EM-guided symmetry modeling procedure:

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Execute Rosetta:

~rosetta/rosetta\_score/bin/minirosetta.macosx

Using the following flags:

-run:protocol symdock

-database ~rosetta/rosetta\_database

-in:file:s input.pdb

-symmetry:symmetry\_definition 25.symm

-symmetry:initialize\_rigid\_body\_dofs

-edensity:mapfile FliF\_map.mrc

-edensity:mapreso 22.0

-edensity:grid\_spacing 5.0

-edensity:score\_symm\_complex true

-packing:ex1

-packing:ex2aro

-use\_input\_sc

-ignore\_unrecognized\_res

-out:nstruct 1000

-out:file:silent phaseI.silent

-out:file:silent\_struct\_type binary

-out:file:fullatom

-residues:patch\_selectors CENTROID\_HA

-use\_incorrect\_hbond\_deriv false

-docking:low\_patch patch\_phaseI

-docking:high\_patch patch\_phaseI

-docking:high\_min\_patch patch\_high\_min\_phaseI

-docking:pack\_patch patch\_phaseI

-docking:dock\_lowres\_filter 15.0 20.0 1500.0

Content of file patch\_phaseI:

elec\_dens\_whole\_structure\_ca = 0.1

Content of file patch\_high\_min\_phaseI:

elec\_dens\_whole\_structure\_ca = 0.1

fa\_rep \*= 4.22

24-fold, 25-fold and 26-fold symmetry files were generated by manually placing the corresponding number of subunits in the FliF EM map, using the following command:

perl $rosettadir/rosetta\_source/src/apps/public/symmetry/make\_symmdef\_file.pl -a A -i B -r 12.0 \

–p input.pdb > input.symm