I dedicate this thesis to my beloved parents (Maa and Deuta), who have been my source of inspiration and my constant pillars of strength. They have been always providing me with their emotional, spiritual, moral, and financial support……

To my wonderful family……

Declaration

I hereby declare that the thesis entitled ***"Computational Investigations on p53-MDM2 Interaction and its Inhibition: a Significant Step in Cancer Therapy"*** has been submitted to Tezpur University in the Department of Molecular Biology and Biotechnology under the School of Sciences for partial fulfillment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology.
I am the sole author of this thesis. This is a true copy of an original work carried out by me including any required final revisions, as accepted by my examiners.

Further, I declare that no part of this work has been reproduced elsewhere for award of any other degree.

Date: **Pundarikaksha Das**

Place: Tezpur University, Tezpur **Registration No.: TZ143774 of 2014**

TEZPUR UNIVERSITY
(A Central University established by an Act of Parliament)
DEPARTMENT OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY
Tezpur-784 028, Assam, India

Dr. Venkata Satish Kumar Mattaparthi
Assistant Professor
E-mail: venakata@tezu.ernet.in

Ph.no. :+91-3712-275443 (O)
+91-8811806866 (M)
Fax :+91-3712-267005/6

CERTIFICATE OF THE PRINCIPAL SUPERVISOR

This is to certify that the thesis entitled **"*Computational Investigations on p53-MDM2 Interaction and its Inhibition: a Significant Step in Cancer Therapy*"** submitted to the School of Sciences, Tezpur University in partial fulfillment for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology is a record of original research work carried out by **Mr. Pundarikaksha Das** under my personal supervision and guidance.

All helps received by her from various sources have been duly acknowledged. No part of this thesis has been reproduced elsewhere for award of any other degree.

Date:

Place: Tezpur University, Tezpur

(Venkata Satish Kumar Mattaparthi)
Supervisor

TEZPUR UNIVERSITY
(A Central University established by an Act of Parliament)
Tezpur-784 028, Assam, India

CERTIFICATE OF THE EXTERNAL EXAMINER

AND ODEC

This is to certify that the thesis entitled **"*Computational Investigations on p53-MDM2 Interaction and its Inhibition: a Significant Step in Cancer Therapy*"** submitted by Mr. Pundarikaksha Das to Tezpur University in the Department of Molecular Biology and Biotechnology under the School of Sciences in partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy in Molecular Biology and Biotechnology has been examined by us on …… and found to be satisfactory.

The committee recommends for the award of the degree of Doctor of Philosophy.

Signature of:

Principal Supervisor **External Examiner**

Date: **Date:**

Acknowledgements

I would not have been able to complete this degree without the support of numerous people, for which I would like to acknowledge their support. First, I would like to express my sincerest gratitude towards my supervisor Dr. Venkata Satish Kumar Mattaparthi, for taking a chance on me and giving me freedom in my research and for his consistent encouragement that I have received throughout the research work. Under his guidance, I successfully overcame many difficulties and learned a lot. He always kept faith in me and guided me through the right direction whenever I needed it the most. At the same time, his constructive criticism and constant vigilance inspired me to perform better. My deep gratitude goes to him for all his dedication and steadiness during the writing of the thesis. I whole-heartedly thank him for everything.

I would also like to thank the Head, Department of MBBT, Tezpur University, and my Doctoral Committee members – Prof. S.K. Ray, Prof. M. Mandal, and Dr. R. Mukhopadhyay, Department of MBBT, Tezpur University, for their insight, comments, and valuable suggestions during my Ph.D. tenure.

I would like to acknowledge all other faculty members in the Department of MBBT, Tezpur University for their help and encouragement and the non-teaching staffs of the Department for their technical support. I would like to take this opportunity to thank Tezpur University for providing me with the state of the art infrastructure and facilities for advanced research. I would like to acknowledge the financial support provided by Tezpur

University and DST (INSPIRE Fellowship).

No word would be enough for expressing my gratitude towards lab mates in MOMO Lab: Mary Ba, Himakshi Ba, Airy Ba, Sushmita Di, Priyanka, Dorothy and Chainee, for their immense help and support. Heartfelt thanks to all the project students (Gariyoshi, Navamallika, Pallav, Krishna, Ambika, and Barsha) for their help and support.

I would like to express my gratitude to my batchmates for supporting me in many occasions during this work and for all the time we spent together.

I would like to thank https://quillbot.com/ for helping me out as a good paraphrasing tool as well as a good citation generator tool.

I would like to thank Mr. Dhairya Jain for helping me out during my thesis writing.

Special thanks to my little sister Jupitara.

Above ground, my biggest thanks of all goes to my mother and my father for their fulltime support and unconditional love and for giving me a meaningful life. Love you both. And finally, I acknowledge my best friend, Supriya, who blessed me with a life full of joy and provided me constant support during my Ph.D. journey.

