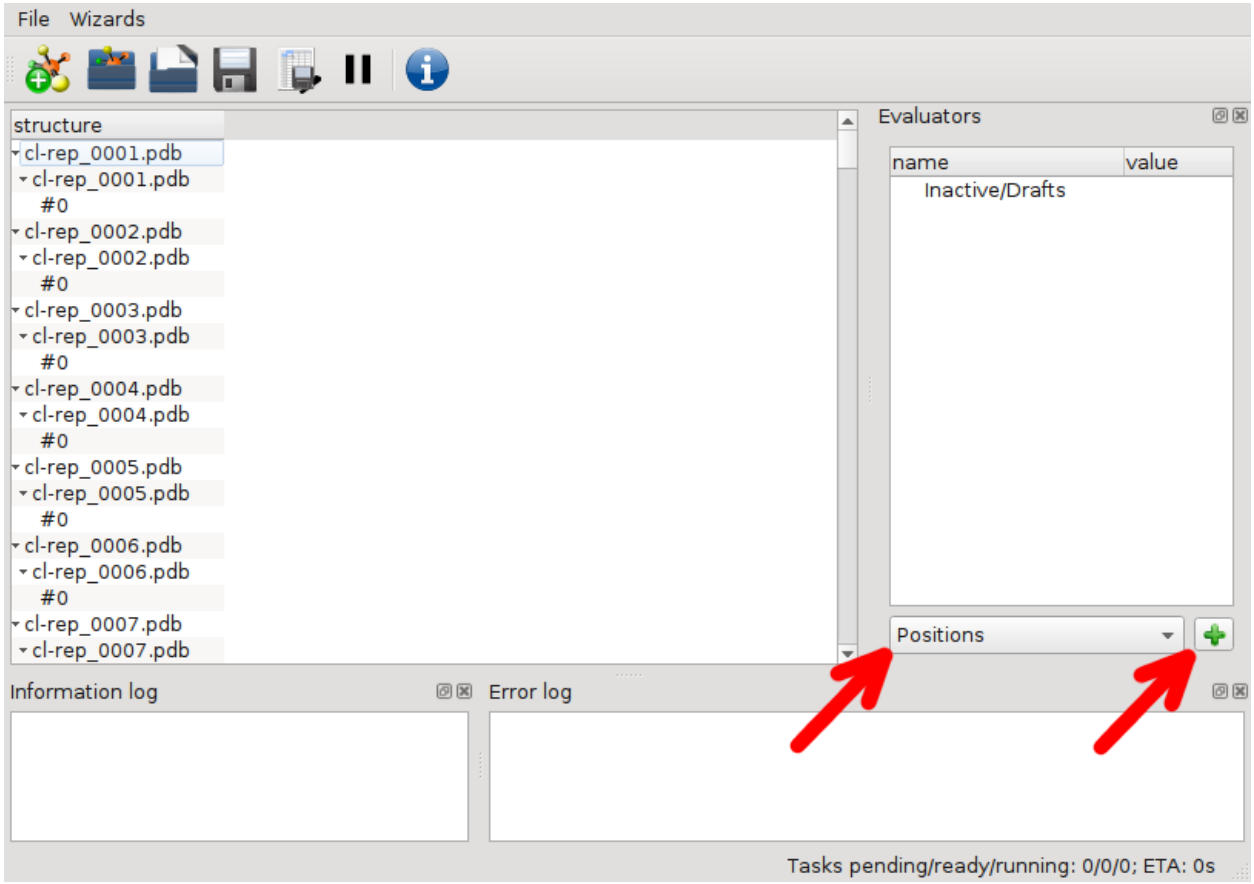
We use T4 lysozyme (T4L) protein as an example. It is recommended go through the screening tutorial first in order to get familiar with the software features.

FRET pair selection

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.



