Protocol Capture:

RosettaTMH:  Membrane Protein Structure Elucidation by Combining EPR Distance Restraints with Assembly of Transmembrane helices

Stephanie H. DeLuca\*, Samuel L. DeLuca\*, Jens Meiler\*

\*Center for Structural Biology, Vanderbilt University, Nashville TN USA

This document contains the protocol capture for the modeling performed for the publication of the same title by Stephanie DeLuca, Sam DeLuca, and Jens Meiler. For this particular publication, input files for folding were generated in Rosetta version 3.4 {Weiner:2013gg}. Rosetta revision numbers d592380 and d7b5a70 were used for RosettaTMH parameter optimization and benchmarking, respectively. For the purpose of this protocol capture, it is recommended that the user download weekly release 2014\_28\_57011, which is available at www.rosettacommons.org.

Information on how this input was generated can be found in the publication’s Supplemental Information. Additionally, EPR restraint simulation, some structural analysis, etc., were performed using the BioChemical Library (BCL) version 3.1.0, revision 4753 or later. Please visit www.meilerlab.org/bclcommonsfor more information on licensing and installation.

Preparation for folding

PDB identifiers (IDs) used for parameter optimization and testing

1FX8A, 1KPLA, 1PY6A, 1U19A, 3B60A, 3GIAA, 3HD6A, 3HFXA, 3O0RB

Benchmark set

1FX8A,1IWGA,1J4NA,1KPLA,1OCCC,1OKCA,1PV6A,1PY6A,1PY7A,1RHZA,1U19A,2BG9A,2BL2A,2BS2A,2IC8A,2K73A,2KSFA,2KSYA,2NR9A,2PNOA,2XQ2A,2XUTA,2YVXA,2ZW3A,3B60A,3GIAA,3HD6A,3HFXA,3KCUA,3KJ6A,3O0RB,3P5NA,3SYOA,4A2NB

FASTA files

1FX8A

>BCL :A|PDBID|CHAIN|SEQUENCE

TLKGQCIAEFLGTGLLIFFGVGCVAALKVAGASFGQWEISVIWGLGVAMA

IYLTAGVSGAHLNPAVTIALWLFACFDKRKVIPFIVSQVAGAFCAAALVY

GLYYNLFFDFEQTHHIVRGSVESVDLAGTFSTYPNPHINFVQAFAVEMVI

TAILMGLILALTDDGNGVPRGPLAPLLIGLLIAVIGASMGPLTGFAMNPA

RDFGPKVFAWLAGWGNVAFTGGRDIPYFLVPLFGPIVGAIVGAFAYRKLI

GRHL

1IWGA

> 1IWGA

SIHEVVKTLVEAIILVFLVMYLFLQNFRATLIPTIAVPVVLLGTFAVLAAFGFSINTLTMFGMVLAIGLLVDDAIVVVENVERVMAEEGLPPKEATRKSMGQIQGALVGIAMVLSAVFVPMAFFGGSTGAIYRQFSITIVSAMALSVLVALILTPALCATMLK

