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## Computational analysis and NGS pipeline for Human GRK2 in complex with G-beta-gamma in cardiovascular disease with Biopython

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### ABSTRACT

This study aims outlines a comprehensive methodology for studying Human GRK2 in complex with G-beta-gamma in cardiovascular disease. g protein-coupled receptor kinases (GRKs) play crucial roles in receptor desensitization. changes in GRK expression have emerged as prominent factors in cardiovascular diseases. Study aims to explore the evolving understanding of GRKs in the context of cardiovascular diseases. The 3D structure of 3KRX is translated into its amino acid sequence using the Molecular Modeling Database (MMDB), while the quality of the protein model is evaluated using ERRAT. Molecular docking studies are performed using CB Dock server to predict potential binding sites for drug discovery. Structural classification is accomplished using the CATH database, categorizing the protein structure into distinct classes and domains, while exploring Gene Ontology terms associated with GRK2 provides insights into its biological functions. Pathway analysis using KEGG tools explores potential interactions and pathways relevant to cardiovascular disease. This comprehensive approach ensures a thorough investigation of Human GRK2 in complex with G-beta-gamma, offering insights into its structural and functional roles in cardiovascular disease research.

Keywords: Drug discovery, Human GRK2, Structure Analysis, Cardiovascular disease, Biopython, NGS

## INTRODUCTION

Cardiovascular diseases (CVDs) pose a formidable worldwide health contest, demanding a nuanced understanding of the molecular intricacies governing their pathophysiology (Samantha L. *et al.*, 2023). Within the intricate signaling networks regulating physiological processes, G protein-coupled receptors (GPCRs) emerge as key players, responding to ligand binding and activating heterotrimeric G proteins (William & Brian, 2018). To fine-tune GPCR responsiveness and prevent sustained activation, G protein-coupled receptor kinases (GRKs), in collaboration with  $\beta$ -arrestins, play crucial roles in receptor desensitization (Pierre-Yves *et al.*, 2017). G protein-coupled receptors (GPCRs) constitute the largest family of membrane receptors, orchestrating a myriad of physiological processes by transducing extracellular signals into intracellular responses (P.-Y. Jean-Charles *et al.*, 2016). The fundamental mechanism involves ligand binding to GPCRs, triggering the activation of associated heterotrimeric G proteins and subsequent intracellular signaling pathways. To prevent hyperactivation of GPCR second-messenger cascades, G protein-coupled receptor kinases (GRKs), in collaboration with  $\beta$ -arrestins, play a pivotal role in desensitizing receptor signal transduction (Erin *et al.*, 2010).

In the realm of cardiovascular pathophysiology, changes in GRK expression have emerged as prominent factors in various conditions, including heart failure, myocardial infarction, hypertension, and cardiac hypertrophy (Packiriswamy & Parameswaran, 2015) (Claudio *et al.*, 2022). The heightened levels and activity of G protein-coupled receptor kinase 2 (GRK2) in these pathological situations contribute to disease progression through multifaceted mechanisms. Additionally, GRK2 has been implicated in interconnected conditions such as obesity, insulin resistance, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD) (Cristina Murga, 2019). Consequently, the intensive exploration of GRKs as potential diagnostic markers and therapeutic targets holds promise for addressing these complex and interrelated health challenges (Jessica *et al.*, 2019).

This study aims to explore the evolving understanding of GRKs, with a focus on the central signaling node, GRK2, in the context of cardiovascular diseases. By unraveling its roles in modulating GPCRs and participating in diverse cellular signaling pathways, we seek to underscore the potential of targeting GRK2 as both a diagnostic marker and a therapeutic avenue. As we delve into the complexities of GPCR signaling mechanisms, including redox signaling, and the conservation of structural and biochemical aspects, we aim to illuminate how GRKs contribute to the selective and specific regulation of biological processes.

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In the relentless pursuit of novel insights into cardiovascular diseases, the amalgamation of computational analysis and cutting-edge Next-Generation Sequencing (NGS) technologies has emerged as an indispensable tool (Mrinmoy *et al.*, 2018). The realm of computational analysis not only facilitates the integration of vast datasets but also enables the discernment of key molecular players and intricate signaling pathways implicated in cardiovascular diseases (Prashant *et al.*, 2021). The utilization of Biopython, a specialized library for computational biology, further enhances analytical capabilities by providing a versatile set of tools for the manipulation and analysis of biological data (Vinita & Uma, 2023). Through the application of Biopython, researchers can delve into genomic sequences, perform sequence alignments, and extract meaningful insights from large-scale biological datasets, thereby refining our understanding of the molecular underpinnings of cardiovascular pathologies (Uma & Kartik, 2023).

NGS facilitates comprehensive genomic and transcriptomic profiling, unraveling intricate genetic variations and expression patterns associated with cardiovascular diseases. This wealth of genomic information not only aids in deciphering the genetic basis of cardiovascular pathologies but also serves as a foundation for identifying potential therapeutic targets (Robert *et al.*, 2018). The synergy between experimental findings and computational approaches extends beyond data analysis to the realm of molecular docking studies. Molecular docking, a computational technique, allows researchers to predict the preferred orientation of one molecule to another when bound together (Priya & Uma, 2024). Applying molecular docking to the study of G protein-coupled receptor kinases 2 (GRK2) provides valuable insights into their interactions with ligands, substrates, and potential inhibitors. This approach facilitates the identification of compounds that may modulate GRK activity, offering prospects for the development of targeted therapeutic interventions in cardiovascular diseases (Helen *et al.*, 2018).

The integration of Biopython, molecular docking studies, and NGS technologies represents a sophisticated approach to cardiovascular research, offering a holistic understanding of the molecular intricacies underlying GRK-mediated signaling. This multidimensional strategy not only refines our comprehension of the complexities associated with cardiovascular diseases but also opens new avenues for the development of precision medicine strategies. As technology advances and these computational tools become more sophisticated, the synergy between experimental and computational methodologies is poised to accelerate breakthroughs in

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cardiovascular research, fostering a new era of targeted therapeutic interventions and personalized medicine (Rajat Mittal *et al.*, 2016).

