Iterative Molecular Dynamics – Rosetta Membrane Protein Structure Refinement Guided by Cryo-EM Densities

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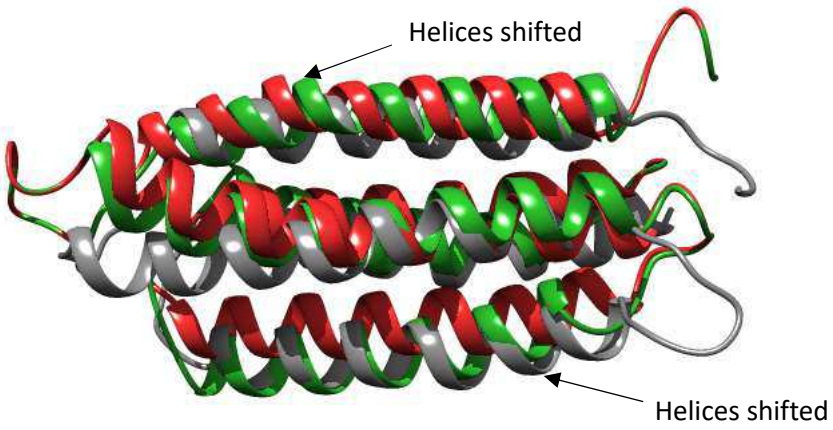
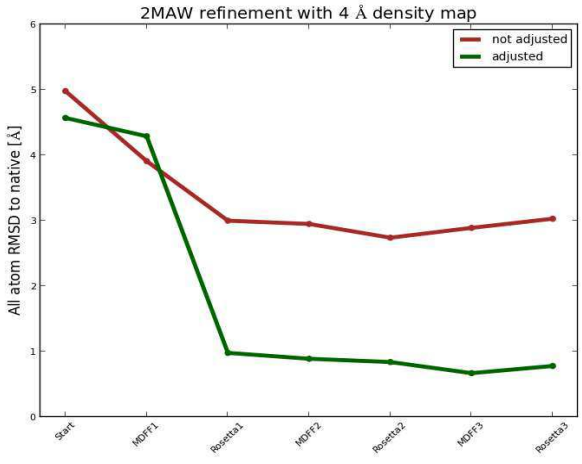
Supporting Information

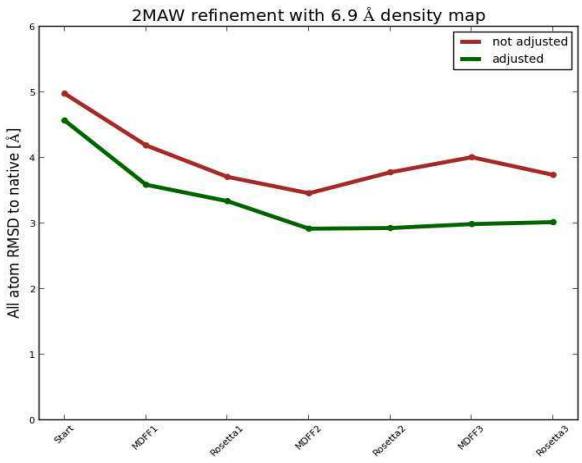
Figure S1: The refinement of the 2MAW ab initio starting structure that was not manually adjusted. (a) Structure alignment; native structure (gray), manually adjusted structure (green) and the best ab initio starting structure (red). Structure overlap shows that by manually adjusting the structure a register shift was introduced that brings the structure closer to the native (b) The RMSDs of 2MAW starting structures along the flow of the iterative Rosetta-MD protocol for the 4 Å density map (c) The RMSDs of 2MAW starting structures along the flow of the iterative Rosetta-MD protocol for the 6.9 Å density map.



(a)



(b)



(c)

Figure S2: Final MDFF step simulated at 3 different gscale parameters (0.2, 0.5 and 1.0) for the membrane proteins refined with a 4 Å density map. The RMSDs of the previous Rosetta step (Rosetta2) and the subsequent Rosetta step (Rosetta3) are also shown.

