RosettaCM: multi-template comparative modeling

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Why comparative modeling with RosettaCM?

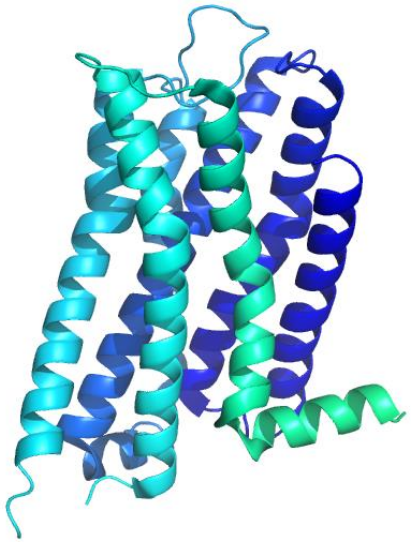
- Deep learning-based methods offer less flexibility – you get what you get and you don't throw a fit
- CM is compatible with NMR, EPR, and electron Density constraints data
- Modeling mutations better with biophysical techniques than MSA based techniques

Sequence

```
>3PBL
DYKDDDDGAPASLSQLSSHLNYTCGAENSTGASQARPHAYYA
LSYCALILAIVFGNGLVCMAVLKERALQTTTNYLVVSLAVAD
LLVATLVMPWVVYLEVTGGVWNFSRICCDVFVTLDVMMCTAS
IWNLCAISIDRYTAVVMPVHYQHGTGQSSCRRVALMITAVWV
LAFAVSCPLLFGFNTTGDPTVCSISNPDFVIYSSVVSFYLPF
GVTVLVYARIYVVLKQRRRKNIFEMLRIDEGLRLKIYKDTEG
YYTIGIGHLLTKSPSLNAAKSELDKAIGRNTNGVITKDEAEK
LFNQDVDAAVRGILRNAKLKPVYDSLDAVRRAALINMVFQMG
ETGVAGFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRV
ITTFRTGTWDAYGVPLREKKATQMVAIVLGAFIVCWLPFFLT
HVLNTHCQTCHVSPELYSATTWLGYVNSALNPVIYTTFNIEF
RKAFLKILSCGRPLEVLFQ
```

?

Homology model



McDonald et al. *Structural Comparative Modeling of Multi-Domain F508del CFTR*, Biomolecules, 2022