## **METHODOLOGY**

To comprehensively study Human GRK2 in complex with G-beta-gamma (3KRX) in cardiovascular disease, an elaborate methodology has been formulated, integrating a variety of tools and databases. Initially, the process begins with retrieving and preparing the biological sample by accessing the relevant PDB entry for the 3KRX protein structure, facilitating the acquisition of its 3D coordinates. Subsequent steps involve analyzing E-values and sequence similarity through BLAST searches across relevant databases to reveal related proteins and evolutionary connections. Utilizing RasMol and PyMOL, structural examinations are conducted to identify domains, active sites, and assess structural differences through RMSD calculations. Moreover, Python scripts utilizing Biopython are employed for detailed structural analyses such as distance and angle calculations. The 3D structure of 3KRX is translated into its amino acid sequence using the Molecular Modeling Database (MMDB), while the quality of the protein model is evaluated using ERRAT. Molecular docking studies are performed using CB Dock server to predict potential binding sites for drug discovery. Structural classification is accomplished using the CATH database, categorizing the protein structure into distinct classes and domains, while exploring Gene Ontology terms associated with GRK2 provides insights into its biological functions. Pathway analysis using KEGG tools explores potential interactions and pathways relevant to cardiovascular disease. This comprehensive approach ensures a thorough investigation of Human GRK2 in complex with G-beta-gamma, offering insights into its structural and functional roles in cardiovascular disease research.

## **RESULTS**

### **SAMPLE**

#### **Chain A, Beta-adrenergic receptor kinase 1**

PDB: 3KRX\_A

>pdb|3KRX|A Chain A, Beta-adrenergic receptor kinase 1

```
ADLEAVLADVSYLMAMEKSKATPAARASKILLPEPSIRSVMQKYLEDRGEVTFEKI
FSQKLGYLLFRDF
CLNHLEEARPLVEFYEEIKKYEKLETEEERVARSRIFDSYIMKELLACSHPFKSATE
HVQGHLGKKQV
PPDLFQPYIEEICQNLRGDVVFQKFIESDKFTRFCQWKNVELNIHMTMNDFSVHRIIGR
GFGEVYGCRKA
DTGKMYAMKCLDKKRIKMKQGETLALNERIMLSLVSTGDCPFIVCMSYAFHTPKL
SFILDLMNGGDLHY
```

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HLSQHGVFSEADMRFYAAEILGLEHMHNRFVVYRDLKPANILLDEHGHVRISDLGL  
ACDFSKKKPHASV  
GTHGYMAPEVLQKGVAYDSSADWFSLGCMLFKLLRGHSPFRQHKTCDKHEIDRMT  
LTMAVELPDSFSPEL  
RSLLEGLLQRDVENRRLGCLGRGAQEVKESPFRLDWQMVFLQKYPPPLIPRGEVN  
AADAFDIGSFDEE  
DTKGIKLLDSDQELYRNFPLTISERWQQEVAETVFDTINAETDRLEARKKAKNKQLG  
HEEDYALGKDCIM  
HGYMSKMGNPFLTQWQRRYFYLFPNRLEWRGEGEAPQSLLTMEEIQSVEETQIKER  
KCLLLKIRGGKQFI  
LQCDSPELVQWKELRDYREAQQLVQRVPMKNKPRSPVVELSKVPLVQRGSA  
NGL

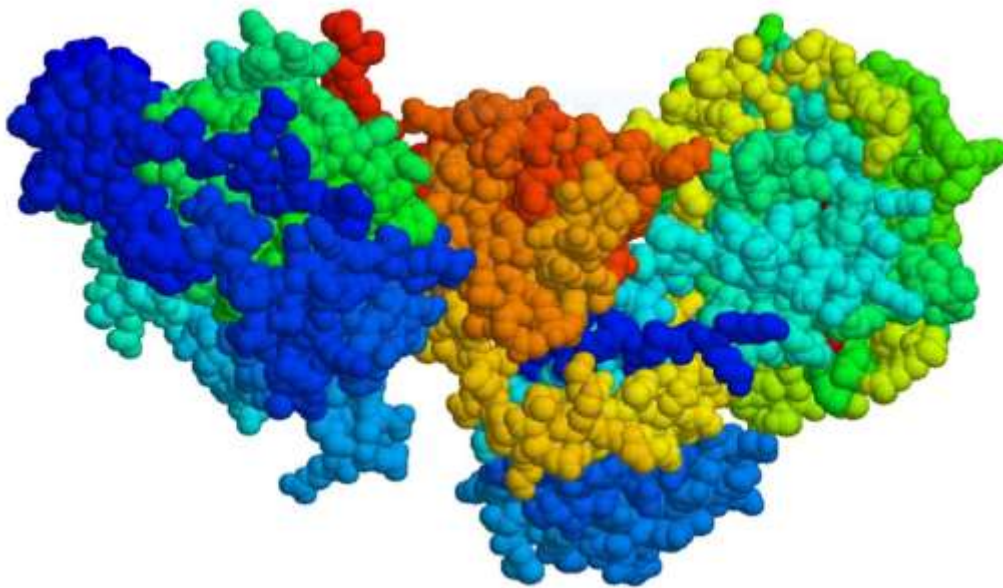


Figure1: Visualization of molecule in space fill representing atoms van der Waals sphere

