DECLARATION BY THE STUDENT

I hereby declare that the thesis “*Developing a Reinforcement Learning Model for Somatic Hypermutations using PD1 and Pembrolizumab*” is a bona fide and genuine research work carried out by me, between 23rd December, 2024 and 4th June, 2024 at IBAB, Bengaluru, under the guidance of *Dr. Nithya Ramakrishnan*, Assistant Professor, Information Theory, Algorithms and Machine Learning in Biology and *Prof. Subha Srinivasan*, IBAB Chair, Genomics.

Date:

Place: Anmol Singh

**CERTIFICATE BY THE SUPERVISOR**

This is to certify that the thesis “*Developing a Reinforcement Learning Model for Somatic Hypermutations using PD1 and Pembrolizumab*” represents research work done by *Anmol Singh* in partial fulfillment of the requirements for M.Sc. in Biotechnology and Bioinformatics at IBAB, Bengaluru, under our guidance.

Date:

Place: Dr. Nithya Ramakrishnan

Prof. Subha Srinivasan

**ACKNOWLEDGMENTS**

**(Not more than one page, double spaced)**

In every thesis, the following acknowledgment must be there in addition to your write-up:

**This work was partially supported by the Department of Electronics, IT, BT, and S&T of the Government of Karnataka.**

The opportunity I had with <IBAB>was a great chance for learning and

professional development. Therefore, I consider myself as a very lucky individual as I was

provided with an opportunity to be a part of it. I am also grateful for having a chance to meet

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Summer Research Fellowship Programme of India's Science Academies at National Centre of Cell Science, Pune I express my deepest gratitude and special thanks to my guide <name>,

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I will strive to use gained skills and knowledge in the best possible way, and I will continue to work on their improvement, in order to attain desired career objectives. Hope to continue cooperation with all of you in the future.

At last, I would also like to thank my family, especially my mother and cousins for their support. I thank all of you for your guidance and support.

**ABSTRACT**

**(Max. 300 words, double spaced)**

The abstract should provide the background (rationale and/or objectives of the study),

methods (resources and parameters used the study), results (major outcomes and their brief

interpretation), and conclusion (summarize the results and provide future perspectives, if

appropriate). The abstract can be written with or without the headings shown in bold (i.e., the

abstract can be structured or unstructured).

**Keywords or phrases (Max. 10):**

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1. **INTRODUCTION**
   1. **Somatic Hyper Mutations**

The B cells have the ability to express cell-surface receptors called as immunoglobins (Ig). These Ig consists of two heavy chains and two light chains. These polypeptides are encoded in three Ig loci, the heavy chain (IgH), the κ-light chain (Igκ), and the λ-light chain (Igλ). These loci consist of variable and constant(C) regions. The variable region of heavy chain is composed of variable(V), diversity(D), and joining(J) genes. On the other hand, the light chain only has V and J genes (Odegard and Schatz, 2006).

Our Immune system have a wonderful system to produce Ig of high specific towards any foreign substance, also called as antigen. This ability is the result of somatic recombination of a small set of gene segments, this process is called V(D)J recombination. This process alone is able to produce around 107 different antibody specificities. But the antibodies created by V(D)J recombination only bind to the antigens by modest affinity, there is a need to fine tune the resultant Ig to make it bind to the antigens with high affinity and specific (Papavasiliou and Schatz, 2002).

The diversification of Ig is caused due to two distinct diversification processes, class switch recombination (CSR) where the C region of IgH changes due to recombination of switch (S) regions, and the somatic hyper mutations (SHM) (Odegard and Schatz, 2006).

The SHM introduces point mutations on the variable regions, the antibodies with higher affinity for the antigen will proliferate and survive. With successive cycles of mutations and proliferation of selected B cells, this results in high affinity antibodies. This process is called affinity maturation (Papavasiliou and Schatz, 2002). The mutations in SHM are mainly point mutations, but insertions and deletions are also observed sometimes (Odegard and Schatz, 2006). For my model, I have only taken point mutations into considerations, on the peptide level rather than gene level.