1J4NA

>BCL :A|PDBID|CHAIN|SEQUENCE

EFKKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQDNVK

VSLAFGLSIATLAQSVGHISGAHLNPAVTLGLLLSCQISVLRAIMYIIAQ

CVGAIVATAILSGITS

1KPLA

>BCL :A|PDBID|CHAIN|SEQUENCE

TPLAILFMAAVVGTLTGLVGVAFEKAVSWVQNMRIGALVQVADHAFLLWP

LAFILSALLAMVGYFLVRKFAPEAGGSGIPEIEGALEELRPVRWWRVLPV

KFIGGMGTLGAGMVLGREGPTVQIGGNLGRMVLDVFRMRSAEARHTLLAT

GAAAGLSAAFNAPLAGILFIIEEMRPQFRYNLISIKAVFTGVIMSSIVFR

IFN

1OCCC

>BCL :C|PDBID|CHAIN|SEQUENCE

HTPAVQKGLRYGMILFIISEVLFFTGFFWAFYHSSLAPTPELGGCWPPTG

IHPLNPLEVPLLNTSVLLASGVSITWAHHSLMEGDRKHMLQALFITITLG

VYFTLLQASEYYEAPFTISDGVYGSTFFVATGFHGLHVIIGSTFLIVCFF

RQLKFHFTSNHHFGFEAGAWYWHFVDVVWLFLYVSIYWWGS

1OKCA

>BCL :A|PDBID|CHAIN|SEQUENCE

DQALSFLKDFLAGGVAAAISKTAVAPIERVKLLLQVQHASKQISAEKQYK

GIIDCVVRIPKEQGFLSFWRGNLANVIRYFPTQALNFAFKDKYKQIFLGG

VDRHKQFWRYFAGNLASGGAAGATSLCFVYPLDFARTRLAADVGKGAAQR

EFTGLGNCITKIFKSDGLRGLYQGFNVSVQGIIIYRAAYFGVYDTAKGML

PDPKNVHIIVSWMIAQTVTAVAGLVSYPFDTVRRRMMMQSGRKGADIMYT

GTVDCWRKIAKDEGPKAFFKGAWSNVLRGMGGAFVLVLYDEI

1PV6A

>BCL :A|PDBID|CHAIN|SEQUENCE

MYYLKNTNFWMFGLFFFFYFFIMGAYFPFFPIWLHDINHISKSDTGIIFA

AISLFSLLFQPLFGLLSDKLGLRKYLLWIITGMLVMFAPFFIFIFGPLLQ

YNILVGSIVGGIYLGFCFNAGAPAVEAFIEKVSRRSNFEFGRARMFGCVG

WALGASIVGIMFTINNQFVFWLGSGCALILAVLLFFAKT

1PY6A

>BCL :A|PDBID|CHAIN|SEQUENCE

TGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITTLVPAIAF

TMYLSMLLGYGLTMVPFGGEQNPIYWARYADWLFTTPLLLLDLALLVDAD

QGTILALVGADGIMIGTGLVGALTKVYSYRFVWWAISTAAMLYILYVLFF

GFTSKAESMRPEVASTFKVLRNVTVVLWSAYPVVWLIGSEGAGIVPLNIE

TLLFMVLDVSAKVGFGLILLRSRAIFG

1PY7A

>BCL :A|PDBID|CHAIN|SEQUENCE

PIYWARYADWLFTTPLLLLDLALLVDADQGTILALVGADGIMIGTGLVGA

LTKVYSYRFVWWAISTAAMLYILYVLFFGFTSKAESMRPEVASTFKVLRN

VTVVLWSAYPVVWLIGSEGAGIV

1RHZA

>BCL :A|PDBID|CHAIN|SEQUENCE

FKEKLKWTGIVLVLYFIMGCIDVYTAGAQIPAIFEFWQTITASRIGTLIT

LGIGPIVTAGIIMQLLVGSGIIQMDLSIPENRALFQGCQKLLSIIMCFVE

AVLFVGAGAFGILTPLLAFLVIIQIAFGSIILIYLDEIVSKYGIGSGIGL

FIAAGVSQTIFVGALG

1U19A

>BCL :A|PDBID|CHAIN|SEQUENCE

EPWQFSMLAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVA

DLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFATLGGEIALWSLVVLA

IERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAPPLVGWSRYIPEG

MQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKE

AAAQQQESATTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSD

FGPIFMTIPAFFAKTSAVYNPVIYIMMN

2BG9A

>BCL :A|PDBID|CHAIN|SEQUENCE

PLYFVVNVIIPCLLFSFLTVLVFYLPTDSGEKMTLSISVLLSLTVFLLVI

VELIPSTSSAVPLIGKYMLFTMIFVISSIIVTVVVINTHHR

2BL2A

>BCL :A|PDBID|CHAIN|SEQUENCE

MVFAVLAMATATIFSGIGSAKGVGMTGEAAAALTTSQPEKFGQALILQLL

PGTQGLYGFVIAFLIFINLGSDMSVVQGLNFLGASLPIAFTGLFSGIAQG

KVAAAGIQILAKKPEHATKGIIFAAMVETYAILGFVISFLLVLNA

2BS2A

>BCL :C|PDBID|CHAIN|SEQUENCE

RMPAKLDWWQSATGLFLGLFMIGHMFFVSTILLGDNVMLWVTKKFELDFI

FEGGKPIVVSFLAAFVFAVFIAHAFLAMRKFPINYRQYLTFKTHKDLMRH

GDTTLWWIQAMTGFAMFFLGSVHLYIMMTQPQTIGPVSSSFRMVSEWMWP

LYLVLLFAVELHGSVGLYRLAVKWGWFDGETPDKTRANLKKLKTLMSAFL

IVLGLLTFGAYVKKGLE

2IC8A

>BCL :A|PDBID|CHAIN|SEQUENCE

ERAGPVTWVMMIACVVVFIAMQILGDQEVMLWLAWPFDPTLKFEFWRYFT

HALMHFSLMHILFNLLWWWYLGGAVEKRLGSGKLIVITLISALLSGYVQQ

KFSGPWFGGLSGVVYALMGYVWLRGERDPQSGIYLQRGLIIFALIWIVAG

WFDLFGMSMANGAHIAGLAVGLAMAFVDSLNA

2K73A

>BCL :A|PDBID|CHAIN|SEQUENCE

MLRFLNQASQGRGAWLLMAFTALALELTALWFQHVMLLKPCVLSIYERAA

LFGVLGAALIGAIAPKTPLRYVAMVIWLYSAFRGVQLTYEHTMLQLYPSP

FATSDFMVRFPEWLPLDKWVPQVFVASGDCAERQWDFLGLEMPQWLLGIF

IAYLIVAVLVVISQ

2KSFA

>BCL :A|PDBID|CHAIN|SEQUENCE

MVQIQGSVVAAALSAVITLIAMQWLMAFDAANLVMLYLLGVVVVALFYGR

WPSVVATVINVVSFDLFFIAPRGTLAVSDVQYLLTFAVMLTVGLVIGNLT

AGVRYQA

2KSYA

>BCL :A|PDBID|CHAIN|SEQUENCE

MVGLTTLFWLGAIGMLVGTLAFAWAGRDAGSGERRYYVTLVGISGIAAVA

YAVMALGVGWVPVAERTVFVPRYIDWILTTPLIVYFLGLLAGLDSREFGI

VITLNTVVMLAGFAGAMVPGIERYALFGMGAVAFIGLVYYLVGPMTESAS

QRSSGIKSLYVRLRNLTVVLWAIYPFIWLLGPPGVALLTPTVDVALIVYL

DLVTKVGFGFIALDAAATLRAEH

2NR9A

>BCL :A|PDBID|CHAIN|SEQUENCE

FLAQQGKITLILTALCVLIYIAQQLGFEDDIMYLMHYPAYEEQDSEVWRY

ISHTLVHLSNLHILFNLSWFFIFGGMIERTFGSVKLLMLYVVASAITGYV

QNYVSGPAFFGLSGVVYAVLGYVFIRDKLNHHLFDLPEGFFTMLLVGIAL

GFISPLFGVEMGNAAHISGLIVGLIWGFIDSKLRKNSLELVP

2PNOA

>BCL :A|PDBID|CHAIN|SEQUENCE

KDEVALLAAVTLLGVLLQAYFSLQVISARRAFRVSPPLTTGPPEFERVYR

AQVNCSEYFPLFLATLWVAGIFFHEGAAALCGLVYLFARLRYFQGYARSA

QLRLAPLYASARALWLLVALAALGLLAHFL

2XQ2A

>BCL :A|PDBID|CHAIN|SEQUENCE