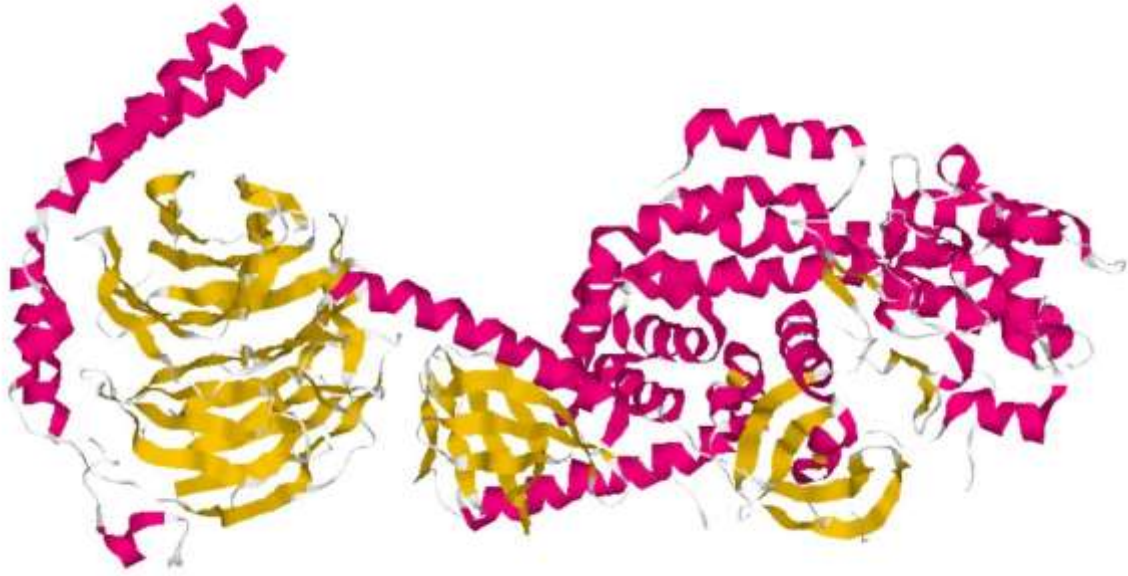


Figure 2: Representation of alpha & beta structure in 3KRX (alpha helix in magenta & beta helix in yellow)

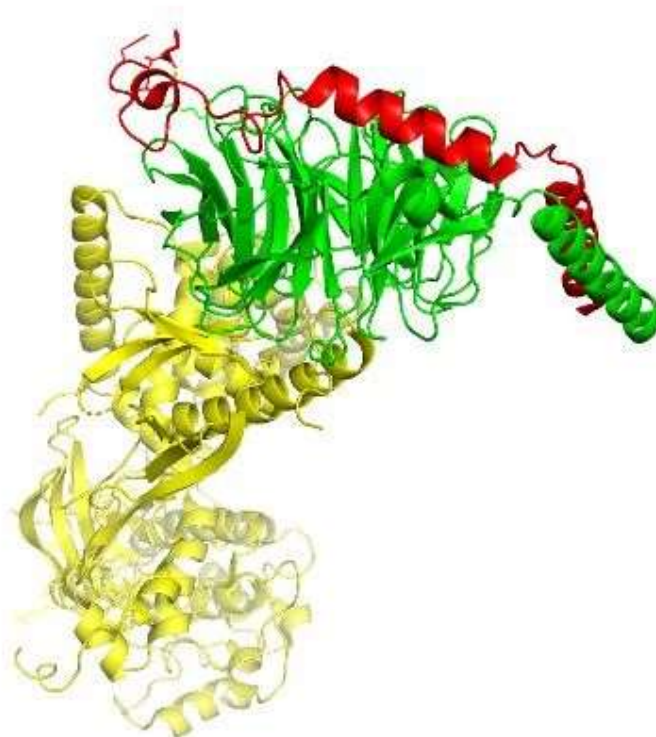


Figure 3: Representation of chain A-yellow, chain B-green, chain c-red in biological sample (representation of domain) in pymol

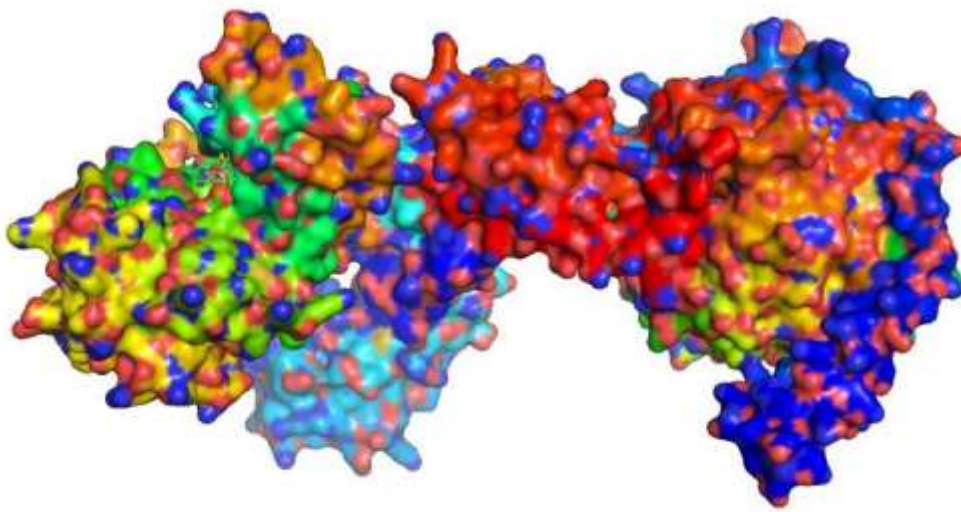


Figure 4: Surface form representation of 3KRX in PyMOL

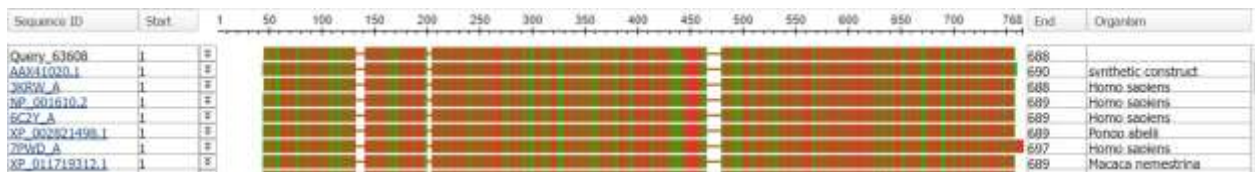


Figure 5: Membrane preference (red-low membrane, green-high) new

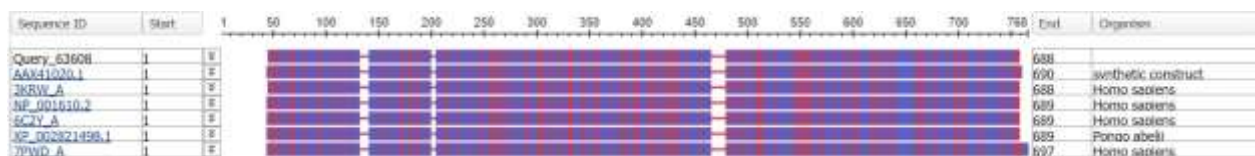


Figure 6: Size of A.A in COBALT (red -smaller side chain blue -larger side chain)