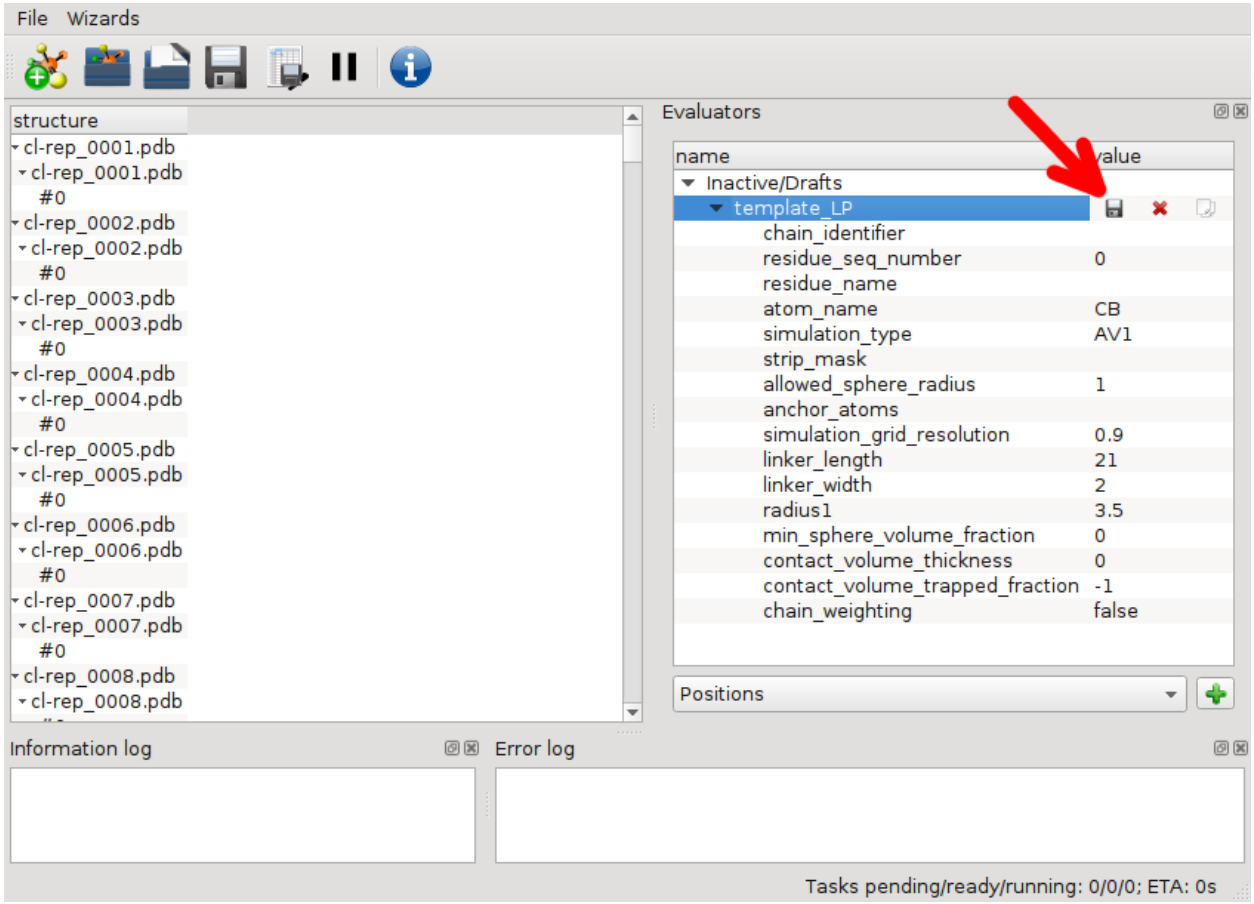
3. Now we need to create a labelling position evaluator that will serve as a template for other positions. In this tutorial we use the same template for donor and acceptor labels, but this is not mandatory. Select "Position" option from the dropdown menu, then press the "+" button.



