**RosettaScripts and commandlines for “*De novo* designed transmembrane domains tune engineered receptor functions”**

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**Fold, Dock, and Design**

<ROSETTASCRIPTS>

 <SCOREFXNS>

 <ScoreFunction name="score0" weights="%%score\_func\_0%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="100"/>

 </ScoreFunction>

 <ScoreFunction name="score1" weights="%%score\_func\_1%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="100"/>

 </ScoreFunction>

 <ScoreFunction name="score2" weights="%%score\_func\_2%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="100"/>

 </ScoreFunction>

 <ScoreFunction name="score3" weights="%%score\_func\_3%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="100"/>

 </ScoreFunction>

 <ScoreFunction name="score5" weights="%%score\_func\_5%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="100"/>

 </ScoreFunction>

 <ScoreFunction name="beta" weights="ref2015\_memb" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="100"/>

 </ScoreFunction>

 <ScoreFunction name="betaNotSymm" weights="ref2015\_memb" symmetric="0">

 <Reweight scoretype="mp\_helicality" weight="100"/>

 </ScoreFunction>

 <ScoreFunction name="helicality" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="1"/>

 </ScoreFunction>

 </SCOREFXNS>

 <TASKOPERATIONS>

 <InitializeFromCommandline name="init"/>

 <RestrictToRepacking name="rtr"/>

 </TASKOPERATIONS>

 <MOVERS>

 <PyMOLMover name="pmm" keep\_history="1"/>

 <SetupForSymmetry name="symm" definition="%%symm\_file%%"/>

 <SymmetricAddMembraneMover name="add\_memb" membrane\_core="%%membrane\_core%%" steepness="%%steepness%%">

 <Span start="%%span\_start\_1%%" end="%%span\_end\_1%%" orientation="%%span\_orientation\_1%%"/>

 <Span start="%%span\_start\_2%%" end="%%span\_end\_2%%" orientation="%%span\_orientation\_2%%"/>

 </SymmetricAddMembraneMover>

 <MembranePositionFromTopologyMover name="init\_pos"/>

 <FastRelax name="fast\_relax" scorefxn="beta"/>

 Fragment movers

 <SingleFragmentMover name="frag9" fragments="%%frags9mers%%" policy="uniform"/>

 <SingleFragmentMover name="frag3" fragments="%%frags3mers%%" policy="smooth"/>

 Fold-and-dock specific movers

 <SymFoldandDockRbTrialMover name="rbtrial" rot\_mag="8.0" trans\_mag="3.0" rotate\_anchor\_to\_x="1"/>

 <SymFoldandDockRbTrialMover name="rbtrial\_smooth" rot\_mag="1.0" trans\_mag="0.1" rotate\_anchor\_to\_x="1"/>

 <SymFoldandDockMoveRbJumpMover name="rbjump"/>

 <SymFoldandDockSlideTrialMover name="slidetrial"/>

 Random movers

 <RandomMover name="early\_stage\_moveset" movers="frag9,rbtrial,rbjump,slidetrial" weights="1.0,0.2,1.0,0.1" repeats="1"/>

 <RandomMover name="final\_stage\_moveset" movers="frag3,rbtrial\_smooth,rbjump,slidetrial" weights="1.0,0.2,1.0,0.1" repeats="1"/>

 Monte Carlo Movers

 <GenericMonteCarlo name="stage1" scorefxn\_name="score0" mover\_name="early\_stage\_moveset" temperature="2.0" trials="200" recover\_low="1"/>

 <GenericMonteCarlo name="stage2" scorefxn\_name="score1" mover\_name="early\_stage\_moveset" temperature="2.0" trials="200" recover\_low="1"/>

 <GenericMonteCarlo name="stage3a" scorefxn\_name="score2" mover\_name="early\_stage\_moveset" temperature="2.0" trials="20" recover\_low="1"/>

 <GenericMonteCarlo name="stage3b" scorefxn\_name="score5" mover\_name="early\_stage\_moveset" temperature="2.0" trials="20" recover\_low="1"/>

 <GenericMonteCarlo name="stage4" scorefxn\_name="score3" mover\_name="final\_stage\_moveset" temperature="2.0" trials="400" recover\_low="1"/>

 Special stage 3 logic

 <ParsedProtocol name="stage3\_cyc">

 <Add mover="stage3a"/>

 <Add mover="stage3b"/>

 </ParsedProtocol>

 <LoopOver name="stage3" mover\_name="stage3\_cyc" iterations="5" drift="1"/>

 Converts the centroid-level pose to fullatom for scoring

 <SwitchResidueTypeSetMover name="fullatom" set="fa\_standard"/>

 <ExtractAsymmetricPose name="extract\_asp"/>

 <!--<SymPackRotamersMover name="soft\_design" scorefxn="beta\_soft" task\_operations="init"/>-->

