

Multi-Template Comparative Modeling with RosettaCM

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Rosetta Workshop 2014

Target: bovine rhodopsin

- PDB ID: 1U19
- Class A G-protein coupled receptor
- No high identity templates
- 7 transmembrane helices
- 3 extracellular loops, 3 intracellular loops
- Highly conserved GPCR residues

Low identity templates

template	PDB ID	score
β2 adrenergic receptor	2RH1	16
β1 adrenergic receptor	2VT4	18
A2A adenosine receptor	3EML	20
CXCR4 chemokine receptor	3ODU	16
Dopamine D3 receptor	3PBL	26
Histamine H1 receptor	3RZE	18
M2 muscarinic acetylcholine receptor	3UON	21
Sphingosine 1-phosphate receptor 1	3V2W	19
M3 muscarinic acetylcholine receptor	4DAJ	22
κ-opioid receptor	4DJH	18
μ-opioid receptor	4DKL	21
N/OFQ opioid receptor	4EA3	21
δ-opioid receptor	4EJ4	19
5-HT1B receptor	4IAR	20
5-HT2B receptor	4IB4	20

Class A GPCR's

Highly conserved
GPCR residues

Similar fold
profiles

Low sequence
identity
(especially loops)

Template PDBs

Human β 2-adrenergic receptor (PDB: 2rh1)

Human A2A adenosine receptor (PDB: 3eml)

Human CXC chemokine 4 receptor (PDB: 3odu)

Find these files at /day06/rosetta_cm/1_setup/

RCSB PDB
PROTEIN DATA BANK

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A Resource for Studying Biological Macromolecules

The PDB archive contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies. As a member of the **wwPDB**, the RCSB PDB curates and annotates PDB data according to agreed upon standards.

The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searches based on annotations relating to sequence, structure and function. These molecules are visualized, downloaded, and analyzed by users who range from students to specialized scientists.

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Structural View of Biology

Molecule of the Month:
Integrin

Our bodies are composed of approximately ten trillion cells, which poses challenging problems for structure and communication. All of these cells must be connected strongly together, to allow us to stand and walk. The infrastructure holding us together, however, must also be malleable enough to allow repairs, to allow us to heal from wounds. These many cells must also communicate with each other, ensuring that each plays its own proper part. Many different molecules in our bodies are involved in this complex

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2011-02-22
Structural Neighbors

Explore structural neighbors lists to find connections between PDB

<http://www.rcsb.org>

Multiple Sequence Alignment

Find this file at /day06/rosetta_cm/2_threading/1u19_2rh1_3eml_3odu.aln

CLUSTAL O(1.2.1) multiple sequence alignment

```
1u19      -----PWQFSM--LAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPLNYILLNLAVADLFM
3ODU_A    ANFNKIFL-----PTIYSIIFLTGIVGNGLVILVMGYQKKLRSMTDKYRLHLSVADLLF
2RH1_A    ---DEVWVVGMGIVMS---LIVLAIVFGNVLVITAIKFERLQTVTNYFITSLACADLVM
3EML_A    -----IMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAV
          :      : : . * *. . ..*: . : *: **: .
```

Clustal Omega

[Input form](#) [Web services](#) [Help & Documentation](#)

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[Tools](#) > [Multiple Sequence Alignment](#) > Clustal Omega

Multiple Sequence Alignment

Clustal Omega is a new multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between **three or more** sequences. For the alignment of two sequences please instead use our [pairwise sequence alignment tools](#).

STEP 1 - Enter your input sequences

Enter or paste a set of **PROTEIN** sequences in any supported format:

Or, upload a file: No file selected.

STEP 2 - Set your parameters

OUTPUT FORMAT **Clustal w/o numbers**

The default settings will fulfill the needs of most users and, for that reason, are not visible.

(Click here, if you want to view or change the default settings.)