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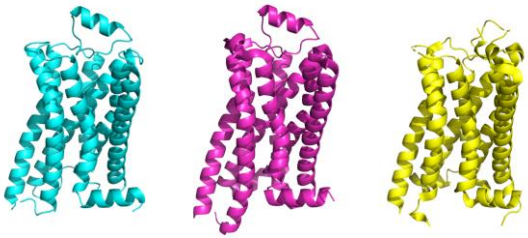
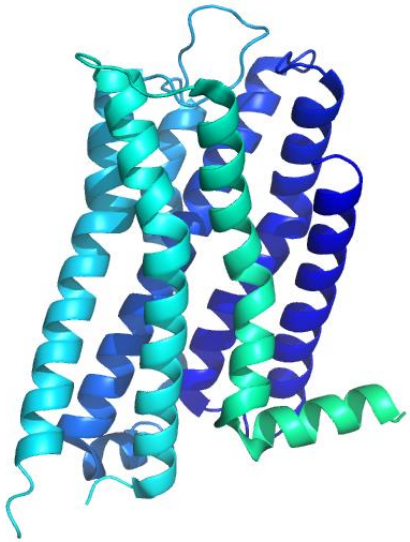
IWNLCAISIDRYTAVVMPVHYQHGTGQSSCRRVALMITAVWV
LAFAVSCPLLFGFNTTGDPTVCSISNPDFVIYSSVVSFYLPF
GVTVLVYARIYVVLKQRRRKNIFEMLRIDEGLRLKIYKDTEG
YYTIGIGHLLTKSPSLNAAKSELDKAIGRNTNGVITKDEAEK
LFNQDVDAAVRGILRNAKLKPVYDSLDAVRRAALINMVFQMG
ETGVAGFTNSLRMLQQKRWDEAAVNLAKSRWYNQTPNRAKRV
ITTFRTGTWDAYGVPLREKKATQMVAIVLGAFIVCWLPFFLT
HVLNTHCQTCHVSPELYSATTWLGYVNSALNPVIYTTFNIEF
RKAFLKILSCGRPLEVLFQ
```

RosettaCM

template PDBs



Homology model



Single template versus multiple template modeling

- Single Template Modeling:
  - Single template as input
  - Uses sequence and template derived fragments
  - Used when available templates have very high identity (>60%)
- Multiple Template Modeling:
  - Multiple templates as input
  - Combine sections of multiple threaded models and sequence derived fragments
  - Used when available templates have low identity (30-50%)
- *Nomenclature Note*
  - Comparative Modeling = Homology Modeling in the land of Rosetta

General workflow for RosettaCM

1. Identification template sequences
2. Preparation of sequence alignments
3. Threading
4. Hybridize
5. Relaxation
6. Scoring and Selection

In the tutorial: Comparative modeling of the Dopamine D3 receptor

Target sequence of dopamine D3 receptor

(PDB: Dopamine D3 receptor 3pbl)

Find this file at */rosetta_cm/demo/input_files/3pbl.fasta*

>3pbl

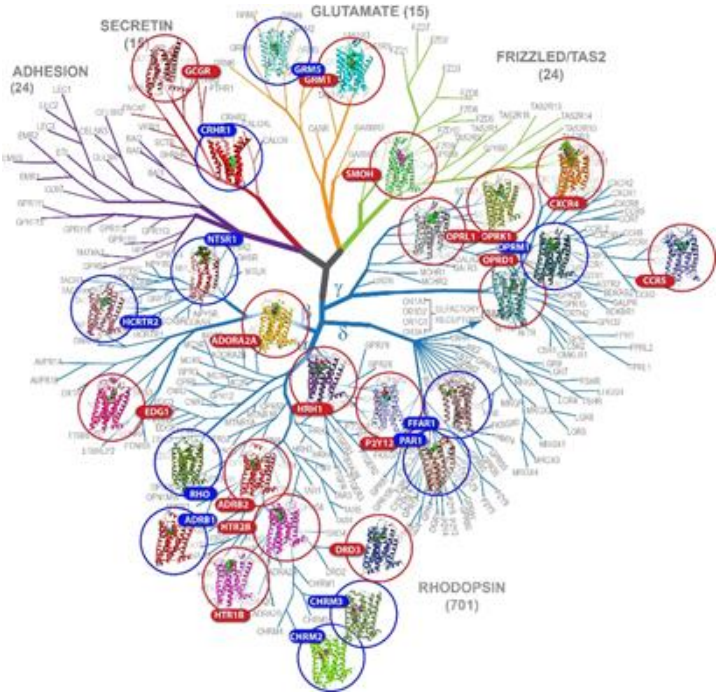
YALSYCALILAIVFGNGLVCMAVLKERALQTTTNYLVVSLAVADLLVATLVMPWVVYLEVTGGVWNFSRICCDVF
VTLDVMMCTASIWNLCAISIDRYTAVVMPVHYQHGTGQSSCRRVALMITAVWVLAFAVSCPLLFGFNTTGDPTVC
SISNPDFVIYSSVVSFYLPFGVTVLVYARIYVVLKQRRRKAAAAAAAAGVPLREKKATQMVAIVLGAFIVCWLPF
FLTHVLNTHCQTCHVSPELYSATTWLGYVNSALNPVIYTTFNIEFRKAFLKILSC

http://www.ncbi.nlm.nih.gov/protein

Template identification for dopamine D3 receptor

- PDB ID: 3pbl
- Class A G-protein coupled receptor (GPCR)
- No high identity templates

- 7 transmembrane helices
- 3 extracellular loops, 3 intracellular loops
- Highly conserved GPCR residues



GPCR phylogenetic tree with crystal structures (2014). Taken from https://katritch.usc.edu/research.html

Template identification for dopamine D3 receptor

- Similarity of Sequences :
  - compare proteins based on amino acid sequences (BLASTP using PDB as search database)
  - suitable templates have ideally >30% sequence identity to the target

- Fold Recognition:
  - using predicted secondary structure information to detect proteins with similar 3D characteristics (DALI, PHYRE)

Template identification for Dopamine D3 receptor

- It is advisable to use multiple templates due to the low sequence identity in available templates

Template	PDB ID	% Seq id
β2-adrenoceptor	3SN6	36
5-HT1B receptor	4IAR	32
β2-adrenoceptor	3D4S	34
5-HT2B receptor	5TVN	32
M1 receptor	5CXV	32
H1 receptor	3RZE	31
M4 receptor	5DSG	29
A2A receptor	2YDO	28
A1 receptor	5N2S	27

Template identification for dopamine D3 receptor

Human 5HT-1B receptor (PDB: 4iar)

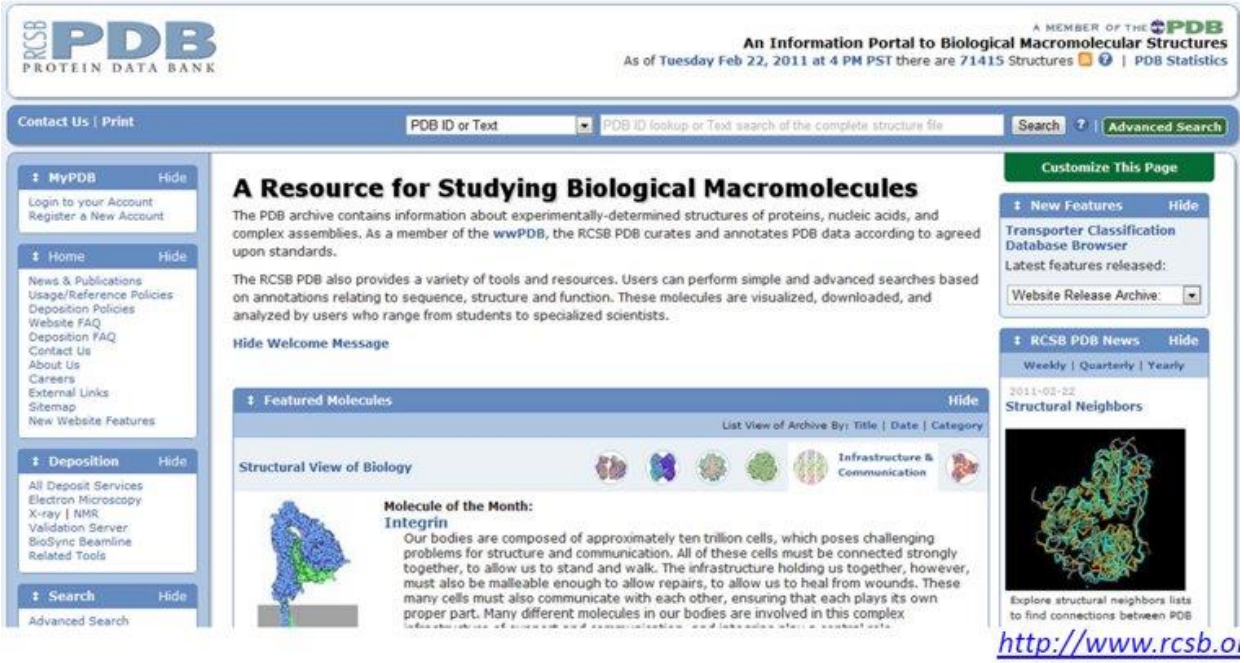
Human beta1-adrenoceptor (PDB: 4bvn)

Human B2-adrenergic receptor (PDB: 2rh1)

Human M4 muscarinic acetylcholine receptor (PDB: 5dsg)

Human M1 muscarinic acetylcholine receptor (PDB: 5cxv)

Find these files at */rosetta_cm/template_pdbs/original_files/*



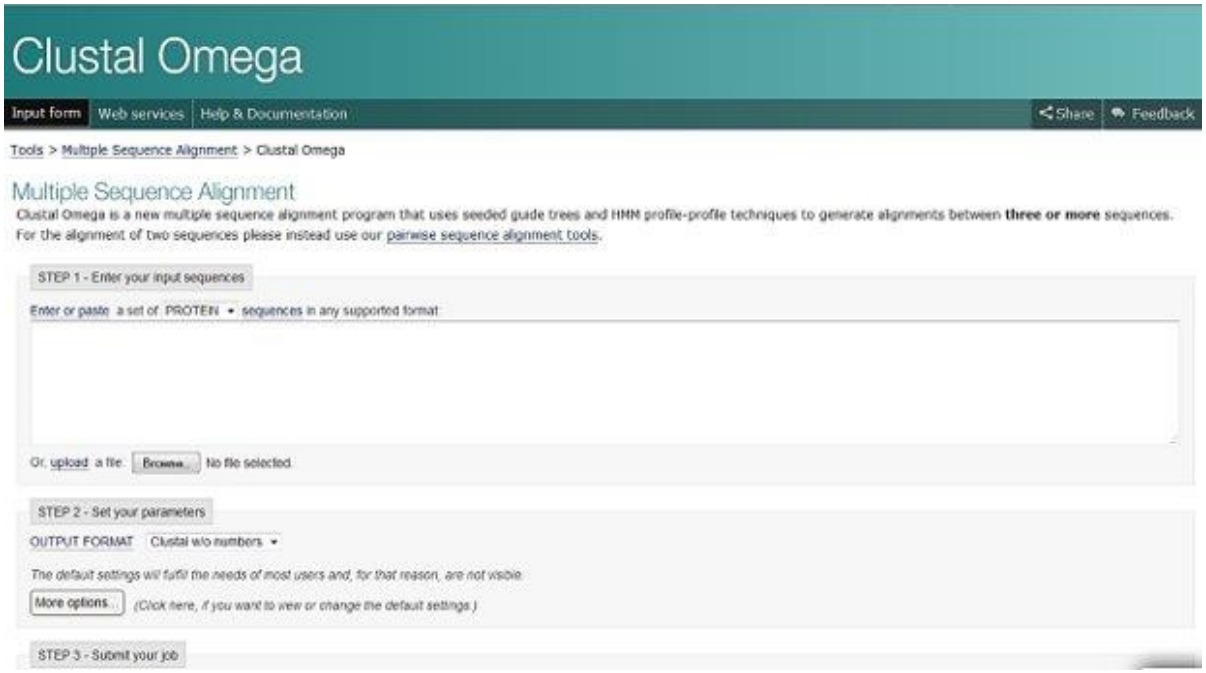
http://www.rcsb.o

Multiple sequence alignment