 <TaskAwareSymMinMover name="hard\_min" scorefxn="beta" chi="1" bb="1" rb="1" task\_operations="init"/>

 <SymPackRotamersMover name="hard\_design" scorefxn="beta" task\_operations="init"/>

 <RotamerTrialsMinMover name="RTmin" scorefxn="beta" task\_operations="init,rtr"/>

 </MOVERS>

 <FILTERS>

 <ScoreType name="total" scorefxn="beta" score\_type="total\_score" confidence="0" threshold="0"/>

 <Sasa name="a\_sasa" confidence="0"/>

 <ResidueLipophilicity name="a\_res\_solv" threshold="1000" confidence="0"/>

 <SpanTopologyMatchPose name="a\_span\_topo" confidence="0"/>

 <Ddg name="a\_ddg" scorefxn="betaNotSymm" chain\_num="2" repeats="5" extreme\_value\_removal="true" confidence="0"/>

 <PackStat name="a\_pack" confidence="0"/>

 <BuriedUnsatHbonds2 name="a\_unsat" scorefxn="beta" confidence="0"/>

 <ShapeComplementarity name="a\_shape" confidence="0"/>

 <TMsSpanMembrane name="a\_tms\_span" confidence="1"/>

 <TMsSpanMembrane name="a\_tms\_span\_fa" confidence="1" min\_distance="25"/>

 <HelixHelixAngle name="a\_hha\_ang" angle\_or\_dist="angle" start\_helix\_1="%%span\_start\_1%%" end\_helix\_1="%%span\_end\_1%%" start\_helix\_2="%%span\_start\_2%%" end\_helix\_2="%%span\_end\_2%%" confidence="0"/>

 <HelixHelixAngle name="a\_hha\_dst\_vec" angle\_or\_dist="dist" dist\_by\_atom="0" start\_helix\_1="%%span\_start\_1%%" end\_helix\_1="%%span\_end\_1%%" start\_helix\_2="%%span\_start\_2%%" end\_helix\_2="%%span\_end\_2%%" confidence="0"/>

 <HelixHelixAngle name="a\_hha\_dst\_atm" angle\_or\_dist="dist" dist\_by\_atom="1" start\_helix\_1="%%span\_start\_1%%" end\_helix\_1="%%span\_end\_1%%" start\_helix\_2="%%span\_start\_2%%" end\_helix\_2="%%span\_end\_2%%" confidence="0"/>

 <ScoreType name="a\_helicality" scorefxn="helicality" score\_type="total\_score" confidence="1" threshold="3"/>

 <TMsAAComp name="a\_tms\_aa\_comp" confidence="0" threshold="0"/>

 </FILTERS>

 <PROTOCOLS>

 <Add mover="symm"/>

 <Add mover="add\_memb"/>

 <Add mover="stage1"/>

 <Add mover="stage2"/>

 <Add mover="stage3"/>

 <Add mover="stage4"/>

 <Add filter="a\_helicality"/>

 <Add mover="fullatom"/>

 <Add filter="a\_tms\_span\_fa"/>

 !!!! design !!!!

 <Add mover="hard\_design"/>

 <Add mover="hard\_min"/>

 <Add mover="hard\_design"/>

 <Add mover="hard\_min"/>

 <Add mover="RTmin"/>

 <Add mover="RTmin"/>

 <Add filter="a\_tms\_span"/>

 <Add mover="fast\_relax"/>

 <Add mover="pmm"/> # DO I WANT THIS HERE?

 <Add filter="total"/>

 <Add filter="a\_sasa"/>

 <Add filter="a\_res\_solv"/>

 <Add filter="a\_span\_topo"/>

 <Add filter="a\_helicality"/>

 # important to remove Symmetry so RMSD can be calculated

 <Add mover="extract\_asp"/>

 <Add filter="a\_res\_solv"/>

 <Add filter="a\_pack"/>

 <Add filter="a\_unsat"/>

 <Add filter="a\_shape"/>

 <Add filter="a\_ddg"/>

 <Add filter="a\_hha\_ang"/>

 <Add filter="a\_hha\_dst\_vec"/>

 <Add filter="a\_hha\_dst\_atm"/>

 <Add filter="a\_tms\_aa\_comp"/>

 </PROTOCOLS>

 <OUTPUT scorefxn="betaNotSymm"/>

</ROSETTASCRIPTS>

**Command line**

~/Rosetta/main/source/bin/rosetta\_scripts.default.linuxgccreleas -database Rosetta/main/database @**flags\_fdd**

flags\_fdd:

# general data

-parser:protocol fold\_dock\_design.xml

-in:file:fasta ploy\_V.fasta

-in:file:native 24.pdb

-overwrite

-use\_input\_sc

-nstruct 12

-jd2:ntrials 10

#-mute all

# fragment stuff

-parser:script\_vars frags9mers=frags\_9ploy\_V\_pdbtm.200.9mers

-parser:script\_vars frags3mers=frags\_3ploy\_V\_pdbtm.200.3mers

-parser:script\_vars symm\_file=C2.symm

# membrane spans:

-parser:script\_vars span\_starts=1A

-parser:script\_vars span\_ends=24A

-parser:script\_vars span\_oris=out2in

-parser:script\_vars span\_start\_1=1

-parser:script\_vars span\_end\_1=24

-parser:script\_vars span\_orientation\_1=out2in

-parser:script\_vars span\_start\_2=25

-parser:script\_vars span\_end\_2=48

-parser:script\_vars span\_orientation\_2=out2in

-parser:script\_vars span\_starts=1A

-parser:script\_vars span\_ends=24A

-parser:script\_vars span\_oris=out2in

-parser:script\_vars score\_func\_0=score0\_memb

-parser:script\_vars score\_func\_1=score1\_memb

-parser:script\_vars score\_func\_2=score2\_memb

-parser:script\_vars score\_func\_3=score3\_memb

-parser:script\_vars score\_func\_5=score5\_memb

# energy function stuff:

-parser:script\_vars energy\_function=ref

# membrane adjustments

-mp:scoring:hbond

-parser:script\_vars steepness=4

-parser:script\_vars membrane\_core=10

**Sequence diversification**

<ROSETTASCRIPTS>

 <SCOREFXNS>

 <ScoreFunction name="ref" weights="ref2015\_memb" symmetric="1">

 <Reweight scoretype="fa\_mpenv\_smooth" weight="0.000001"/>

 <Reweight scoretype="coordinate\_constraint" weight="0.4"/>

 </ScoreFunction>

 <ScoreFunction name="ref\_no\_cst" weights="ref2015\_memb" symmetric="1">

 <Reweight scoretype="fa\_mpenv\_smooth" weight="0.000001"/>

 </ScoreFunction>

 <ScoreFunction name="helicality" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="1"/>

 </ScoreFunction>

 </SCOREFXNS>

 <RESIDUE\_SELECTORS>

 <ResidueName name="gly" residue\_name3="GLY"/>

 <ResidueName name="asn\_gln" residue\_name3="ASN,GLN"/>

 <Index name="all\_aas" resnums="1-48"/>

 <Not name="not\_aas" selector="all\_aas"/>

 </RESIDUE\_SELECTORS>

 <TASKOPERATIONS>

 <OperateOnResidueSubset name="keep\_gly" selector="gly">

 <RestrictToRepackingRLT/>

 </OperateOnResidueSubset>

 <OperateOnResidueSubset name="keep\_qn" selector="asn\_gln">

 <RestrictToRepackingRLT/>

 </OperateOnResidueSubset>

 <OperateOnResidueSubset name="freeze\_not\_aas" selector="not\_aas">

 <PreventRepackingRLT/>

 </OperateOnResidueSubset>

 <RestrictToRepacking name="rtr"/>

 <RestrictAbsentCanonicalAAS name="to\_restrict\_avlimfwyst" resnum="0" keep\_aas="GAVIFSTYW"/>

 <InitializeFromCommandline name="init"/>

 </TASKOPERATIONS>

 <FILTERS>

 <ResidueLipophilicity name="a\_res\_lipo" confidence="0"/>

 <ScoreType name="a\_total" scorefxn="ref" score\_type="total\_score" confidence="0" threshold="0"/>

 <ScoreType name="a\_total\_final" scorefxn="ref" score\_type="total\_score" confidence="1" threshold="-140"/>

 <Sasa name="a\_sasa" confidence="0"/>

 <SpanTopologyMatchPose name="a\_span\_topo" confidence="0"/>

 <Ddg name="a\_ddg" scorefxn="ref\_no\_cst" confidence="0" />

 <PackStat name="a\_pack" confidence="1" threshold="0.3"/>

 <BuriedUnsatHbonds2 name="a\_unsat" scorefxn="ref" confidence="0"/>

 <ShapeComplementarity name="a\_shape" confidence="0"/>

 <TMsSpanMembrane name="a\_tms\_span" confidence="0"/>

 <TMsSpanMembrane name="a\_tms\_span\_fa" confidence="0" min\_distance="25"/>

 <HelixHelixAngle name="a\_hha\_ang" angle\_or\_dist="angle" start\_helix\_1="%%s1%%" end\_helix\_1="%%e1%%" start\_helix\_2="%%s2%%" end\_helix\_2="%%e2%%" confidence="0"/>

