Analysis of the Structure-Function-Dynamics Relationship of G-Protein Coupled Receptors

Augustus J. Olthafer & Dr Sanchita Hati
Department of Chemistry – University of Wisconsin Eau Claire – Eau Claire, WI 54702

Abstract

The presence of G-proteins and G-protein coupled receptors (GCPRs) are highly ubiquitous in upper eukaryotic organisms. With nearly 60% of all modern pharmaceuticals targeting GCPRs, the understanding of these proteins' dynamical-functional-structural relationship is critical for designing better drug molecules. A wide variety of GCPR proteins exist, differing by the ligands they bind, their cellular responsive mechanisms and their intrinsic properties. These proteins are classified into six classes based on their sequence and functional similarities. In an attempt to strengthen our understanding of the structure-function relationships of various types of GPCRs, the intrinsic dynamics of GPCR proteins were explored. A variety of computational programs were employed to simulate protein dynamics and compare/contrast the intrinsic dynamical patterns of various GPCRs. More precisely, the role of GPCRs' active site dynamics in recognizing and binding the appropriate ligand and how these intrinsic dynamics differ between different classes of GPCRs have been the main focus of the current study. The preliminary results of this study will be presented.

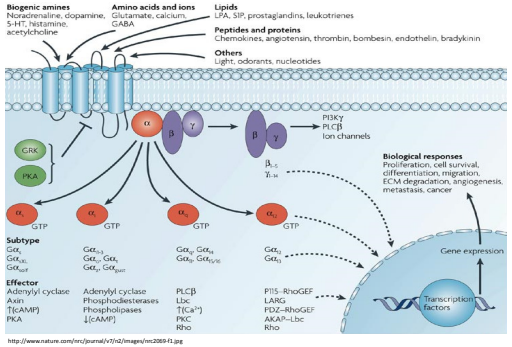
Background

G-Protein Coupled Receptors (GPCRs)

GPCRs are 7-pass transmembrane proteins, which are coupled to a G-protein and are responsible for countless functions within our bodies. Each GPCR has an extracellular ligand binding domain and an intracellular domain which work in tandem to mediate G-protein coupled interactions. GPCRs are grouped into six classes based on sequence homology and functional similarity [1]. These classes being: Rhodopsin-like, Secretin Receptors, Metabotropic, Fungal Mating Receptors, Cyclic AMP Receptors, and Frizzled/Smoothened.

The Basic Mechanism of GPCRs: An Extracellular Signal Eliciting an Intracellular Response

Upon ligand binding on the extracellular side of the plasma membrane, the GPCR undergoes a conformational change which alters the intracellular portion of the protein's structure. This will cause a three-dimensional change that can either bind, or release a G-protein. The G-proteins and their abilities to which they become sequestered or freed of its GPCR are termed G_i or G_s respectively. The binding of a ligand can cause the release of a G-protein or the binding of a G-protein can 'kick off' a bound ligand.



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Objectives

- To better understand the structure-function-dynamic relationship of GPCRs including elements such as:
  - Ligand binding
  - Intracellular conformational changes
  - Correlations between intra- and extracellular domains
- Confirmatory (or novel) classifications of GPCRs based on their dynamic characteristics

Methodology

•Computational, Simulation, and Visual Rendering Programing including:

- •Visual Molecular Dynamics (VMD) [2]
  - Protein Visualizations
- •Normal Mode Analysis (WebNMA) [3]
  - Dynamic Cross Correlation Matrices (DCCM)

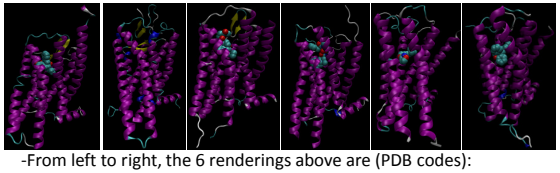
- Crystal structures of 6 GPCRs were obtained from PDB.org and visualized through VMD software. Any proteins containing chimeric domains where truncated to display only GPCR associated residues.

-WebNMA was used to obtain DCCMs of the 6 truncated proteins under study.

- Delta Opioid GPCR (4N6H)
- Opsin GPCR (4J4Q)
- Mu Opioid GPCR (4DKL)
- Serotonin GPCR (4IB4)
- M2 Muscarinic GPCR (4MQS)
- H1 Histamine GPCR (3RZE)

-DCCMs were used in tandem with VMD to observe and highlight the correlated motions within the proteins.