```
CLUSTAL O(1.2.4) multiple sequence alignment


5cxv      -------KGPWQVAFIGITTGLLSLATVTGNLLVLISFKVNTELKTVNNYFLLSLACADL
5dsg      GPSSHNRYETVEMVFIATVTGSLSLVTVVGNILVMLSIKVNRQLQTVNNYFLFSLACADL
3pbl      ---------------YALSYCALILAIVFGNGLVCMAVLKERALQTTTNYLVVSLAVADL
4iar      YIYQDSISLPWKV-LLVMLLALITLATTLSNAFVIATVYRTRKLHTPANYLIASLAVTDL
2rh1      -------DEVWVV-GMGIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADL
4bvn      ------LSQQWEA-GMSLLMALVVLLIVAGNVLVIAAIGSTQRLQTLTNLFITSLACADL
```

Find this file at */demo/alignment_files/3pbl_alignments.txt*



http://www.ebi.ac.uk/Tools/msa/clustalo/

Adjusting multiple sequence alignments

- Experimental expectations:
  - Highly conserved residues
  - Secondary structure elements

Raw ClustalO alignment:

```
3pbl  ---------------YALSYCALILAIVFGNGLVCMAVLKERALQTTTNYLVVSLAVADL
5cxv  -------KGPWQVAFIGITTGLLSLATVTGNLLVLISFKVNTELKTVNNYFLLSLACADL
5dsg  GPSSHNRYETVEMVFIATVTGSLSLVTVVGNILVMLSIKVNRQLQTVNNYFLFSLACADL
4iar  YIYQDSISLPWKV-LLVMLLALITLATTLSNAFVIATVYRTRKLHTPANYLIASLAVTDL
2rh1  -------DEVWVV-GMGIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADL
4bvn  ------LSQQWEA-GMSLLMALVVLLIVAGNVLVIAAIGSTQRLQTLTNLFITSLACADL
```

Adjusted alignment:

```
3pbl  ---------------YALSYCALILAIVFGNGLVCMAVLKE-RALQT-TTNYLVVSLAVADL
5cxv  -------KGPWQVAFIGITTGLLSLATVTGNLLVLISFKVN-TELKT-VNNYFLLSLACADL
5dsg  GPSSHNRYETVEMVFIATVTGSLSLVTVVGNILVMLSIKVN-RQLQT-VNNYFLFSLACADL
4iar  -YIYQDSISLPWKVLLVMLLALITLATTLSNAFVIATVYRT-RKLHT-PANYLIASLAVTDL
2rh1  --------DEVWVVGMGIVMSLIVLAIVFGNVLVITAIAKF-ERLQT-VTNYFITSLACADL
4bvn  -------LSQQWEAGMSLLMALVVLLIVAGNVLVIAAIGST-QRLQT-LTNLFITSLACADL
```

helix regions

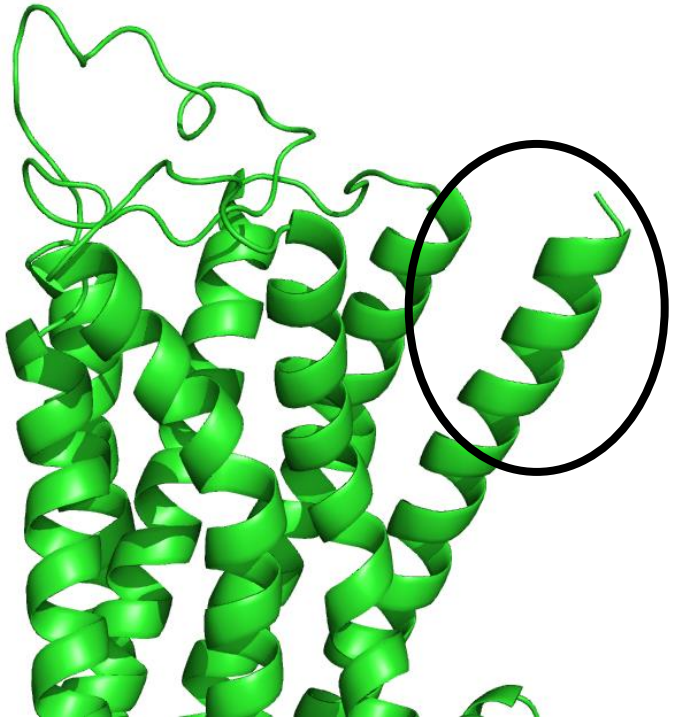
highly conserved residues

Alignment issues to be resolved

predicted membrane spanning region from OCTOPUS

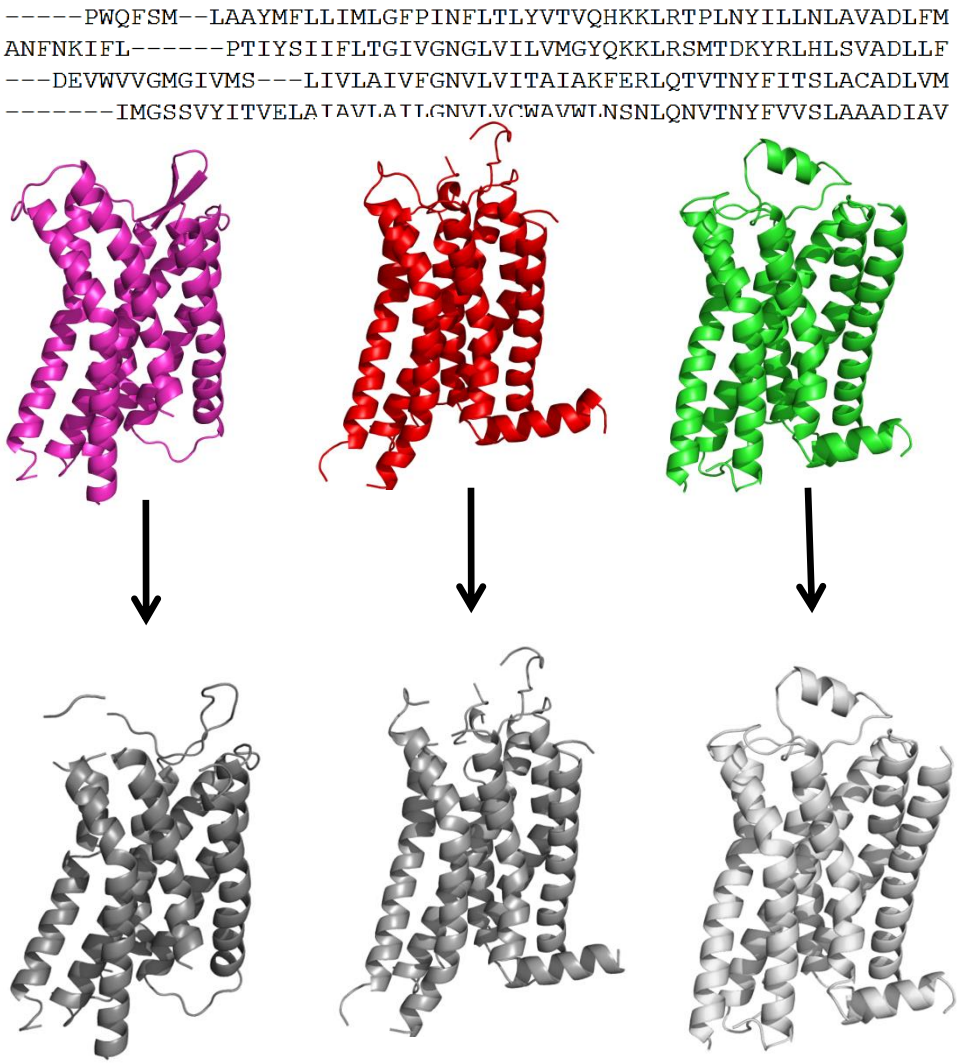
Adjusted multiple sequence alignments result in improved modeling perfomance

Example model using raw alignment

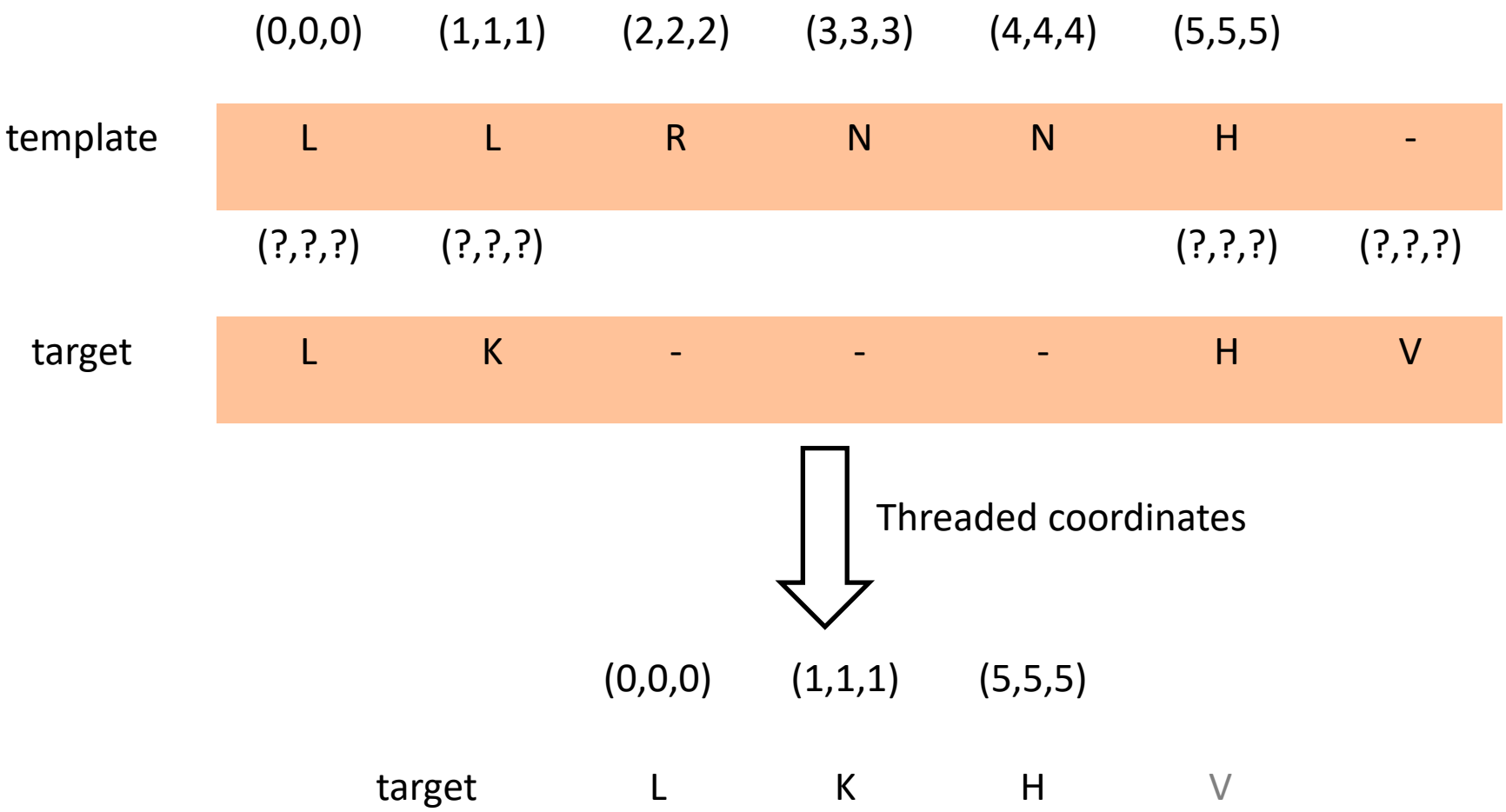


Example model using raw alignment

Partial Thread



Partial Thread



Partial thread only excepts alignments in grishin format

- ClustalO format:
  - All sequences in one file
  - Sequences broken up over several lines
- Grishin format:
  - One file per alignment pair