STEP 3 - Submit your job

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

Adjusting multiple sequence alignment

Experimental expectations:

- Highly conserved residues
- Secondary structure elements

Raw ClustalO alignment

1u19	-	-	-	-	-	P	W	Q	F	S	M	-	-	L	A	A	Y	M	F	L	L	I	M	L	G	F	P	I	N	F	L	T	L	Y	V	T	V	Q	H	K	K
3ODU_A	A	N	F	N	K	I	F	L	-	-	-	-	-	P	T	I	Y	S	I	I	F	L	T	G	I	V	G	N	G	L	V	I	L	V	M	G	Y	Q	K	K	
2RH1_A	-	-	-	D	E	V	W	V	V	G	M	G	I	V	M	S	-	-	-	L	I	V	L	A	I	V	F	G	N	V	L	V	I	T	A	I	A	K	F	E	R
3EML_A	-	-	-	-	-	-	-	I	M	G	S	S	V	Y	I	T	V	E	L	A	I	A	V	L	A	I	L	G	N	V	L	V	C	W	A	V	W	L	N	S	N

Adjusted alignment

1u19	-	-	-	-	-	-	P	W	Q	F	S	M	L	A	A	Y	M	F	L	L	I	M	L	G	F	P	I	N	F	L	T	L	Y	V	T	V	Q	H	
3ODU_A	A	N	F	-	-	-	-	-	-	N	K	I	F	L	P	T	I	Y	S	I	I	F	L	T	G	I	V	G	N	G	L	V	I	L	V	M	G	Y	Q
2RH1_A	-	-	-	D	-	-	-	E	V	W	V	V	G	M	G	I	V	M	S	L	I	V	L	A	I	V	F	G	N	V	L	V	I	T	A	I	A	K	F
3EML_A	-	-	-	-	-	-	-	I	M	G	S	S	V	Y	I	T	V	E	L	A	I	A	V	L	A	I	L	G	N	V	L	V	C	W	A	V	W	L	N

helix regions

highly conserved residues

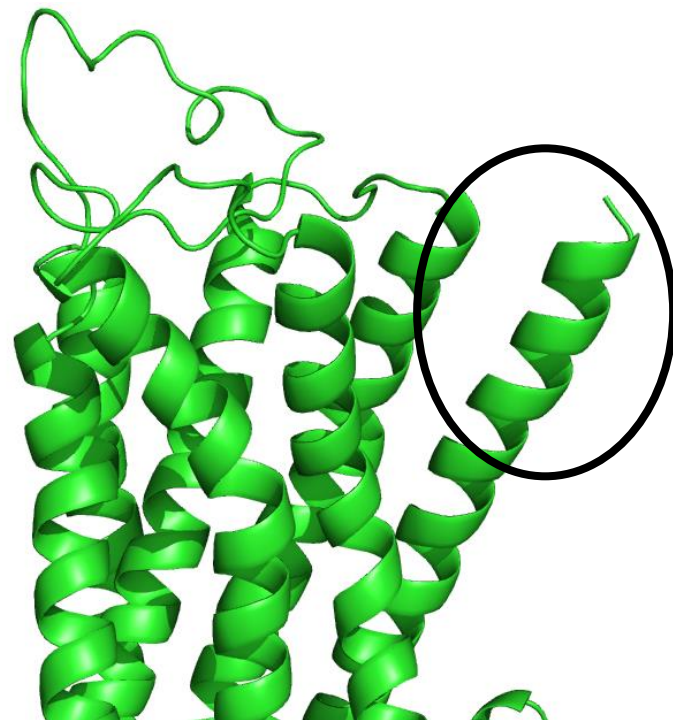
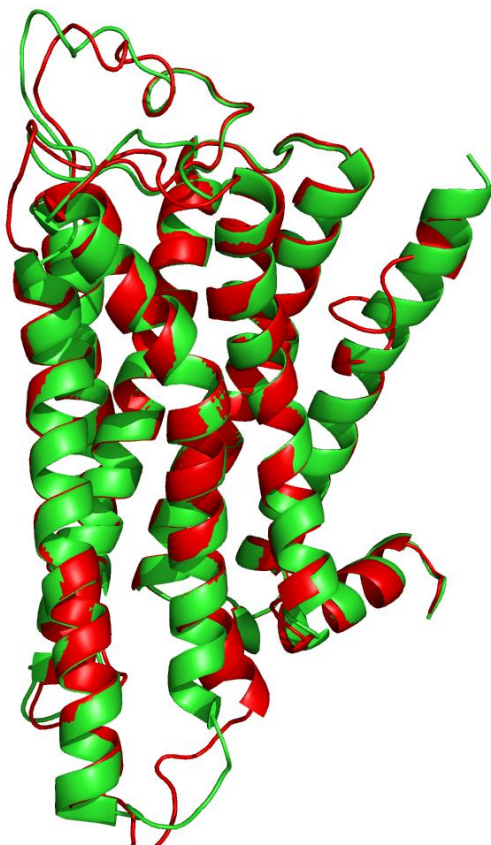
Alignment issues to be resolved