 <MembAccesResidueLipophilicity name="a\_marl" confidence="0" verbose="0"/>

 <TMsAAComp name="a\_tms\_aa\_comp" confidence="0" threshold="0"/>

 <BindingStrain name="a\_bind" scorefxn="ref\_no\_cst" jump="1" confidence="1" threshold="5"/>

 <ScoreType name="a\_helicality" scorefxn="helicality" score\_type="total\_score" confidence="1" threshold="3"/>

 <Sigmoid name="a\_total\_sig" filter="a\_total" steepness="0.5" offset="20" negate="0" confidence="0"/>

 <Sigmoid name="a\_ddg\_sig\_10" filter="a\_ddg" steepness="1" offset="10" negate="0" confidence="0"/>

 <Sigmoid name="a\_ddg\_sig\_2" filter="a\_ddg" steepness="1" offset="3" negate="0" confidence="0"/>

 <Sigmoid name="a\_comp\_sig" filter="a\_tms\_aa\_comp" steepness="50" offset="0.05" negate="0" confidence="0"/>

 <Operator name="a\_obj\_func\_ddg\_10" filters="a\_total\_sig,a\_ddg\_sig\_10,a\_comp\_sig" operation="PRODUCT" logarithm="1" threshold="100000" confidence="0" negate="1"/>

 <Operator name="a\_obj\_func\_ddg\_2" filters="a\_total\_sig,a\_ddg\_sig\_2,a\_comp\_sig" operation="PRODUCT" logarithm="1" threshold="100000" confidence="0" negate="1"/>

 </FILTERS>

 <MOVERS>

 <SymmetricAddMembraneMover name="add\_memb" membrane\_core="%%memb\_core%%" steepness="%%steepness%%">

 <Span start="%%s1%%" end="%%e1%%" orientation="%%o1%%"/>

 <Span start="%%s2%%" end="%%e2%%" orientation="%%o2%%"/>

 </SymmetricAddMembraneMover>

 <RandomMutation name="mutate" task\_operations="freeze\_not\_aas,init,to\_restrict\_avlimfwyst,keep\_gly,keep\_qn" scorefxn="ref"/>

 <VirtualRoot name="virt\_root"/>

 <AtomCoordinateCstMover name="atom\_cst" coord\_dev="0.5" bounded="false" native="false"/>

 <TaskAwareSymMinMover name="min\_mover" scorefxn="ref" chi="1" bb="1" rb="1" task\_operations="init,rtr"/>

 <ParsedProtocol name="mutate\_min">

 <Add mover="mutate"/>

 <Add mover="min\_mover"/>

 </ParsedProtocol>

 <GenericMonteCarlo name="gmc\_sigs\_1" filter\_name="a\_obj\_func\_ddg\_10" preapply="0" mover\_name="mutate\_min" temperature="0.1" trials="240" recover\_low="1" reset\_baselines="1"/>

 <GenericMonteCarlo name="gmc\_sigs\_2" filter\_name="a\_obj\_func\_ddg\_2" preapply="0" mover\_name="mutate\_min" temperature="0.1" trials="80" recover\_low="1" reset\_baselines="1"/>

 <GenericSimulatedAnnealer name="sim\_anneal" mover\_name="mutate\_min" filter\_name="a\_obj\_func\_ddg\_2" trials="120" sample\_type="low" recover\_low="1" preapply="0" reset\_baselines="1" history="10"/>

 <SetupForSymmetry name="symm" definition="%%symm\_file%%" preserve\_datacache="false"/>

 </MOVERS>

 <PROTOCOLS>

 <Add mover="symm"/>

 <Add mover="add\_memb"/>

 <Add mover="atom\_cst"/>

 <Add mover="min\_mover"/>

 <Add mover="sim\_anneal"/>

 <Add mover="min\_mover"/>

 <Add filter="a\_total\_sig"/>

 <Add filter="a\_total\_final"/>

 <Add filter="a\_ddg\_sig\_2"/>

 <Add filter="a\_ddg\_sig\_10"/>

 <Add filter="a\_comp\_sig"/>

 <Add filter="a\_obj\_func\_ddg\_2"/>

 <Add filter="a\_obj\_func\_ddg\_10"/>

 <Add filter="a\_tms\_aa\_comp"/>

 <Add filter="a\_res\_lipo"/>

 <Add filter="a\_marl"/>

 <Add filter="a\_sasa"/>

 <Add filter="a\_total"/>

 <Add filter="a\_ddg"/>

 <Add filter="a\_bind"/>

 <Add filter="a\_pack"/>

 <Add filter="a\_unsat"/>

 <Add filter="a\_shape"/>

 <Add filter="a\_tms\_span"/>

 <Add filter="a\_helicality"/>

 <Add filter="a\_tms\_aa\_comp"/>

 </PROTOCOLS>

 <OUTPUT scorefxn="ref"/>

</ROSETTASCRIPTS>

**Command line**

~/Rosetta/main/source/bin/rosetta\_scripts.default.linuxgccreleas -database Rosetta/main/database @**flags\_seq\_divers**

flags\_seq\_divers:

-parser:protocol GMC\_seq\_diversifier.xml

-overwrite

-parser:script\_vars memb\_core=10

-parser:script\_vars steepness=4

-mute all

-nstruct 10

-parser:script\_vars s1=1

-parser:script\_vars s2=24

-parser:script\_vars e1=25

-parser:script\_vars e2=48

-parser:script\_vars o1=out2in

-parser:script\_vars o2=out2in

-mp:scoring:hbond

-jd2:ntrials 1000000

-use\_input\_sc

-s seed\_INPUT.pdb

-parser:script\_vars symm\_file=seed.symm

**FilterScan**

<ROSETTASCRIPTS>

 <SCOREFXNS>

 <ScoreFunction name="ddg\_sfx" weights="%%scorefxn%%" symmetric="1"/>

 <ScoreFunction name="full" weights="ref2015\_memb" symmetric="1">

 <Reweight scoretype="coordinate\_constraint" weight="%%cst\_value%%"/>

 </ScoreFunction>

 <ScoreFunction name="soft" weights="ref2015\_soft" symmetric="1">

 <Reweight scoretype="mp\_res\_lipo" weight="1"/>

 <Reweight scoretype="coordinate\_constraint" weight="%%cst\_value%%"/>

 </ScoreFunction>

 </SCOREFXNS>

 <TASKOPERATIONS>

 <InitializeFromCommandline name="init"/>

 <RestrictToRepacking name="rtr"/>

 <DesignAround name="des\_around" design\_shell="0.1" resnums="%%current\_res%%" repack\_shell="8.0"/>

 <OperateOnResidueSubset name="restrict\_res">

 <Index resnums="%%res\_to\_restrict%%"/>

 <RestrictToRepackingRLT/>

 </OperateOnResidueSubset>

 <OperateOnResidueSubset name="fix\_res">

 <Index resnums="%%res\_to\_fix%%"/>

 <PreventRepackingRLT/>

 </OperateOnResidueSubset>

 </TASKOPERATIONS>

 <MOVERS>

 <SetupForSymmetry name="symm" definition="%%symm\_file%%"/>

 <SymmetricAddMembraneMover name="add\_memb" membrane\_core="10" steepness="4">

 <Span start="1" end="24" orientation="in2out"/>

 <Span start="25" end="48" orientation="in2out"/>

</SymmetricAddMembraneMover>

 <TransformIntoMembraneMover name="tramsform" />

 <AtomCoordinateCstMover name="atom\_cst" coord\_dev="0.5" bounded="false" native="false"/>

 <MinMover name="min\_all" scorefxn="ddg\_sfx" chi="1" bb="1" jump="%%jump%%"/>#scorefxn\_full

 <SymPackRotamersMover name="soft\_repack" scorefxn="soft" task\_operations="init,rtr"/>

 <SymPackRotamersMover name="hard\_repack" scorefxn="full" task\_operations="init,rtr"/>

 <SymRotamerTrialsMover name="RTmin" scorefxn="full" task\_operations="init,rtr"/>

 <SymMinMover name="soft\_min" scorefxn="soft" chi="1" bb="1" jump="0"/>

 <SymMinMover name="hard\_min" scorefxn="full" chi="1" bb="1" jump="0"/>

 <ParsedProtocol name="refinement\_block"> #10 movers

 <Add mover\_name="soft\_repack"/>

 <Add mover\_name="soft\_min"/>

 <Add mover\_name="soft\_repack"/>

 <Add mover\_name="hard\_min"/>

 <Add mover\_name="hard\_repack"/>

 <Add mover\_name="hard\_min"/>

 <Add mover\_name="hard\_repack"/>

 Add mover\_name="RTmin"/>

 Add mover\_name="RTmin"/>

 <Add mover\_name="hard\_min"/>

 </ParsedProtocol>

 <LoopOver name="iter4" mover\_name="refinement\_block" iterations="4"/>

 </MOVERS>

 <FILTERS>

 <Ddg name="ddg" scorefxn="ddg\_sfx" threshold="0" repeats="5"/>#chain\_num="2"