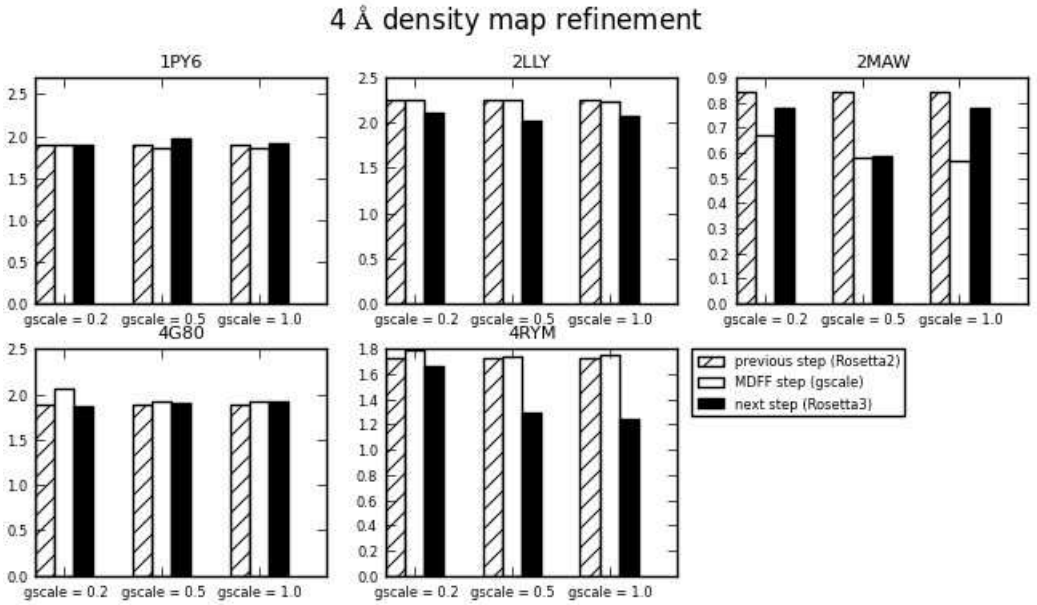
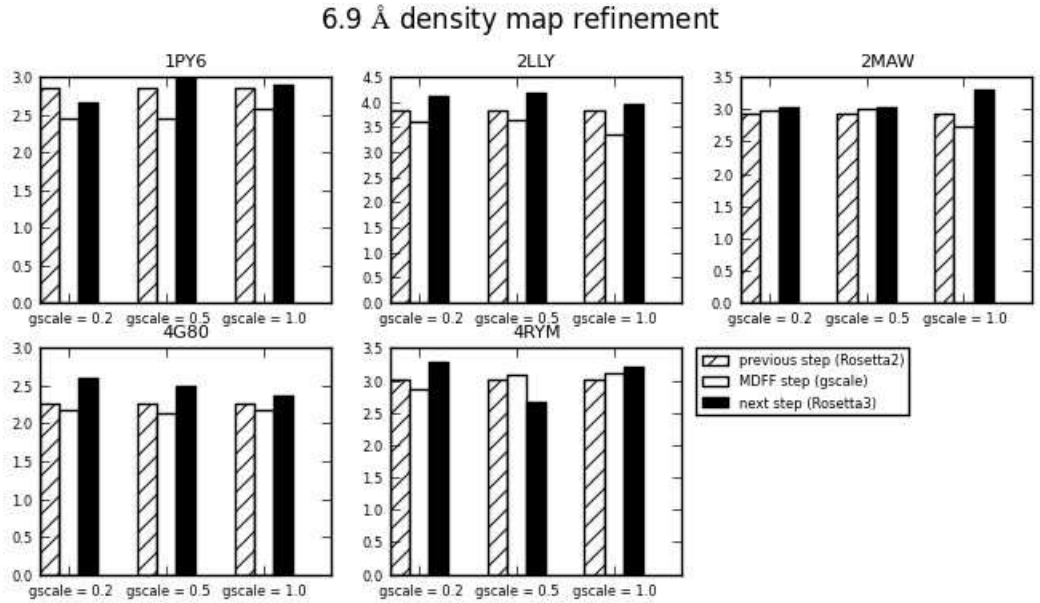


Figure S3: Final MDFF step simulated at 3 different gscale parameters (0.2, 0.5 and 1.0) for the membrane proteins refined with a 6.9 Å density map. The RMSDs of the previous Rosetta step (Rosetta2) and the subsequent Rosetta step (Rosetta3) are also shown.



Procedure to simulate density maps

The density maps were generated from the OPM-aligned native protein structures of each membrane protein using the pdb2vol program in the Situs package (situs.biomachina.org)[1].

Pdb2vol native.pdb densitymap.mrc

The values for arguments are collected after the above command is used. One third of the target resolution was used as the voxel spacing. For the 4 Å and 6.9 Å resolution density map simulations voxel spacings of 1.33 Å and 2.3 Å were used respectively. The atoms are not mass-weighted or selected based on B-factor. Gaussian smoothing kernel was used with an amplitude of 1.

Adding noise to density maps:

Noise was introduced to density maps using BCL density protocol[2].

bcl-apps-static.exe density:FromPDB native.pdb -resolution 6.9 -voxel_size 2.3 -kernel GaussianSphere -noise 0.8 -random_seed

Protocol Capture

Rosetta refinement (2016 version with talaris2014 scoring function)

/rosetta/main/source/bin/rosetta_scripts.linuxgccrelease -database /rosetta/main/database/ -in::file::s Rosetta1_start.pdb -parser::protocol xml_local_rebuilding1_mem.xml -parser::script_vars denswt=25 rms=1.5 reso=4.0 map=1PY6_native_4A.mrc testmap=1PY6_native_4A.mrc -ignore_unrecognized_res -edensity::mapreso 4.0 -default_max_cycles 200 -edensity::cryoem_scatterers -out::suffix _1 -crystal_refine -nstruct 1

NAMD (namd-2.11)

run_namd melting-01.conf
run_namd protein_fixed-02.conf

Generate the MDFF inputs using the tcl script:
last_frame_02_prepare_input.tcl

run_namd last_frame_02_MDFF-step1.namd (starting from the last frame of the previous NAMD simulation).

*All input files, input structures, ab initio starting structures, density maps and the final refined models are included as zip file.

1. Wriggers, W., Conventions and workflows for using Situs. *Acta Crystallographica Section D: Biological Crystallography* **2012,** *68* (4), 344-351.
2. Woetzel, N.; Lindert, S.; Stewart, P. L.; Meiler, J., BCL:: EM-Fit: Rigid body fitting of atomic structures into density maps using geometric hashing and real space refinement. *Journal of structural biology* **2011,** *175* (3), 264-276.