predicted membrane spanning region from OCTOPUS

Removing helix gaps



Raw alignment



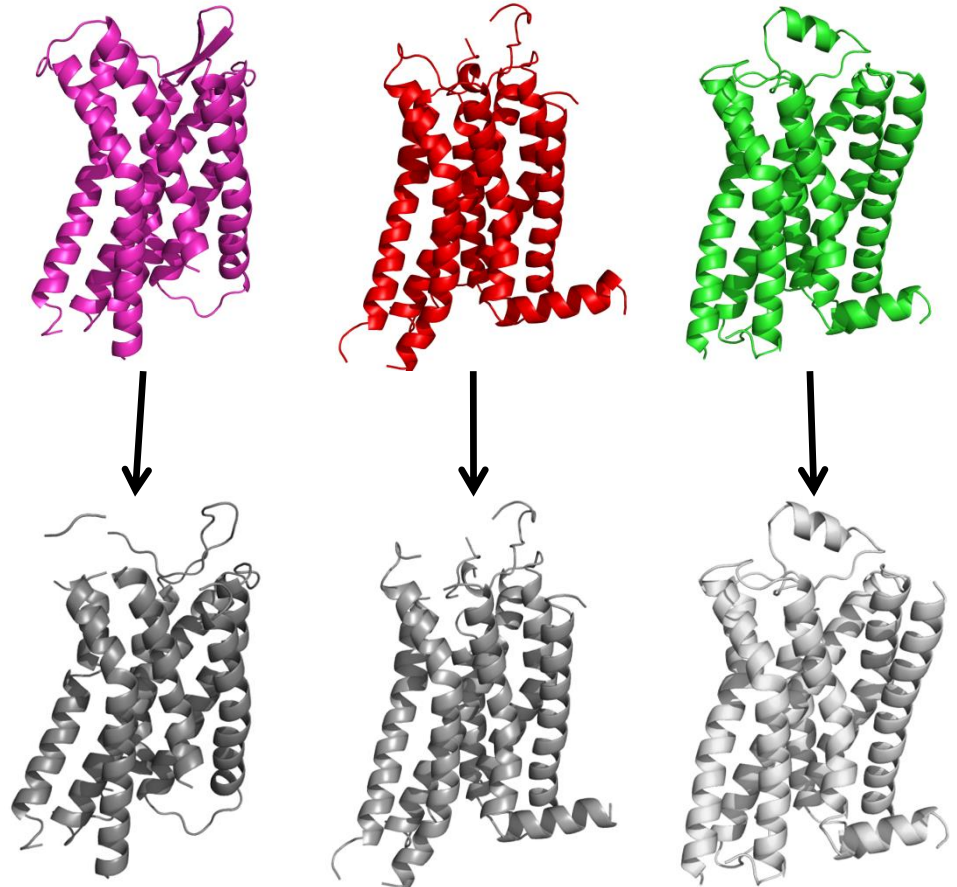
Adjusted alignment

Comparative Modeling Protocol

- **Step 1:** Align target sequence with template sequences
- **Step 2:** Partial-thread the target sequence onto template structures
- **Step 3:** Combine pieces from different templates using RosettaCM Hybridize
- **Step 4:** Full-atom refinement and relax

```
-----PWQFSM--LAAYMFLLIMLGFPINFLTLYVTVQHKKLRTPNLYILLNLAVADLFM  
ANFNKIFL-----PTIYSIIFLTGIVGNGLVILVMGYQKKLRSMTDKYRLHLSVADLLE  
---DEVWVVGMGIVMS---LIVLAIVFGNVLVITAIKFERLQTVTNYFITSLACADLVM  
-----IMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAV
```

+



Threading

Template:

(0,0,0)	(1,1,1)	(2,2,2)	(3,3,3)	(4,4,4)	(5,5,5)	
L	K	R	N	N	H	-
(?,?,?)	(?,?,?)				(?,?,?)	(?,?,?)
L	K	-	-	-	H	V

Target:

*Thread
Coordinates*



Target:

(0,0,0) (1,1,1) (5,5,5)
L K H V

Grishin Format

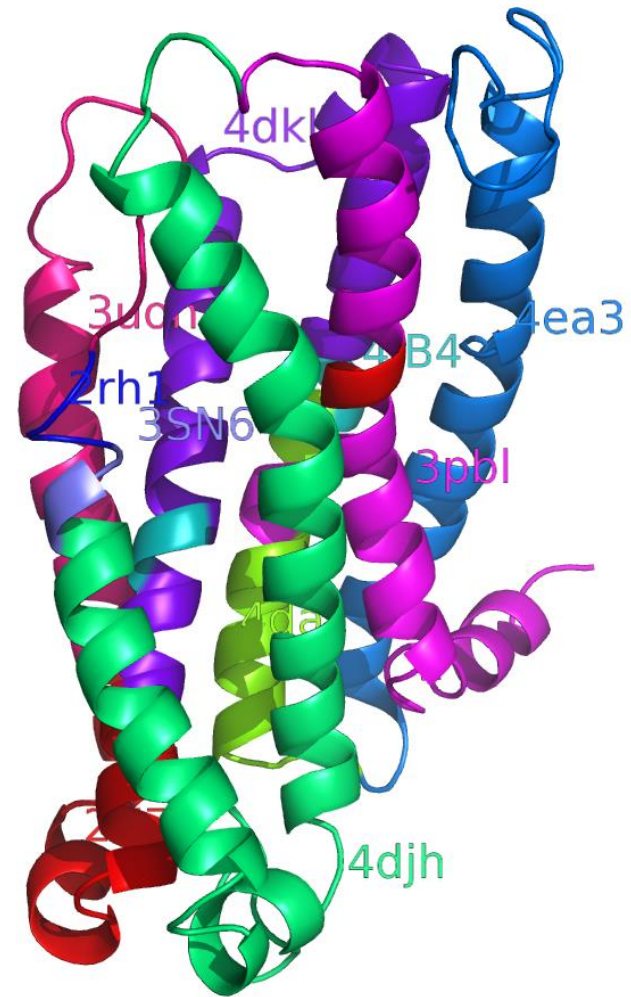
- ClustalO:
 - all sequences in one file
 - Sequences broken up over several lines
- Grishin:
 - one file per alignment pair
 - sequences continuous over one line each
 - Contains header information

Find converted alignment files at /day06/rosetta_cm/2_threading/

(1u19_2rh1.grishin, 1u19_3eml.grishin, 1u19_3odu.grishin)

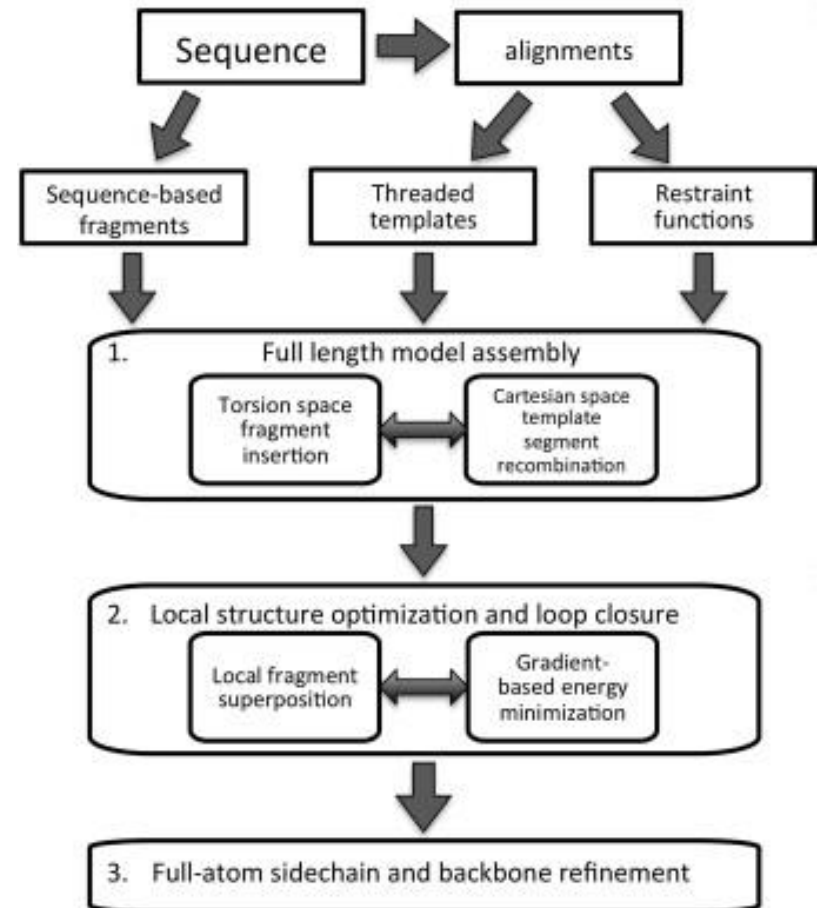
Comparative Modeling Protocol

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RosettaCM: Three Stages

1. Generate initial models from template alignments
- 2. Explore deviations from templates and close loops with 2-step MC:**
 - Randomly select de novo or template-based fragment and substitute into current conformation
 - Cartesian space full-backbone minimization
3. Full atom refinement and final relax