SFIDIMVFAIYVAIIIGVGLWVSRDKKGTQKSTEDYFLAGKSLPWWAVGA

SLIAANISAEQFIGMSGSGYSIGLAIASYEWMSAITLIIVGKYFLPIFIE

KGIYTIPEFVEKRFNKKLKTILAVFWISLYIFVNLTSVLYLGGLALETIL

GIPLMYSILGLALFALVYSIYGGLSAVVWTDVIQVFFLVLGGFMTTYMAV

SFIGGTDGWFAGVSKMVDAAPGHFEMILDQSNPQYMNLPGIAVLIGGLWV

ANLYYWGFNQYIIQRTLAAKSVSEAQKGIVFAAFLALIVPFLVVLPGIAA

YVITSDPQLMASLGDIAATNLPSAANADKAYPWLTQFLPVGVKGVVFAAL

AAAIVSSLASMLNSTATIFTMDIYKEYISPDSGDHKLVNVGRTAAVVALI

IAALIAPMLGGIGQCFQYIQEYTGLVSPGILAVFLLGLFWKKTTSKGAII

GVVASIPFALFLKFMPLSMPFMDQMLYTLLFTMVVIAFTSLSTSINDDDP

KGISVTSSMFVTDRSFNIAAYGIMIVLAVLYTLFWVNADAEITLIIFGVM

AGVIGTILLISYGIK

2XUTA

>BCL :A|PDBID|CHAIN|SEQUENCE

QIPYIIASEACERFSFYGMRNILTPFLMTALLLSIPEELRGAVAKDVFHS

FVIGVYFFPLLGGWIADRFFGKYNTILWLSLIYCVGHAFLAIFEHSVQGF

YTGLFLIALGSGGIKPLVSSFMGDQFDQSNKSLAQKAFDMFYFTINFGSF

FASLSMPLLLKNFGAAVAFGIPGVLMFVATVFFWLGRKRYIHMPPEPKDP

HGFLPVIRSALLTKVEGKGNIGLVLALIGGVSAAYALVNIPTLGIVAGLC

CAMVLVMGFVGAGASLQLERARKSHPDAAVDGVRSVLRILVLFALVTPFW

SLFDQKASTWILQANDMVKPQWFEPAMMQALNPLLVMLLIPFNNFVLYPA

IERMGVKLTALRKMGAGIAITGLSWIVVGTIQLMMDGGSALSIFWQILPY

ALLTFGEVLVSATGLEFAYSQAPKAMKGTIMSFWTLSVTVGNLWVLLANV

SVKSPTVTEQIVQTGMSVTAFQMFFFAGFAILAAIVFA

2YVXA

>BCL :A|PDBID|CHAIN|SEQUENCE

HKLGAVDVPDLVYSEAGPVALWLARVRWLVILILTGMVTSSILQGFESVL

EAVTALAFYVPVLLGTGGNTGNQSATLIIRALATRDLDLRDWRRVFLKEM

GVGLLLGLTLSFLLVGKVYWDGHPLLLPVVGVSLVLIVFFANLVGAMLPF

LLRRLGVDPALVSNPLVATLSDVTGLLIYLSVARLLLE

2ZW3A

>BCL :A|PDBID|CHAIN|SEQUENCE

DWGTLQTILGGVNKHSTSIGKIWLTVLFIFRIMILVVAAKEVWGDEQADF

VCNTLQPGCKNVCYDHYFPISHIRLWALQLIFVSTPALLVAMHVAYRRHE

KKRKFIKGEIKSEFKDIEEIKTQKVRIEGSLWWTYTSSIFFRVIFEAAFM

YVFYVMYDGFSMQRLVKCNAWPCPNTVDCFVSRPTEKTVFTVFMIAVSGI

CILLNVTELCYLLIRY

3B60A

>BCL :A|PDBID|CHAIN|SEQUENCE

WQTFRRLWPTIAPFKAGLIVAGIALILNAASDTFMLSLLKPLLDDGFGKT

DRSVLLWMPLVVIGLMILRGITSYISSYCISWVSGKVVMTMRRRLFGHMM

GMPVAFFDKQSTGTLLSRITYDSEQVASSSSGALITVVREGASIIGLFIM

MFYYSWQLSIILVVLAPIVSIAIRVVSKRFRSISKNMQNTMGQVTTSAEQ

MLKGHKEVLIFGGQEVETKRFDKVSNKMRLQGMKMVSASSISDPIIQLIA

SLALAFVLYAASFPSVMDSLTAGTITVVFSSMIALMRPLKSLTNVNAQFQ

RGMAACQTLFAILDSEQEK

3GIAA

>BCL :A|PDBID|CHAIN|SEQUENCE

LKNKKLSLWEAVSMAVGVMIGASIFSIFGVGAKIAGRNLPETFILSGIYA

LLVAYSYTKLGAKIVSNAGPIAFIHKAIGDNIITGALSILLWMSYVISIA

LFAKGFAGYFLPLINAPINTFNIAITEIGIVAFFTALNFFGSKAVGRAEF

FIVLVKLLILGLFIFAGLITIHPSYVIPDLAPSAVSGMIFASAIFFLSYM

GFGVITNASEHIENPKKNVPRAIFISILIVMFVYVGVAISAIGNLPIDEL

IKASENALAVAAKPFLGNLGFLLISIGALFSISSAMNATIYGGANVAYSL

AKDGELPEFFERKVWFKSTEGLYITSALGVLFALLFNMEGVASITSAVFM

VIYLFVILSHYILIDEVGGRKEIVIFSFIVVLGVFLLLLYYQWITNRFVF

YGIIATFIGVLIFEIIYRKVTKRTFSNNMYVKS

3HD6A

>BCL :A|PDBID|CHAIN|SEQUENCE

SAWNTNLRWRLPLTCLLLQVIMVILFGVFVRYDFENEFYYRYPSFQDVHV

MVFVGFGFLMTFLQRYGFSAVGFNFLLAAFGIQWALLMQGWFHFLQDRYI

VVGVENLINADFCVASVCVAFGAVLGKVSPIQLLIMTFFQVTLFAVNEFI

LLNLLKVKDAGGSMTIHTFGAYFGLTVTRILYRRNLEQSKERQNSVYQSD

LFAMIGTLFLWMYWPSFNSAISYHGDSQHRAAINTYCSLAACVLTSVAIS

SALHKKGKLDMVHIQNATLAGGVAVGTAAEMMLMPYGALIIGFVCGIIST

LGFVYLTPFLESRLHIQDTCGINNLHGIPGIIGGIVGAVTAASDWTARTQ

GKFQIYGLLVTLAMALMGGIIVGLILRLPFWGQPSDENCFEDAVYWEMPE

GNS

3HFXA

>BCL :A|PDBID|CHAIN|SEQUENCE

PKVFFPPLIIVGILCWLTVRDLDAANVVINAVFSYVTNVWGWAFEWYMVV

MLFGWFWLVFGPYAKKRLGNEPPEFSTASWIFMMFASCTSAAVLFWGSIE

IYYYISTPPFGLEPNSTGAKELGLAYSLFHWGPLPWATYSFLSVAFAYFF

FVRKMEVIRPSSTLVPLVGEKHAKGLFGTIVDNFYLVALIFAMGTSLGLA

TPLVTECMQWLFGIPHTLQLDAIIITCWIILNAICVACGLQKGVRIASDV

RSYLSFLMLGWVFIVSGASFIMNYFTDSVGMLLMYLPRMLFYTDPIAKGG

FPQGWTVFYWAWWVIYAIQMSIFLARISRGRTVRELCFGMVLGLTASTWI

LWTVLGSNTLLLIDKNIINIPNLIEQYGVARAIIETWAALPLSTATMWGF

FILCFIATVTLVNACSYTLAMSTCREVRDGEEPPLLVRIGWSILVGIIGI

VLLALGGLKPIQTAIIAGGCPLFFVNIMVTLSFIKDAKQNWKD

3KCUA

>BCL :A|PDBID|CHAIN|SEQUENCE

KHPLKTFYLAITAGVFISIAFVFYITATTGTGTMPFGMAKLVGGICFSLG

LILCVVCGADLFTSTVLIVVAKASGRITWGQLAKNWLNVYFGNLVGALLF

VLLMWLSGEYMTANGQWGLNVLQTADHKVHHTFIEAVCLGILANLMVCLA

VWMSYSGRSLMDKAFIMVLPVAMFVASGFEHSIANMFMIPMGIVIRDFAS

PEFWTAVGSAPENFSHLTVMNFITDNLIPVTIGNIIGGGLLVGLTYWVIY

LR

3KJ6A

>BCL :A|PDBID|CHAIN|SEQUENCE

GMGIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADLVMGL

AVVPFGAAHILMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAVDRYFA

ITSPFKYQSLLTKNKARVIILMVWIVSGLTSFLPIQMHWYRATHQEAINC

YAEETCCDFFTNQAYAIASSIVSFYVPLVIMVFVYSRVFQEAKRQLQKID

KSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKEHKALKTLGIIMGTFTL

CWLPFFIVNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRI

AFQELLCLRRS

3O0RB

>BCL :B|PDBID|CHAIN|SEQUENCE

FASQAVAKPYFVFALILFVGQILFGLIMGLQYVVGDFLFPAIPFNVARMV

HTNLLIVWLLFGFMGAAYYLVPEESDCELYSPKLAWILFWVFAAAGVLTI

LGYLLVPYAGLARLTGNELWPTMGREFLEQPTISKAGIVIVALGFLFNVG

MTVLRGRKTAISMVLMTGLIGLALLFLFSFYNPENLTRDKFYWWWVVHLW

VEGVWELIMGAILAFVLVKITGVDREVIEKWLYVIIAMALISGIIGTGHH

YFWIGVPGYWLWLGSVFSALEPLPFFAMVLFAFNTINRRRRDYPNRAVAL

WAMGTTVMAFLGAGVWGFMHTLAPVNYYTHGTQLTAAHGHMAFYGAYAMI

VMTIISYAMPRLRGIGEAMDNRSQVLEMWGFWLMTVAMVFITLFLSAAGV

LQVWLQRMPADGAAMTFMATQDQLAIFYWLREGAGVVFLIGLVAYLLSF

3P5NA

>BCL :A|PDBID|CHAIN|SEQUENCE

QQNKRLITISMLSAIAFVLTFIKFPIPFLPPYLTLDFSDVPSLLATFTFG

PVAGIIVALVKNLLNYLFSMGDPVGPFANFLAGASFLLTAYAIYKNKRST

KSLITGLIIATIVMTIVLSILNYFVLLPLYGMIFNLADIANNLKVIIVSG

IIPFNIIKGIVISIVFILLYRRLANFLKR

3SYOA

>BCL :A|PDBID|CHAIN|SEQUENCE

YRYLTDIFTTLVDLKWRFNLLIFVMVYTVTWLFFGMIWWLIAYIRGDMDH

IEDPSWTPCVTNLNGFVSAFLFSIETETTIGYGYRVITDKCPEGIILLLI

QSVLGSIVNAFMVGCMFVKISQ

4A2NB

>BCL :B|PDBID|CHAIN|SEQUENCE

MNENLWKICFIVMFIIWVFVRKVYGTRAMKNKSKKKVRPNFEKSLVFLNF

IGMVFLPLTAVFSSYLDSFNINLPDSIRLFALIVTFLNIGLFTKIHKDLG

NNWSAILEIKDGHKLVKEGIYKNIRHPMYAHLWLWVITQGIILSNWVVLI

FGIVAWAILYFIRVPKEEELLIEEFGDEYIEYMGKTGRLFPK

Fragment files

The fragment files and secondary structure prediction files are the same ones used for the modelling published in reference {Weiner:2013gg}. Information on how this input was generated can be found in the publication’s Supplemental Information.

# Example using 1U19A. Was repeated for all nine proteins in benchmark set

# command line from Weiner, et al. 2013

## make\_fragments.pl performed 3 times, each time, adding PDB IDs to the homolog files, that occur in more than 30% of the fragments.

## CVS information:

## $Revision: 5280 $

## $Date: 2004-10-08 17:21:30 -0500 (Fri, 08 Oct 2004) $

## $Author: dylan $

make\_fragments.pl -id 1U19A -psipredfile 1U19A.psipred\_ss2 -jufofile 1U19A.jufo -nosam -verbose 1U19A.fasta -nohoms -nojufo –nopsipred >& log &

# Now using Rosetta-3.4

/rosetta/rosetta-3.4/rosetta\_tools/fragment\_tools/make\_fragments.pl -id 1U19A -verbose -nohoms -psipredfile 1U19A.psipred\_ss2 -nopsipred 1U19A.fasta >& log &

Generation of spanfiles and lipophilicity files

The spanfiles and lipophilicity files are the same ones used for the modelling published in reference {Weiner:2013gg}. Information on how this input was generated can be found in the publication’s Supplemental Information. Spanfiles were generated using the Rosetta version 3.4 octopus2span.pl script and the SPOCTOPUS prediction as input. Lipophilicty files were generated using run\_lips.pl script.

# Example running octopus2span.pl using 1U19. Was repeated for all nine proteins in benchmark set

/rosetta-3.4/rosetta\_source/src/apps/public/membrane\_abinitio/octopus2span.pl 1U19A.octo\_topo > 1U19A.span

# Example spanfile – 1U19A

TM region prediction for 1U19A.octo\_topo predicted using OCTOPUS

7 278

antiparallel

n2c

7 27 7 27

43 63 43 63

82 102 82 102

120 140 120 140

171 191 171 191

221 241 221 241

255 275 255 275

# Example of running run\_lips.pl on 1U19A – produces 1U19A.lips4

/rosetta-3.4/rosetta\_source/src/apps/public/membrane\_abinitio/run\_lips.pl 1U19A.fasta 1U19A.span /my\_dir/blastpgp /rosetta-3.4/rosetta\_source/src/apps/public/membrane\_abinitio/alignblast.pl >& log &

# Example lipophilicity file – first 10 lines of 1U19A.lips4

Lipid exposed data: resnum mean-lipo lipophil entropy

12 -1.000 2.302 3.334

15 -1.000 3.410 3.819

16 -1.000 3.064 6.190

19 -1.000 0.894 2.784

22 -1.000 2.158 4.783

23 -1.000 0.382 1.011

26 -1.000 1.748 2.160

8 -1.000 2.230 5.054

9 -1.000 1.424 5.436

Generation of Topology Broker “rigid” files for computing RMSD100SSE

Taken from secondary structure element definitions from DSSP and are similar to that used for evaluation of models in reference {Weiner:2013gg}. The RIGID definitions indicate the beginning and end points of the region of the protein over which RMSD100 will be computed.

Residues over which RMSD100SSE was computed

All native PDBs were renumbered starting at residue 1, as are all models folded with Rosetta. This is what the following lists of residues assume.