Pundarikaksha Das

List of Figures

List of Figures

List of Figures

List of Figures

List of Figures

List of Tables

List of Tables

List of Tables

List of Abbreviations

Å	Angstrom
ACE	Atomic contact Energy
AD	Acidic Domain
AMBER	Assisted Model Building with Energy Refinement
BFE	Binding free energy
CHARMM	Chemistry at HARvard Macromolecular Mechanics
CID	compound identifier
CircRNA	Circular RNA
CoM	Centre of Mass
CPPTRAJ	A rewrite of PTRAJ in C++
CRPC	Castration-resistant prostate cancer
CTD	C-Terminal Domain
3-D	3-Dimensional
DBD	DNA Binding Domain
DNA	Deoxyribonucleic Acid
EGCG	Epigallocatechin gallate
FF99SB	Force-field 99 Stony Brook
GAFF	General Amber force field
GB	Generalized Born
GBSA	Generalized Born Surface Area
I-TASSER	Iterative Threading ASSembly Refinement
MD	Molecular Dynamics
MDM2	Murine Double Minute 2
MDMX	Murine Double Minute 4
miRNA	MicroRNA
MM	Molecular Mechanics
MSM	Markov State Model
ns	nanosecond
NES	Nuclear Export Signal
NLS	Nuclear Localization Signal
NoLS	Nucleolar Localization Signal
NMR	Nuclear Magnetic Resonance Spectroscopy
NTD	N-Terminal Domain
OD	Oligomerization Domain
p53	Protein 53
ps	picosecond
PaCS	Parallel Cascade Selection
PB	Poisson-Boltzmann
PBC	Periodic boundary conditions
PBSA	Poisson-Boltzmann Surface Area
PDB	Protein Data Bank
PME	Particle Mesh Ewald
PMF	Potential of Mean Force
PPG	Photoremovable Protecting Group
PPI	Protein-protein interaction

List of Abbreviations

PRED	Per-residue energy decomposition
PRR	Proline-Rich Region
PTRAJ	Short for Process TRAJectory
RCSB	Research Collaboratory for Structural Bioinformatics
REG	C-Terminal (Regulatory) Domain
REMD	Replica Exchange Molecular Dynamics
Rg	Radius of Gyration
RING	Really Interesting New Gene
RITA	Reactivating p53 and Inducing Tumor Apoptosis
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SASA	Solvent-accessible surface area
TAD	Transactivation Domain
TET	Tetramerization Domain
TM	Template modeling
TIP3P	Transferable Intermolecular Potential Three-point
UCSF	University of California, San Francisco
UniProt	Universal Protein Resource
US	Umbrella Sampling
VMD	Visual Molecular Dynamics
WHAM	Weighted Histogram Analysis Method

List of Publications

This thesis is partly based on the following original communications:

1. **Das, P.** and Mattaparthi, V.S.K. Computational Investigation on the MDM2-Idasanutlin Interaction Using the Potential of Mean Force Method. *Current Chemical Biology*, 15(3): 262-270, 2021. DOI: http://dx.doi.org/10.2174/2212796815666210716151211

2. **Das, P.** and Mattaparthi, V.S.K. Computational Investigation on the p53–MDM2 Interaction Using the Potential of Mean Force Study. *ACS omega*, 5(15): 8449-8462, 2020. DOI: https://doi.org/10.1021/acsomega.9b03372

3. **Das, P.** and Mattaparthi, V.S.K. Computational investigation on the molecular interactions between MDM2 and its photoactivatable inhibitor. *Biointerface Research in Applied Chemistry*, 9(6): 4671 – 4684, 2019. DOI: https://doi.org/10.33263/BRIAC96.671684

List of Publications

Other publications:

1. Naik, B., Mattaparthi, V.S.K., Gupta, N., Ojha, R., **Das, P.**, Singh, S., Prajapati, V.K., and Prusty, D. Chemical system biology approach to identify multi-targeting FDA inhibitors for treating COVID-19 and associated health complications. *Journal of Biomolecular Structure and Dynamics*, 1-25, 2021. DOI: https://doi.org/10.1080/07391102.2021.1931451

2. Kakati, M., Das, D., **Das, P.**, Sanjeev, A., and Mattaparthi, V.S.K. Effect of ethanol as molecular crowding agent on the conformational dynamics of α-synuclein. *Letters in Applied NanoBioScience*, 9: 779-783, 2020. DOI: https://doi.org/10.33263/LIANBS91.779783

3. Pradhan, S., **Das, P.**, and Mattaparthi, V.S.K. Characterizing the binding interactions between DNA-binding proteins, XPA and XPE: a molecular dynamics approach. *ACS omega*, 3(11) 15442-15454, 2018. DOI: https://doi.org/10.1021/acsomega.8b01793

In addition, this thesis also contains unpublished data.

Conference Proceedings

1. **Das, P.** and Mattaparthi, V. S. K. "*In silico* Investigation on the p53–MDM2 Interaction Using the Potential of Mean Force Study." 8th International Symposium on "Current Trends in Drug Discovery Research; Ageing Associated Metabolic & CNS Disorders" held at CSIR-Central Drug Research Institute, Lucknow, India on 12th-14th March, 2022. (Poster Presentation, Online Mode)

2. **Das, P.** and Mattaparthi, V. S. K. "*In silico* Investigation on the p53–MDM2 Interaction Using the Potential of Mean Force Study." National Seminar on "Advances in Basic and Translational Research in Biology (ABTRiB)" held at Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Assam, India on 11th-12th March, 2022. (Oral Presentation)

3. Pradhan, S., **Das, P.**, and Mattaparthi, V. S. K. "*Characterizing the binding interactions between DNA binding proteins, XPA and XPE: A molecular dynamics approach*." Assam Science Festival 2019, held at Tezpur University, Napaam, Assam, India on 23rd-25th March, 2019, organized by Assam Science, Technology and Environment Council in collaboration with Tezpur University, India. (Poster Presentation)