Song, et al. 2013

Hybridize input

- Partial-threads
- Fragment files (3mer and 9mer)
- Membrane spanning regions (span file)
- Mover definition and options
- Weight patches

Fragment Files

Find these files at

`/day06/rosetta_cm/3_hybridize/1u19_3.frag`

`/day06/rosetta_cm/3_hybridize/1u19_9.frag`



www.bakerlab.org

Structure Prediction Fragment Libraries Alanine Scanning DNA Interface Scan
[Queue] [Submit] [Queue] [Submit] [Queue] [Submit] [Queue] [Submit]
[Register / Update] [Docs / FAQs] [Login]

Submit a job to the Fragment Server

*Please submit one job at a time

Required

[Registered Username:](#) or [Registered Email Address:](#)

Target Name:

Paste [Fasta](#)

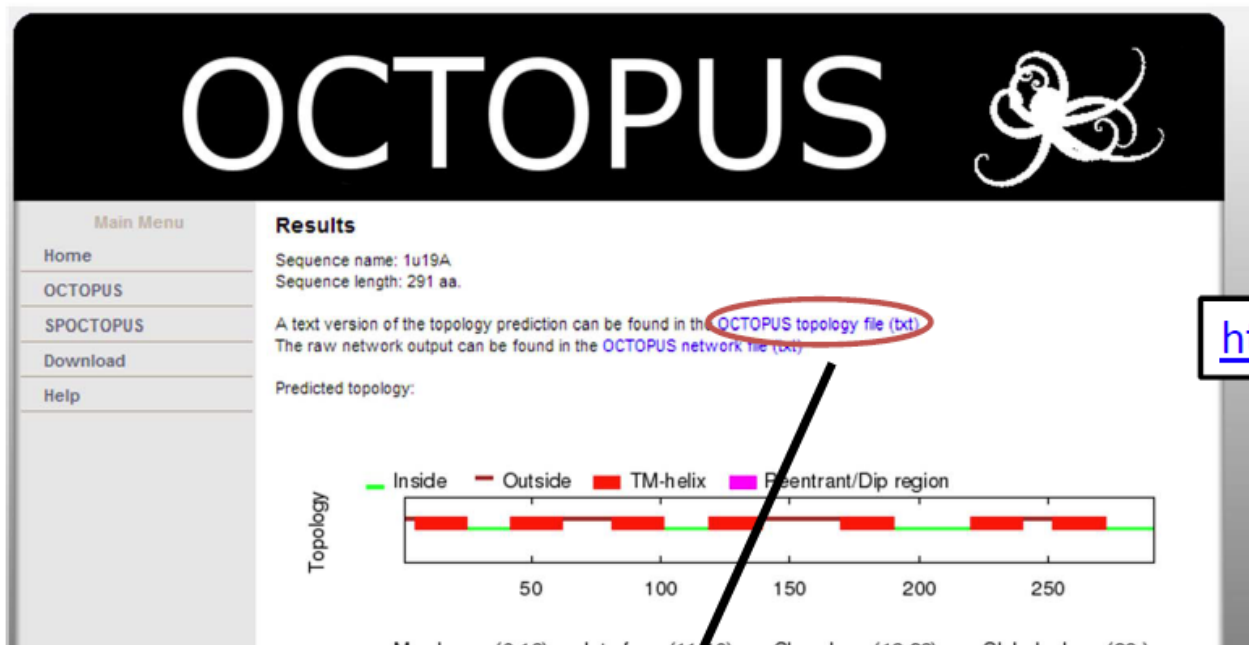
or Upload [Fasta](#): No file chosen