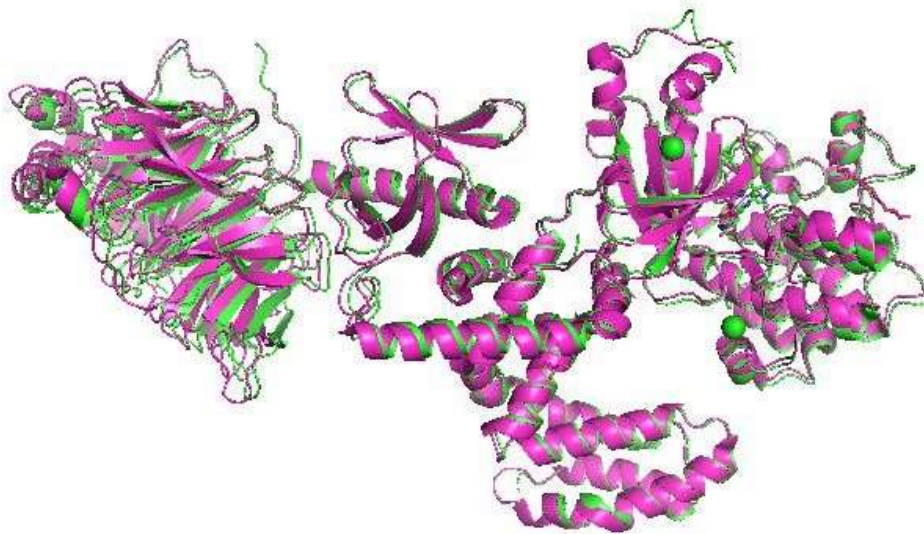


Figure 7: RMSD value score calculation(3krx,7pwd) square root of the mean showing the distance between the matched atoms determining the RMSD values. The analysis observed by python command-based work RMSD values is 0.675 (7096 to 7096 atoms) i.e.; closer to 0 the

#### MOLECULAR DOCKING BY WEB SERVER CB-Dock (captopril)

**ChainA:**LYS663

**Chain B:** TYR59 ALA60 MET61 HIS62 THR102 CYS103 SER147 CYS148 ARG150 MET188 SER189 LEU190 SER191 LEU192 ALA193 PRO194 ASN230 ALA231 ILE232 CYS233 PHE234 PHE235 PRO236 THR274 SER275 VAL276 SER277 PHE278 SER279 LYS280 SER316 CYS317 LEU318 GLY319 VAL320 THR321 ASP322

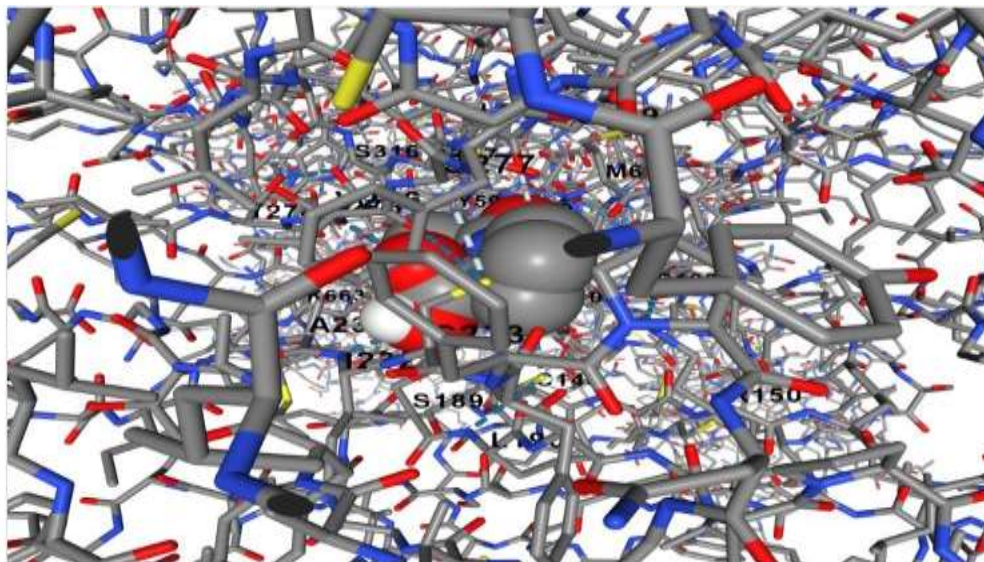


Figure 8: Molecular docking analysis in protein-ligand interaction with docking score of -5.6  
**MOLECULAR DOCKING BY WEB SERVER CB-Dock (mexiletine)**

**Chain A:** ILE197 GLY198 ARG199 VAL205 LYS215 MET216 TYR217 ALA218 LYS220 VAL255 LEU271 ASP272 LEU273 MET274 ASN275 GLY276 GLY277 ASP278 ALA321 ASN322 LEU324 ASP326 GLU327 HIS328 ARG332 SER334 ASP335 ARG516