 <ScoreType name="stability\_score\_full" scorefxn="full" score\_type="total\_score" threshold="0.0"/>

 <Delta name="delta\_score\_full" filter="stability\_score\_full" upper="1" lower="0" range="0.5"/> #upper and lower are booleans. Delta filters out all the mutations that are worse or better by less than -0.55R.E.U

 Delta name="delta\_score\_full" filter="stability\_score\_full" upper="1" lower="0" range="0.5"/> #upper and lower are booleans. Delta filters out all the mutations that are worse or better by less than -0.55R.E.U

 FilterScan name="filter\_scan" scorefxn="ddg\_sfx" relax\_mover="min\_all" keep\_native="%%keep\_n%%" task\_operations="init,des\_around,fix\_res,restrict\_res" delta\_filters="delta\_score\_full" delta="true" resfile\_name="%%resfiles\_path%%/res\_%%current\_res%%" report\_all="1" delta\_filter\_thresholds="%%fs\_thresholds%%" score\_log\_file="%%scores\_path%%/res%%current\_res%%\_score\_full.log" dump\_pdb="1" />

 <FilterScan name="filter\_scan" scorefxn="full" relax\_mover="iter4" keep\_native="%%keep\_n%%" task\_operations="init,des\_around,fix\_res,restrict\_res" delta\_filters="delta\_score\_full" delta="true" resfile\_name="%%resfiles\_path%%/res\_%%current\_res%%" report\_all="1" delta\_filter\_thresholds="%%fs\_thresholds%%" score\_log\_file="%%scores\_path%%/res%%current\_res%%\_score\_full.log" dump\_pdb="0" />

 </FILTERS>

 <PROTOCOLS>

 <Add mover="symm"/>

 <Add mover="add\_memb"/>

 Add mover="tramsform"/>

 <Add mover="atom\_cst"/>

 <Add filter="filter\_scan"/>

 </PROTOCOLS>

</ROSETTASCRIPTS>

**Command line**

~/Rosetta/main/source/bin/rosetta\_scripts.default.linuxgccreleas -database Rosetta/main/database @**flags\_filterscan**

Flags\_filterscan:

# general data

-parser:protocol filterscan\_auto\_refine\_SYMM.xml

#-database #path to database

-overwrite

# membrane spans: #changed to in2out 23Feb17 for experimental reasons

-parser:script\_vars res\_to\_fix=1A

-parser:script\_vars res\_to\_restrict=1A

-parser:script\_vars cst\_value=0.4

-parser:script\_vars jump=1

-parser:script\_vars scorefxn=ref2015\_memb

-parser:script\_vars span\_orientation\_2=in2out

-parser:script\_vars fs\_thresholds=0.0,0.5,1.0,1.5,2.0,2.5,3.0,3.5,4.0,4.5,5.0,5.5,6.0,6.5,7.0,8.0,9.0,10.0,11.0,12.0

-parser:script\_vars keep\_n=1

#-score::elec\_memb\_sig\_die

#-corrections::beta\_nov16

#-score:memb\_fa\_sol

-mp:scoring:hbond

-use\_input\_sc

-parser:script\_vars current\_res=3

-parser:script\_vars pdb\_dump=#filterscan/pdbs/3\_

-out:path:score score

-out:path:pdb pdbs

-parser:script\_vars resfiles\_path=./

-parser:script\_vars scores\_path=./

-parser:script\_vars symm\_file=seed.symm

***ab-initio* structure prediction**

<ROSETTASCRIPTS>

 <TASKOPERATIONS>

 <InitializeFromCommandline name="init"/>

 <RestrictToRepacking name="rtr"/>

 </TASKOPERATIONS>

 <SCOREFXNS>

 <ScoreFunction name="score0" weights="%%score\_func\_0%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 </ScoreFunction>

 <ScoreFunction name="score1" weights="%%score\_func\_1%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 <Reweight scoretype="mp\_nonhelix" weight="0"/>

 </ScoreFunction>

 <ScoreFunction name="score2" weights="%%score\_func\_2%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 <Reweight scoretype="mp\_nonhelix" weight="0"/>

 </ScoreFunction>

 <ScoreFunction name="score3" weights="%%score\_func\_3%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 <Reweight scoretype="mp\_nonhelix" weight="0"/>

 </ScoreFunction>

 <ScoreFunction name="score5" weights="%%score\_func\_5%%" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 <Reweight scoretype="mp\_nonhelix" weight="0"/>

 </ScoreFunction>

 <ScoreFunction name="ref" weights="ref2015\_memb" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 </ScoreFunction>