<http://rosetta.bakerlab.org/fragmentsubmit.jsp>



Membrane spanning regions

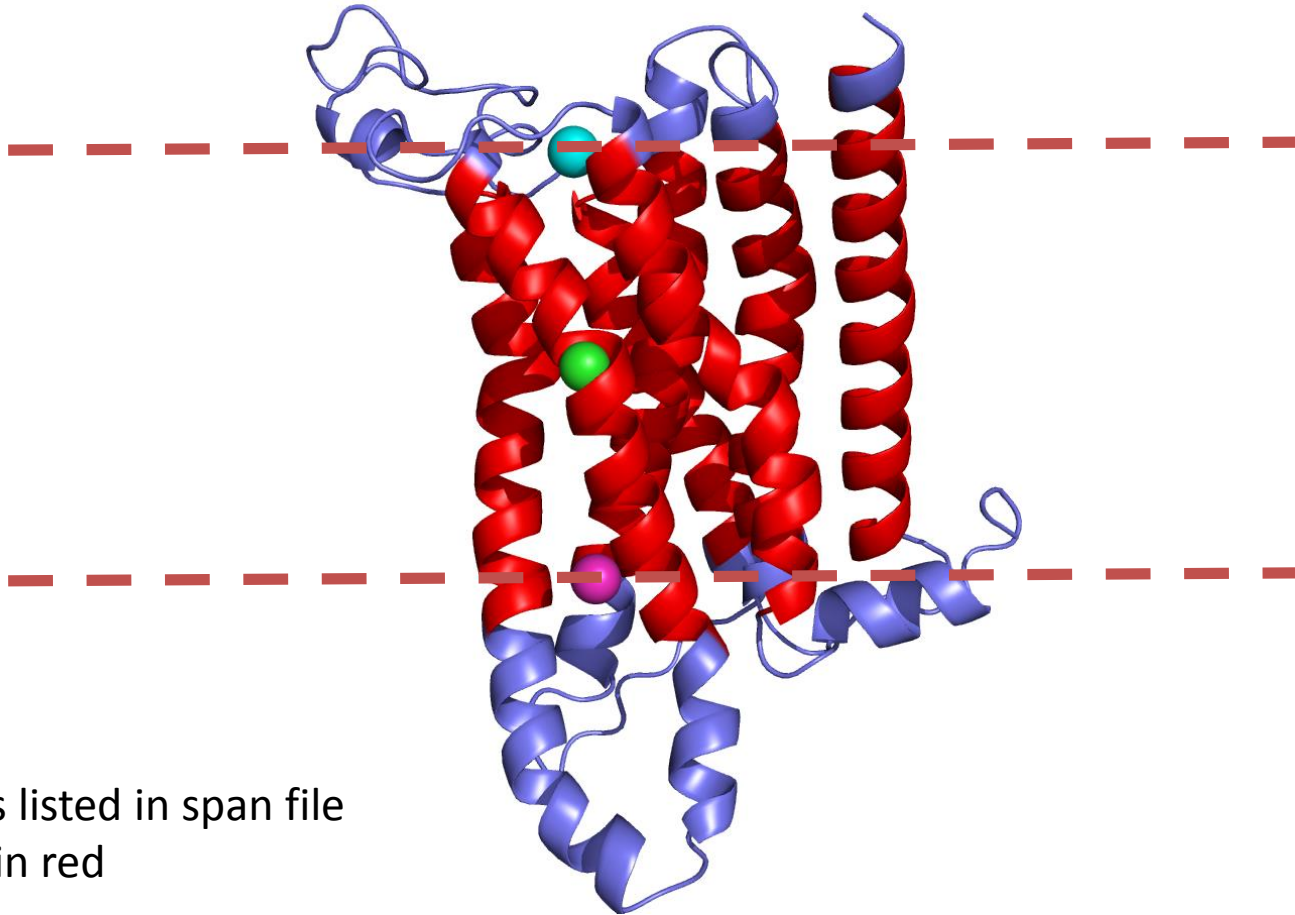
Find this file at /day06/rosetta_cm/3_hybridize/1u19.span



<http://octopus.cbr.su.se/>

```
octopus2span.pl 1u19A.octopus
```


Rosetta Membrane



Residues listed in span file
colored in red

Hybridize Mover

/day06/rosetta_cm/3_hybridize/rosetta_cm.xml

```
<SCOREFXNS>
  <stage1 weights="stage1_membrane.wts" symmetric=0>
    <Reweight scoretype=atom_pair_constraint weight=1/>
  </stage1>
  <stage2 weights="stage2_membrane.wts" symmetric=0>
    <Reweight scoretype=atom_pair_constraint weight=0.5/>
  </stage2>
  <fullatom weights="stage3_rlx_membrane.wts" symmetric=0>
    <Reweight scoretype=atom_pair_constraint weight=0.5/>
  </fullatom>
</SCOREFXNS>
```