Results (VMD Rederings)



-From left to right, the 6 renderings above are (PDB codes):
4N6H, 4J4Q, 4DKL, 4IB4, 4MQS, 3RZE

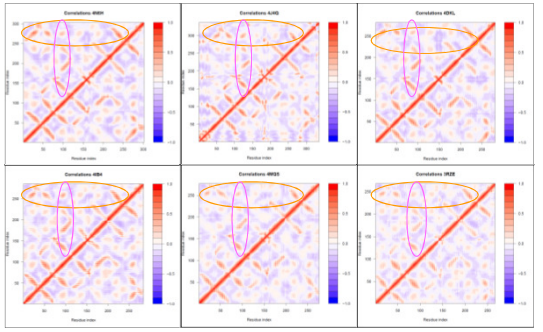
-A structural similarity can be observed across the 6 proteins in question. Their functions also include the binding of a ligand (or cofactor in the instance of 4I4Q) and the elicitation of an intracellular response.

Results (DCCM Analysis)

-DCCMs were obtained using WebNMA website using PDB files and sequence identities were obtained using BLAST (table below).

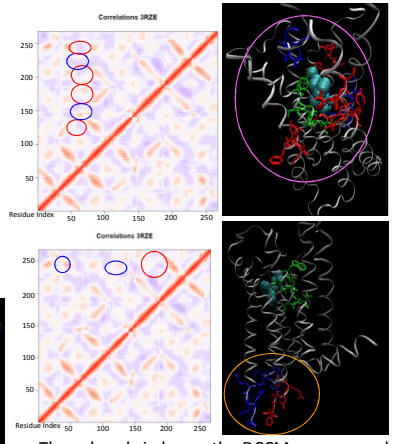
Seq IDs	3RZE	4DKL	4IB4	4J4Q	4MQS	4N6H
3RZE	1.00	0.31	0.32	0.19	0.36	0.29
4DKL	XXXXXXX	1.00	0.27	0.22	0.27	0.70
4IB4	XXXXXXX	XXXXXXX	1.00	0.20	0.30	0.26
4J4Q	XXXXXXX	XXXXXXX	XXXXXXX	1.00	0.23	0.20
4MQS	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	1.00	0.27
4N6H	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	XXXXXXX	1.00

-The correlations seen in each protein share a similar patterns, indicating similar dynamical movements within the protein.



-Red cross-correlations indicate correlated motion (motion in the same direction).
-Blue cross-correlations indicate anti-correlated motions (motion in the opposite direction).
-Strong similarities found in all matrices are encircled in orange and pink ovals.

DCCM Analysis to VMD Visualization



- Two DCCMs and two VMD renderings are shown to the left. The top two figures show the DCCM and VMD focusing on the residues surrounding the ligand binding site. The bottom two figures show the DCCM and VMD rending, highlighting the intracellular residues with respects to the active site. Green, red and blue colors correspond to: the residues in question, residues shown to have correlated motion, and residues with anti-correlated motion respectively.
- The colored circles on the DCCMs correspond to the colored residues in the VMD renderings.
- Correlated motion of the active site and intracellular residues could be indicative of residues responsible for G-protein interactions.
- Despite structural and sequential differences, dynamics are very similar.

Conclusions and Future Directives

- A very similar mobility patterns were observed in the six GPCRs studied despite sequential differences in amino acids; correlated motions exist between the active site and intracellular residues.
- Further investigations utilizing more computational intensive methods and employing experimental techniques could provide better insight into the dynamic-structure-function relationships in GPCRs.

References:
1) Attwood, T K., and J B. Findlay. "Fingerprinting G-protein-coupled receptors." *Protein Engineering* 7.2 (1994). 1 Apr. 2014.
2) Humphrey, W., Dalke, A. and Schulten, K., "VMD - Visual Molecular Dynamics", J. Mol. Graphics, 1996, vol. 14, pp. 33-38.
http://www.ks.uiuc.edu/Research/vmd/
3) Hollup SM, Salensminde G, Reuter N. WEBnm@: a web application for normal mode analysis of proteins BMC Bioinformatics. 2005 Mar 11;6(1):52

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