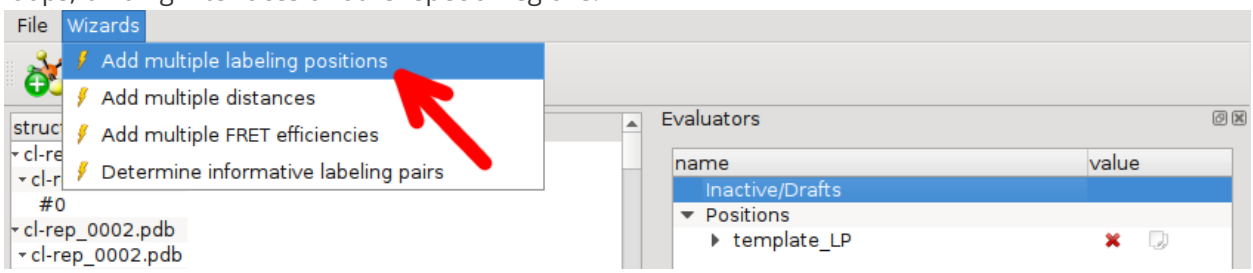
New labelling position will appear in the evaluators panel. Unfold it to see its settings.

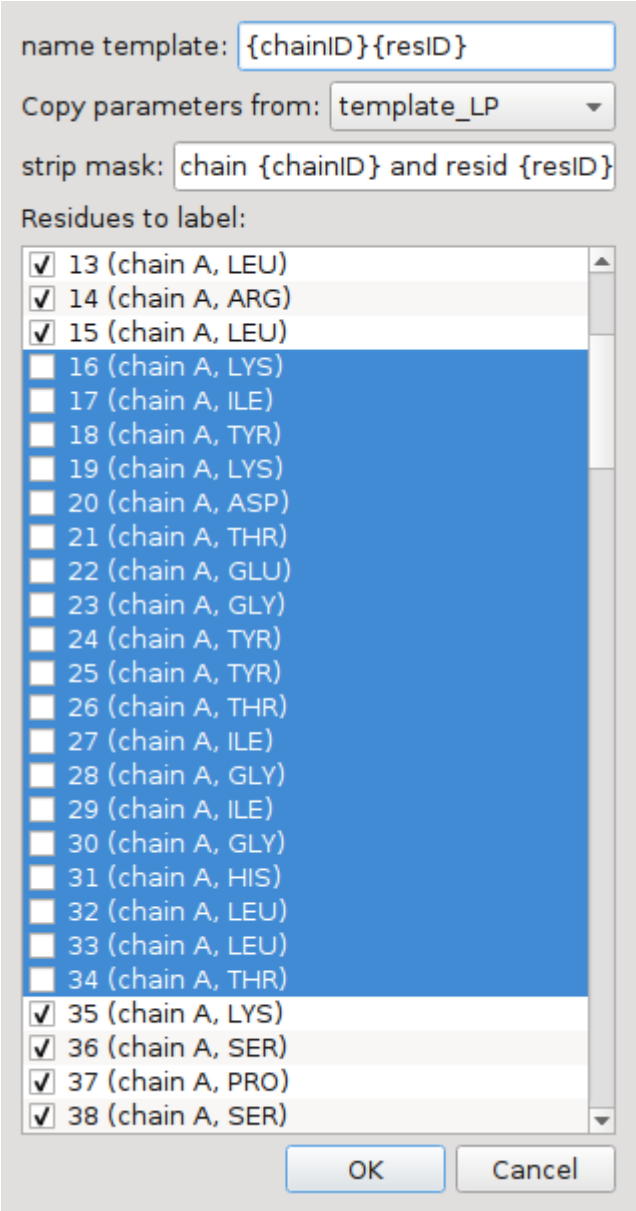
4. Now Labelling Position properties can be filled out. In this tutorial parameters are chosen to represent both Alexa 488 and Alexa 647 dyes. First one has to choose the simulation type (here AV1 is selected). Chromophore moiety approximated by a sphere with the radius of 3.5 Angstrom (`radius1 = 3.5`). It's linker has length of 21 Angstrom at maximum extension (`linker_length = 21`). We use linker width of 2 Angstrom; digitization step is set to 0.9 Angstrom (`simulation_grid_resolution = 0.9`). For the template chain ID (`chain_identifier`), residue ID (`residue_seq_number`), residue name (`residue_name`) should not be changed, they will be set automatically later. 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 Cβ 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. `template_LP` by double-clicking on its name. Activate the template labelling position draft by

pressing "save" button.