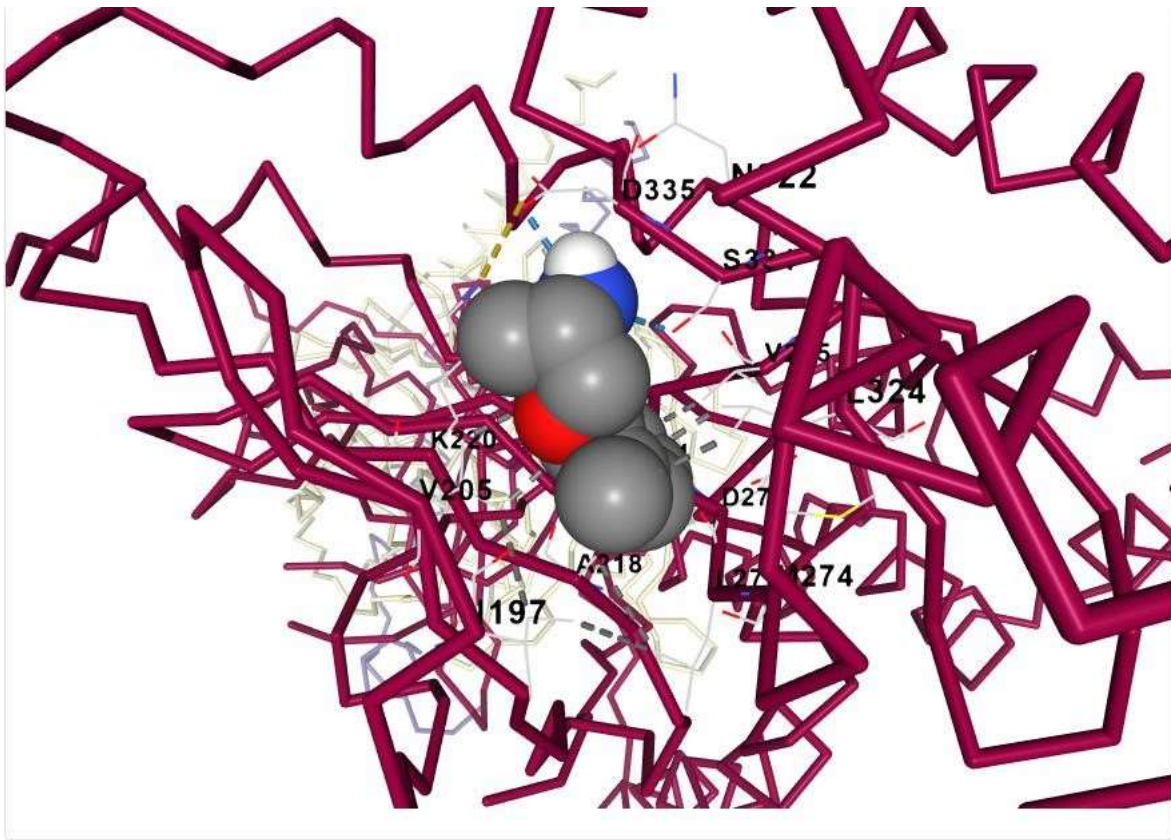


Figure 9: Protein-Ligand interaction with score -5.7 with mexiletine ligand with CB-Dock showing very good binding and considered as the cure drug for human cardiovascular biological sample.

S.No.	Drug Name	Vina Score	Centre			Docking Size		
			x	y	z	x	y	z
1.	Captopril	-5.7	65	38	112	18	35	18
2.	mexiletine	-5.6	33	1	51	18	18	18

