Identification of core amino acids stabilizing rhodopsin

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Outline

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- Computational Methods
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- Relation to Research Project
Protein folding is the process by which a protein assumes its functional shape or conformation from the primary amino acid sequence.

Membrane proteins are different from soluble proteins due to their hydrophobic nature.
The current paradigm for helical membrane protein folding: The 2-stage hypothesis based on studies of bacteriorhodopsin
GPCRs

- Largest family of cell-surface receptors.
- Characterized by 7 transmembrane helices
- Biological functions include smell, taste, vision, neurotransmission, hormones.
- Prototype: Rhodopsin as the only GPCR with a known 3D structure.
Mutations in rhodopsin’s extracellular and transmembrane domains cause misfolding.

Misfolded rhodopsin also contains a wrong disulfide bond.

Correct disulfide bond located at the interface between the TM and EC domains.

The two stage model can’t explain the mutations causing misfolding in mammalian rhodopsin.
Impact

Misfolding is the mechanism for a large number of diseases

- Membrane proteins:
  - Retinitis pigmentosa (RP)
  - Cystic fibrosis
  - Arrhythmias
  - Hearing loss
  - Charcot-Marie-Tooth

- Soluble Proteins:
  - Creutzfeld-Jacob
  - Alzheimer
  - etc.
What is the goal?

- Identifying core amino acids stabilizing rhodopsin based on the rhodopsin structure to explain misfolding mutations of rhodopsin and possibly in misfolding diseases of other membrane proteins.
In this study...

- Can we obtain information on the mechanism of folding and stability of rhodopsin from the crystal structure?

- Application of two computational methods
  - Floppy Inclusions and Rigid Substructure Topography Model (FIRST)
  - Gaussian Network Model (GNM)
Computational Methods

- **Input**: crystal structure of rhodopsin: Protein Data Bank (PDB) ID code 1L9H, molecule ‘A’

- **Floppy Inclusions and Rigid Substructure Topography Model** (FIRST) to predict a folding core using simulated unfolding by breaking H-bonds from strongest to weakest.

- **Gaussian Network Model** (GNM) to predict amino acids important for stability from the network of interactions in the crystal structure.
Methods – FIRST

- The FIRST software uses techniques from graph theory to analyze and quantify the rigidity or flexibility of proteins.

- The output identifies regions of the protein that are collectively rigid or in other words *stable* connected by *unstable* flexible joints.
Results

- Simulated thermal unfolding with FIRST
Methods - GNM

- In the GNM the protein is modeled as an elastic network composed of beads and springs connecting all interacting residues.

- A connectivity, or Kirchhoff, matrix is constructed, which identifies the residues that are within a certain cutoff distance with respect to one another.
Results

Mode decomposition of the GNM dynamics
The amino acids predicted to be important for stability and folding of rhodopsin are located at the interface between the loop and transmembrane helices.
Paradigm Shift?

- The current paradigm suggests that the individual helices are sufficiently hydrophobic to be stable within the lipid bilayer on their own, without the contacts to the other helices.

- Importance of the loop regions for the folding of membrane proteins.
The New Paradigm

Unfolded → Long-range interactions intermediate → Folded

Long-range helix-helix contact
Long-range loop-loop contact
The old paradigm was based on experimental studies of bacteriorhodopsin.

Goal of the project is to test the two models – FIRST and possibly GNM - so far applied to mammalian rhodopsin on bacteriorhodopsin.
References


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