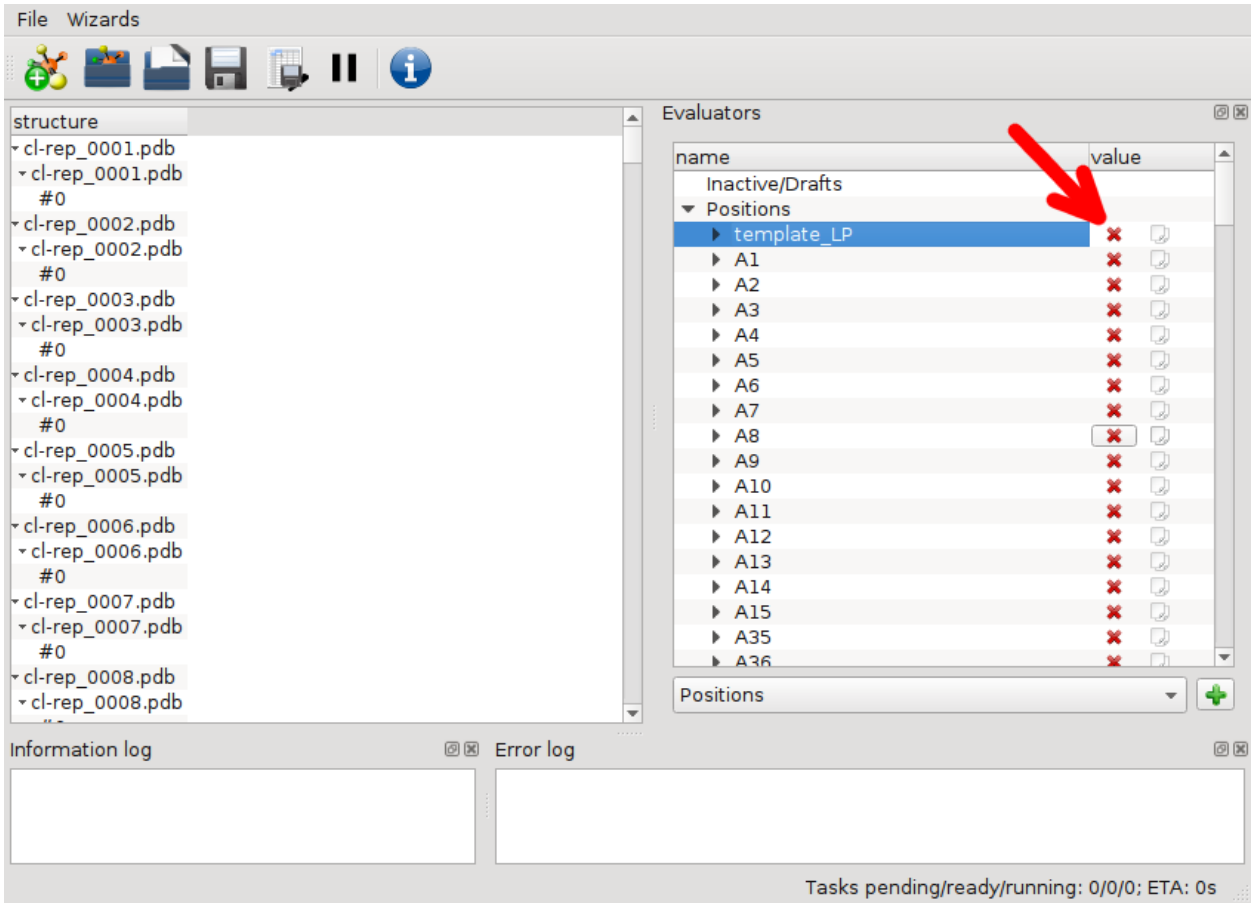


5. Next, we will use `Wizards -> Add multiple labeling positions` to automatically populate the labelling positions list with all residues that could be labelled. In this case we include all residues, except the 16-34 piece, since it contains the catalytic region, could be critical for protein activity and should not be mutated and labelled. One could exclude any residues necessary, e. g. N- and C- terminal loops, binding interfaces or other special regions.