*Find all **.wts** files in /day06/rosetta_cm/3_hybridize/*

```
<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>
    <Fragments 3mers="1u19_3.frgs" 9mers="1u19_9.frgs"/>
    <Template pdb="1u19_on_2rh1.pdb" cst_file="AUTO" weight= 1.000 />
    <Template pdb="1u19_on_3eml.pdb" cst_file="AUTO" weight= 1.000 />
    <Template pdb="1u19_on_3odu.pdb" cst_file="AUTO" weight= 1.000 />
  </Hybridize>
</MOVERS>
```

RosettaCM Options

/day06/rosetta_cm/3_hybridize/rosetta_cm.options

i/o

-in:file:fasta 1u19.fasta
-parser:protocol rosetta_cm.xml

relax options

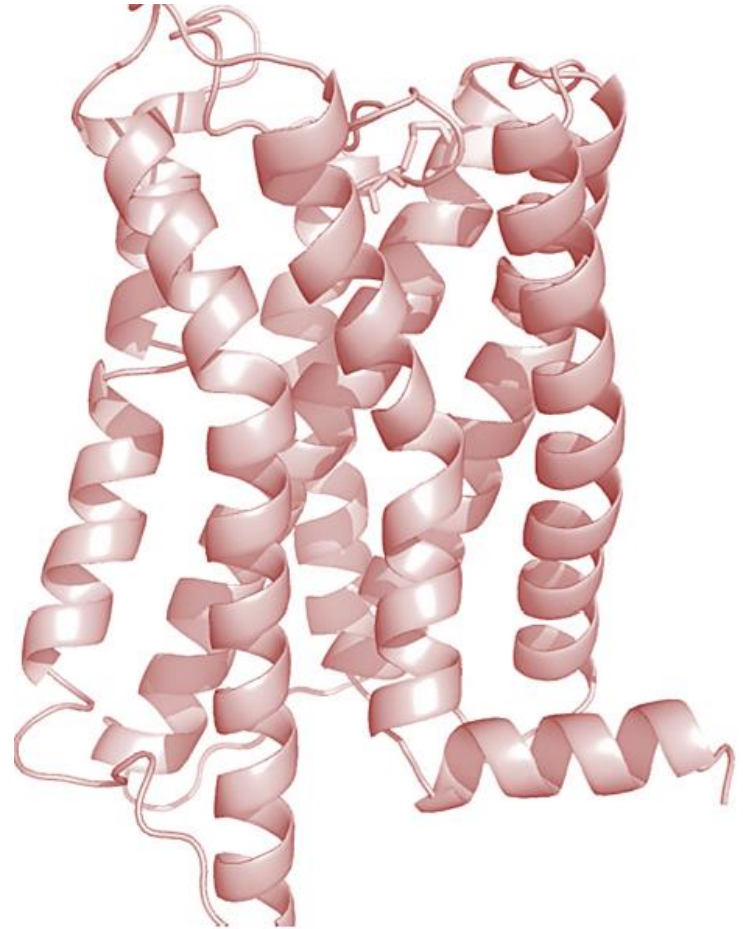
-relax:minimize_bond_angles
-relax:minimize_bond_lengths
-relax:jump_move true
-default_max_cycles 200
-relax:min_type lbfgs_armijo_nonmonotone
-score:weights stage3.wts
-use_bicubic_interpolation
-hybridize:stage1_probability 1.0

#Initialize membrane

-in:file:spanfile 1u19.span
-membrane:no_interpolate_Mpair
-membrane:Menv_penalties
-rg_reweight .1

