Simple Models Characterize the Activation Mechanism of G Protein-Coupled Receptors

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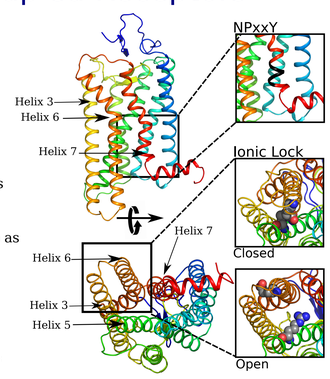
tinyurl.com/go-go-gpcr

Abstract

G protein-coupled receptors (GPCRs), the largest family of proteins in the human genome, are integral membrane proteins responsible for transducing signals across the cell membrane. They play vital roles in vision, olfaction, taste and many other signaling processes. They are major drug targets, so understanding their activation mechanism may lead to better drug design. In this investigation, we studied the activation and deactivation of two class-A GPCRs, rhodopsin and β_2 Adrenergic Receptor (β_2AR), using computational methods. Specifically, we used structure-based potentials (Gō-models) to rapidly simulate the transition from the inactive state to the active state, as well as the reverse process. We monitored the transition using a experimentally motivated quantity, as well as a generalized technique based on principal component analysis of inter-residue contacts. The latter approach proved to be more informative, and helped us develop novel insights into the difference in activation pathway for these structurally similar proteins.

G Protein-Coupled Receptors

- Largest family of integral membrane proteins
- Biomedically important
  - -Targeted by one-third of FDA approved drugs
- Rhodopsin
  - -Mammalian dim-light receptor
  - -Activated by its covalently bound ligand, retinal
  - -Structurally similar to other GPCRs
- β_2-Adrenergic Receptor (β_2AR)
  - -Role in cardiac response
  - -Activated by hormone ligands such as adrenaline
- Conserved motifs in class A GPCRs
  - -NPxxY (7.49-7.53)
  - -(E)DRY (3.49-3.51)
  - -Ionic lock: between 3.50 & 6.30
  - -Notation: helix.residue number, where 50 is the most conserved residue in the helix

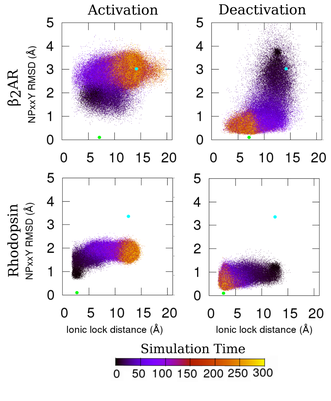


Structure-Based Potentials (Gō Models)

- Simulate transition between active and inactive structures for both GPCRs
- Forcefield constructed from the crystal structures (defined by SMOG)
- Ending structure represents global energy minimum
- Structure-based models paired with the forcefield of opposite state
  - -Start in inactive state, simulate activation
  - -Path taken to reach global energy minimum
- Molecular dynamics using gromacs 4.5.4
  - -1000 independent simulations for each transition
  - -Computationally inexpensive

GPCR	Rhodopsin	β2AR
Inactive (PDB ID)	1U19	3NY8
Active (PDB ID)	3PXO	3P0G

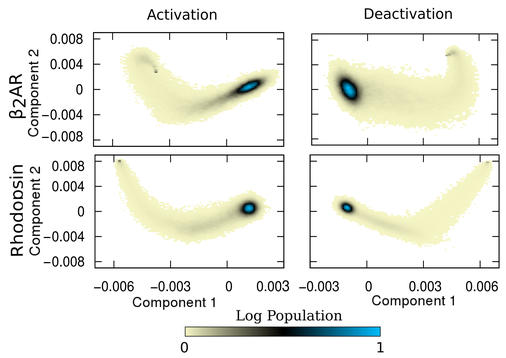
Experimentally Motivated Coordinate



- Pick reaction coordinate based on previous experiments
  - -Ionic lock
  - -NPxxY motif
- Reference structures:
  - -Active (cyan circle) and inactive (green circle) crystal structure
- β_2AR Activation
  - -NPxxY transitions 1st, followed by ionic lock
- β_2AR Deactivation
  - -Reverse of activation
  - -NPxxY transitions 1st
  - -Similar to Dror et al.,2011
- Rhodopsin
  - -Ionic lock transitions
  - -NPxxY does not
- Need a better tool to characterize transition