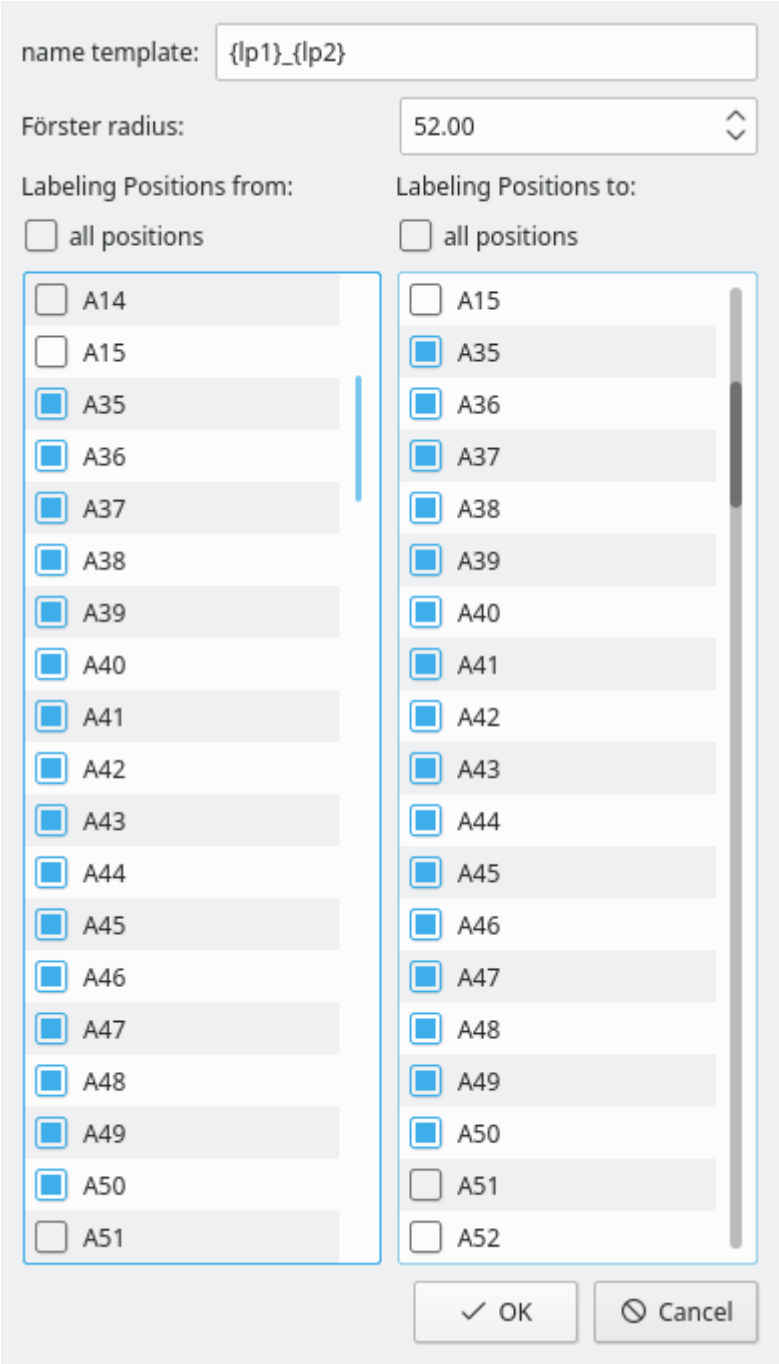


You can use `Ctrl + A` combination to select all residues and `Space` to toggle the check boxes. Once all needed residues are checked, press OK button. Positions will appear in the Evaluators widget:

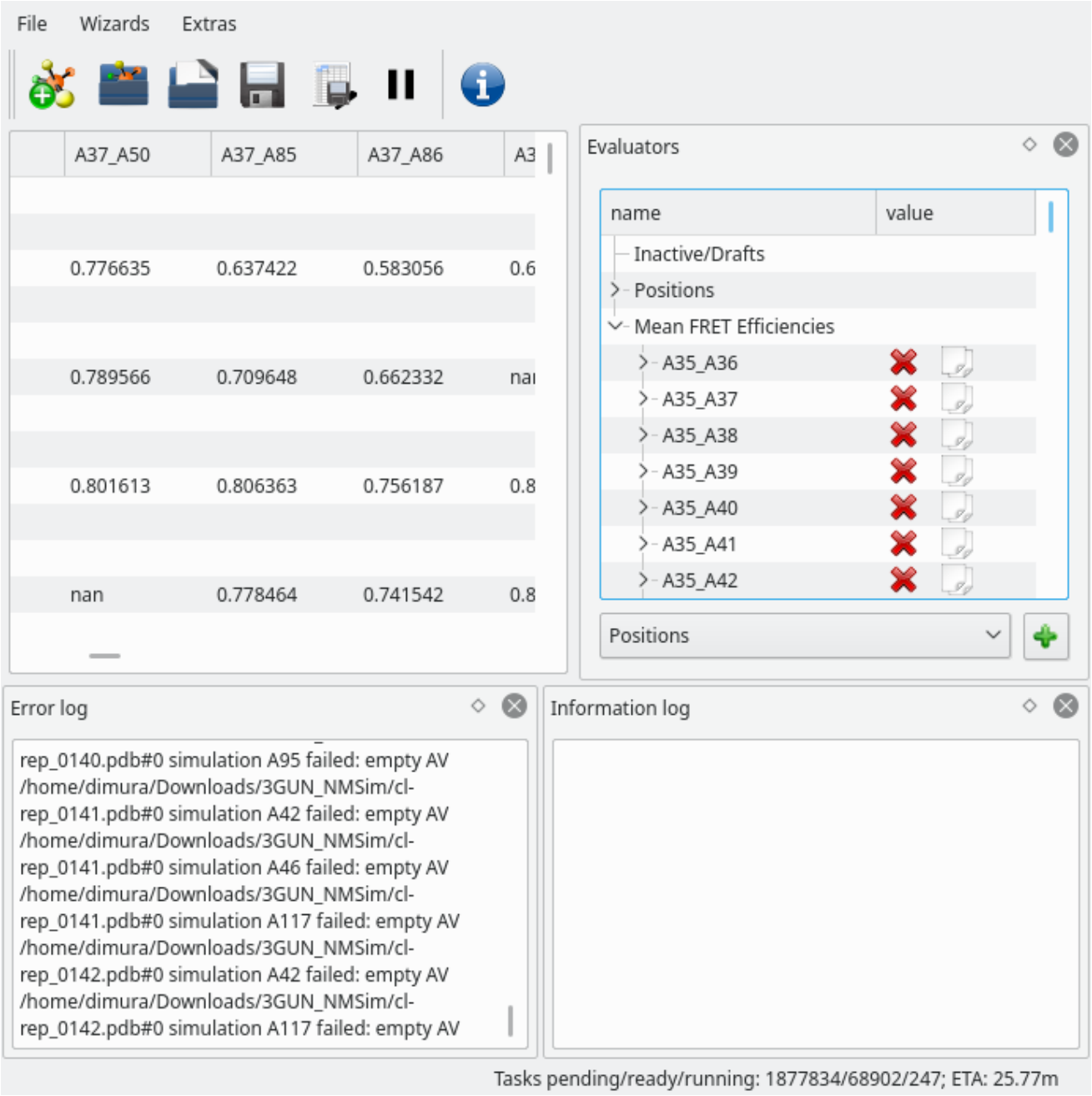


`template_LP` is not needed anymore and can be removed using "delete" button.

