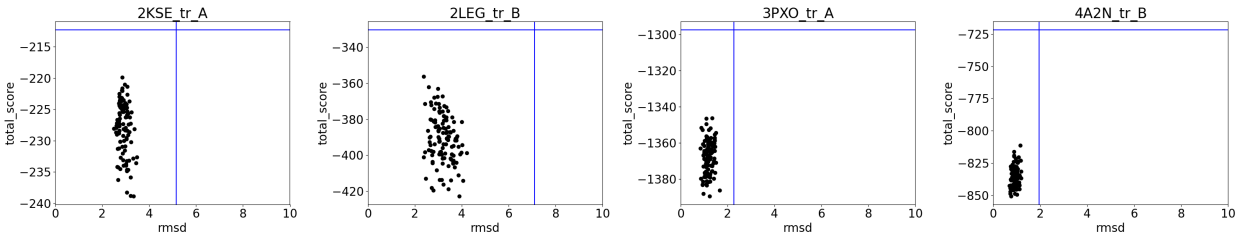
Scientific test: mp_relax

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, but Julia Koehler Leman is now in the lab of Richard Bonneau.

PURPOSE OF THE TEST

We test whether how much flexibility we can sample with mp_relax. The mp_relax app runs FastRelax under the hood but by using the membrane framework RosettaMP.

BENCHMARK DATASET

The benchmark set contains 4 test proteins that were run for mp_relax from the RosettaMP framework paper (Alford, Koehler Leman et. al, PlosCompBio, 2015). Note that the mp_relax application could be further optimized or compared to the newer protocol mp_range_relax that hasn't been published yet. The paper describes mp_relax and other applications as a proof-of-concept.

Input PDBs were downloaded from the PDBTM database (which have better membrane embedding than PDBs from the OPM database). This means that the proteins are transformed into membrane coordinates and have the correct biomolecular assembly, even though we are only looking at dimers for this application. PDBs were cleaned using clean_pdb.py from the Rosetta/ tools/protein_tools/scripts directory. Spanfiles were created using the mp_span_from_pdb application in Rosetta.

The PDB files were used as natives for RMSD calculations. Detail command lines are described in the Supplement to (Alford, Koehler Leman et. al, PlosCompBio, 2015).

PROTOCOL

The mp_relax application runs in RosettaScript and uses the FastRelax protocol as a mover but adjusted with the membrane framework RosettaMP. We create 100 models per protein to sample protein conformations. We use the mp_framework_smooth_2012 scorefunction for scoring until something better becomes available.

[Note that the natives should have a membrane residue (MEM), otherwise Rosetta crashes during RMSD calculation.]

On average for this benchmark set, mp_relax creates a decoy in ~350s; that is dependent on size of the protein. That makes 350s x 4 proteins x 100 decoys = 140,000s which are <40 CPU hours.

PERFORMANCE METRICS

We use the total Rosetta score and RMSD to the native structure as we are interested in the sampling range and the score ranges in sampling. A passed test constitutes 90% of the decoys generated have a smaller than the defined score and RMSD cutoffs. The cutoffs were defined by looking at these ranges in a single run without outlier decoys. Specifically, for RMSD we use the maximum RMSD + stdev. For score, we use the maximum score + stdev.

KEY RESULTS

We use crystal and structures as natives for comparison. The sampling range is up to 4 Angstrom in our examples.

2kse is an NMR structure of a histidine kinase, which normally has 2 chains but is here modeled as a single chain. That's bad. Not sure why this was chosen.

2leg is the NMR structure of DsbB, not a great structure and has a long loop which flops around quite a bit

3pxo is the crystal structure of metarhodopsin, a decent structure

4a2n is a crystal sructure with relatively long loops which don't seem to be super floppy - Rosetta predicts a pretty narrow range

DEFINITIONS AND COMMENTS

LIMITATIONS

The mp_relax app needs to be improved (or moved to mp_range_relax) and to benchmark that properly, a larger dataset is needed. From that, a collection of good, intermediate and outlier proteins should be picked for continuous scientific benchmarking.

The protein embedding in the membrane should be optimized during relax. Since the FoldTree in mp_relax is such that the MEM is relaxed around the

protein, doing that and superimposing the structures leads to MEM residues all over the place. The protein set is not very well picked. We need a broader set including beta-barrels and larger ranges of protein size with high-quality structures. There are NMR structures in here, which we might or might not want to avoid. We need to test multi-chain proteins in relax. And we need to test against range_relax and mp_range_relax which runs much master and provides better results.