 <ScoreFunction name="refNotSymm" weights="ref2015\_memb" symmetric="0">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 </ScoreFunction>

 <ScoreFunction name="helicality" symmetric="1">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 </ScoreFunction>

 <ScoreFunction name="helicality\_notsymm" symmetric="0">

 <Reweight scoretype="mp\_helicality" weight="0"/>

 <Reweight scoretype="mp\_span\_ang" weight="0"/>

 </ScoreFunction>

 </SCOREFXNS>

 <MOVERS>

 <SetupForSymmetry name="symm" definition="%%symm\_file%%"/>

 <SymmetricAddMembraneMover name="add\_memb" membrane\_core="%%membrane\_core%%" steepness="%%steepness%%" span\_starts\_num="%%span\_starts%%" span\_ends\_num="%%span\_ends%%" span\_orientations="%%span\_oris%%"/>

 <MembranePositionFromTopologyMover name="init\_pos"/>

 <FastRelax name="fast\_relax" scorefxn="%%energy\_function%%" task\_operations="init"/>

 Fragment movers

 <SingleFragmentMover name="frag9" fragments="%%frags9mers%%" policy="uniform">

 <MoveMap>

 <Span begin="1" end="24" chi="1" bb="1"/>

 </MoveMap>

 </SingleFragmentMover>

 <SingleFragmentMover name="frag3" fragments="%%frags3mers%%" policy="smooth">

 <MoveMap>

 <Span begin="1" end="24" chi="1" bb="1"/>

 </MoveMap>

 </SingleFragmentMover>

 Fold-and-dock specific movers

 <SymFoldandDockRbTrialMover name="rbtrial" rot\_mag="8.0" trans\_mag="3.0" rotate\_anchor\_to\_x="1"/>

 <SymFoldandDockRbTrialMover name="rbtrial\_smooth" rot\_mag="1.0" trans\_mag="0.1" rotate\_anchor\_to\_x="1"/>

 <SymFoldandDockMoveRbJumpMover name="rbjump"/>

 <SymFoldandDockSlideTrialMover name="slidetrial"/>

 Random movers

 <RandomMover name="early\_stage\_moveset" movers="frag9,rbtrial,rbjump,slidetrial" weights="1.0,0.2,1.0,0.1" repeats="1"/>

 <RandomMover name="final\_stage\_moveset" movers="frag3,rbtrial\_smooth,rbjump,slidetrial" weights="1.0,0.2,1.0,0.1" repeats="1"/>

 Monte Carlo Movers

 <GenericMonteCarlo name="stage1" scorefxn\_name="score0" mover\_name="early\_stage\_moveset" temperature="2.0" trials="200" recover\_low="1"/>

 <GenericMonteCarlo name="stage2" scorefxn\_name="score1" mover\_name="early\_stage\_moveset" temperature="2.0" trials="200" recover\_low="1"/>

 <GenericMonteCarlo name="stage3a" scorefxn\_name="score2" mover\_name="early\_stage\_moveset" temperature="2.0" trials="20" recover\_low="1"/>

 <GenericMonteCarlo name="stage3b" scorefxn\_name="score5" mover\_name="early\_stage\_moveset" temperature="2.0" trials="20" recover\_low="1"/>

 <GenericMonteCarlo name="stage4" scorefxn\_name="score3" mover\_name="final\_stage\_moveset" temperature="2.0" trials="400" recover\_low="1"/>

 Special stage 3 logic

 <ParsedProtocol name="stage3\_cyc">

 <Add mover="stage3a"/>

 <Add mover="stage3b"/>

 </ParsedProtocol>

 <LoopOver name="stage3" mover\_name="stage3\_cyc" iterations="5" drift="1"/>

 Converts the centroid-level pose to fullatom for scoring

 <SwitchResidueTypeSetMover name="fullatom" set="fa\_standard"/>

 <ExtractAsymmetricPose name="extract\_asp" clear\_sym\_def="1"/>

 <MinMover name="min\_mover" scorefxn="refNotSymm" chi="1" bb="1" jump="1"/>

 <PackRotamersMover name="pack" scorefxn="refNotSymm" task\_operations="init,rtr"/>

 <RotamerTrialsMinMover name="RTmin" scorefxn="refNotSymm" task\_operations="init,rtr"/>

 <DumpPdb name="dump\_pdb" fname="dump.pdb" scorefxn="%%energy\_function%%"/>

 <SwitchChainOrder name="switch" chain\_order="123"/>

 <DeleteChain name="delete\_mem" chain="4" />

 </MOVERS>

 <FILTERS>

 <ScoreType name="total" scorefxn="%%energy\_function%%" score\_type="total\_score" confidence="1" threshold="0"/>