# 1FX8A

RIGID 2 29

RIGID 36 58

RIGID 64 73

RIGID 78 114

RIGID 121 130

RIGID 140 162

RIGID 173 192

RIGID 199 212

RIGID 227 253

# 1IWGA

RIGID 2 23

RIGID 31 51

RIGID 57 85

RIGID 93 122

RIGID 129 160

# 1J4NA

RIGID 2 31

RIGID 48 70

RIGID 76 85

RIGID 90 115

# 1KPLA

RIGID 2 40

RIGID 48 70

RIGID 79 87

RIGID 97 111

RIGID 117 136

RIGID 141 160

RIGID 163 174

RIGID 185 202

# 1OCCA

RIGID 3 36

RIGID 59 83

RIGID 86 113

RIGID 122 154

RIGID 163 189

# 1OKCA

RIGID 3 36

RIGID 72 98

RIGID 107 141

RIGID 175 198

RIGID 208 239

RIGID 272 290

# 1PV6A

RIGID 7 38

RIGID 42 70

RIGID 74 101

RIGID 104 136

RIGID 140 164

RIGID 166 186

# 1PY6A

RIGID 5 29

RIGID 33 58

RIGID 76 97

RIGID 101 123

RIGID 127 158

RIGID 161 187

RIGID 197 221

# 1PY7A

RIGID 4 25

RIGID 29 51

RIGID 55 86

RIGID 89 115

# 1RHZA

RIGID 2 20

RIGID 53 67

RIGID 79 107

RIGID 115 141

RIGID 147 165

# 1U19A

RIGID 2 32

RIGID 39 68

RIGID 75 108

RIGID 118 140

RIGID 168 193

RIGID 210 245

RIGID 253 277

# 2BG9A

RIGID 2 28

RIGID 33 60

RIGID 65 90

# 2BL2A

RIGID 2 36

RIGID 41 68

RIGID 75 112

RIGID 117 144

# 2BS2A

RIGID 2 33

RIGID 56 80

RIGID 101 129

RIGID 148 174

RIGID 182 216

# 2IC8A

RIGID 5 24

RIGID 58 79

RIGID 81 103

RIGID 111 127

RIGID 137 152

RIGID 161 180

# 2K73A

RIGID 12 36

RIGID 42 63

RIGID 68 96

RIGID 142 163

# 2KSFA

RIGID 7 25

RIGID 35 48

RIGID 55 66

RIGID 80 103

# 2KSYA

RIGID 3 28

RIGID 33 56

RIGID 70 91

RIGID 95 117

RIGID 122 152

RIGID 154 180

RIGID 190 222

# 2NR9A

RIGID 7 24

RIGID 60 81

RIGID 83 105

RIGID 112 129

RIGID 140 151

RIGID 163 189

# 2PNOA

RIGID 5 32

RIGID 43 73

RIGID 75 98

RIGID 101 129

# 2XQ2A

RIGID 2 21

RIGID 45 72

RIGID 74 101

RIGID 116 150

RIGID 154 172

RIGID 178 204

RIGID 247 267

RIGID 272 305

RIGID 340 378

RIGID 384 409

RIGID 415 439

RIGID 445 464

RIGID 471 493

RIGID 514 536

RIGID 538 564

# 2XUTA

RIGID 3 29

RIGID 39 69

RIGID 73 92

RIGID 97 125

RIGID 132 162

RIGID 165 187

RIGID 217 240

RIGID 246 270

RIGID 283 316

RIGID 326 345

RIGID 361 388

RIGID 397 421

RIGID 430 451

RIGID 468 487

# 2YVXA

RIGID 18 45

RIGID 61 84

RIGID 92 121

RIGID 127 155

RIGID 164 182

# 2ZW3A

RIGID 22 47

RIGID 72 105

RIGID 125 155

RIGID 184 215

# 3B60A

RIGID 15 45

RIGID 52 101

RIGID 112 154

RIGID 156 204

RIGID 214 262

RIGID 274 314

# 3GIAA

RIGID 8 35

RIGID 38 63

RIGID 82 114

RIGID 120 140

RIGID 142 170

RIGID 182 208

RIGID 215 244

RIGID 268 303

RIGID 319 335

RIGID 338 362

RIGID 371 395

RIGID 397 421

# 3HD6A

RIGID 9 29

RIGID 37 62

RIGID 67 92

RIGID 104 124

RIGID 130 155

RIGID 163 181

RIGID 197 219

RIGID 225 253

RIGID 261 281

RIGID 285 314

RIGID 322 342

RIGID 345 377

# 3HFXA

RIGID 4 20

RIGID 41 60

RIGID 77 106

RIGID 117 149

RIGID 176 209

RIGID 218 237

RIGID 244 266

RIGID 301 327

RIGID 333 364

RIGID 395 422

RIGID 436 455

RIGID 458 490

# 3KCUA

RIGID 3 28

RIGID 36 57

RIGID 79 107

RIGID 133 156

RIGID 160 177

RIGID 182 198

RIGID 219 250

# 3KJ6A

RIGID 2 24

RIGID 33 56

RIGID 75 102

RIGID 113 129

RIGID 174 197

RIGID 233 252

RIGID 274 292

# 3O0RB

RIGID 2 33

RIGID 44 75

RIGID 82 105

RIGID 132 154

RIGID 160 180

RIGID 186 221

RIGID 225 250

RIGID 258 285

RIGID 296 321

RIGID 333 362

RIGID 371 408

RIGID 417 448

# 3P5NA

RIGID 2 21

RIGID 51 69

RIGID 75 95

RIGID 100 122

RIGID 145 175

# 3SYOA

RIGID 16 45

RIGID 92 121

# 4A2NB

RIGID 3 30

RIGID 39 62

RIGID 75 99

RIGID 127 143

RIGID 146 175

Topology Broker setup files

# Using 1U19A as an example

# folding from an extended chain

# protocol\_capture/demo/broker\_setup/extended\_epr/1U19A\_s00\_b000\_0000.cst.tpb

CLAIMER MembraneTopologyClaimer

END\_CLAIMER

# folding from an extended chain with using EPR restraints (extended chain + EPR)

# protocol\_capture/demo/broker\_setup/extended\_epr/1U19A\_s01\_b001\_0000.cst.tpb

CLAIMER MembraneTopologyClaimer

END\_CLAIMER

CLAIMER ConstraintClaimer

FILE protocol\_capture/input\_files/1U19A\_s01\_b001\_0000.cst

END\_CLAIMER

# folding with RosettaTMH

# protocol\_capture/demo/broker\_setup/tmh\_epr/1U19A\_s00\_b000\_0000.cst.tpb

CLAIMER MembraneTopologyClaimer

END\_CLAIMER

CLAIMER TMHTopologySamplerClaimer

END\_CLAIMER

# folding with RosettaTMH with EPR restraints (RosettaTMH + EPR)

# protocol\_capture/demo/broker\_setup/tmh\_epr/1U19A\_s01\_b001\_0000.cst.tpb

CLAIMER MembraneTopologyClaimer

END\_CLAIMER

CLAIMER TMHTopologySamplerClaimer

END\_CLAIMER

CLAIMER ConstraintClaimer

FILE protocol\_capture/input\_files/1U19A\_s01\_b001\_0000.cst

END\_CLAIMER

Simulating EPR distance restraints using the BCL

# converted Rosetta native PDB file to BCL format

# NOT NECESSARY if have PDB file already in BCL format (pdb\_bcl.pdb)

#!/bin/csh

# BCL v3.1.0, r4753, compiled on Mon Feb 10 03:23:31 2014

# given a list of PDB IDs (pdb.ls)

foreach pdb ( `cat ../pdb.ls` )