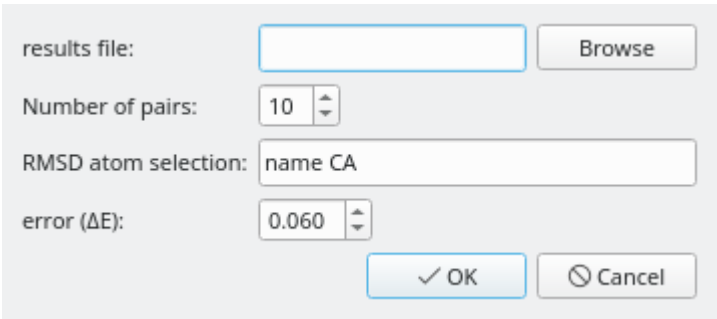
6. Next, we add FRET efficiency evaluators for all possible pairwise combinations of labeling positions. Go to `Wizards -> Add multiple FRET efficiencies` to show the corresponding dialog. Normally one would select all positions in this dialog. However, time needed to calculate FRET efficiencies is proportional to the number of pairs added. In this case it would take several hours to calculate FRET efficiencies for all pairs. In order to save time in this demonstration we only select positions 35-50, 85-95 and 115-120.



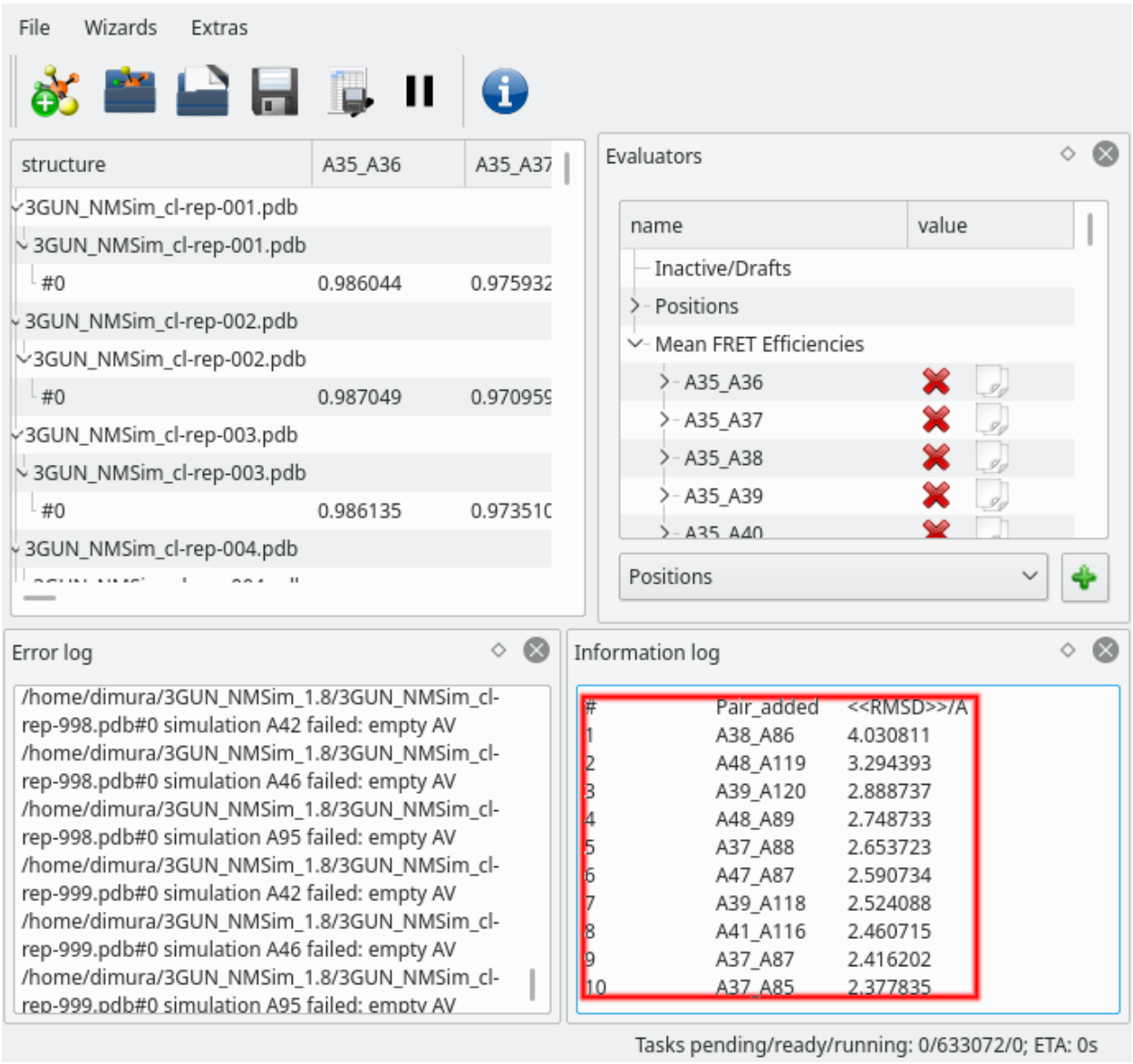
Set the Förster radius, then check labelling positions you want to be included in the ranking (we select all), and press OK. It may take a couple of minutes to add all FRET pairs. Once pairs are added, the program will start calculating efficiencies for each loaded conformer, which might take about 30 min.