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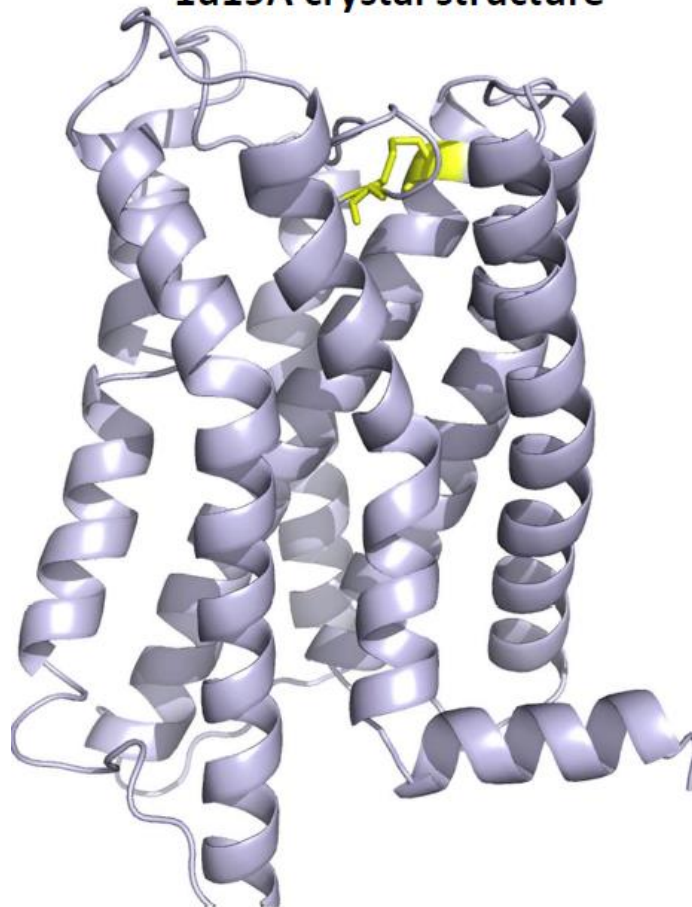


Disulfide constraints

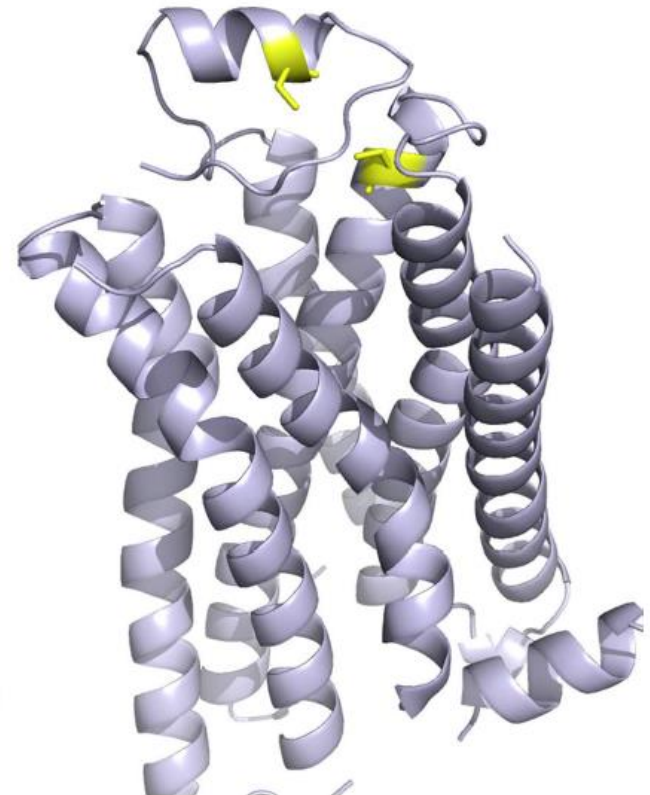
Find this file at /day06/rosetta_cm/4_relax/1u19.disulfide

77 154

1u19A crystal structure



1u19A threaded onto 2rh1A



Relax options

/rosetta_cm/4_relax/relax.options

i/o

-in:fix_disulf 1u19.disulfide
-out:file:fullatom
-out:pdb

Dualspace settings

-relax:dualspace
-relax:minimize_bond_angles
-set_weights cart_bonded .5 pro_close 0
-default_max_cycles 200

#Initialize membrane

-in:file:spanfile 1u19.span
-membrane:no_interpolate_Mpair
-membrane:Menv_penalties
-score:weights membrane_highres_Menv_smooth.wts

Tutorial

Comparative modeling of rhodopsin with three class A GPCR's

Five stages:

1. Setup
2. Threading
3. RosettaCM hybridize
4. Dualspace relax
5. Final model selection

References

- **Rosetta 3.5 User Guide**

https://www.rosettacommons.org/manuals/archive/rosetta3.5_user_guide/index.html

- **Modeling Membrane Proteins**

https://www.rosettacommons.org/manuals/archive/rosetta3.5_user_guide/db/d38/membrane_abinitio.html

- **RosettaCM**

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

Initial
alignment

Adjusted
alignment