**Table:** Representing the Binding Score of the Effective drugs.

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```
!drug-db show-drug Mexiletine
```

Scaling factors for drug  
Mexiletine and FPC 1

Name	Value
scale_drug_INa	0.9991
scale_drug_INaL	0.7491
scale_drug_Ito	1.0
scale_drug_ICaL	0.904
scale_drug_IKr	0.9938
scale_drug_IKs	1.0
scale_drug_IK1	1.0
scale_drug_IKb	1.0
scale_drug_INab	1.0
scale_drug_ICab	1.0
scale_drug_IpCa	1.0
scale_drug_Isacns	1.0
scale_drug_Isack	1.0

Figure 10: DRUG DISCOVERY USING BIOPYTHON CODE TO FETCHING DRUG.

Scaling factors for drug Mexiletine and FPC 1

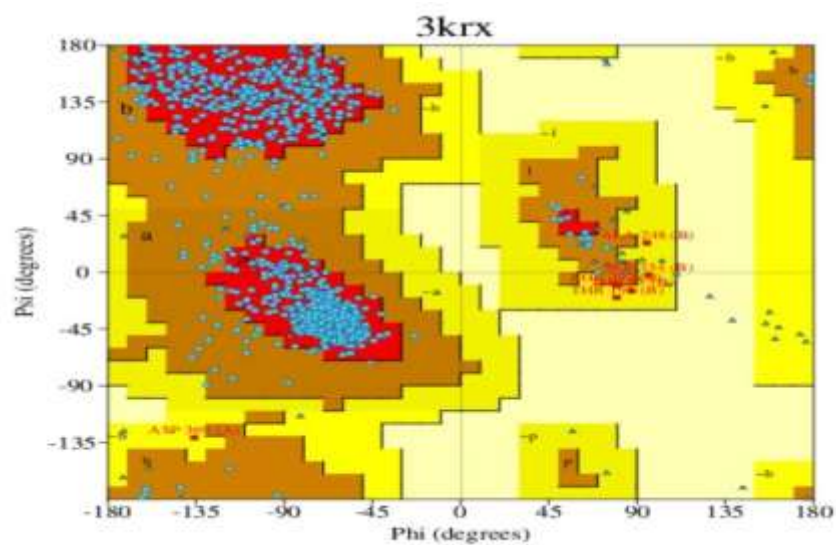


Figure 11: Ramachandran plot generated by PROCHECK validation server showing the analysis of result

## Side-chain parameters

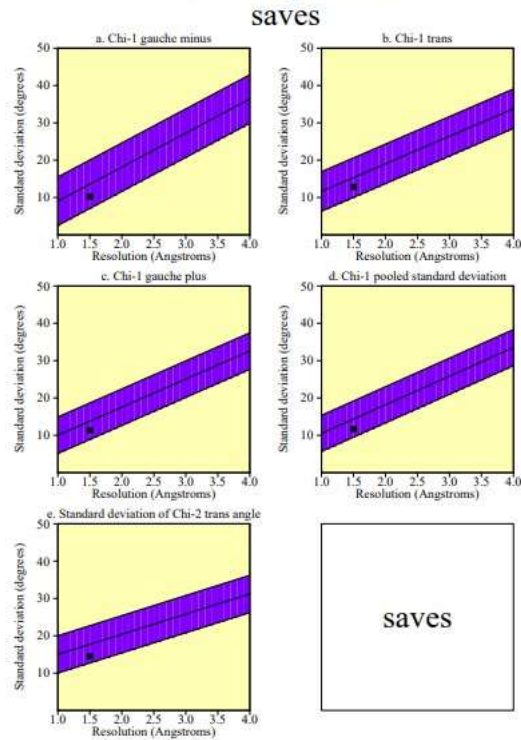


Figure 12: SIDE CHAIN RESULT IN PROCHECK.

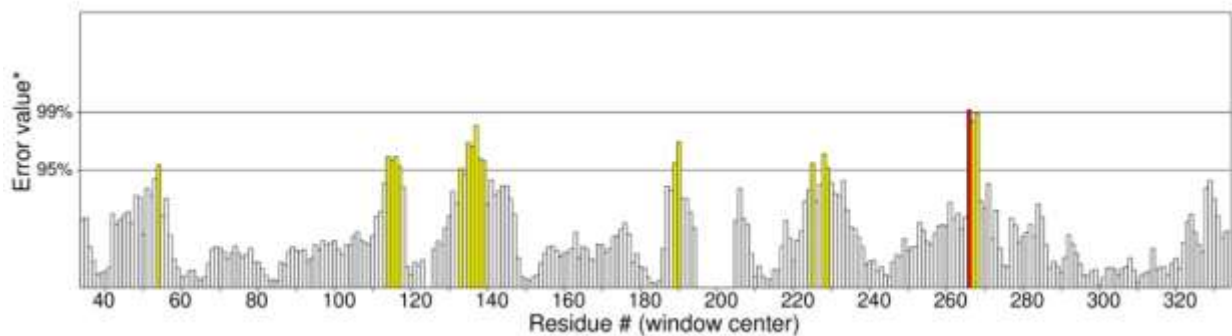


Figure 13: Structure Validation in ERRATE chain A with Overall quality factor: 91.483

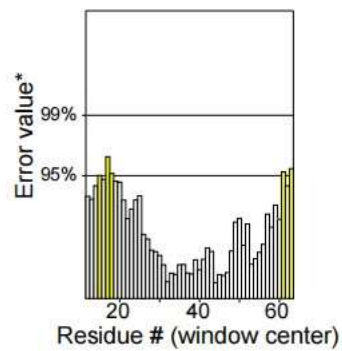


Figure 14: Chian G final model (Errate)

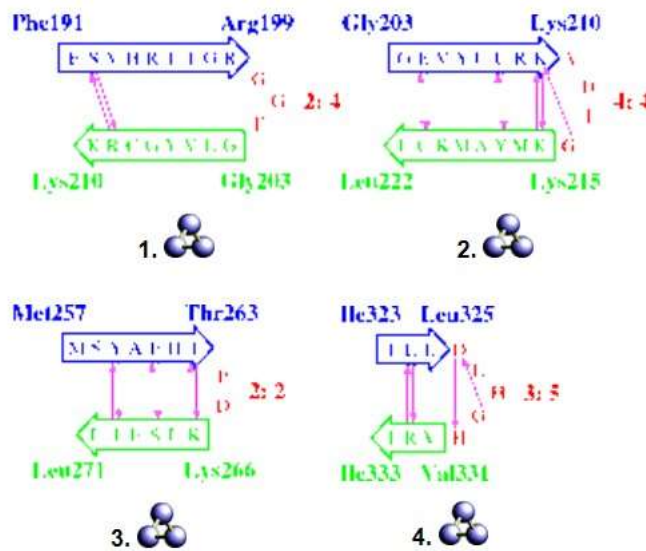


Figure 15: 9beta hairpins

No.	Strand 1			Strand 2			Hairpin class
	Start	End	Length	Start	End	Length	
* 1.	Phe191	Arg199	9	Gly203	Lys210	8	2:4
* 2.	Gly203	Lys210	8	Lys215	Leu222	8	4:4
* 3.	Met257	Thr263	7	Lys266	Leu271	6	2:2
* 4.	Ile323	Leu325	3	Val331	Ile333	3	3:5
5.	Met561	Lys567	7	Gln577	Phe584	8	0:0
6.	Gln577	Phe584	8	Arg587	Arg591	5	2:2
7.	Arg587	Arg591	5	Ser599	Thr602	4	8:8
8.	Ile606	Gln613	8	Lys618	Ile624	7	2:4
9.	Lys618	Ile624	7	Gln629	Leu632	4	3:5 IG

Figure 16: table of 9 beta hairpins PDB web server

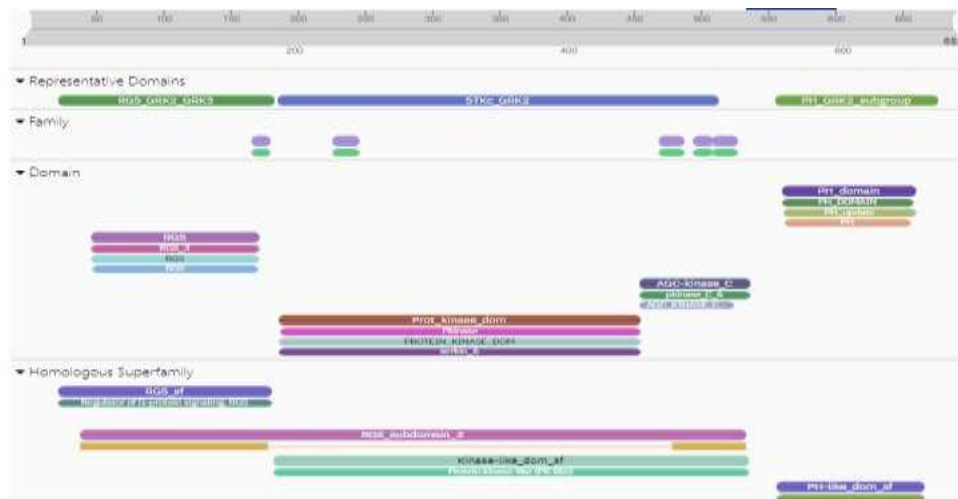


