PONDICHERRY UNIVERSITY
PUDUCHERRY-605014

EXECUTIVE SUMMARY OF FINAL REPORT OF THE WORK DONE ON THE PROJECT

1.	Title of the Project	Structure of Q-beta replicase in different ionic strengths using single particle analysis
2.	Name & Address of the Principal Investigator	Dr. A. Murali Center for Bioinformatics, Pondicherry University, Puducherry-605014.
3.	Name & Address of the Institution	Center for Bioinformatics, Pondicherry
4.	UGC approval letter no. and date	F. No. 41-665/2012 (SR), dated 23.7.12
5.	Date of Implementation	27/08/2012
6.	Tenure of the project	3 yrs
7.	Total Grant Allocated	13,37,542/-
8.	Total Grants Received	12,33,568/-
9.	Final Expenditure	10,10,839/-
10.	Objectives of the Project	1) Molecular Modeling studies of Qbeta replicase subunits with the help of TEM and *in silico* methods 2) Comparison of the conformational differences of T7 RNA Polymerase (T7RNAP) at different pH conditions using Transmission Electron Microscope (TEM) and Single Particle Analysis (SPA) and *in silico* tools. 3) Finding inhibitors for T7RNAP. 4) Structural investigation of LGP2 (Laboratory of Genetics and Physiology 2) helicase domain using Single Particle Analysis (SPA) data and *in silico* tools.
11.	Whether objectives were achieved (give details)	Yes
12.	Achievements from the project	• The polymerase of Qbeta replicase was modeled including its constituting sub-domains. These models were compared with the density maps reconstructed from EM images. A manuscript based on these results is in progress. • Another replicase from T7 bacteriophage (T7RNAP) was modeled using homology modeling

		approach and its interactions with its known inhibitors were analyzed to understand the inhibition mechanism. These findings are published in Bioinformatics and Biology Insights. • The pH dependence of T7RNAP structure and its interaction of T7Lysozyme were studied using *in silico* tools and the results were correlated with EM observations. These results were published in Scientific Reports and BMC: Structural Biology. • Investigated the structural details of LGP2 (Laboratory of Genetics and Physiology 2) helicase domain. LGP2, a member of Retinoic acid Inducible Gene–I like receptors (RLR), is one of the essential protein that induce antiviral response against many RNA viruses. The findings are published in International Journal of Biochemistry and Biophysics. • The project assistant (Mr. Subhomoi Borkotoky) was trained in these techniques. Based on these results he submitted his Ph.D. thesis and expected to receive his Ph.D. degree soon.
13.	Summary of the findings (in 500 words)	The individual and full length models of Qbeta replicase subunits were modeled through in silico tools. The model structures were validated by fitting them with their respective density maps obtained from transmission electron microscopy results. We have also analyzed the pH induced conformational change of both T7RNAP and transcriptional inhibitor T7 lysozyme with molecular dynamics approach. Further, we have also analyzed how these conformational changes effect on the interactions on T7RNAP and T7 lysozyme with molecular docking approach. Upon docking the individual representatives of T7L and T7RNAP at different pH strengths, it was observed that the T7L interacts with T7RNAP more strongly at both pH 5 and pH 7.9 rather than neutral pH with pH 7.9 representative

		having a higher KDa value. Hence, it can be proposed that the structural changes observed in both pH 5 and pH 7.9 in T7L as well as T7RNAP are favourable for both proteins. As an experimental support to the pH dependent models of T7RNAP we used single particle analysis with TEM data at pH 5 and pH 7.9 maintained in simulation studies. We have also proposed a possible mode of inhibition of T7RNAP by heparin. In addition to the above work we also investigated the details of LGP2 (Laboratory of Genetics and Physiology 2) helicase domain. LGP2, a member of Retinoic acid Inducible Gene–I like receptors (RLR), is one of the essential protein that induce antiviral response against many RNA viruses. The LGP2 shares a considerable similarity to the crystal structure of Hef Helicase Domain, which is involved in RNA binding. Our study provides a more detailed insight into the LGP2 RNA binding scenario. Though the crystal structure of CTD bound with RNA and the NMR structure without RNA were solved, there is no full-length structure available for LGP2, and information regarding the active site of LGP2 is also limited. In this study we have modeled the full-length structure of LGP2 through homology modeling approach. We have also predicted the active site residues for LGP2 with in-silico tool, which was supported by the sequence analysis of Hef helicase domain.
14.	Contribution to the society (give details)	The study of bacteriophage has been of immense importance from understanding biology of virus to phage therapy. Many studies demonstrates that phages as natural and self-amplifying antibacterial drugs could be used to safely and effectively treat or prevent many common human diseases of bacterial etiology. Hence, it is expected that the studies conducted on the two important proteins from the bacteriophage Qbeta and T7 phage, Qbeta replicase and T7 RNA polymerase, will enhance the existing knowledge on bacteriophage biology. The

		function of LGP2 has been found to be more relevant to the field of innate antiviral immunity. LGP2 has been found to be essential for producing effective antiviral responses against many viruses that are recognized by RIG-I and MDA5. This knowledge will help in the endeavors of phage research or antiviral research to help the society.
15.	Whether any Ph.D. enrolled/produced out of the project	Mr. Subhomoi Borkotoky - Project fellow appointed in this project is enrolled for PhD at Centre for Bioinformatics, Pondicherry University and awarded Ph.D. in 2017.
16.	No. of publications out of the project	5

Principal Investigator

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