  - Sequences continuous over one line each
  - Contains header information
  - Due to complicated format, we have provided a script for conversion `make_alignment_files.sh` for your use back home

Find converted Grishin alignment files at */rosetta_cm/demo/alignment_files/*

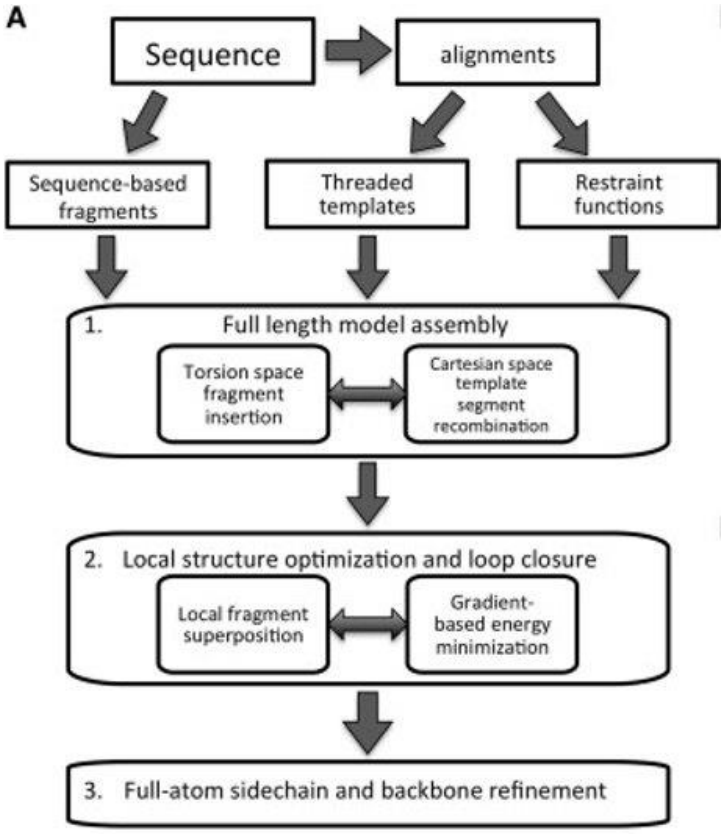
(2rh1.aln 4bvn.aln 4iar.aln 5cxv.aln 5dsg.aln)

Hybridize protocol contains three stages

1. Generate initial models from template alignments

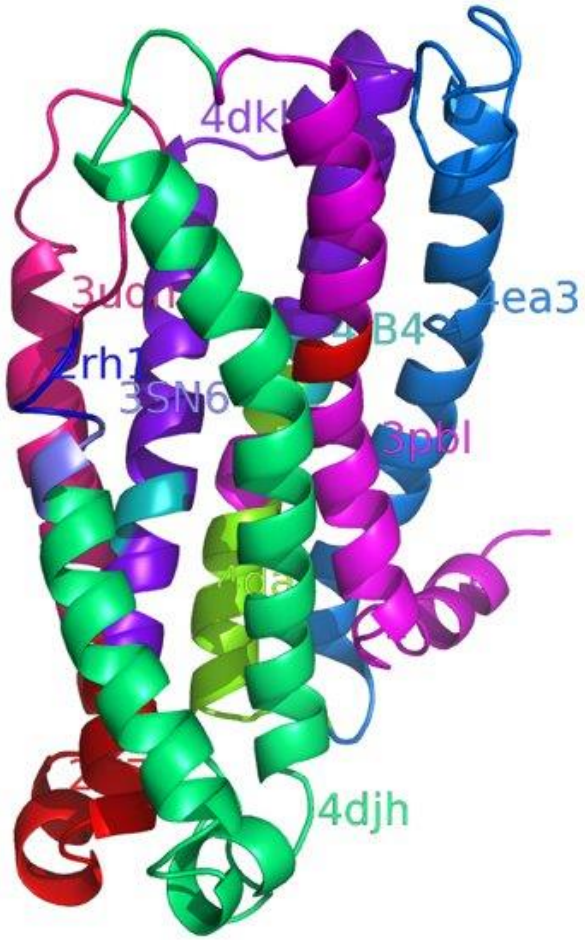
2. Explore deviations from templates and close loops in 2 steps :
   - MC: Randomly select de novo or template-based fragment and substitute into current conformation
   - Cartesian space full-backbone minimization

3. Full atom backbone and side chain refinement and final relax



Song, Y.; *et al.* Structure, ***2013***

Final models contain template information from multiple templates



Input files for RosettaCM

Bare minimum:

- Partial-threaded structures
- Mover definition and options

Specific to membrane proteins (not needed if modeling soluble proteins):