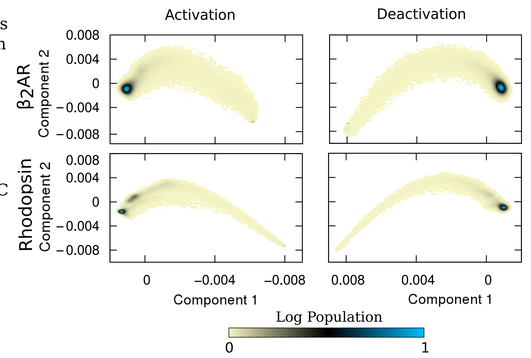
Data-Derived Reaction Coordinates

Cartesian PCA



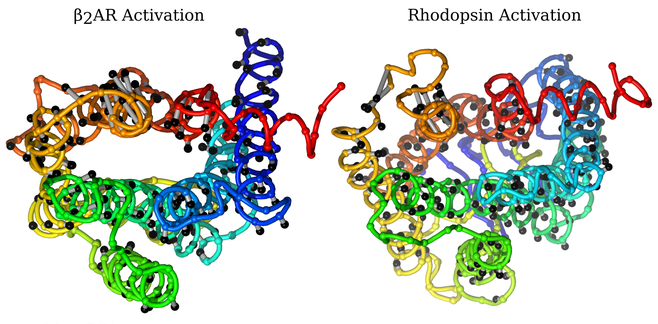
- Principal Component Analysis using transmembrane region
- Cartesian PCA
  - -Standard technique
  - -Measures collective changes in α-carbons positions
- Contact-Based PCA
  - -Unique contacts formed and broken during transition
- Trajectory projected along PC 1 and 2 (most concerted motion)
- Non-monotonic pathway
  - -PC2 overshoots average value
- β_2AR:
  - -Wider path and end-point

Contact-Based PCA



Visual Interpretation Of Transitions

Residue Movements During Activation



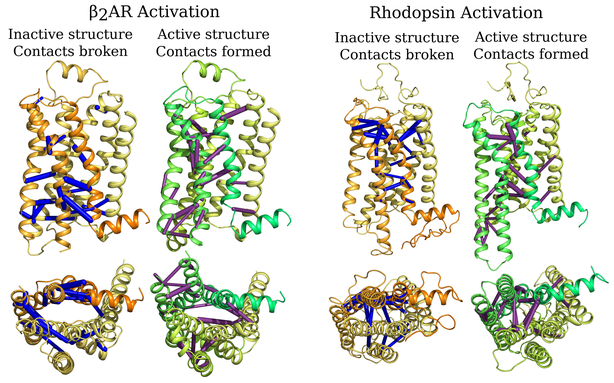
- Map PC1 onto structure
- Models: inactive structures of rhodopsin and β_2AR
- Black lines drawn on α-carbons
  - -Length shows contribution of residue to PC1
  - -Direction of most concerted motion

- Key movements during activation
  - -Helix 6 outward rotation
  - -Larger movement of NPxxY region in β_2AR
  - -Helix 5 elongation

Crucial Contacts Are Widely Distributed

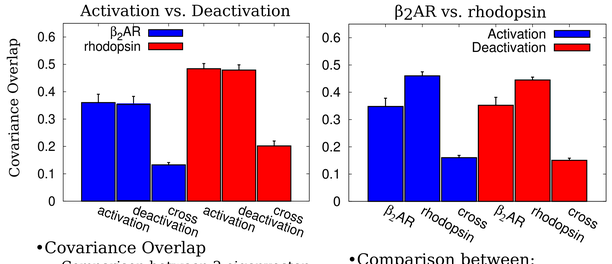
- Contacts drawn between α-carbons
  - -Thickness shows contribution to PC1
- Contributing contacts differ in all cases, spread throughout GPCR

- Model:
  - -Yellow-orange (inactive structure)
  - -Yellow-green (active structure)
- Sticks:
  - -Blue (formed contacts)
  - -Purple (broken contacts)



