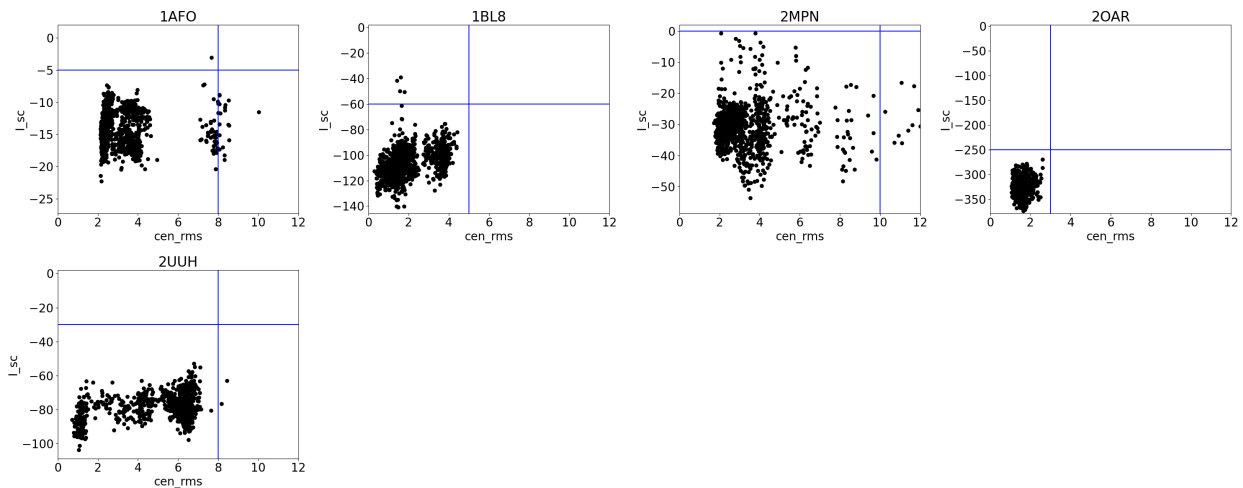


# Scientific test: mp\_symdock

## FAILURES

None

## RESULTS



## ## AUTHOR AND DATE

The benchmark was set up by Julia Koehler Leman (julia.koehler.leman@gmail.com) in March 2019.

The research was performed in Jeff Gray's lab, before Julia Koehler Leman moved to the lab of Richard Bonneau.

## ## PURPOSE OF THE TEST

We test Rosetta's ability to dock symmetric membrane proteins, i.e. how well the symmetry framework and membrane framework RosettaMP interface with the docking protocol. We basically check how well Rosetta can recapitulate native subunit interfaces in symmetric membrane proteins, how much the symmetry framework samples in the membrane and how well the membrane scorefunction scores the decoys.

## ## BENCHMARK DATASET

We test on 5 proteins that were part of the original benchmark set for testing RosettaMP described in the paper (Alford, Koehler Leman et. al. PlosCompBio 2015). This benchmark set tests C2, C3, C4 and C5 symmetries.

The input files were downloaded from the OPM database (1BL8 didn't have the correct bioassembly in PDBTM), cleaned, spanfiles were created with `mp_span_from_pdb`, then I used `mp_range_relax` to refine the structure. The outer helices in 1BL8 often came off the rest of the structure and started

flopping around, the closest RMSD structures had high scores due to clashes, but I took one of those anyway as the starting structure.

## **## PROTOCOL**

The protocol is described in (Alford, Koehler Leman et. al. PlosCompBio 2015) with command lines in the paper supplement. However, some flags had to be adjusted to make this run properly.

Note that for -in:file:native I am using the relaxed native but the symmetry framework starts with the asymmetric unit as the input structure. Therefore, the RMSD will never be super close to the native (even though with the adjusted not-optimal flags the RMSDs are actually amazing compared to what I see in the figures in the paper supplement). Not-optimal flags means that to get decoys in a reasonable amount of time, I had to use some flags that should not be used (like one for docking prepack for instance). I can imagine that some code has changed for low-res and high-res filtering steps as well, the docking:ppk flag makes sure the models pass these filters which they otherwise would not.

The protocol is the MPSymDock protocol, which runs the SymDock protocol underneath, which is based on the DockingProtocol. So we have a low-res docking step before it does high-res docking. Because of this, some decoys wouldn't pass the low-res stage, therefore the score file can have a mixture of centroid and high-res decoys with differing headers. That's the reason why the plotting script goes by index and not by column tag.

Runtimes are <100s per decoys: 100s x 5 proteins x 1000 decoys = 500,000 CPU secs which is <140 CPU hours. Note that the runtimes for these are with the docking:ppk flag and will likely increase (and possibly substantially) once better flags are found.

## **## PERFORMANCE METRICS**

We use interface score I\_sc vs. RMSD to the native. The native here is the relaxed OPM structure, not the symmetrized version created by the make\_symdef\_file script.

Cutoffs were defined by looking at the I\_sc vs. RMSD distribution of the first run. The I\_sc vs RMSD plots look substantially better than in the paper, not quite sure why. Differences are inclusion of the '-score:weights mpframework\_docking\_fa\_2015.wts' flag and the '-docking::dock\_ppk true'. Cutoffs need to be redone once the protocol has been optimized and better flags are found.

Note that the I\_sc in a normal range would be <0, maybe in the range between -20 to 0, not in negative hundreds as we sometimes see here.

## **## KEY RESULTS**

The baseline are relaxed structures from the OPM database.

1afo - C2 symmetry of single TM helix

1bl8 - C4 symmetry of potassium channel

2mpn - C2 symmetry of 2TMspan helix - looks like a handshake

2oar - C5 symmetry of a channel

2uuh - C3 symmetry of leukotriene synthase

The RMSDs that are sampled are pretty good but the interface scores are sometimes a little too good and it needs to be figured out where this is coming from. Note that this is a proof-of-concept protocol that could and should be improved code-wise and properly benchmarked.

## **## DEFINITIONS AND COMMENTS**

The original benchmarking was done by Rebecca Alford and this benchmark set, even though the same proteins, was set up by JKLeiman, so there are slight differences. For instance, while relaxing the structures, I noticed that mp\_relax moved 1 protein out of the membrane. Using mp\_range\_relax the 1BL8 structure of the potassium channel had the outer helices flopping around, meaning they didn't keep their interface with the other helices. This makes me wonder how stable these structures are in the first place and should be played around with more!!!

## **## LIMITATIONS**

The protocol should certainly be improved code-wise. This protocol is a proof-of-concept so far, needs to be benchmarked on a larger dataset with more diverse structures and symmetries. I'd like to see some beta-barrels in the benchmark set. Quality measures are standard, so they are fine.

In an earlier version of this protocol the funnel plots looked pretty great, but again, these are docking\_ppk structures and had horrible total scores due to clashes. I also noticed that the models (without docking\_ppk flag) were too close and tight and therefore have horrible scores compared to the natives. So adjusting the protocol to move the structures slightly further apart (perpendicular to the symmetry axis) should give better scoring models that are closer to the native without needing the docking\_ppk flag.