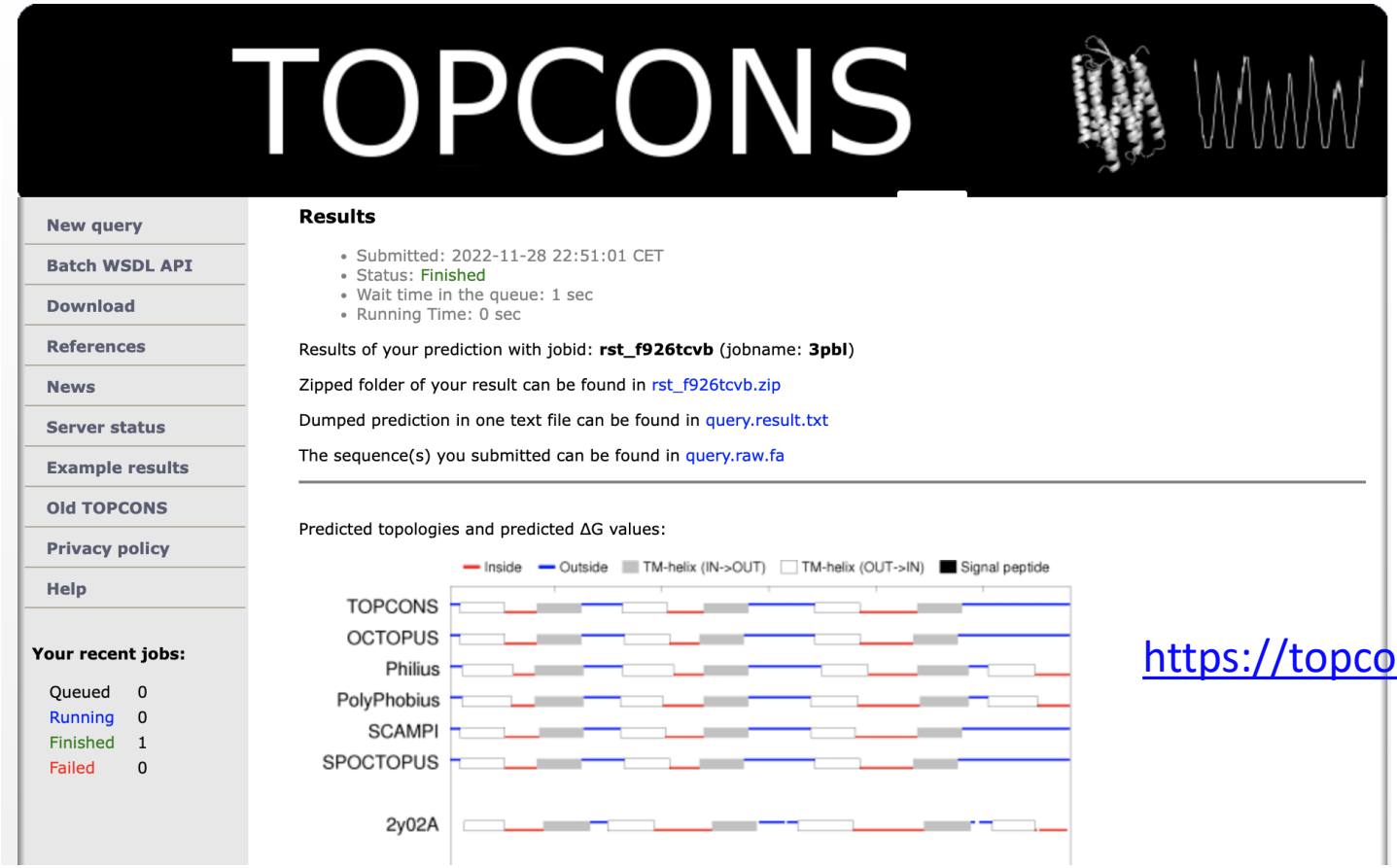
- Membrane spanning regions (span file)
- Membrane weight patches

Optional files based on available information:

- Constraint information (eg. atom pair connectivity)
- Disulfide Connectivity

Membrane spanning regions

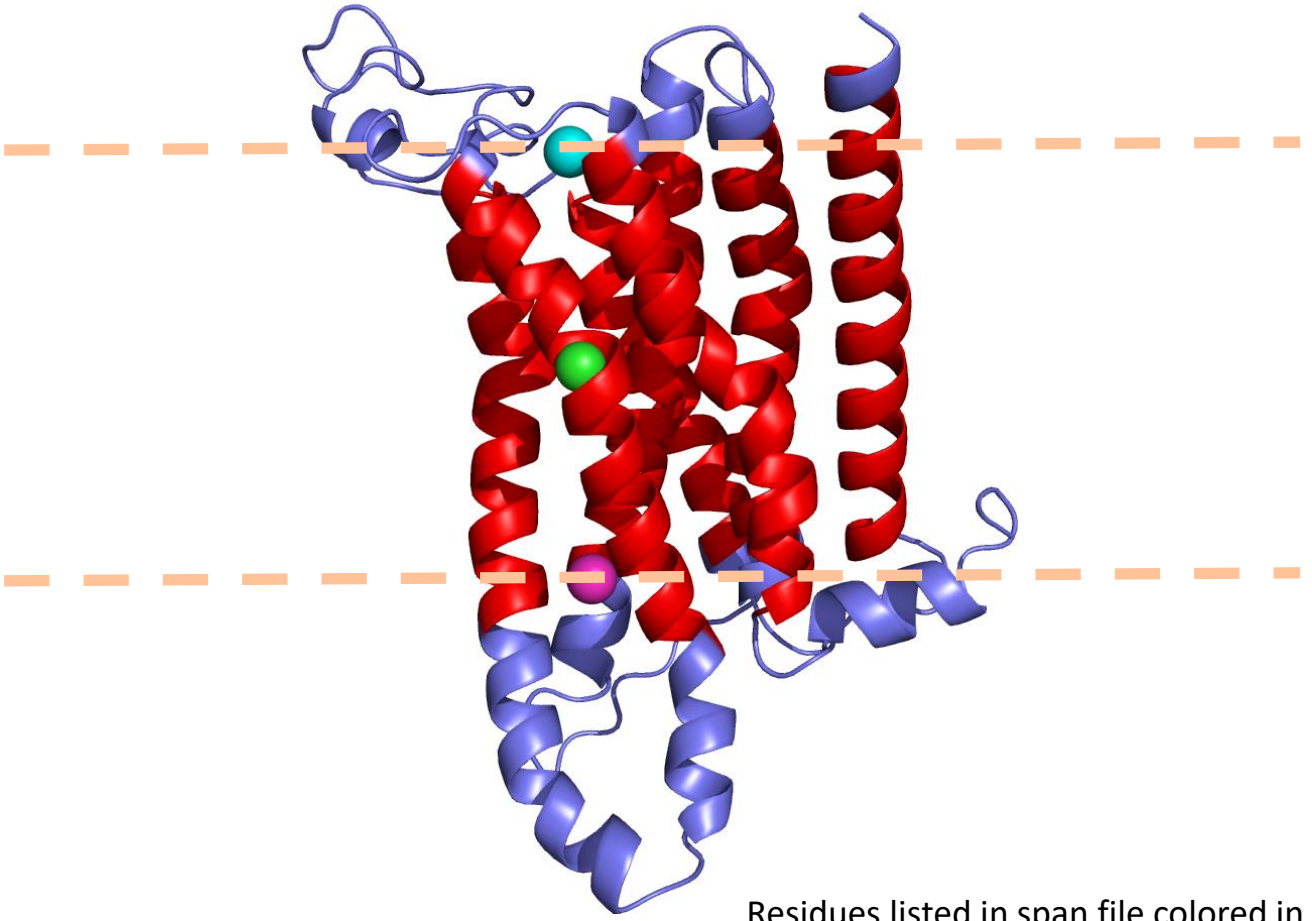
Find this file at */rosetta_cm/demo/input_files/3pbl.span*



https://topcons.cbr.su.se/pred/

query.result.txt

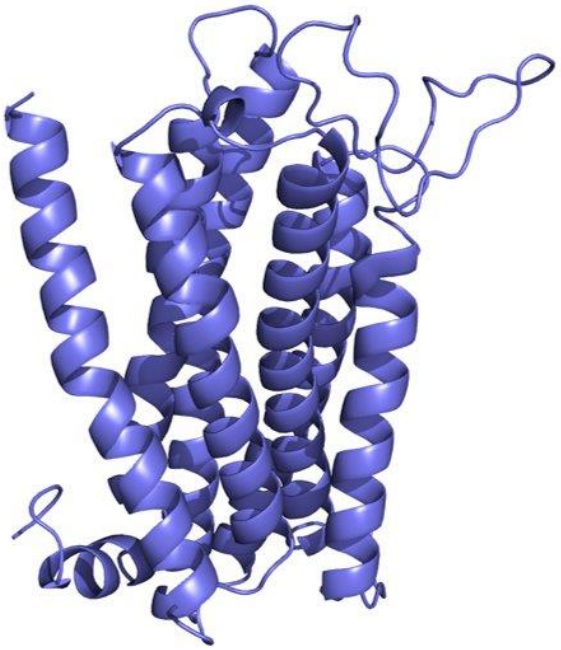
The span file helps RosettaMembrane to define transmemebrane regions



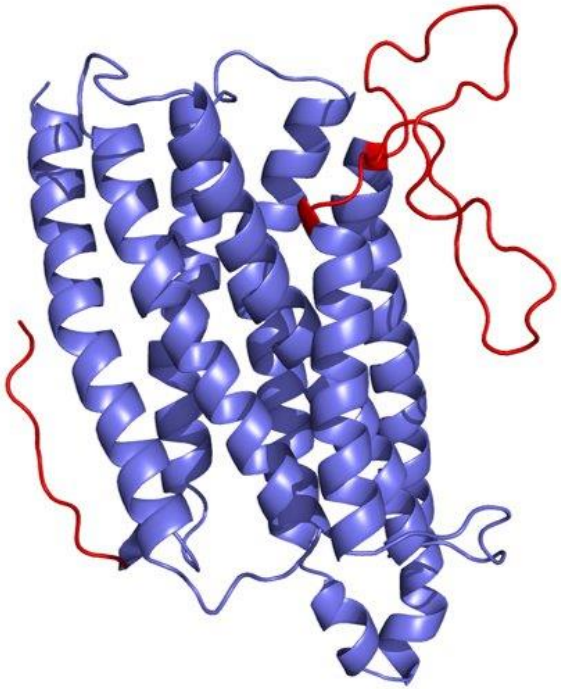
Residues listed in span file colored in red

Why are membrane scoring terms important?

With membrane penalties/weights



Without membrane penalties/weights

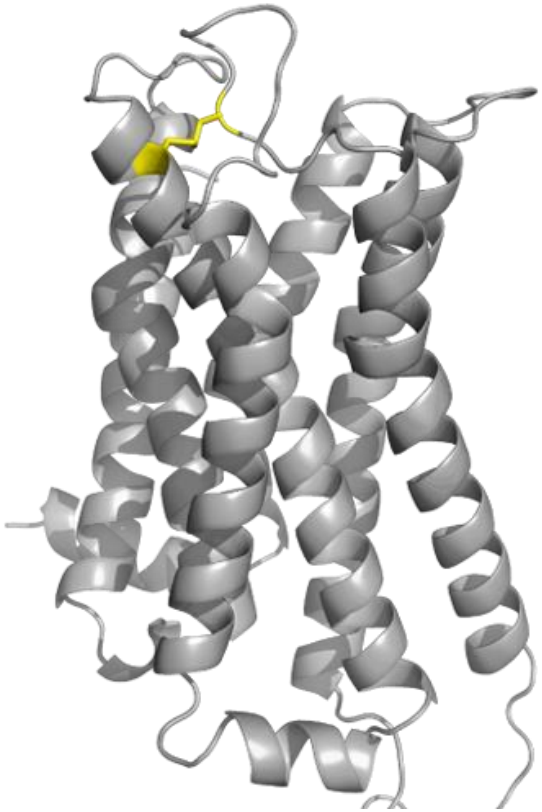


Disulfide constraints

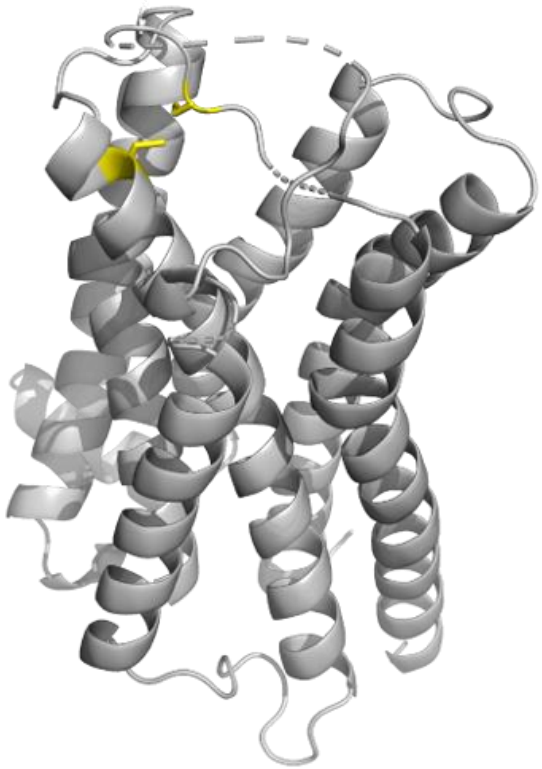
Find this file at */rosetta_cm/demo/input_files/3pbl.disulfide*

72 150

3pbl crystal structure



3pb1 thread into 2rh1



RosettaCM XML

/rosetta_cm/demo/input_files/rosetta_cm.xml