sed -i '/CEN/d' ${pdb}.pdb

bcl.exe protein:PDBConvert ${pdb}.pdb -bcl\_pdb -output\_prefix ${pdb}\_ >& ${pdb}\_bcl.log

end

# mutate.wts for generating EPR distance restraints using the BCL

bcl::storage::Table<**double**>    add\_all add\_single filter\_aa\_type\_excl filter\_sse\_size remove\_single       swap distance\_range\_0 filter\_exposure\_0

weights 0 1 0 0 1 1 0 0

# score.wts for generating EPR distance restraints using the BCL

bcl::storage::Table<**double**> data\_density aa\_type\_excl    seq\_sep data\_set\_size sse\_connection   sse\_size   sse\_term    bipolar sse\_center triangulation\_0 distance\_range\_0 exposure\_0

weights 0 0 1 1 1 0 0 0 0 0 10000 10000

# SSE pool for 1U19A – required to run bcl.exe restraint:OptimizeDataSetPairwise

# All pool files based on spanfiles (TMH definitions)

bcl::assemble::SSEPool

HELIX 1 1 PRO A 2 GLN A 32 1 31

HELIX 2 2 PRO A 39 HIS A 68 1 30

HELIX 3 3 GLY A 74 VAL A 107 1 34

HELIX 4 4 GLU A 118 LEU A 140 1 23

HELIX 5 5 ASN A 168 GLN A 193 1 26

HELIX 6 6 THR A 210 THR A 245 1 36

HELIX 7 7 PRO A 253 MET A 276 1 24

END

# command line for restraint picking

# BCL v3.1.0, r4753, compiled on Mon Feb 10 03:23:31 2014

bcl.exe restraint:OptimizeDataSetPairwise -fasta protocol\_capture/demo/input\_files/1U19A.fasta -pool\_min\_sse\_lengths 3 0 -pool protocol\_capture/demo/input\_files/1U19A\_native.pool -distance\_min\_max 10 50 -nc\_limit 10 -ensembles pdb.ls -mc\_number\_iterations 10000 10000 -prefix 1U19A -nmodels 10 -read\_scores\_optimization ./score.wts -read\_mutates\_optimization mutate.wts -read\_mutates\_start ./mutate.wts -message\_level Standard -pymol\_output -data\_set\_size\_range 10 40 -data\_set\_size\_fraction\_of\_sse\_resis 0.2 >& log &

### scores.wts and mutate.wts in demo are different because some scores do not exist in more recent versions of BCL. Namely, filter\_sse size (mutate.wts) and sse\_connect, sse\_size, sse\_term, bipolar, sse\_center (score.wts). If using a newer version of the BCL to simulate EPR distance restraints, contact Jens Meiler (jens.meiler@vanderbilt.edu) for best practices.

# adding spin label uncertainty

# BCL v3.1.0, r4753, compiled on Mon Feb 10 03:23:31 2014

bcl.exe SimulateDistanceRestraints -pdb 1U19A\_bcl.pdb -simulate\_distance\_restraints -output\_file 1U19A\_s01\_b001\_0000.cst -add\_distance\_uncertainty sl-cb\_distances.histograms -restraint\_list 1U19A\_final\_0000.data 0 1 5 6 -random\_seed 0123456 -write\_rosetta\_mini\_restraints

Convert restraint files to fold membrane proteins in Rosetta

# example using 1U19A from within protocol\_capture/simulate\_epr\_restraints/1U19A directory

# original cst file name: 1U19A\_final\_0000.cst; final name: 1U19A\_s01\_b001\_0000.cst

protocol\_capture/demo/1U19A\_bcl\_sim\_epr/1U19A\_make\_mp\_cst\_file.sh

# replace HA2 to 2HA

sed -i 's/HA2/2HA/g' 1U19A\_s01\_b001\_0000.cst

Rosetta restraint file format

# weighting EPR KBP by 10.0 and quadratic penalty by 1.0

# if have Gly in AtomPair, replace CB with 1HA or 2HA

AtomPair CB 67 CB 255 SCALARWEIGHTEDFUNC 1.0 SPLINE EPR\_DISTANCE 28.9577 1.0 0.5

AtomPair CB 67 CB 255 SCALARWEIGHTEDFUNC 1.0 BOUNDED 16.9577 40.9577 1.0 NOE ;dist

Building loops on BCL and RosettaTMH files (for the purposes of measuring RMSD between BCL and Rosetta models)

# Loops file format – residues defined in loops are all residues not covered by spanfile

# EXAMPLE: 1FX8A

LOOP 1 6

LOOP 26 37

LOOP 57 82

LOOP 102 141

LOOP 161 172

LOOP 192 229

LOOP 249 254

# convert BCL files to Rosetta files and make loops files for Rosetta loop building

# BCL v3.1.0, r4753, compiled on Mon Feb 10 03:23:31 2014

# from the protocol\_capture/demo/build\_loops/input\_pdbs directory:

# given a list of PDB files formatted for use in the BCL (without .pdb extension:

foreach pdb (`cat pdb.ls`)

bcl.exe protein:PDBConvert ${pdb}.pdb -loop\_file\_rosetta CCD -write\_zero\_coordinates -bcl\_pdb Split -output\_prefix ../loops/${pdb} >& ${pdb}\_loops.log

end

# Build loops in Rosetta (with options file (see fill\_gaps.options) – from a PDB file

# used to build loops on RosettaTMH models and BCL models

# weekly release 2014\_28\_57011

# example in protocol\_capture/demo/build\_loops

/Rosetta/main/source/bin/loopmodel.default.linuxgccrelease -database /Rosetta/main/database/ @fill\_gaps.options -in:file:l pdb.ls -out:pdb\_gz -out:prefix example\_ -out:no\_nstruct\_label -out:file:scorefile example\_build\_loops.sc >& log &