Figure 17: Interproscan provides an integrative classification of protein sequences into families, and identifies functionally important domains and conserved sites

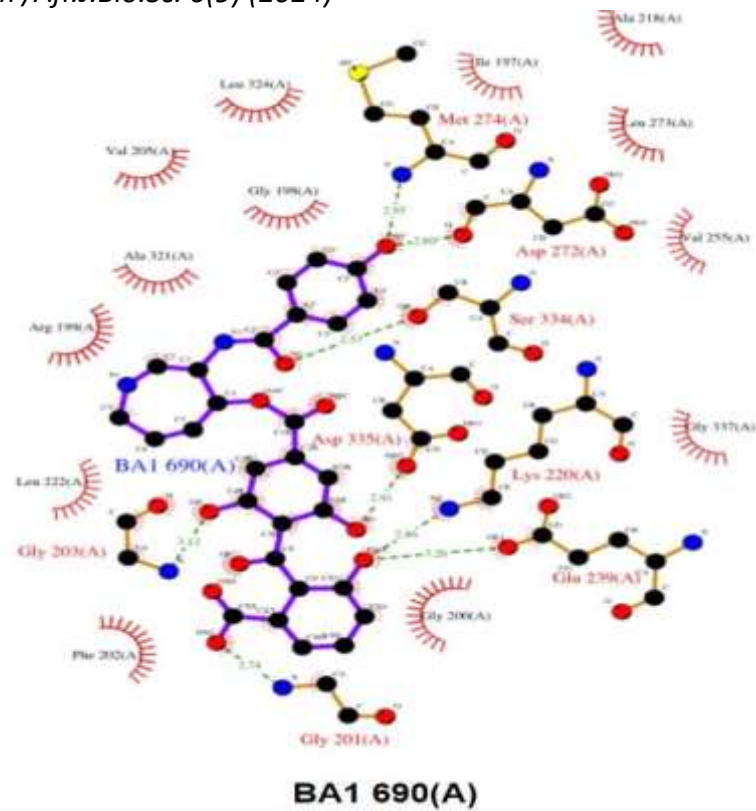


Figure 18: LIGPLOT of interactions involving ligand BA1

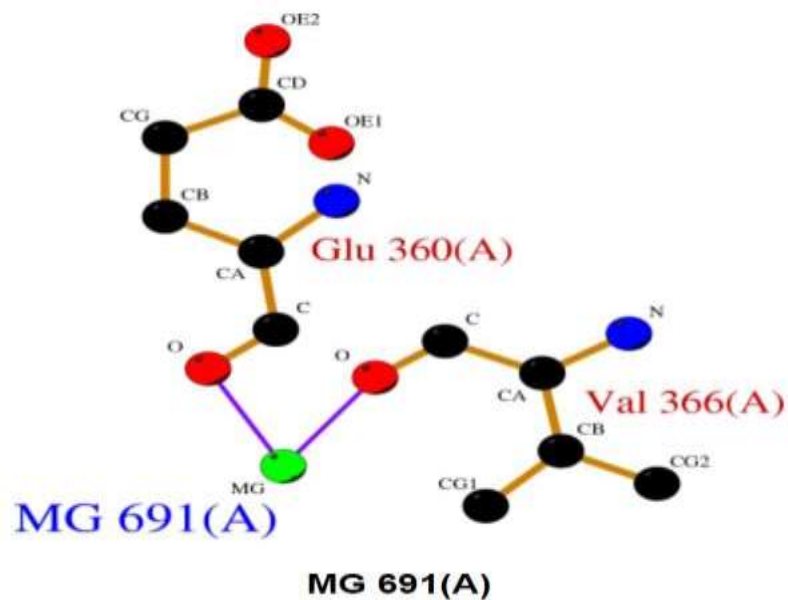
**Metal ion MG - Magnesium ion****LIGPLOT of interactions involving metal MG**

Figure 19: MG (metal) interaction with Protein sample

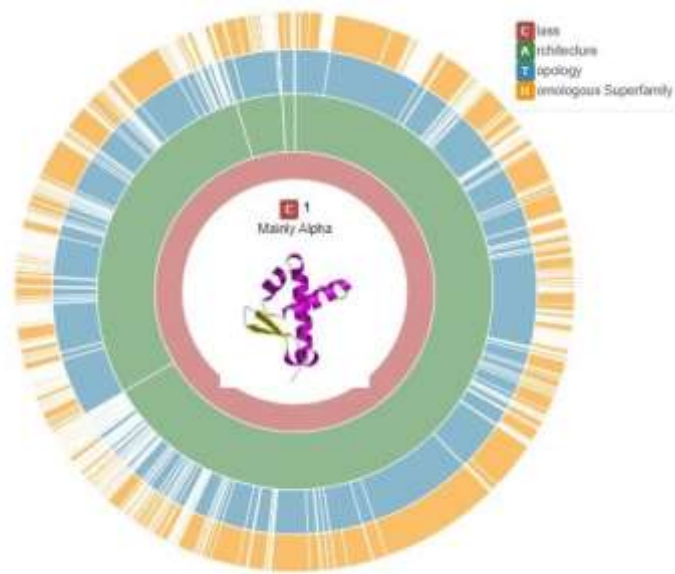
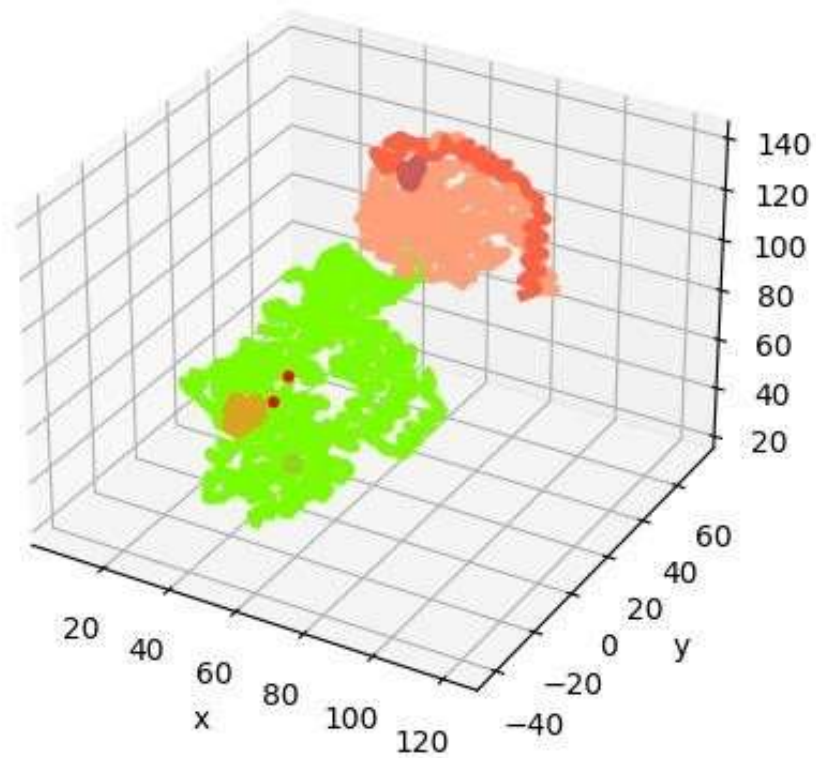


Figure 20: Class identification of 3KRX IN CATH SERVER

CATH Classification		
Level	CATH Code	Description
	<b>1</b>	Mainly Alpha
	<b>1.10</b>	Orthogonal Bundle
	<b>1.10.287</b>	Helix Hairpins
	<b><u>1.10.287.1270</u></b>	

Figure 21: Table of Hierarchical Classification of 3KRX



**Figure 22:** Structure Visualization of the protein (3KRX) with the help of Bio.PDB in BioPython.

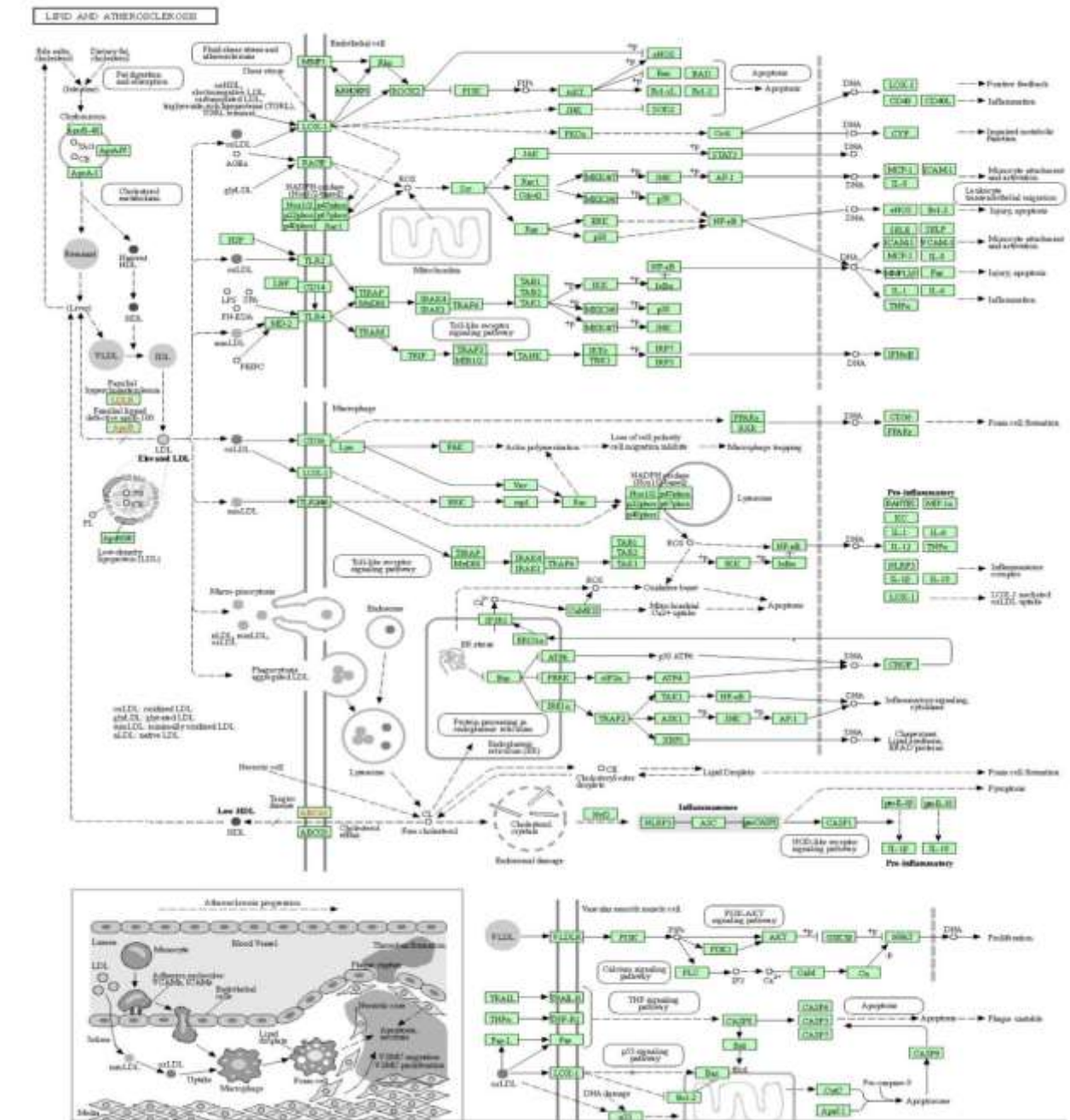


Figure 23: Pathway Analysis

CONCLUSION

In conclusion, our study employed a comprehensive array of computational tools and techniques to analyze various aspects of protein structure and function in the context of cardiovascular research. Utilizing a computational tool, we visualized the protein structure of Human GRK2 in complex with G-beta-gamma in cardiovascular disease with PDB code 3KRX, highlighting key features such as atom representation, secondary structure elements, and active sites. The representation of different protein chains in biological samples provided

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insights into domain organization, with color coding indicating chain identity. Furthermore, the membrane preference analysis shed light on potential interactions with lipid bilayers. Additionally, our investigation involved the assessment of amino acid size preferences using COBALT, revealing variations in chain size within the protein structure. Sequence similarity analysis using BLAST uncovered conserved regions across related protein sequences, enhancing our understanding of evolutionary relationships. RMSD calculations offered valuable insights into the structural deviations between protein models, aiding in the evaluation of structural integrity.

Furthermore, molecular docking analysis provided valuable insights into protein-ligand interactions, potentially informing drug discovery efforts. Ramachandran plot analysis via the structural validation server offered a comprehensive assessment of protein structure quality, aiding in the identification of structural irregularities. LIGPLOT analysis elucidated the interactions involving ligands, providing insights into molecular recognition events. The identification of metal interactions with protein samples further enriched our understanding of protein function and stability. Class identification through the CATH server facilitated the categorization of protein structures based on their structural features.

Overall, our integrative approach, combining computational analysis, molecular modeling, and bioinformatics tools, yielded valuable insights into the structure, function, and evolutionary relationships of proteins relevant to cardiovascular diseases. These findings have implications for the development of targeted therapeutic interventions and further research in the field.

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