```
 <SCOREFXNS>
      <ScoreFunction name="stage1" weights="input_files/stage1_membrane.wts" symmetric="0">
            <Reweight scoretype="atom_pair_constraint" weight="1"/>
      </ScoreFunction>
      <ScoreFunction name="stage2" weights="input_files/stage2_membrane.wts" symmetric="0">
            <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
      </ScoreFunction>
      <ScoreFunction name="fullatom" weights="input_files/stage3_rlx_membrane.wts"
symmetric="0">
            <Reweight scoretype="atom_pair_constraint" weight="0.5"/>
      </ScoreFunction>
      <ScoreFunction name="membrane" weights="membrane_highres_Menv_smooth" symmetric="0">
            <Reweight scoretype="cart_bonded" weight="0.5"/>
            <Reweight scoretype="pro_close" weight="0"/>
      </ScoreFunction>
</SCOREFXNS>
```

*Find all **.wts** files in */rosetta_cm/ demo/input_files*

RosettaCM XML

/rosetta_cm/demo/input_files/rosetta_cm.xml

```
<MOVERS>
    <Hybridize name="hybridize" stage1_scorefxn="stage1" stage2_scorefxn="stage2"
fa_scorefxn="fullatom" batch="1" stage1_increase_cycles="1.0" stage2_increase_cycles="1.0"
linmin_only="1" realign_domains="0"  disulf_file="input_files/3pbl.disulfide"
fa_cst_file="fullatom.cst">
          <Template pdb="threaded_pdbs/4iar_out.pdb" cst_file="AUTO" weight="1.000" />
          <Template pdb="threaded_pdbs/4bvn_out.pdb" cst_file="AUTO" weight="1.000" />
          <Template pdb="threaded_pdbs/2rh1_out.pdb" cst_file="AUTO" weight="1.000" />
          <Template pdb="threaded_pdbs/5dsg_out.pdb" cst_file="AUTO" weight="1.000" />
          <Template pdb="threaded_pdbs/5cxv_out.pdb" cst_file="AUTO" weight="1.000" />
    </Hybridize>
    <ClearConstraintsMover name="clearconstraints"/>
    <FastRelax name="relax" scorefxn="membrane" repeats="1" dualspace="1" bondangle="1"/>
</MOVERS>
<OUTPUT scorefxn="membrane"/>
```

RosettaCM Options

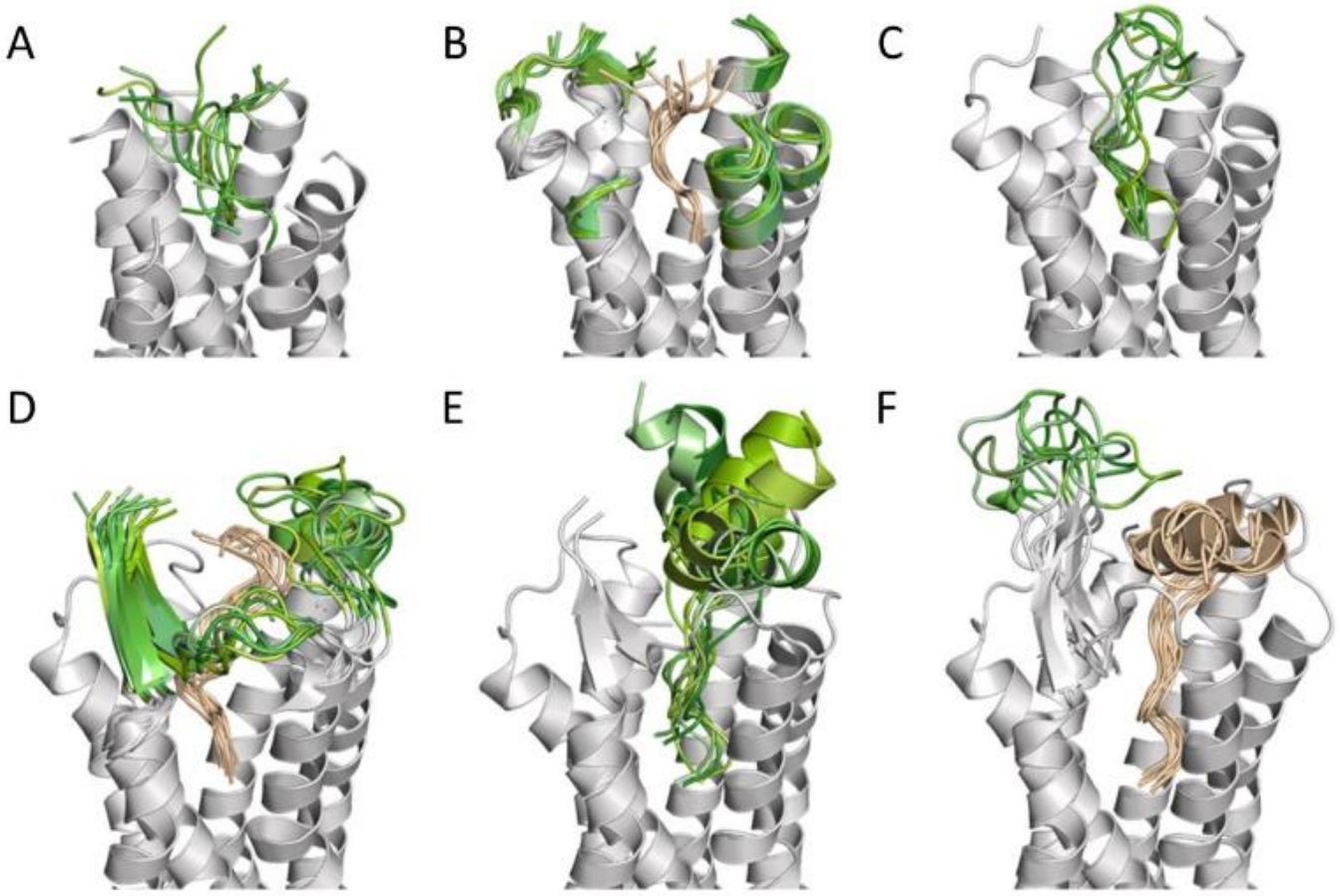
/rosetta_cm/3_hybridize/rosetta_cm.options