* 1. **Immunotherapy and Pembrolizumab**

Immunotherapy is the ability to utilize a patient’s immune system to target

cancer has resulted in many novel therapeutic ways. But even though these approaches are useful in many cases there are still many challenges in the clinical scenarios.

The tumor-host interactions are heterogenous and based on these interactions the immunotherapy responsiveness can differ. The tumor microenvironment (TME), can affect the immunotherapeutic response and the immune evasion.

There is multiple type of immunotherapies used currently. These are immune checkpoint inhibition (ICI), adoptive cellular therapy (CAR T-cell therapy) and cancer vaccination. Pembrolizumab comes under the ICI, thus let us understand it in more details.

The T cells contain evolutionarily conserved regulatory markers that are like checkpoints to regulate activation of T cells. After the early activation, the T cells upregulate the inhibitory receptor cytotoxic T lymphocyte antigen 4 (CTLA4) and then after programmed cell death 1 (PD-1) which bind to ligands B7-1, B7-2 and PD-L1 or PD-L2. These ligands are presented by tumor cells, myeloid cells, regulatory T cells (Tregs), and antigen-presenting cells (APCs), which reduce the cytotoxic T-cell activation, resulting in immune suppression and tumor growth. But after treatment with ICI, inhibition is released and cancer cells are targeted and destroyed by the primed and activated cytotoxic T cells.

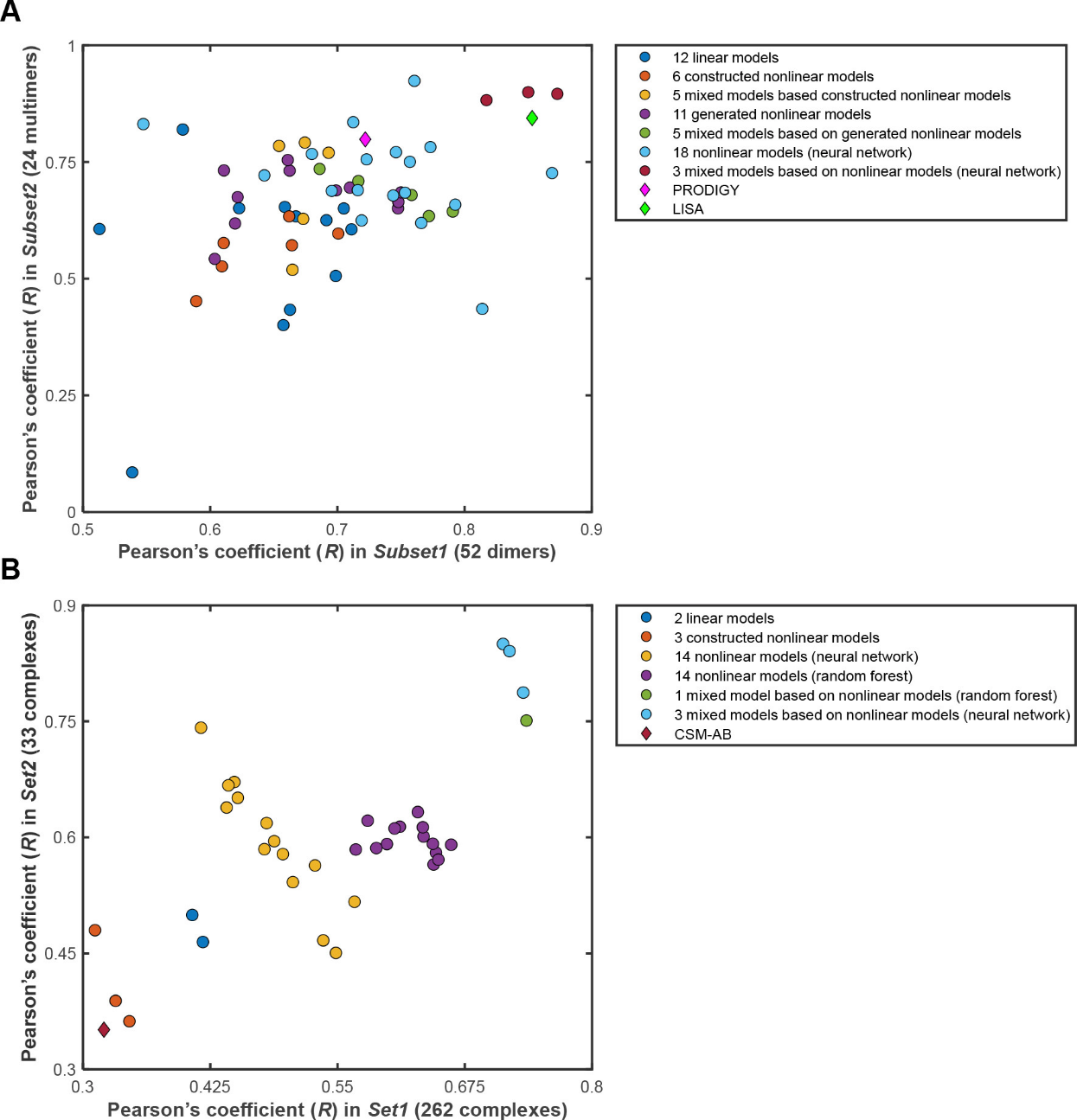
Pembrolizumab (pembro), a IgG4 anti PD1 checkpoint inhibitor antibody, was one of the first FDA-approved therapy for melanoma. Pembro was very successful in the melanoma patients and is still used in ICI therapy. I am using pembrolizumab as a model IgG antibody to perform SHM and possibly find a higher affinity antibody (Peterson et al., 2022).