 <Sasa name="a\_sasa" confidence="0" threshold="300"/>

 <ResidueLipophilicity name="a\_res\_lipo" threshold="1000" confidence="0"/>

 <SpanTopologyMatchPose name="a\_span\_topo" confidence="0"/>

 <Ddg name="a\_ddg" scorefxn="%%energy\_function%%NotSymm" chain\_num="2" repeats="5" extreme\_value\_removal="true" confidence="0" threshold="-5"/>

 <PackStat name="a\_pack" confidence="0" threshold="0.3"/>

 <BuriedUnsatHbonds2 name="a\_unsat" scorefxn="%%energy\_function%%" confidence="0"/>

 <ShapeComplementarity name="a\_shape" confidence="0"/>

 <TMsSpanMembrane name="a\_tms\_span" confidence="1" min\_distance="25"/>

 <TMsSpanMembrane name="a\_tms\_span\_fa" confidence="0" min\_distance="25"/>

 <HelixHelixAngle name="a\_hha\_ang" angle\_or\_dist="angle" start\_helix\_1="%%span\_start\_1%%" end\_helix\_1="%%span\_end\_1%%" start\_helix\_2="%%span\_start\_2%%" end\_helix\_2="%%span\_end\_2%%" confidence="0"/>

 <HelixHelixAngle name="a\_hha\_dst\_vec" angle\_or\_dist="dist" dist\_by\_atom="0" start\_helix\_1="%%span\_start\_1%%" end\_helix\_1="%%span\_end\_1%%" start\_helix\_2="%%span\_start\_2%%" end\_helix\_2="%%span\_end\_2%%" confidence="0"/>

 <HelixHelixAngle name="a\_hha\_dst\_atm" angle\_or\_dist="dist" dist\_by\_atom="1" start\_helix\_1="%%span\_start\_1%%" end\_helix\_1="%%span\_end\_1%%" start\_helix\_2="%%span\_start\_2%%" end\_helix\_2="%%span\_end\_2%%" confidence="0"/>

 <MembAccesResidueLipophilicity name="a\_marl" confidence="0" verbose="0"/>

 <ScoreType name="a\_helicality" scorefxn="helicality\_notsymm" score\_type="mp\_helicality" confidence="0" threshold="10"/>

 <ScoreType name="a\_helicality\_symm" scorefxn="helicality" score\_type="mp\_helicality" confidence="0" threshold="10"/>

 <MPSpanAngle name="a\_angle\_1" tm="1" ang\_min="0" ang\_max="50" confidence="0"/>

 <MPSpanAngle name="a\_angle\_2" tm="2" ang\_min="0" ang\_max="50" confidence="0"/>

 <BindingStrain name="a\_bind" scorefxn="%%energy\_function%%" jump="1" confidence="0" threshold="5"/>

 <PoseInfo name="info"/>

 </FILTERS>

 <PROTOCOLS>

 <Add mover="symm"/>

 <Add mover="add\_memb"/>

 <Add mover="stage1"/>

 <Add mover="stage2"/>

 <Add mover="stage3"/>

 <Add mover="stage4"/>

 <Add filter="a\_helicality\_symm"/>

 <Add filter="a\_angle\_1"/>

 <Add filter="a\_angle\_2"/>

 <Add mover="fullatom"/>

 <Add filter="a\_tms\_span"/>

 <Add mover="fast\_relax"/>

 <Add filter="total"/>

 <Add filter="a\_sasa"/>

 <Add filter="a\_span\_topo"/>

 <Add mover="extract\_asp"/>

 <Add mover="pack"/>

 <Add mover="min\_mover"/>

 <Add mover="RTmin"/>

 <Add mover="RTmin"/>

 <Add filter="a\_tms\_span"/>

 <Add filter="total"/>

 <Add filter="a\_sasa"/>

 <Add filter="a\_span\_topo"/>

 <Add filter="a\_res\_lipo"/>

 <Add filter="a\_pack"/>

 <Add filter="a\_unsat"/>

 <Add filter="a\_shape"/>

 <Add filter="a\_ddg"/>

 <Add filter="a\_hha\_ang"/>

 <Add filter="a\_hha\_dst\_vec"/>

 <Add filter="a\_hha\_dst\_atm"/>

 <Add filter="a\_marl"/>

 <Add filter="a\_tms\_span\_fa"/>

 <Add filter="a\_helicality"/>

 <Add filter="a\_angle\_1"/>

 <Add filter="a\_angle\_2"/>

 <Add filter="a\_bind"/>

 </PROTOCOLS>

 <OUTPUT scorefxn="%%energy\_function%%NotSymm"/>

</ROSETTASCRIPTS>