Restraint weights for folding in Rosetta (CST\_WEIGHT in options file)

pdb weight

1FX8A 10.5596

1IWGA 8.8708

1J4NA 8.3960

1KPLA 9.7660

1OCCA 9.6317

1OKCA 13.1527

1PV6A 8.6969

1PY6A 9.8134

1PY7A 8.0013

1RHZA 10.4518

1U19A 10.9355

2BG9A 6.8484

2BL2A 8.1081

2BS2A 10.9428

2IC8A 10.7754

2K73A 11.0377

2KSFA 9.1686

2KSYA 9.4516

2NR9A 11.0417

2PNOA 7.9325

2XQ2A 13.5716

2XUTA 12.7241

2YVXA 10.2313

2ZW3A 12.4219

3B60A 10.5270

3GIAA 12.2199

3HD6A 12.0958

3HFXA 14.0806

3KCUA 11.5959

3KJ6A 14.9617

3O0RB 11.9658

3P5NA 10.9224

3SYOA 10.9717

4A2NB 11.0417

Options files for de novo folding in Rosetta

# assume the options file is in the protocol\_capture/demo directory

# MembraneAbinitio

-in

-file

-native input\_files/1U19A.pdb

-fasta input\_files/1U19A.fasta

-frag3 input\_files/aa1U19A03\_05.200\_v1\_3

-frag9 input\_files/aa1U19A09\_05.200\_v1\_3

-spanfile 1U19A.span

-lipofile 1U19A.lips4

-residues

-patch\_selectors CENTROID\_HA

-score

-find\_neighbors\_3dgrid

# -use\_membrane\_rg #### use this flag if using MP-specific RG score

-membrane

-no\_interpolate\_Mpair

-Menv\_penalties

-abinitio

-membrane

-explicit\_pdb\_debug # if want to output at stages 0-4

-rg\_reweight 4.25

-stage2\_patch input\_files/score\_membrane\_s2.wts\_patch

-stage3a\_patch input\_files/score\_membrane\_s3a.wts\_patch

-stage3b\_patch input\_files/score\_membrane\_s3b.wts\_patch

-stage4\_patch input\_files/score\_membrane\_s4.wts\_patch

-evaluation

-gdtmm

-rmsd NATIVE \_tm\_sse input\_files/1U19A\_tm\_sse\_052814.txt

-out

-output

-file

-output\_virtual

-silent\_struct\_type binary

-overwrite

# extended chain (and extended chain + EPR when CST\_WEIGHT ≠ 0.0)

# assume the options file is in the protocol\_capture/demo directory

-in

-file

-native input\_files/1U19A.pdb

-fasta input\_files/1U19A.fasta

-frag3 input\_files/aa1U19A03\_05.200\_v1\_3

-frag9 input\_files/aa1U19A09\_05.200\_v1\_3

-spanfile input\_files/1U19A.span

-lipofile input\_files/1U19A.lips4

-residues

-patch\_selectors CENTROID\_HA

-broker

-setup broker\_setup/extended\_epr/1U19A\_s00\_b000\_0000.cst.tpb #### will follow format of broker setup file above

# broker\_setup/extended\_epr/1U19A\_s01\_b001\_0000.cst.tpb if using EPR restraints

-run

-protocol broker

-score

-find\_neighbors\_3dgrid

-membrane

-no\_interpolate\_Mpair

-Menv\_penalties

-abinitio

-membrane

-explicit\_pdb\_debug # if want to output at stages 0-4

-rg\_reweight 4.25

-stage2\_patch input\_files/score\_membrane\_s2.wts\_patch

-stage3a\_patch input\_files/score\_membrane\_s3a.wts\_patch

-stage3b\_patch input\_files/score\_membrane\_s3b.wts\_patch

-stage4\_patch input\_files/score\_membrane\_s4.wts\_patch

-constraints

-cst\_file input\_files/1U19A\_s00\_b000\_0000.cst # or 1U19A\_s01\_b001\_0000.cst

-cst\_weight 0.0 # or 10.9355

-epr\_distance

-fold\_cst

-force\_minimize

-seq\_sep\_stages 1.0 1.0 1.2

-evaluation

-gdtmm

-rmsd NATIVE \_tm\_sse input\_files/1U19A\_tm\_sse\_052814.txt

-out

-output

-file

-output\_virtual

-silent\_struct\_type binary

-overwrite

# RosettaTMH (and RosettaTMH + EPR when cst\_weight != 0.0)

# assume the options file is in the protocol\_capture/demo directory

-in

-file

-native input\_files/1U19A.pdb

-fasta input\_files/1U19A.fasta

-frag3 input\_files/aa1U19A03\_05.200\_v1\_3

-frag9 input\_files/aa1U19A09\_05.200\_v1\_3

-spanfile input\_files/1U19A.span

-lipofile input\_files/1U19A.lips4

-residues

-patch\_selectors CENTROID\_HA

-broker

-setup broker\_setup/tmh\_epr/1U19A\_s00\_b000\_0000.cst.tpb #### will follow format of broker setup file above

# broker\_setup/tmh\_epr/1U19A\_s01\_b001\_0000.cst.tpb if using EPR restraints

-large\_frag\_mover\_stage1\_weight 0.0

-small\_frag\_mover\_stage1\_weight 0.0

-rb\_mover\_stage1\_weight 5.0

-run

-protocol broker

-score

-find\_neighbors\_3dgrid

# -use\_membrane\_rg #### use this flag if using MP-specific RG score

-membrane

-fixed\_membrane

-no\_interpolate\_Mpair

-Menv\_penalties

-abinitio

-membrane

-explicit\_pdb\_debug # if want to output at stages 0-4

-rg\_reweight 4.25

-stage2\_patch input\_files/score\_membrane\_s2.wts\_patch

-stage3a\_patch input\_files/score\_membrane\_s3a.wts\_patch

-stage3b\_patch input\_files/score\_membrane\_s3b.wts\_patch

-stage4\_patch input\_files/score\_membrane\_s4.wts\_patch

-constraints

-cst\_file input\_files/1U19A\_s00\_b000\_0000.cst # or 1U19A\_s01\_b001\_0000.cst

-cst\_weight 0.0 # or 10.9355

-epr\_distance

-fold\_cst

-force\_minimize

-seq\_sep\_stages 1.0 1.0 1.2

-rigid

-rotation 0.1

-translation 0.5

-evaluation

-gdtmm

-rmsd NATIVE \_tm\_sse input\_files/1U19A\_tm\_sse\_052814.txt

-out

-output

-file

-output\_virtual

-silent\_struct\_type binary

-overwrite

# loop building onto BCL and RosettaTMH models

# (from the protocol\_capture/demo/build\_loops directory)

-in

-file

-native 1U19A/1U19A.pdb

-spanfile 1U19A/1U19A.span

-lipofile 1U19A/1U19A.lips4

-residue\_type\_set centroid

-chemical

-patch\_selectors CENTROID\_HA

-score

-find\_neighbors\_3dgrid

-evaluation

-rmsd NATIVE \_tm\_sse 1U19A/1U19A\_tm\_sse\_052814.txt

-membrane

-no\_interpolate\_Mpair

-Menv\_penalties

-loops

-loop\_file 1U19A/1U19A.loops

-frag\_sizes 9 3 1

-frag\_files 1U19A/aa1U19A09\_05.200\_v1\_3 1U19A/aa1U19A03\_05.200\_v1\_3 none

-remodel quick\_ccd

-cen\_weights score\_membrane

-cen\_patch 1U19A/score\_membrane\_s4.wts\_patch

-out

-output

-no\_nstruct\_label

-nstruct 1

-file

-silent\_struct\_type binary # only if outputting silent files, not pdb or pdb\_gz files