7. Once all FRET efficiencies are calculated, we can rank them by how informative they could be. Go to `Wizards -> Determine informative labeling pairs` to show the pair selection dialog.



Enter the number of pairs you would like to select, atoms you would like to consider for RMSD calculation and expected error in FRET efficiency. Optionally, it is possible to specify a file where the results should be saved. If no results file is selected, pair list will be printed in the "Information log" widget. Press OK to start the calculation.


File
Wizards
Extras
structure
A35_A36
A35_A37
3GUN_NMSim_cl-rep-001.pdb
3GUN_NMSim_cl-rep-001.pdb
#0
0.986044
0.975932
3GUN_NMSim_cl-rep-002.pdb
3GUN_NMSim_cl-rep-002.pdb
#0
0.987049
0.970959
3GUN_NMSim_cl-rep-003.pdb
3GUN_NMSim_cl-rep-003.pdb
#0
0.986135
0.973510
3GUN_NMSim_cl-rep-004.pdb
Evaluators
name
value
Inactive/Drafts
Positions
Mean FRET Efficiencies
A35_A36
A35_A37
A35_A38
A35_A39
A35_A40
Positions
Error log
/home/dimura/3GUN_NMSim_1.8/3GUN_NMSim_cl-rep-998.pdb#0 simulation A42 failed: empty AV
/home/dimura/3GUN_NMSim_1.8/3GUN_NMSim_cl-rep-998.pdb#0 simulation A46 failed: empty AV
/home/dimura/3GUN_NMSim_1.8/3GUN_NMSim_cl-rep-998.pdb#0 simulation A95 failed: empty AV
/home/dimura/3GUN_NMSim_1.8/3GUN_NMSim_cl-rep-999.pdb#0 simulation A42 failed: empty AV
/home/dimura/3GUN_NMSim_1.8/3GUN_NMSim_cl-rep-999.pdb#0 simulation A46 failed: empty AV
/home/dimura/3GUN_NMSim_1.8/3GUN_NMSim_cl-rep-999.pdb#0 simulation A95 failed: empty AV
Information log
Pair_added <<RMSD>>/A
1 A38_A86 4.030811
2 A48_A119 3.294393
3 A39_A120 2.888737
4 A48_A89 2.748733
5 A37_A88 2.653723
6 A47_A87 2.590734
7 A39_A118 2.524088
8 A41_A116 2.460715
9 A37_A87 2.416202
10 A37_A85 2.377835
Tasks pending/ready/running: 0/633072/0; ETA: 0s