Building a Homology Model of the Transmembrane Domain of the Human Glycine α-1 Receptor

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Glycine Alpha 1 Receptor (GlyRa1)

• Member of the superfamily of ligand-gated ion channels
  – Essential to the transfer of information within the nervous system
  – Very sensitive to anesthetics and alcohol
• Anesthetics trigger alterations in transmembrane domain
• Structural problem
  – Does not crystallize well (transmembrane)
Nicotinic Acetylcholine Receptor (nAChR)

- Same pentameric superfamily
- Facilitates fast transmission of electrical signals
- Found in high concentrations at the nerve-muscle synapse
- Known 4Å structure

nAChR Structure

- Assembled from a ring of five homologous subunits
- Two binding sites
- Extracellular, transmembrane, intracellular domains
- Subunit-subunit interfaces provide potential pathways for diffusing ions
Transmembrane Domain

- Four $\alpha$-helical segments M1-M4
- Pore shaped by inner ring of M2 helices
- Outer shell of helices (M1, M3, M4) shield the inner ring from lipids

nAChR Gating

- Acetylcholine binds at both sites
- Local disturbance causes $\alpha$-subunits to rotate
- Unlocks interactions that restrict the rotational movements of the pore-lining helices
- Destabilizes weak hydrophobic interactions holding the gate together
- Lumen widens causing the pore to open
Sequence Alignment

- Search protein databases to find which protein to model after
- Align sequences of nAChR δ chain and GlyRa1 using ClustalW

Model Membranes

- For each transmembrane, use Modeller to create 10 models
- Choose the models with the lowest Modeller Objective Function
- Use VMD Multiple Sequence Alignment to align each model with the corresponding segment of nAChr δ chain
- Manually merge the separate pdb files
**Fill Gaps**

- Use Modeller to create 10 models – different alignment file
- Choose model with lowest Modeller Objective Function

**Analyze Results**

- Evaluate Discrete Optimized Potential Energy (DOPE) scores
Refine Model

- Refine loop using Modeller
- Choose model with lowest Modeller Objective Function as best model

Analyze Results

- Compare DOPE scores for each residue of the two models
Analyze Results

Ramachandran Plot

GlyR\_7

Plot Statistics

73 residues in most favored regions (93.6%)
4 residues in additional allowed regions (5.1%)
1 residue in generally allowed regions (1.3%)
0 residues in disallowed regions (0.0%)

Direct Modeling

• Align complete sequences of nAChR \( \alpha_5 \) subunit and GlyRa1 using ClustalW
• Use Modeller to generate 10 models transmembrane domain of the subunit
• Choose model with lowest Modeller Objective Function
• Duplicate subunit four times and rotate each copy 72° to create pentameric structure
Final Model

405 residues in most favored regions (96.4%)
15 residues in additional allowed regions (3.6%)
0 residues in generally allowed regions (0.0%)
0 residues in disallowed regions (0.0%)

Analyze Results

Plot Statistics
References


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