-residue\_type\_set centroid

-overwrite

Score patches

# score\_membrane\_s2.wts\_patch

pair = 0.0

Mpair = 1.0

env = 0.0

Menv = 2.019

cbeta = 0.0

Mcbeta = 0.0

Menv\_non\_helix = 2.019

Menv\_termini = 2.019

Menv\_tm\_proj = 2.019

Mlipo = 1.0

# score\_membrane\_s3a.wts\_patch

pair = 0.0

Mpair = 1.0

env = 0.0

Menv = 2.019

cbeta = 0.0

Mcbeta = 0.5

Menv\_non\_helix = 2.019

Menv\_termini = 2.019

Menv\_tm\_proj = 2.019

Mlipo = 1.0

# score\_membrane\_s3b.wts\_patch

pair = 0.0

Mpair = 1.0

env = 0.0

Menv = 2.019

cbeta = 0.0

Mcbeta = 0.5

Menv\_non\_helix = 2.019

Menv\_termini = 2.019

Menv\_tm\_proj = 2.019

Mlipo = 1.0

# score\_membrane\_s4.wts\_patch

pair = 0.0

Mpair = 1.0

env = 0.0

Menv = 2.019

cbeta = 0.0

Mcbeta = 2.5

Menv\_non\_helix = 2.019

Menv\_termini = 2.019

Menv\_tm\_proj = 2.019

Mlipo = 1.0

Command lines for *de novo* folding

MembraneAbinitio

# Rosetta revision numbers d592380 and d7b5a70.

# For 1U19A, takes approximately 15 minutes

/Rosetta/main/source/bin/membrane\_abinitio2.default.linuxgccrelease -database /Rosetta/main/database/ @memabrlx\_no\_cst.options -out::nstruct 1 -out:file:silent output\_files/1U19A\_memabrlx\_no\_cst.out -out:sf output\_files/1U19A\_memabrlx\_no\_cst.sc

Extended chain (and Extended chain + EPR if CST\_WEIGHT ­≠ 0.0)

# Rosetta revision numbers d592380 and d7b5a70.

# Note that for rmsd values, look in the silent file, not the score file

# For 1U19A with no restraints, takes approximately 3 minutes

# For 1U19A with restraints, takes approximately 9.5 minutes

/Rosetta/main/source/bin/minirosetta.default.linuxgccrelease -database /Rosetta/main/database/ @mp\_extended.options -out::nstruct 1 -out:file:silent output\_files/1U19A\_extended\_no\_cst.out -out:file:scorefile output\_files/1U19A\_extended\_no\_cst.sc >& output\_files/extended\_no\_cst.log &

RosettaTMH (and RosettaTMH + EPR if CST\_WEIGHT != 0.0)

# Rosetta revision numbers d592380 and d7b5a70.

# Note that for rmsd values, look in the silent file, not the score file

# For 1U19A with no restraints, takes approximately 75 seconds

# For 1U19A with restraints, takes approximately 5 minutes

/Rosetta/main/source/bin/minirosetta.default.linuxgccrelease -database /Rosetta/main/database/ @mp\_rosettatmh.options -out::nstruct 1 -out:file:silent output\_files/1U19A\_rosettatmh\_no\_cst.out -out:file:scorefile output\_files/1U19A\_rosettatmh\_no\_cst.sc >& output\_files/1U19A\_rosettatmh\_no\_cst.log &

Weighting schemes tested

Radius of gyration (RG) score weights tested

Both default and membrane protein (MP)-specific RG scores were weighted by 0.0, 0.01, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.00 when testing effect of MP-specific RG score.

EPR restraint weights tested. KBP = knowledge-based potential.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Quadratic Penalty** | | | | | | |
| **EPR KBP** |  | 0.0 | 1.0 | 10.0 | 20.0 | 30.0 | 40.0 | 50.0 |
| 0.0 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 1.0 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 10.0 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 20.0 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 30.0 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 40.0 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| 50.0 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

Analysis of Results

Generation of RMSD­100SSE histograms

# format for files for input into rmsd\_to\_rmsd100.py found in protocol capture scripts directory

# doesn’t matter how many fields are between field 1 and description, but “SCORE:” must be first, and “description” last, also total score is “score”

# example is protocol\_capture/demo/analysis/1U19A\_s01\_b001\_0000\_example.sc

SCORE: score atom\_pair\_constraint rms\_tm\_sse description

SCORE: -124.890 -368.904 17.8586 S\_0001

SCORE: 96.155 -329.637 16.3458 S\_0002

SCORE: -71.851 -372.237 13.7901 S\_0003

SCORE: -149.703 -360.364 15.2572 S\_0004

SCORE: -169.673 -364.787 17.3802 S\_0005

# convert Rosetta-computed RMSD values to RMSD100

# type rmsd\_to\_rmsd100.py –h for commandline options

protocol\_capture/scripts/rmsd\_to\_rmsd100.py --membrane --silent 1U19A\_s01\_b001\_0000\_example.sc -n 278 --outfile 1U19A\_s01\_b001\_0000\_example.sc.RMSDSSE100 --rms\_tag=tm\_sse

# Generate histograms and summary

perl ../scripts/Smbins\_RMSD\_dist\_from\_score.pl 1U19A\_s01\_b001\_0000\_example.sc.RMSDSSE100 5 | awk '{print($2"\t"$4)}' | head -n21 > 1U19A\_s01\_b001\_0000\_example.sc.rmsdSSE100.txt

Convert Rosetta silent files to PDB files

/Rosetta/main/source/bin/format\_converter.default.linuxgccrelease -database /Rosetta/main/database -in:file:silent 1U19A\_rosettatmh\_cst.out -in:file:silent\_struct\_type binary -in:file:residue\_type\_set centroid -out:pdb -out:file:residue\_type\_set centroid

Calculating contact order

Downloaded script from Baker laboratory website (http://depts.washington.edu/bakerpg/contact\_order/)

# options: -c = cutoff, default is 6; -a = absolute contact order

./scripts/contactOrder.pl –c 8 –a S\_0001\_0001.pdb