```
# i/o
-in:file:fasta input_files/3pbl.fasta                                    #### your target
sequence
-parser:protocol input_files/rosetta_cm.xml
-out:path:all output_files/

#Initialize membrane                                              #### only if modeling a membrane
protein
-in:file:spanfile input_files/3pbl.span
-membrane:no_interpolate_Mpair
-membrane:Menv_penalties
-rg_reweight .1
-restore_talaris_behavior

# relax options
-relax:minimize_bond_angles
-relax:minimize_bond_lengths
-relax:jump_move true
-default_max_cycles 200
-relax:min_type lbfgs_armijo_nonmonotone
-score:weights input_files/stage3_rlx_membrane.wts          #### use ref2015_cart if soluble
protein
-use_bicubic_interpolation
-hybridize:stage1_probability 1.0
-sog_upper_bound 15
```

Consecutive modeling of the Ghrelin/GHSR complex



Bender, B.J.; *et al.* Structure, ***2019***

Tutorial

Comparative modeling of D3 receptor with five class A GPCR templates

Four steps:
1. Setup
2. Threading
3. RosettaCM hybridize
4. Final model selection

References

- RosettaCM documentation
  https://www.rosettacommons.org/docs/latest/application_documentation/structure_prediction/RosettaCM

- RosettaCM: Multi-template

  Yifan Song, et al. (2013). High-Resolution Comparative Modeling with RosettaCM. Structure, *21*(10), 1735-1742.