Quantitative Comparison Of Pathways

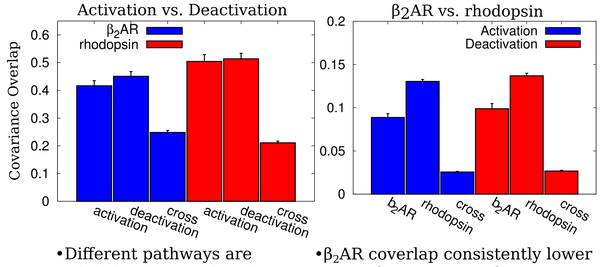
Cartesian PCA



- Covariance Overlap
  - -Comparison between 2 eigenvector sets
  - -Weighted by eigenvalues
  - -Coverlap=1, data sets are similar
  - -Coverlap=0, data sets are orthogonal

- Comparison between:
  - -Activation and deactivation
  - -β_2AR and rhodopsin

Contact-Based PCA

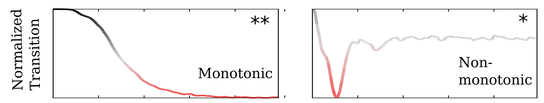


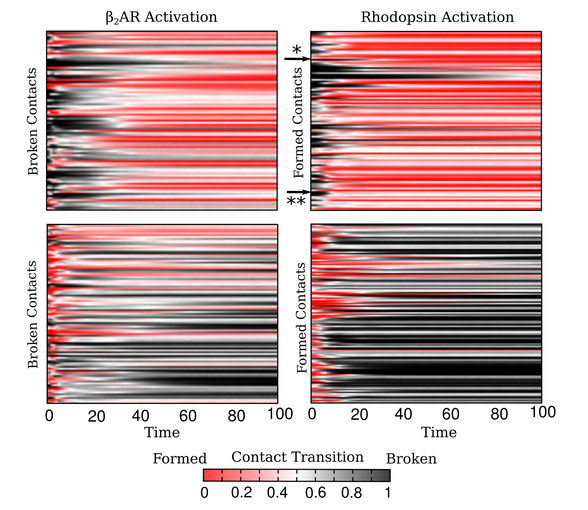
- Different pathways are statistically significant
  - -Activation and deactivation
  - -β_2AR and rhodopsin

- β_2AR coverlap consistently lower
  - -Broader transition pathway
  - -Pathway less well-defined
  - -Basal activity

Pathways Are Non-Linear

- Monitor unique contacts for the first-third of all trajectories
- Plot transition coefficient time series for individual contacts, averaged over ensemble
  - -Each row is an individual unique contact
  - -Red (Formed contact)
  - -Black (Broken contact)
- Contacts fluctuate between formed and broken states



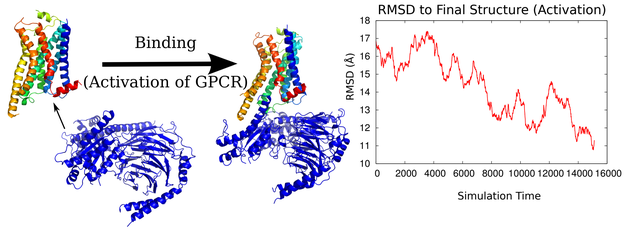


Conclusions

- Simulated activation
- Experimentally motivated reaction coordinate not always best
- Data-derived reaction coordinate
  - -Identifies diverse set of important contacts and residue movements
  - -Allows quantification of pathways
- Non-linear pathway
  - -Some contacts peak during transition
- Activation and deactivation pathways different
- β_2AR and rhodopsin different
  - -Contributing residue pairs differ
  - -β_2AR path broader

Future Directions

- Simulate binding of G Protein to GPCR
- Apply to other GPCRs as crystal structures become available
- Implement a double-minimum potential model



LOOS (Lightweight Object Oriented Structure analysis library), a project of the Grossfield Lab, is an open-source library using C++ and BOOST to provide an easy to use and easy to extend framework for rapidly developing analytical tools for molecular simulations. LOOS is available through sourceforge at: **http://loos.sourceforge.net**