* 1. **Binding Affinity**

For us to evaluate the antibody and antigen interactions quantitatively and qualitatively and to do that we need to understand the type of interactions which happen between Ab-Ag and their binding affinity.

Binding affinity can be determined experimentally, but it isn’t feasible to do so, thus we use computational methods to estimate binding affinities. To choose which method is best for our use depends on: time taken to estimate binding affinity, accessibility of the method (if it is available via web-server or can be installed on local machines), accuracy and relevance of the method, and the metadata provided by the method. I compared multiple methods including PRODIGY (Xue L et al., 2016), LISA (Raucci R et al.,2018), CSM-AB (Myung Y et al., 2022), AREA-AFFINITY (Yang Y et al, 2023) and DG-Affinity (Yuan Y et al,2023) by literature review and comparing them based on the parameters mentioned before.

PRODIGY uses a linear model consisting of type of inter-molecular non covalent contacts and also non interacting surfaces (NIS) to predict the binding affinity. LISA utilizes a non-linear model to estimate the protein-protein interactions. Both tools considered good for protein-protein interactions (PPI) (Yang Y et al, 2023). They are locally installable and fast. But LISA has not been maintained and not dependable for future. PRODIGY is maintained (<https://github.com/haddocking/prodigy>) and also provides metadata on type of contacts the proteins are making.



**Figure 1**. Performance of different methods used in AREA-AFFINITY along with CSM-AB, PRODIGY and LISA. **A**. For protein-protein interaction methods. **B.** For Ab-Ag models. (Yang Y et al, 2023).

CSM-AB, AREA-AFFINITY and DG-Affinity are web-server based tools and are specifically made for Antibody and antigen interaction. But AREA-AFFINITY and DG-Affinity do not provide an API for easy accessibility. CSM-AB is slow in response and the web-server goes off-service occasionally.

PRODIGY was considered the best for our use case after the assessment of tools.

* 1. **Reinforcement Learning**
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1. **MATERIALS AND METHODS**
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